Department of Imaging and Applied Physics

Centre for Marine Science and Technology

An investigation into active and passive acoustic techniques to study aggregating fish species

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Abstract

Techniques of single- and multi-beam active acoustics and the passive recording of fish vocalisations were employed to evaluate the benefits and limitations of each technique as a method for assessing and monitoring fish aggregations. Five species, Samson fish (*Seriola hippos*), mulloway (*Argyrosomus japonicus*), West Australian dhufish (*Glaucosoma hebraicum*), Bight redfish (*Centroberyx gerrardi*) and pink snapper (*Pagrus auratus*) were investigated on the basis of their abundance, ecological importance and differing behaviour. The primary focus was on *S. hippos,* a large non-vocal schooling fish, and *A. japonicus,* a large vocal fish, with each species forming aggregations for spawning purposes.

Simrad EQ60 single-beam echosounder assessments of mid-water, *S. hippos* aggregations at seven sites west of Rottnest Island illustrated the relative biomass increase, stabilisation and decrease between the months of October and March each year from October 2004 to March 2007. Surveys highlighted the preferred sites for spawning, spatial extents of each aggregation, as well as a decline in aggregation stability at full moon and end of season periods. Regular Department of Fisheries surveys displayed the relative ease with which single-beam techniques could be deployed, used and data analysed to monitor large, comparatively stable, deep (>50 m) aggregations of large swimbladdered fish. Acquired acoustic data illustrated the limitations of single-beam surveys conducted on a mobile school of fish.

RESON 8125 and 7125 multi-beam sonar (MBS) surveys of *S. hippos* at Rottnest Island locations, some conducted simultaneously with the Simrad EQ60 single-beam, illustrated the improved spatial resolution of midwater targets achievable with MBS systems. The identification of individual *S. hippos* targets in MBS data facilitated the confirmation of *S. hippos* undetected by single-beam transects, due to relative sampling volumes. The MBS surveys showed evidence of possible fishing effects on *S. hippos*

aggregations with school structure varying after a two hour period of fishing and video tows. Relative decline in aggregation stability towards the end of the season and possible avoidance behaviour from approaching vessels was observed as successive MBS transects, over a short space of time, recorded school movement around the wreck above which *S. hippos* aggregations sit.

P. auratus spawning in the Cockburn Sound, Fremantle illustrated the limitations of single-beam acoustics to monitor aggregations of mobile fish in shallow water, due to vessel avoidance behaviour. Similar sampling issues were observed in MBS surveys despite the inherent geometric advantages of the wide acoustic swath and increased sample volume. It was anticipated that adjusting the MBS mounting position, such that nadir beams were orientated laterally athwartships (rather then vertically downwards), increased the lateral distance at which the fish could be observed, thus reducing vessel avoidance implications. However, due to time constraints and equipment availability, remounting the MBS was not possible at the time of survey and the effects of MBS orientation could not be verified.

Single-beam and passive acoustic surveys of *G. hebraicum* illustrated the complexity of acoustic investigation of comparatively sedentary, demersal fish which often spawn in small groups. Discrimination of individuals using single-beam techniques was often restricted by the fish proximity to the seafloor and the footprint of the single-beam. Single-beam species identification of small groups of fish is impractical without simultaneous visual confirmation, due to the stochastic nature of fish reflectance. However, single-beam acoustics could provide information on *G. hebraicum* spawning related essential fish habitat using seafloor classification. While biological assessment of *G. hebraicum* otoliths, swimbladder and related muscular structure imply a soniferous species, as yet no vocal behaviour of any of the Glaucosomatidae has been reported, despite attempts here to detect vocalisations. Thus the characteristics of this species presented the greatest limitations for study using active or passive acoustic techniques.

Passive acoustic techniques were shown to be ideally suited for monitoring the low density, benthic aggregations of *A. japonicus* in the Swan River. Spawning related

vocalisations of *A. japonicus* were recorded *in situ* and *in aquaria* (Mosman Bay, Swan River and TAFE, Fremantle aquaculture centre respectively) each spawning season between October and May for four spawning seasons. *A. japonicus* calls, produced by the contraction of bi-lateral paired sonic muscles around the posterior two thirds of a heavily damped swimbladder, were classified into three categories relating to differing spawning functions. Category 1 calls ('Bup') of 2-4 swimbladder pulses were believed to function to gather males together in temporary broadcasting territories and to announce readiness to spawn. Category 2 calls comprised 11-32 pulses in a single audible tone ('Baarp'), which could also be broken into two or more parts ('Ba-Baarp') with a believed function as a call of attraction, predominantly from males to females. The third Category comprised calls produced in quick succession at increasing call rate to a point of cessation. Series of Category 3 calls ('Thup') were recorded only at times associated with spawning, in fewer numbers than other call categories and consisted of between 1 and 4 pulses. Pulse repetition and spectral peak frequencies of Category 3 calls were notably higher than those of Category 1 and 2, both *in situ* and *in aquaria,* despite the similar number of pulses. For example, in situ pulse repetition frequencies of up to 114 Hz for Category 3 calls compared with approximately 59 Hz for other categories. It is suggested that the increased pulse repetition frequencies of Category 3 calls require greater, unsustainable levels of energy (corroborated by the decreasing pulse rate as these calls progress) and such calls are therefore reserved for specific, uncommon events, possibly episodes of courtship. Ground truth *in aquaria* calls exhibited similar call structure to those recorded *in situ*, however, pulse repetition rates and occurrence were significantly lower (respective pulse repetition frequencies of 41.74 and 58.68 Hz for captive and *in situ* Category 2 calls).

Season-long monitoring of sound production in Mosman Bay determined spawning commencement was correlated with a daytime water temperature threshold at, or above 18.5 °C, occurring between October and November. Generalized Additive Models showed sound pressure levels (SPLs) and, by proxy spawning throughout the season, were correlated with temperature, salinity, sunset and tidal effects with decreasing order of effect. Increases in short-term sound production were observed on a semi-lunar basis, occurring at the new and full moons. Local chorus level maxima were found to occur on a 3.97 day basis (s.d. = 1.8), similar to that found from egg collection *in aquaria* and previous *in situ* SPLs in local studies of *A. japonicus*. Comparisons between Mosman Bay tidal related afternoon/evening activity and nocturnal behaviour of alternative populations in captivity suggest that *A. japonicus* exhibits adaptive vocal behaviour, and by proxy spawning activities, dependent on environmental variables.

Individual *A. japonicus* were localised during spawning within and close to an array of hydrophones by using vocalisation arrival-time differences, surface reflection and comparative energy level techniques to analyse vocalisations. Several individual *A. japonicus* were followed for periods ranging from seconds to several minutes as they called repetitively. Monitoring individual movement and separation distances between calling fish confirmed low mobility over long periods, indicative of lekking behaviour. The determination of call source levels employed calls of known range using data from the localisation study. Mean squared pressure source levels and 95 % confidence limits of the three call categories were measured as: 163 (147.7, 178.6), 172 (168.4, 176.0) and 157 (154.0, 160.3) dB re 1µPa for Categories 1, 2 and 3, respectively.

During periods of low density calling in the 2006-7spawning season, techniques of call counting produced absolute abundance estimates for *A. japonicus* present within the hydrophone detection range of approximately 500 m, observing a maximum of 15 calling individuals. Assuming a 1.3:1 sex ratio this implies a detectable spawning population of 26 fish within approximately 100, 000 m^2 (range restricted across stream by depth) equivalent to approximately 3, 850 m^2 per fish (assuming a random distribution of callers and recipients). However, during high density 'continuous chorus' calling the maximum number of callers able to be discerned using call counting techniques was exceeded. The application of call counting techniques and call contributions to overall SPLs to estimate biomass during 'chorus' calling, where calls merge together, requires further investigation. Recorded chorus levels were not a simple function of animals calling within the receiver proximity, but were strongly influenced

by source-receiver range. A preliminary model to estimate minimum numbers of callers within derived range boundaries has been laid out.

Recording of *A. japonicus* vocalisations illustrated the developing capabilities of passive acoustics to monitor soniferous fish species. A suggested set of protocols has been laid out to standardise the reporting of fish calls together with supplementary data relating environmental variables to their subsequent effects on the acoustic characteristics of the call. Standardisation of reporting will facilitate future spatial and temporal comparison of inter- and intra-species sound production.

This study has illustrated that the features of each acoustic technique endear them to particular species-specific characteristics. For example, although *S. hippos* did not vocalise they formed midwater aggregations of large fish (107 cm mean fork length) and were thus amenable to active acoustic monitoring. In contrast, *A. japonicus* form low density, benthic aggregations and hence are not suited to study by active acoustics, but vocalised profusely rendering them suitable for passive acoustic monitoring. In many cases a combination of techniques both acoustic and non-acoustic is required to monitor the particular species, in order to ground truth the data.

Table of Contents

List of Figures

List of Tables

CHAPTER 1

1. Introduction

The following study presents an acoustic investigation into spawning aggregations of differing fish species inhabiting the coastal waters of Western Australia, in order to monitor biomass and better understand each species and the habitats they spawn in. The study was conducted between 2005 and 2008 using active and passive acoustic techniques.

1.1. The importance of sustainable fisheries

Fishing provides livelihood and/or food security for around 200 million people worldwide. Around one in six people on the planet depend on fish as a primary source of protein (Anonymous, 2003, Helfman, 2007). In an environment where fishing pressures have become so great that 90% of large pelagic predatory fish have gone (Myers and Worm, 2003), and changing climatic conditions will have a further as yet unquantified impact on fish stocks (Hopday *et al.*, 2006), the mitigation of further negative anthropogenic effects on already declining fisheries is paramount. Determining optimum management policies, together with the development of existing and innovative fishery monitoring techniques is key to the sustainability of these crucial food and economic resources. The long-term success of fishery management relies upon the knowledge of exploited populations, the size and distribution of which may vary annually due to a variety of natural and anthropogenic causes (Simmonds and MacLennan, 2005). Such fish populations must be consistently and objectively monitored over years to observe and understand variation due to gradual climatic changes.

1.2. Management and monitoring of spawning aggregations within the West Coast Bio-region.

In order to optimise spawning success and survival of offspring, fishes have developed many techniques of reproduction. One such strategy is the spawning aggregation;

defined by Domeier & Colin (1997) as, 'a group of conspecific fish gathered for the purpose of spawning with fish densities or numbers significantly higher than those found in the area of aggregation during non-reproductive periods'. This is not always the case; however, as a species may aggregate to spawn in numbers lower than those found in schools of the same species. Therefore a more pertinent description from a management perspective is that reproductively active fish are grouped together in a manner which increases their vulnerability to fishing activities (Mackie *et al.*, 2009.).

The discovery of spawning aggregations, with their predictably high yields from commercial fishing, has made many species susceptible to over-fishing (Claydon, 2004). By 2004 aggregation fishing was estimated to have eradicated around one third of local spawning aggregations in the Caribbean, and similarly resulted in the elimination of some aggregations in the Indo-Pacific region. Such fishing related losses of spawning aggregations are often associated with the collapse of the fisheries they supported (Claydon, 2004). Management of spawning aggregations has now globally become a crucial element, addressed at the 3rd World Conservation Congress, 2004 and in article 6.8 of the United Nations Food and Agriculture Organisation's Code of Conduct for Responsible Fisheries (Mackie *et al.*, 2009.).

Coastal waters of Western Australia and their associated habitats are home to many species of demersal fish that migrate to form short-lived aggregations in order to spawn. These spawning aggregations may form at the same sites over successive, predictable spawning seasons. It is important to identify what condition the aggregations are in, quantify species biomass levels, and consider monitoring and controlling of anthropogenic effects on the aggregations. This management process was raised as an important issue and initiated a Department of Fisheries project entitled the 'Management and monitoring of fish spawning aggregations within the west coast bioregion of Western Australia' supported by the Commonwealth Government, Fisheries Research and Development Corporation (Mackie *et al.,* 2009.).

1.3. The role of acoustics in fisheries

As pressures on fish stock levels increase, so does the necessity for accurate quantification of abundance. To accurately assess the biomass, distribution, behaviour and ecological importance of spawning aggregations, techniques are required which are non-invasive, incite as little behavioural bias as possible, can repetitively acquire high resolution data for periods up to entire spawning seasons and are comparatively easy and cost effective to deploy. While conventional methods of stock assessment such as catch per unit effort (CPUE), (Quinn and Deriso, 1999), yield and egg per recruit models (YPRM and EPRM, respectively), (Haddon, 2001) and biological sampling of age structure (Mackie *et al*., 2009) offer good assessments of overall productivity and relative abundance variations they are often labour intensive and involve subjective data from numerous sources (Luczkovich *et al*,. 1999a). Video techniques have become increasingly employed to monitor the presence and populations of fish and the habitat over which they aggregate/reside (Watson *et al*, 2005, 2007, Delacy, *in prep*.). However, video techniques possess their own limitations, such as high and variable attenuation of light in water, varying sampling volumes when using baits, and behavioural bias generated by the equipment. Additionally, many species inhabit or aggregate at places or times which are not conducive to conventional sampling strategies, such as deep water or remote aggregations, or fish which spawn at night or inhabit turbid waters.

Although not without their own limitations (see Section 5), acoustic techniques offer unique, complementary methods to other monitoring techniques. Sound waves propagate efficiently through water (Urick, 1983), allowing acoustic ‘observation’ of marine animals at considerably greater ranges and conditions than visual techniques (Simmonds and MacLennan, 2005). As such, the acquisition and analysis of marine acoustic backscatter data using single-beam echosounders has become a comparatively well established tool in the assessment of fish stock levels (Simmonds and MacLennan, 2005). More recently, the development of multi-beam sonar (MBS) swath mapping techniques to acquire data from within the whole water column, rather than just the seabed, has extended its use to fisheries related surveys (Gallaudet and de Moustier, 2002). In addition to these ‘active’ acoustic techniques the passive recording of fish vocalisations offers a rapidly developing technique to ‘observe’ soniferous fish (Section 2.3.2).

1.4. Thesis Aims

The primary thesis aims are to assess acoustic data collected from fish spawning aggregations by means of active (single-beam echosounder and MBS) techniques and the passive acoustic recording of fish vocalisations. This will enable the evaluation of each technique for relative benefits and limitations, with respect to abundance and behaviour (both spawning related and in response to anthropogenic activity), exhibited by species of differing biological and behavioural characteristics.

More specifically, the objectives of this thesis are to:

- Assess spawning aggregation acoustic backscatter acquired with single-beam and MBS systems to estimate biomass and school structure over temporal scales of multiple seasons.
- Record vocalisations, primarily from mulloway (*Argyrosomus japonicus)*, during and around aggregation periods, on single and multiple hydrophones. Identify call functions and their occurrence to classify spawning times, levels and response to environmental variables on temporal scales ranging from seconds to seasons.
- Develop innovative new methods to monitor soniferous species from their vocalisations, such as passive acoustic arrays to monitor individual behaviour.
- Estimate numbers of fish present from passive recordings of their calls.
- Evaluate each method (single-beam, MBS and passive acoustics) for its advantages and limitations in species identification, biomass estimation and behavioural observation to develop a reference for assigning a specific combination of techniques for locating and investigating future aggregations of known species.
- Generate baseline knowledge for interpreting effects of increased anthropogenic activity on spawning aggregations.

1.5. Thesis outline

Chapter 2 outlines acoustic theory pertinent to each technique and the effects of biological species-specific characteristics. Data from the following research are presented in the form of papers designed to lead the reader through two disciplines of fisheries acoustics. As such, each paper has been designed for individual publication and overlap somewhat in methods and introduction. The first data section (Chapter 3) describes the active acoustic techniques of single-beam echosounding and MBS surveys with particular focus on mid-water aggregations of Samson fish (*Seriola hippos*). Chapter 4 describes the steps involved in monitoring soniferous (sound producing) species from the characterisation of vocalisations to absolute abundance estimates. Spawning aggregations of *A. japonicus* in Mosman Bay, Swan River have been used to highlight the application of passive acoustics and the need for standardisation of reporting fish sounds. In Chapter 5 the thesis discusses the implications for present fisheries and possible applications for management from the perspective of each technique and its relevance to species specific biological and behavioural characteristics. Finally, Chapter 6 outlines anticipated future work necessary to address issues and develop techniques highlighted by this research.

CHAPTER 2

2. Background to monitoring fishes using acoustic techniques

In terms of active acoustic analysis, an aggregation of fish has been defined as a backscatter patch where individual targets cannot be resolved (Kieser *et al.*, 1993). In recent years, the resolution of active acoustic techniques has improved such that individual fish within a group can often be resolved. However, the sentiment remains that an aggregation displays an overall acoustic density of fish significantly greater than at the same location, outside of the spawning season. Similarly, from the perspective of passive acoustics, an aggregation could be described as a spawning-related chorus produced by a group of con-specific, soniferous fish where calls from multiple fish overlap (Cato, 1978).

This chapter aims to give a brief theoretical background to fisheries acoustics and the ecology and physiology of the target species pertinent to their acoustic properties. A more in-depth introduction to relevant marine and fisheries acoustics can be found in Urick (1983), Medwin and Clay (1998), and Simmonds and MacLennan (2005). Useful texts on sound production and hearing in fishes include Fine *et al*. (1977), Tavolga (1971) and Popper *et al*. (1982). Where it has been deemed prudent further relevant detail regarding specific theory and biology is presented in the appropriate chapters.

This chapter is divided into the following subsections:

- an overview of underwater acoustic propagation and measurement (Section 2.1.);
- a description of the basic anatomy of teleosts which affect their acoustic properties pertinent to active and passive acoustics (Section 2.2);
- an outline of the theory and history behind each technique (Section 2.3);
- an evaluation of target species biological and behavioural characteristics known prior to this research, and identifies their anticipated effects on the acquisition of acoustic data (Section 2.4).

2.1. Sound measurement and propagation in water

Sound is the longitudinal vibration of particles within a medium. This vibration occurs about a rest position, propagating across adjacent particles. Propagation results in zero gross movement of the medium's particles after the sound wave has passed. The back and forth particle motion generates pressure variations, defined by instantaneous particle velocity and acceleration, as the sound wave passes. It is this pressure variation which is measured in most studies (McCauley, 2001). The intensity I of the sound wave is the energy flux per unit time, given by:

$$I = p^2 / \rho c \tag{2.1}$$

where p is the pressure, ρ is the medium's density and c is the sound speed (Simmonds and MacLennan, 2005). Assuming the density and sound speed remain the same the intensity of the sound is proportional to the pressure squared. Hydrophones measure the pressure and convert it into variations about a mean voltage output such that sound pressure is proportional to voltage. The magnitude of signal energy content, often expressed as intensity, can be perceived over a large range covering many orders of magnitude. As such it is treated on a logarithmic scale (Simmonds and MacLennan, 2005). Given a medium of near constant density the sound pressure level (SPL) in decibels can be simplified to:

$$SPL(dB) = 10\log_{10}\left(\frac{p_m^2}{p_0^2}\right) = 20\log_{10}\left(\frac{p_m}{p_0}\right) \tag{2.2}$$

where p_m and p_0 are the measured and reference pressures respectively. Sound pressure levels are given with a reference unit (typically re 1 µPa for water and 20 µPa in air).

Propagation of energy from a single point source of sound in infinite space spreads out across a spherical wave front. The variation in intensity between that at a reference location I_0 (usually 1 m from the source) and that at distance r, I_r is given by:

$$I_r = I_0 / r^2 \tag{2.3}$$

Therefore as the wave front expands the intensity over a given area reduces at the rate of $20\log_{10}(r)$ and every time range is doubled an equivalent 6 dB re 1 μPa loss is observed (Urick, 1983). Although sounds are not produced by point sources, for simplicity measurements are assumed at ranges sufficient that all sources act as points. The effective intensity at 1 m is then calculated from the calibrated, recorded level and an estimation of losses. Over long distances, or near the plane of differing mediums, reflection and refraction can play an important role in transmission losses (Section 2.3.2). However, in general, active acoustics are concerned with short-range or near vertical transmission and therefore only spherical spreading is considered.

However, spreading is not the only cause of losses during underwater sound propagation. Acoustic energy is converted to heat via absorption. This occurs at differing rates for differing frequencies and is due to molecular frictional and chemical processes (Simmonds and MacLennan, 2005). This is typically described by:

$$I(x) = I_0 10^{\left(\frac{-\alpha x}{10}\right)} \tag{2.4}$$

where I_0 and $I_{(x)}$ are the reference intensity and intensity at distance x respectively, and α is the expression for the absorption coefficient, as the energy loss at a given frequency in dB per unit distance (typically km). The frequency (Hz), or the vibration of particles per second about the rest position, is a function of the sound speed and wavelength (λ). Absorption is typically greater at higher frequencies and does not become significant over the ranges associated with fish calls until calls of a few kHz (McCauley, 2001). It does, however, become a considered loss in the use of active acoustics, where fisheries echosounders typically operate at frequencies between 12 and 200 kHz (Simmonds and MacLennan, 2005), and MBS systems can function up to 455 kHz.

Sound speed (*c*) is a function of temperature, salinity and depth (Urick, 1983). Therefore, a variation in any of these variables over a plane, at any angle other than normal to the propagation, causes refraction of the sound wave. At vertical or near vertical directions this has negligible refractive impacts; however, at other angles effects can be as great as surface ducting. For further detail on refraction see Medwin and Clay (1998).

2.2. Teleost physiology of acoustic significance.

Many species of fish aggregate to spawn in habitats where communication through visual stimuli is greatly inhibited by turbidity or lack of light (for example nocturnal or estuarine spawning). Thus species have developed alternative methods to vision for communicating, in this case, acoustic communication. A brief outline of fish hearing is presented. For a more detailed introduction to this topic see Popper (1980), Popper and Fay (1973) and Popper *et al.* (1982).

2.2.1. Auditory system

Fish inner ears possess three semicircular canals and three otolithic organs, similar to other vertebrates (Popper & Lu, 2000). However, due to the speed of sound in water and the small separation and coupling between the ears it is unlikely that fishes use the same binaural cues for sound source localisation as most terrestrial vertebrates (Lu *et al.*, 1996). Hearing involves the inner ear, an auditory section of the central nervous system and, in some species, peripheral structures such as the swimbladder (Popper and Coombs, 1980). Located within the three otolithic organs, otoliths are calcareous structures associated with sound detection functions (Popper and Fay, 1993). The otolithic organs contain a sensory epithelium which is oriented in three dimensions around the otolith, and possesses a narrow strip covered with a large number of sensory hair cells lying against the inner edge of the otolith. Projecting from the hair cells, towards the otolith are ciliary bundles comprising stereocilia and kinocilium. The hair cells populate the otolithic membrane and are coupled to the otolith by a gel. Cilia bending occurs because the sensory cells and the otolith move differentially. Bending of the ciliary bundle towards, or away from the kinocilium creates polarisation of the hair cell (Popper *et al.*, 1982) and it is this stimulation with which the inner ear detects changes due to a sound source.

Underwater sound comprises two components, directional particle motion and propagating scalar pressure waves (Medwin and Clay, 1998). These two physically linked components reach the inner ear of a fish in different ways to induce cilia bending (Fay & Popper, 1975, Horodysky *et al.*, 2008). As otoliths are roughly three times as dense as water, a fish body may move with a displacement in water mass while the otolith displacement lags behind (Dijkgraaf, 1960). This amplitude and phase differential between sensory epithelia and otoliths provides fish with a biological accelerometer which directly detects particle motion (Popper *et al.*, 1982, Popper & Fay, 1993, 1999). Such 'direct' detection works primarily at frequencies below 500-600 Hz (Popper & Fay, 1999, Ramcharitar *et al.*, 2006a). However, a swimbladder containing gas of a different density from the body could respond to changes in pressure produced by sound waves. These pressure changes are translated into displacement energy and can be transmitted to the inner ear where hair cells are stimulated. Many fishes have developed accessory structures to maximise this 'indirect' pathway, reradiating sound pressure waves towards the otoliths in the form of particle displacement (Fay & Popper, 1974, Popper & Fay, 1993). The application of these two mechanisms means that several species of fish are capable of detecting acoustic signals via both pressure variation and particle displacement, over a wide range of frequencies. Some example ranges of fish hearing thresholds can be found in Appendix 2.2.

2.2.1.1. Hearing 'generalists' and 'specialists'

Fishes are classed as either hearing 'specialists' or 'generalists' based on their anatomy, ability to detect sound pressure waves, and detectable bandwidth range (Horodysky *et* *al.*, 2008). Hearing specialists of often unrelated taxa have typically evolved accessary hearing structures to extend bandwidths to higher frequencies and lower the hearing thresholds and do so by connecting the swimbladder to the ear. These structures may come in different forms such as the anterior swimbladder diverticulae of the weakfish (*Cynoscion regalis*; Connaughton *et al.*, 2000), the Weberian ossicles (characteristic bones which connect the swimbladder to the inner ear; Popper *et al.*, 1982) of ostariophysines such as the goldfish (*Cassius auratus*; Ladich & Wysocki, 2003) or the suprabranchial chambers (labyrinths) of anabatoids (Wysocki et al., 2009). Fishes without these peripheral structures are described as hearing generalists and typically exhibit higher hearing thresholds and a smaller frequency bandwidth. However, in species where the anterior of the swimbladder terminates closer to the ear lower thresholds over extended frequencies are often observed (Wysocki *et al.*, 2009). Species which do not possess a swimbladder are only sensitive to particle motion only as unaided the inner ear is insensitive to indirect transduction of sound pressure (Enger and Andersen, 1967, Chapman & Sand, 1974, Wysocki *et al.*, 2009). However, the possession of peripheral hearing structures does not necessarily result in increased frequency bandwidth. The catfish (*Arius felis*), for example, is an ostariophysine which can only detect sounds below 1000 Hz. By contrast the squirrelfish (*Holocentrus ascensionus)* is a non-ostariophysine with good sensitivity in excess of 2000 Hz (Popper *et al.*, 1982, Popper and Fay, 1973).

Many species appear to have developed their hearing structures independently and no single fish ‘ear’ can be applied to a taxonomic group. Sciaenids, for example, display at least three types of swimbladder-ear configurations (Ramcharitar *et al.*, 2006). Sciaenids such as the spot (Leoisomtus xanthurus) and black drum (*Pogonias chromis*) are considered hearing generalist and have swimbladders which terminate some distance from the ear (Ramcharitar & Popper, 2004, Ramcharitar *et al.*, 2009). By comparison, the weakfish (*Cynoscion regalis*), spotted seatrout (*Cynoscion nebulosus*) and silver perch (*Bairdiella chrysoura*) have developed anterior horns on the swimbladder which project forwards, close to the ear (Chao, 1978, Ramcharitar, *et al.*, 2004). In between these species, Atlantic croaker (*Micropogonias undulatus*) swimbladders possess anteriorly directed diverticulae which approach, but do not touch the ear (Ramcharitar et al., 2006).

Until recently very few studies had been conducted to determine whether a species uses the sound pressure or particle motion for detecting sound and the hearing thresholds associated with each path (Popper & Lu, 2000, Horodysky *et al.*, 2008) and due to a lack of commercially available sensors audiograms are often described in terms of sound pressure levels (Wysocki *et al.*, 2009). As hydrophones sensitive to particle motion become more available and accurate *in situ* and *ex situ* studies can be conducted hearing thresholds related to particle velocity and acceleration are being increasingly reported (Myberg & Spires, 1980, Casper & Mann, 2006, Lu *et al.*, 196, Ramcharitar *et al.*, 2005, Horodysky *et al.*, 2008, Wysocki et al., 2009, Kojima *et al.*, 2010). However, due to time constraints and the lack of availability of a hydrophone sensitive to particle motion the propagation of sound in this study has been considered predominantly as scalar pressure waves.

Auditory characteristics have implications for the ability of a fish to discern sounds of differing frequencies at differing ranges and levels of ambient noise. Given an estimated critical hearing ratio (Fay, 1974, Tavolga, 1974), McCauley (2001) was able to estimate masking levels for sounds of differing source level by hearing specialists and non-specialists. Therefore the biological examination of a species' auditory system provides an indication of hearing sensitivity and frequency. For example, it has been suggested that relative otolith to body size correlates to species hearing acuity and, as there is a positive relationship between sensitivity and communication, the likelihood of sound production (Gauldie, 1988, Paxton, 2000, Montgomery & Pankhurst 1997, Lychakov & Rebane, 2000, 2002, Ladich and Popper, 2001, Cruz and Lombarte, 2004). The species hearing frequency range illustrates some of the acoustic characteristics a recipient fish should be able to determine, and by inference, those produced by a caller.

2.2.2. Sound production

Sound production by fishes can eventuate from diverse methods, such as bubble release from the mouth, or vibration of bubbles at the anal cavities (Wahlberg and Westerberg, 2003). However, the two chief mechanisms of fish sound production are via stridulation (high frequency, wide-bandwidth, usually of short duration), such as the catfish (*Bagre marinus*; Diogo *et al.,* 2001), or the vibration of the swimbladder (McCauley, 2001). Stridulation is the rubbing or knocking of body parts together creating a noise similar to that of marine invertebrates. This may be from pectoral fins such as sea catfish (*Felichthys felis*) or skeletal bones like the pipefish (*Syngnathus louisanae*) (Burkenroad, 1931, Fish, 1953). Contraction of the 'sonic' muscles creates vibrations in the swimbladder chamber. Since the acoustic impedance of the gas inside the swimbladder and the surrounding water differs greatly the swimbladder is highly effective at generating sound (Simmonds and MacLennan, 2005). Variations in frequency can be created by altering chamber volume. For example, McCauley (2001) recorded two similar, but different calls from *Terapon theraps* and postulated that differences were produced by the opening state of a sphincter allowing gas exchange between the two chambered swimbladder. Some species, such as the catfish (*Ictalurus punctatus*), generate sound by a combination of stridulation and swimbladder vibration (Vance, 2000). Production of sound by vibration of a swimbladder is considered in more detail in Chapter 4.

2.2.3. Buoyancy

Although the swimbladder is involved in sound production, hearing and detecting pressure change, its primary function is buoyancy control (Pelster, 2004, Sand and Hawkins, 1973). At moderate pressures the density of gas inside a swimbladder is near negligible, compared with that of the water (Evans, 1998) and, as the majority of a fish body is of similar density to water (Strand *et al*., 2005), a fish requires a volume of low density to offset the denser skeleton and proteins for overall neutrally buoyancy. In most species a swimbladder that accounts for approximately 8 and 5% of the whole-body volume in freshwater and coastal (shallow water) marine fish respectively, is sufficient to provide neutral buoyancy (Denton, 1962, Evans, 1998, Jones & Marshall, 1953).

For fish possessing a swimbladder this difference in density between the enclosed gas and surrounding water represents the dominant scattering target for an acoustic ensonifying ‘ping’ used in active acoustics. Species not possessing a swimbladder, such as Atlantic mackerel (*Scomber scombrus*), comprise a body predominantly similar in density to the surrounding water, and therefore possess different acoustic characteristics from those with swimbladders (Juell *et al.*, 1996).

From Boyle’s law, an unimpinged swimbladder volume is inversely proportional to the pressure p outside the swimbladder in the form of:

$$V \propto \frac{1}{p} \tag{2.5}$$

Thus if a swimbladder were to be isolated, the volume, and therefore target strength from an ensonifying acoustic pulse (TS) (see Section 2.3.1.2), would vary significantly with vertical migration. As the fish ascends for example, the pressure outside the swimbladder decreases, the volume inside increases, and therefore so does the reflected energy of an acoustic ping.

Swimbladders are not isolated within the fish and species which possess a swimbladder can be broken into two groups. Physostomous species typically take air from the surface to fill the swimbladder for maintaining buoyancy and release air through a pneumatic duct, thus these species are generally surface dwelling (Schmidt-Nielson, 1997). Physoclists have a closed swimbladder and must reabsorb or secrete gas through a specialised organ (*Rete Mirabile*) with a vascular countercurrent system for enhanced gas secretion or absorption in order to maintain neutral buoyancy (Steen, 1970, Pelster, 2004). It is through this gas secretion/absorption that these fish are able to maintain a swimbladder of constant volume and thus remain neutrally buoyant. However, if a vertical passage of migration is too fast for the fish to compensate the swimbladder internal pressure to the surrounding water pressure the volume will change.

Although the spectral characteristics of a call are affected by the internal pressure of the swimbladder, they are also affected by other factors such as the swimbladder shape, wall stiffness, muscle tension and environmental conditions (these factors are discussed in further detail in Chapter 4) and predominantly, the volume. Were a fish to make a call during a sudden vertical rush the change in swimbladder volume due to the reduction in water pressure would significantly affect the resonant frequency of the swimbladder and therefore that of the call.

2.3. Application of sound propagation in fisheries acoustics

The use of acoustic techniques to detect aquatic organisms is a centuries old process, dating from Chinese fishermen following the sound of soniferous fish through the hulls of their wooden boats (Moulton, 1964). In 1492 Leonardo da Vinci acknowledged the ability to hear ships at a great distance, through a tube, with one end placed in the water (Urick, 1983), and over a century ago Lord Rayleigh investigated acoustic scattering by fluid spheres (Barr and Coombs, 2005). Since then, technological advancements in the remote detection of submerged bodies have evolved rapidly.

2.3.1. Active acoustic techniques

The first technological application of hydro-acoustics for underwater organisms was to detect fish in a tank (Kimura, 1929). Transferring these techniques to detect fish in the marine environment, trials were conducted in the Barents Sea, Norway (Sund, 1935) and later in the North Sea, UK (Balls, 1948), to show the qualitative abundance and distribution of fish life. Such methods involved ensonifying a volume of water with an acoustic pulse (a ‘ping’) and recording the returned response with time. Targets within the ensonified volume of water, and of a different density to water, returned a reflection.

In the context of this thesis echosounding has been considered as the ensonification of a volume of water by a beam with a central axis directed vertically downwards. Receive arrays in multi-beam sonar (MBS) systems simultaneously sample volumes using a number of effective beams, directed at various angles from vertical and have therefore been classed not as echosounders, but sonar (SOund Navigation And Ranging).

2.3.1.1. *Single-beam echosounders*

Since the Second World War single-beam echosounders have become comparatively well established in the estimation of marine biomass (Simmonds and MacLennan, 2005). The main advantages of single-beam techniques are the logistical ease of use and availability of the system. The majority of commercial vessels are now fitted with echosounders as standard, allowing high accessibility for broad-scale temporal coverage of stock abundance. In addition to raw acoustic backscatter, modern scientific echosounders log transducer position and often vessel motion and heading data, thus allowing complex post processing to be applied in order to account for transducer orientation.

In single-beam fisheries echosounders an acoustic beam is usually transmitted vertically towards the seabed, generating short (~0.2 - 1.0 ms) single or repetitive pulses, at frequencies between around 12 kHz and 200 kHz (Simmonds and MacLennan, 2005). Active acoustic transducers possess inherent beam directivity as illustrated in Figure 2.3.1. This directivity is derived in terms of intensity B at a point of angles (ϑ, ϕ) relative to the acoustic axis as:

$$B(\vartheta, \phi) = \frac{I(\theta, \phi)}{I(0,0)} \tag{2.6}$$

where $I(0,0)$ is the intensity on the acoustic axis and $I(\theta, \phi)$ is the intensity at given angles from the axis.

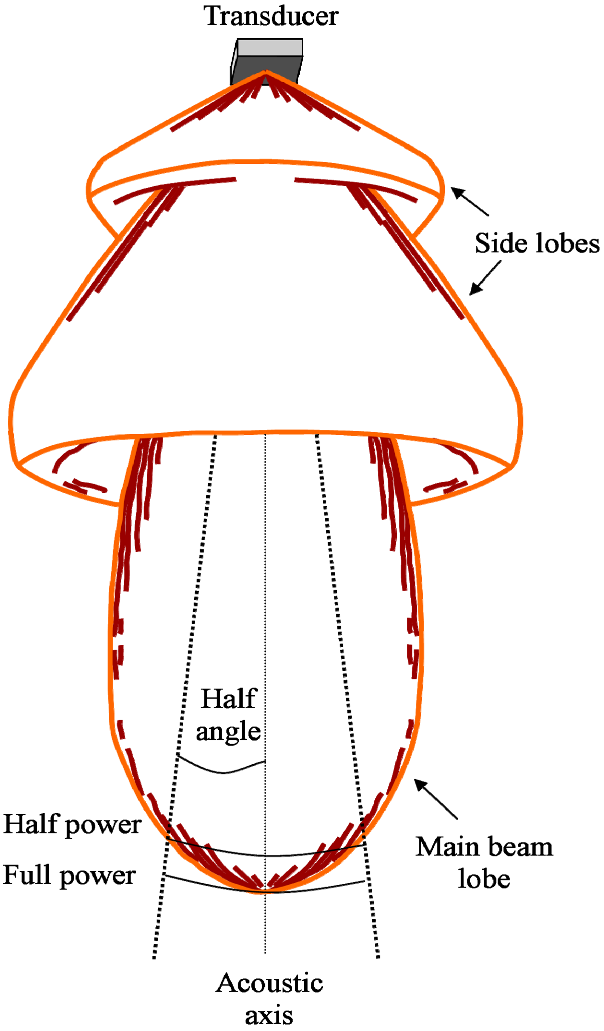

Figure 2.3.1. *Example schematic of the three-dimensional beam pattern of a single-beam transducer.*

Similarly to single-beam, dual-beam systems produce a ‘narrow’ acoustic beam. However, in dual-beam systems a concentric arrangement of elements can be operated to receive two different beam patterns (one wide and one narrow beam) of the same acoustic axis. For a single target, the ratio (called the beam factor) of the two receive intensities, as determined by the two beams, is a function of the target’s angle from the central acoustic axis. This ‘off-axis’ angle allows the TS to be calculated as if the target had been positioned on the central axis. In split-beam systems a more complex technique is created where the acoustic beam-echo is received in defined quadrants to calculate a target's horizontal position within the emitted beam. Further detail on dual- and split-beam systems can be found in Ona (1999) and Simmonds and MacLennan (2005).

To simplify the transducer directivity an ideal beam is calculated where a solid angle beam width of flat response is defined. The beam width is the angle between opposite sides of the main lobe where the intensity is 3 dB less, or half the power than that on the acoustic axis (Simmonds and MacLennan, 2005). The half angle is the angle between the acoustic axis and the point at which the intensity is 3 dB less, the value of the intensity is the half power.

Abundance estimates using single-, dual-, or split-beam echosounders can be conducted via target counting if the density of fish is low enough. If targets are sufficiently separated in range individual echoes can be counted to estimate fish numbers. For the echoes not to overlap, a transmitted pulse, of duration $c\tau$ (where c is the sound speed and τ is the pulse duration), travels away from the transducer and encounters two targets at range R_1 and R_2 the ranges must differ by:

$$R_2 - R_1 > c\tau / 2 \tag{2.7}$$

However, where there are multiple targets separated by ranges less than half the pulse length target counting is no longer an option to estimate biomass and techniques of echo-integration are employed (Johannesson and Mitson, 1983).

Echo-integration has been experimentally shown to be linear (Foote, 1983), and therefore the resulting echo integrator output can be converted to biomass estimates over a required depth by use of the Target Strength (TS) of fish within the integration region. If echosounders are calibrated to current standards, where the absolute sample volumes of the transmitted and received pings are known (Foote *et al.*, 1987), then abundance results from echo-integration can be reported as absolute (Foote and Knudsen, 1994), if the TS is known.

2.3.1.2. *Target strength*

The TS relates to the effective backscattering cross section of the fish (Urick, 1983). Target strength is the logarithmic measure of the proportion of incident energy which is backscattered by the target, at 1 m from the target. Scatter occurs when there is an impedance (ρ*c*) variation between two mediums. For an object of length d where $d^3 \equiv$ volume, and $d << \lambda$ where λ is the wavelength, the object can be assumed to scatter as a point source. If an acoustic pulse is assumed to be long and I_i refers to the intensity at the midpoint of the pulse, then I_b is the backscattered intensity at the midpoint of the backscattered pulse. The backscattering cross section σ at range r is then given by:

$$\sigma_{bs} = r^2 \frac{I_b}{I_i} \tag{2.8}$$

For spherical spreading σ_{bs} is the same for a given target. When considering the off axis backscattering where (θ, φ) describe the direction of the receiver to the transmitter the differential backscattering cross-section is:

$$\sigma(\theta,\phi) = r^2 \frac{I_{scat}(\theta,\phi)}{I_i} \tag{2.9}$$

where $I_{scat}(\theta,\phi)$ is the backscattered intensity in the direction of the receiver.

The target strength (TS) of a fish is related to its backscattering cross section by the simplified expression:

$$TS = 10\log_{10}(\sigma_{bs}) \tag{2.10}$$

where σ_{bs} is specific to the sampling frequency (Horne, 2000). It is this length relationship on which many regression target strength models have been based, in the form of;

$$TS = a\log L - b \tag{2.11}$$

where a and b are constants specific to each species and frequency, and *L* is the length of an individual fish in centimetres, (Urick 1983, Johannesson & Mitson 1983).

Where targets are small, compared to the sample volume, and there are many contained within the volume, their echoes combine to form a continuous received signal of varying amplitude (Simmonds and MacLennan, 2005). Echo-integration is experimentally linear, thus acoustic and real density are equal (Foote 1983). The volume backscattering coefficient s_V is defined as:

$$s_V = \sum \sigma_{bs} / V_0 \tag{2.12}$$

where the sum is taken over all discrete targets contributing to echoes from the sample volume, V_0. Volume backscattering is calculated in the linear domain and presented as a logarithmic scale.

If the volume is greater than $c\tau/2$ the Nautical area scattering coefficient, s_A ($m^2/(n.mi)^2$) (also known as NASC), of the scatterers maybe calculated, through integration of the amplitude of returned signals over defined along track intervals (Misund, 1997). This is computed as:

$$s_A = 4\pi(1852)^2 \int_{Z_1}^{Z_2} s_V(z)dz \tag{2.13}$$

where s_V is the volume backscattering coefficient (m^2/m^3) and z is the range from transducer (depth). The echo integrator output can therefore be converted to biomass estimates, over a required depth, by use of the TS of fish within the integration region (Foote and Traynor, 1988)

The area scattering can be related to fish density ρ_A by:

$$\rho_A = \frac{s_A}{\langle \sigma \rangle} \tag{2.14}$$

where $\langle \sigma \rangle$ is the average acoustic backscattering cross section (m^2) and ρ_v is the volumetric density, from a depth layer Δz (Ona, 1999)

$$\rho_v \frac{s_a}{\langle \sigma \rangle \Delta z} \tag{2.15}$$

For a spherical target TS is isotropic. However, a fish swimbladder can contribute in excess of 90% of the overall TS (Foote, 1980). Neither fish, nor swimbladder is a uniform object and morphology is species-specific, as shown in Figure 2.3.2, where the swimbladder acoustic reflectance varies with angle. Thus TS can vary considerably based on a number of variables.

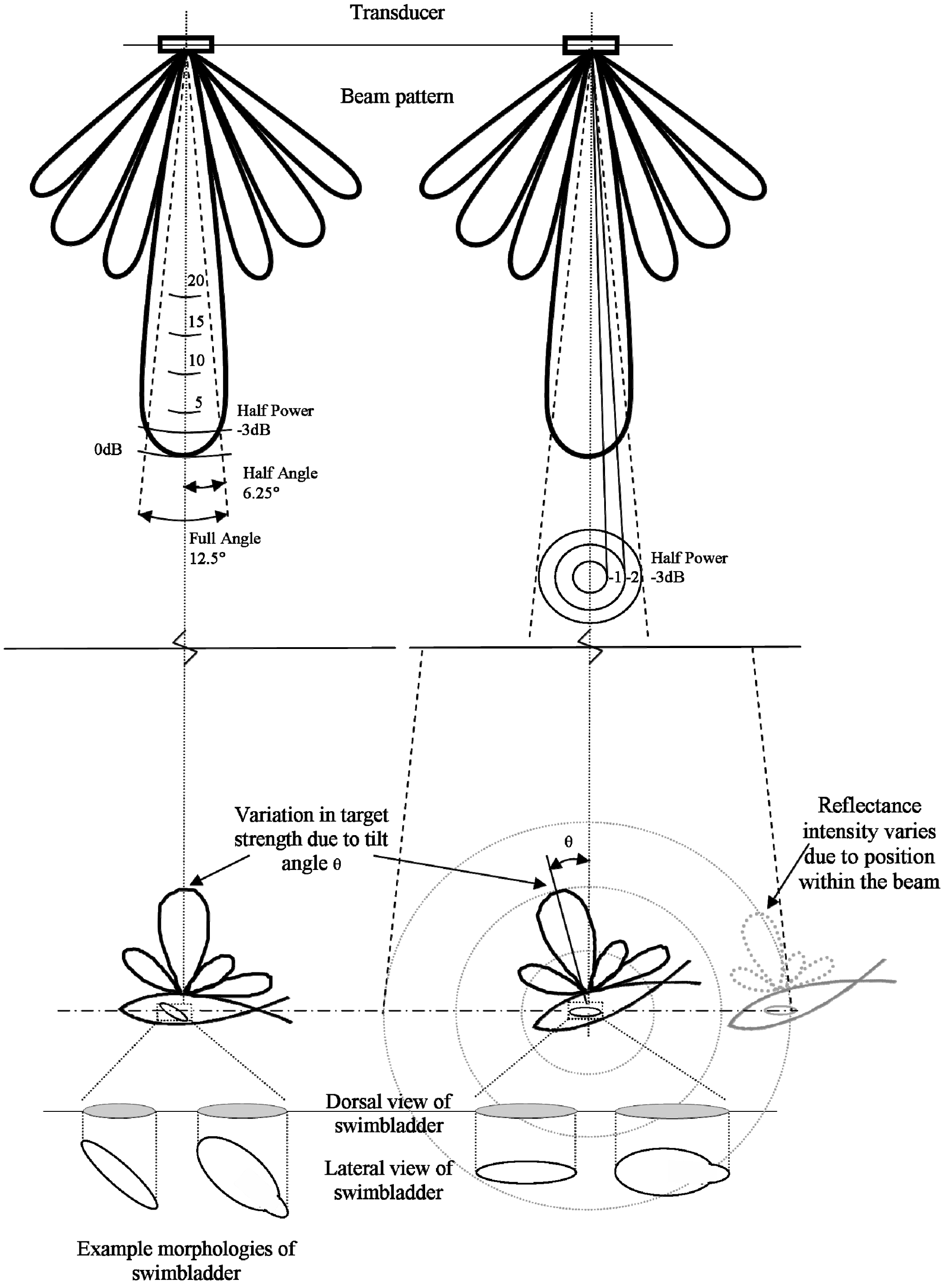

Figure 2.3.2. *Transducer beam pattern and fish reflectance pattern illustrating the effects of fish tilt angle and position within the beam on reflectance and swimbladder backscattering area. Adapted from Johannesson and Mitson (1983).*

The swimbladder back scattering cross-section of a given species increases by approximately the square of the fish length, leading to variations in up to 25 dB in TS with length (Nakken and Olsen, 1977). The swimbladder exhibits varying levels of directional complexity (Johanesson and Mitson, 1983). TS varies considerably with swimbladder tilt angle θ (for example see Figure 2.3.2), shown to be up to 10-15 dB for a 5° tilt (McClatchie *et al.*, 1999) and in some cases 30 dB at a 45° tilt in certain species (Foote, 1980). The stochastic nature of fish TS is shown in Figure 2.3.2, along with the conceptual effects of swimbladder tilt angle.

In addition to tilt angle various distortions of the swimbladder account for a large proportion of the changes detected in TS of an individual. Factors which distort the swimbladder include: spawning maturity (Ona, 1990), which during reproductive stages alter swimbladder size with an effect on TS almost as significant as length (Machias and Tsimenides, 1996); rapid vertical movement with insufficient time to compensate swimbladder volume for pressure change (Ona, 1984); feeding (Ona, 1990); reverberation (Johannesson and Mitson, 1983) and to a lesser extent body fat content (Ona, 1990, 2003, Reynisson, 1993). Factors external to the fish such as position within the acoustic beam may also lead to variations in TS, though this can be accounted for in dual- and split-beam systems. Therefore TS variability is species, length, relative orientation, depth, time of day, spawning maturity and behaviourally dependent, requiring significant *in situ* TS data to model the true mean value (Korneliussen *et al.*, 2007). In quantitative analysis of TS, stochastic models are often used to deal with variability.

Methods of measuring TS can be divided into *in situ* and *ex situ* (Foote, 1991). A thorough review of these methods, can be found in Foote (1991) and MacLennan & Simmonds (2005). The most valuable TS values are those that reflect the specific situation of the fish under survey (Foote, 1987). As such the *in situ* methods provide estimates of the fish in their natural environment. Indirect methods using single-beam echosounders to count single targets comprise inherent difficulties created by the beam pattern and the inability to accurately distinguish the location and orientation of the fish within the footprint (Urick, 1983; Simmonds and MacLennan, 2005). This can be alleviated, not without difficulty, with video ground truth techniques, or by the use of split or dual beam echosounders. It is also a necessity that the population density of the selected aggregations be low enough to correctly identify sufficient echoes from single targets to obtain unbiased measurements of *in situ* TS (Foote *et al*., 1986). However, without confirming the length relationship of individual targets it is impossible to create a regressive TS-length relationship and so no model can be created for future surveys. Thus the *in situ* measurements would have to be repeated for each survey of the aggregations, due to maturation of the members or seasonal declines in mean size of spawning fish (consistent with over-fishing). *In situ* TS measurements could, however, be used for verification of an existing model.

The most common *ex situ* method involves measuring the TS of caged fish of known lengths and weights. Detailed examples can be found in Gautier and Rose (2001), Ona (2003) and Knudsen *et al.* (2004). This involves removing the fish from their natural habitat, thus capture and transport stress may affect behavioural patterns and consequentially TS measures (although these adverse effects are moderated as the fish becomes acclimatised). Such extraction of fish from their habitats may require consideration of mortality rates and logistics, if for example the species is large, is raised from depth and suffers from barotrauma, or resides a significant distance from the holding location. These behavioural drawbacks are often outweighed by the comparative ease of qualifying biological aspects such as tilt angle, distress reactions, avoidance, pressure effects, spawning maturity and stomach content of individual targets.

Modelling of species TS is frequently conducted using Kirchoff-Ray Methods (KRM) to calculate the reflectance of different structures within the fish body, such as the swimbladder, backbone and skull (Clay and Horne, 1994). However, such techniques often rely on re-inflation of a swimbladder or freezing immediately after capture to retain swimbladder volume. The natural swimbladder volume at depth is uncertain.

Measurements of a range of TS readings attributed to a single species can only be definitively related to TS in the surveyed environment and period (Ona, 1990). However, with samples of TS at various stages of spawning maturity, it may be possible to relate time of year to a particular model, when combined with estimated seasonal changes in fat content (Iles and Wood, 1965). Assumptions of recurring behavioural characteristics, such as daytime and night time tilt angle variations, would also be necessary in order to maintain model use.

Variations in derived and true mean TS have significant implications for biomass estimates. Biomass estimates calculated from the same survey, but employing independently derived TS models of the same species, have shown significant variation in final biomass estimates (Kloser *et al.*, 2005). Thus estimates employing TS require sufficient quantities of *in situ*, length verified data, at the time of survey to determine the survey true mean TS value and limit temporal, behavioural and system generated TS variations.

2.3.1.3. Additional considerations for single-beam acoustics

Off-axis target position within the acoustic beam of a single-beam transducer is uncertain. Therefore differences of up to 3 dB can be observed between two responses of the same target (between positions on-axis and at the edge of the ideal, half power beam angle). Dual- and split-beam systems are able to account for target position by the timing of echoes as received by differing sections of the transducer. A dual-beam system provides an off-axis angle and therefore TS, while the split-beam provides target position within the beam and thus not only TS, but limited ability to track individual fish (for further details see Simmonds and MacLennan, 2005).

The linearity principle (the received reflected ping is the sum of energy from all scatterers within the beam) assumes a random distribution of fish within the acoustic sample volume (Foote, 1983); however, this may not be the case. In extreme examples, if targets were equally spaced a half wavelength apart, the echo intensity would increase more rapidly than the actual target density. With a very dense layer a shadowing effect occurs and the echo intensity increases less rapidly than the target density, due to the shielding of targets (Simmonds and MacLennan, 2005). Therefore changes in fish behaviour may alter the acoustic response of the sample volume.

The acoustic dead zone near the seabed can be obstructive when surveying targets which occur close to the seafloor. Targets within the dead zone will be detected as part of the bottom, as shown in Figure 2.3.3. Any on-axis fish, less than $c\tau/2$ above the bottom (off axis this distance increases), returns an echo which overlaps with that of the floor.

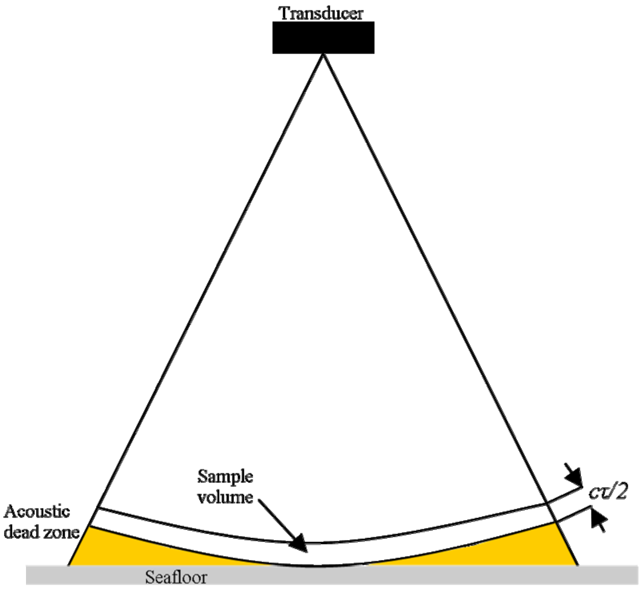

Figure 2.3.3. *Schematic description of the position of the sample volume upon first detection of the seafloor, together with the effective acoustic dead zone (yellow) in relation to the seafloor.*

2.3.1.4. Multi-beam sonar (MBS) systems

Underwater exploration in the modern age has been inextricably linked to the development of sonar systems (Mayer *et al.*, 2002). Primarily designed for acquiring high-resolution bathymetry data (Simmonds and MacLennan, 2005) and more recently seabed backscatter data (de Moustier, 1986, Hughes-Clarke *et al.*, 1996), multi-beam sonar (MBS) systems are employed as a seafloor characterisation tool (Holmes *et al.*, 2005, Parnum, 2008).

Typically a transmit array generates a single acoustic beam which is wide across the vessel track with athwartships coverage in excess of 90° (see Figure 2.3.4, red). A receive array forms a large number of receive beams typically wide in the along track direction and fine athwartships (Figure 2.3.4, blue). Therefore a swath of numerous thin beams (generally < 1° alongships and athwartships), typically positioned athwartships, sample a large volume of water.

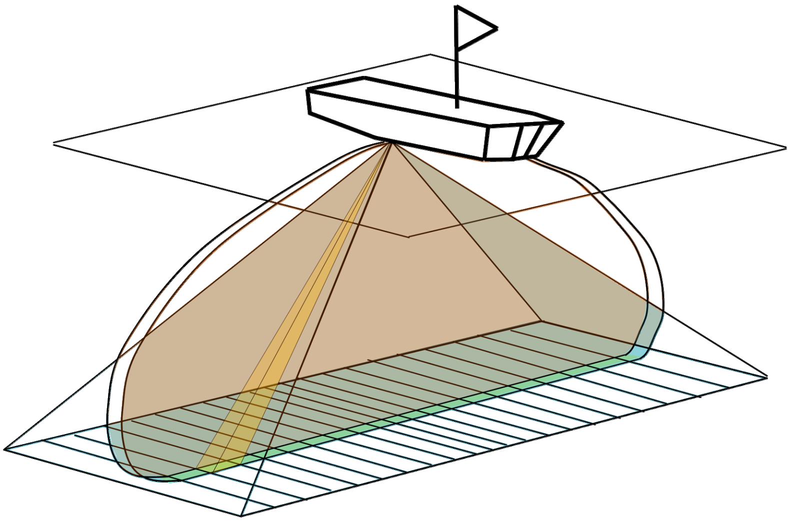

Figure 2.3.4. *Schematic of a vessel mounted multi-beam sonar system with transmit beam (red), received beam forming (blue) and an example receive beam (yellow).*

The use of MBS systems in place of single-beam echosounders for bio-acoustic surveys is warranted by their increased coverage (Figure 2.3.4). The increased sampling volume can improve acoustic estimates of biomass and abundance and better characterize spatial distributions of organisms (Gallaudet and de Moustier, 2002). However, MBS systems have been inhibited in collecting water column backscatter by the sheer volume of information entailed and the data processing capabilities available. As a result, since their inception, the systems were designed to record only the returns from the seafloor. Advances in hardware and processing technology alleviated this somewhat and allowed the introduction of MBS systems as a fisheries assessment tool, recording the complete water column (Mayer *et al.*, 2002), although processing and large data storage requirements have so far limited its practical applications. Modern MBS are capable of acquiring Gigabytes per minute if sampling a 100 m water column, leading to Terabytes per day of a survey (Malzone *et al.*, 2007, 2008, author *pers. obs.*).

Calculations of received fish backscatter become more complex as they involve greater angles of off-axis incidence requiring precise positioning of the sonar swath. The additional beams and angles require application of the techniques involved in single-beam acoustics across the MBS swath. Further detail on calibration and quantification of MBS systems can be found in Foote *et al.* (2005), Cochrane *et al.* (2003) and Chu *et al.* (2002).

With the addition of a third dimension in the MBS data (resolution across the vessel track), detecting objects such as fish schools is no longer a straightforward procedure (Buelens *et al.*, 2005). MBS data is less intuitive, since visualisation of such data is a challenging computational task. A data mining approach leading to useful data products is required (Buelens *et al.*, 2005). However, once fish targets are detected, they are located in space and time, allowing visualization within a four-dimensional environment (x, y, z and time), providing detailed data on school structure and vessel avoidance or migratory characteristics (Wilson *et al.*, 2005).

Modern shallow water MBS systems such as the RESON 8125 and 7125 operate at frequencies in the hundreds of kHz (455 and 400 kHz respectively), thus due to attenuation at such frequencies, the range of MBS systems is considerably less than echosounders. In order to observe deeper groups of fish and maintain the resolution that such systems can operate at, it may be necessary to fit the system to an underwater vehicle for remote operation.

2.3.1.5. Mobility and vessel avoidance behaviour in active acoustics

A basic assumption of the conventional echo-integration method for fish abundance estimation is that the presence of a research vessel has an insignificant influence on fish behaviour (Johannesson and Mitson, 1983). However, if fish escape from the path of the echosounder, significant underestimates of fish densities may occur (Olsen, 1990). Beam geometry and vessel speed suggest horizontal avoidance is a more critical factor than other directions (Misund and Aglen, 1992). In the past fish have been thought to react to noise stimuli by swimming radially downwards (Olsen *et al.,* 1983).

Modern vessels generate low-frequency sound which is within the hearing range of teleosts (Hawkins, 1986), possibly causing avoidance behaviour (repulsion) up to hundreds of metres ahead of the research vessel (Soria *et al*., 1996, Handegard *et al*., 2003), whereas vessel shadow, hull pressure waves, trawl sweep lines or towed objects are the most likely factors causing closer avoidance reactions (Freon *et al*., 1992; Ona, 1999). It has also been suggested that noise directivity pattern of the vessel and hull shadowing create lobes of maximum intensity, either side of the vessel, and that these lobes may contribute to herding effects (Urick, 1983).

Analysis of avoidance behaviour via the use of single-beam echosounders alone is severely restricted by the geometry of the beam. However, as with aggregation mobility, beam geometry of MBS, indicate increased ease in quantifying fish avoidance reactions as documented by Misund and Aglen (1992), Soria *et al*. (1996) and Gerlotto *et al*. (1999).

2.3.1.6. Species classification of active acoustic returns

Due to the stochastic nature of fish acoustic reflectance, individual responses are not always species specific. Many aggregations and/or schools of fish are packed at such densities that single sample volumes include response from multiple fish rendering individual TS unusable as a discriminator of species. Therefore other methods of classification are required. Species identification is an integral component of modern fisheries assessments, and yet there is still a relative scarcity of assessments that implement stock identification requirements (Begg *et al.*, 1999, Begg and Waldman, 1999). Scrutiny of echograms, based on human experience, is a subjective method of determining species in an area of high species diversity (Reid *et al.*, 1999). Therefore increasing the reliability of acoustic fish identification has become a much researched area (Reid *et al.*, 2000, Lawson *et al.*, 2001, Kang *et al.*, 2002). Several protocols are now in use to characterise aggregation species by image analysis techniques and comprise a catalogue of acoustic, morphological and positional data (Iglesias *et al.*, 2003).

The specification of standard protocols for the extraction of parameters from acoustic surveys of fish aggregations at a school and Elementary Distance Sampling Unit (EDSU) level (typically 0.05 nautical miles) was undertaken by 'C.L.U.S.T.E.R.', a European Union research project (Reid *et al.*, 2000) and a similar approach was undertaken at the Memorial University of Newfoundland, culminating in the development of a computer application FASIT (Fisheries Assessment and Species Identification Toolkit) to identify schools (LeFeuvre *et al.*, 2000). The foundation behind these processes is that morphometric characteristics of the fish 'school' in two-dimensional echograms, such as altitude, height, width and edge roughness, are species-specific. Intuitively, this technique is more useful in areas of low species diversity and homogenous aggregations, such as the north Atlantic. Confidence in identification of species decreases in areas of greater bio-diversity with heterogeneous aggregations.

Alternative methods for species classification, such as wide-band acoustics and multi-frequency techniques (Scalabrin *et al.*, 1996), have shown promise in experimental studies (Koslow and Kloser, 1999, LeFeuvre *et al.*, 2000).

2.3.1.7. Alternative techniques

Active acoustic techniques have been adapted in several innovative configurations to minimise avoidance effects including the Bergen Acoustic Buoy (Handegard *et al.*, 2003), toroidal sector scanners (Gallaudet and de Moustier, 2002), offset multi-beam and sonar (Soria *et al.*, 1996), single-beam arrays (Godø *et al.*, 2005), and multi frequency techniques, such the MUFTI (Kloser and Horne, 2003). Transducers may be placed at the head of a trawl net ('netsonde') to observe fish entering or escaping the trawl (Simmonds and MacLennan, 2005). DIDSON acoustic cameras have been shown to provide short-range high resolution imagery of fish (Simmonds and MacLennan, 2005, Holmes *et al*., 2009).

2.3.2. Passive acoustic techniques

Winn (1964) and Fine *et al*. (1977) summarised sounds produced by fish as associated with one of several categories including; aggressive encounters (usually territorial); reproductive; echolocation; schooling; recognition; feeding; migration; exploration; distress; and not-understood. Often species are characterized by their specialisation in acoustic communication. An example is the Sciaenidae family, often calle drums or croakers, (Fish and Mowbray, 1970) which are typically signified by their well-developed, fast acting muscles that they use to vibrate the swimbladder (Moulton, 1963). Many of such sounds are associated with reproductive behaviour (Guest, 1978, Mok and Gilmore, 1983, Saucier and Baltz, 1993, Connaughton, 1996, Luczkovich *et al*., 1999b, Holt, 2002, McCauley, 2001).

Calling by spawning fish has been commonly reported (Mok and Gilmore, 1983), and recently techniques have been employed to use calling behaviour to locate aggregations (Saucier and Baltz, 1993, Luczkovich *et al*., 1999a, Holt, 2002). Although many species utilise sound as part of their reproductive behaviour (Mok and Gilmore, 1983, Saucier and Baltz, 1993, McCauley, 2001), the production of sound by aggregating fishes may serve several functions and requires elucidation. The male haddock *Melanogrammus aeglefinus*, for example, produces sounds of varying characteristics in the lead up to, and during courtship (Hawkins and Amorim, 2000) while the male Atlantic Cod (*Gadus morhua*) exhibits different call types with associated visual displays (Nilsson, 2004). McCauley (2001) speculated that the habit of fish calling "en masse" in Terapontidae and Sciaenidae choruses in Eastern Australian waters may function as one or more of: increasing the 'catchment area' of the aggregation; to 'prime' nearby fish for spawning; or to assist in mate selection and mediating gamete release. However, as spawning in these species is invariably in a dark environment after dusk, specific confirmation is difficult to obtain. By comparison, correlations have been shown between calls and spawning related events for species that have been observed by diver or video, with simultaneous acoustic monitoring, either *in situ,* (Lobel, 1992, Mann and Lobel, 1995, 1998, McCauley, 2001, Sprague and Luczkovich, 2004) or *in aquaria* (Allen and Demer, 2003).

Many fish sounds contain species-specific pulse rates, spectral peak frequencies and structures (Lobel and Macchi, 1995; Mann and Lobel, 1998) and are repeated with little change, allowing the identification of a sound by simple parameters, such as duration, peak frequency, repetition frequency and bandwidth (Mann, 2002). The identification of a single spawning call has been recorded on many occasions (Mok and Gilmore, 1983, Luczkovich *et al.*, 1999a, McCauley, 2001, Hawkins, 2002, Sprague and Luczkovich, 2004). The application of such techniques to estimate absolute biomass has, as yet, not been conducted.

The characteristics of fish calls such as spectral peak frequency can be individually specific (Parsons *et al.*, 2006a) and reportedly discerned by recipients (Myrberg and Spires, 1972, Myrberg *et al.,* 1993). Animal calls contain energy at many frequencies, the measurement of which involves the application of frequency filters or Fast Fourier Transforms (FFT) to break down the full frequency band into finite bands. Similarly, the thresholds at which animals can perceive acoustic energy vary with frequency (Ladich and Popper, 2001). It follows that differing levels of energy may be encompassed in differing frequency bands and as such it is important to report the frequency range over which a measurement is taken. If results are presented with no bandwidth is given, it is implied that all energy associated with the signal is included in the measurement (McCauley, 2001).

FFT analysis divides the signal into specified linear frequency bins with optional filtering of bin edges and can therefore provide any required frequency resolution, within the available sampling constraints. As human and many animals' perception of frequency are logarithmic, frequency filter techniques often use 1/3 octave sample bands (McCauley, 2001). Reporting of such bands are presented as dB re 1 μPa over the 1/3 octave, with a specified centre frequency. A further measure of intensity is given by the spectral level, for example if a signal is divided into 1Hz bins the intensity is measured as dB re 1 $\mu Pa^2/Hz$. If this were to be expressed over the frequency band f_b the spectrum level (L_s in dB re $1\mu Pa^2/Hz$) is equal to:

$$L_s = L_b - 10\log_{10}(f_b) \tag{2.16}$$

where L_b is the broadband intensity level across the frequency band (McCauley, 2001).

Fish calls are often emitted and received in shallow waters, over distances greater than the water depth. In such cases horizontal propagation of underwater sound in shallow water effectively occurs along a duct between the water's surface and the sea floor. The two surfaces may reflect sound as it propagates along or in the case of the sea floor sound may enter the bottom, but be refracted back into the water column at another location in the form of a head wave (Figure 2.3.5, McCauley, 2001). Reflectance and refraction by the sea floor varies with the nature of the substrate and the incident angle of the signal path (Jensen, 1997). A calm water surface, by comparison, may act as a perfect reflector (albeit with a complete phase inversion), if surface motion is significantly smaller than the signal wavelength. Therefore a hydrophone will receive not only the direct signal from the source, but shortly after a series of multi-path reflections (Figure 2.3.5), or in some environments a head wave preceding the water borne arrival (since sound speeds in the seabed may be significantly faster than in water). These multi-path effects become more important at shallower depths.

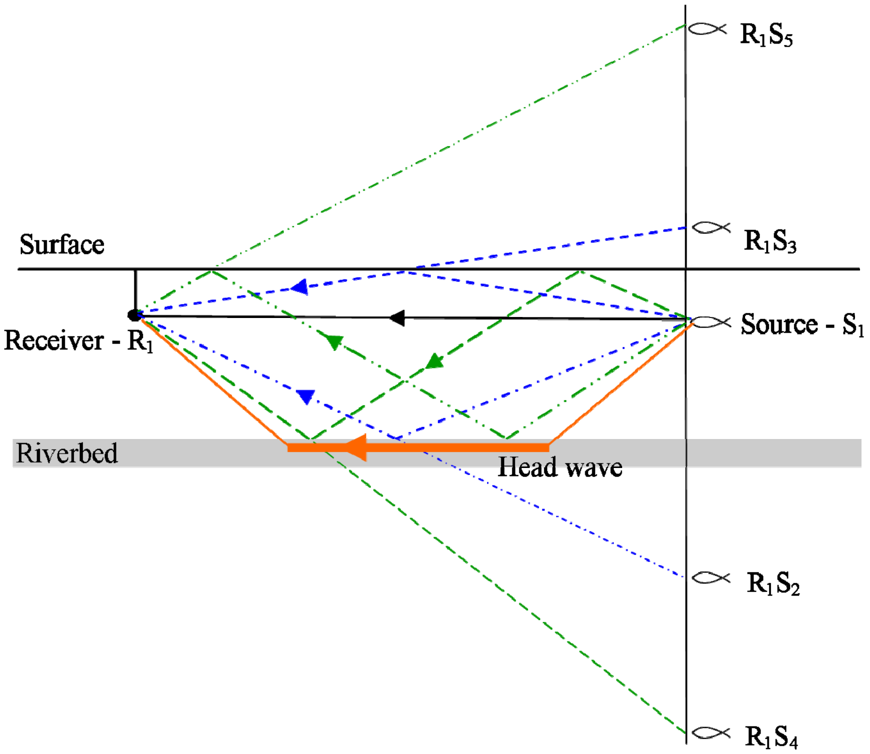

Figure 2.3.5. *Schematic of possible signal paths between source (S_1) and receiver (R_1) in shallow water, redrawn from McCauley (2001). Direct path is shown in black. Multi-paths and imaginary sources for four examples of surface reflection (one surface reflection – blue, two surface reflections – green) and a possible example of bottom surface head wave refraction (orange) are shown.*

Exact measurements of propagation losses depend on the acoustic properties of the location; however, minimum losses due to geometric spreading can be estimated (Sprague and Luczkovich, 2004).

2.3.3. Ground truthing of species presence and length distribution

Two important conditions of the spawning aggregation which require ground truth evidence are the species present within the sampled waters and proof of reproductive behaviour by the surveyed species.

One method of ground truthing acoustic surveys is the use of trawlers to sample surveyed schools (McClatchie *et al.*, 2000, Simmonds and MacLennan., 2005). Trawlers are common fishing vessels and the technique produces large sample sizes, although even trawl samples do not always provide reliable composition data (Hammond and Swartzman, 2001). However, the size of the aggregations of interest in this study are relatively small and numbers are such that trawling could wipe out the aggregation. Therefore ground truthing of species identification and spawning maturity in this study was conducted through video techniques and direct sampling (line fishing) during active acoustic surveys. Comparison of waveforms and spectral content of *in situ* passive acoustic recordings with control *in aquaria* calls from individuals of known size and species was sufficient to ground truth passive acoustic surveys at locations where inter species vocal diversity was small and animals could be maintained *in aquaria*.

Unless ground-truthed by video, proof of reproductive activity within spawning grounds have to be inferred from either the distribution of pelagic eggs or capture of ripe spawners (Hawkins, 2002, Holt, 2002). However, many species migrate immediately before spawning (Holt, 2002), and/or spawn in locations where tidal motion or currents affect egg location subsequent to release (Farmer, 2008) thus lack of eggs and/or larvae does not always preclude spawning behaviour at that location. Surveys of soniferous spawners such as red drum (*Sciaenops ocellata*) have shown that spatial and temporal distributions of egg and larval abundance can correlate highly to calling numbers (Gilmore, 2002). Thus if eggs are located near a soniferous aggregation it is a useful ground truth tool to associate calling with spawning behaviour (Luczkovich *et al*, 1999b).

2.4. General species characteristics and their implications for acoustic monitoring

The applicability of acoustic monitoring of a fishery is dependent on the biological and behavioural characteristics of the species targeted. Prior biological and ecological knowledge is therefore required to determine the most appropriate monitoring tool for a particular species.

2.4.1. Samson fish *(Seriola hippos,* Carangidae*)*

S. hippos is a pelagic member of the Carangidae family endemic to Australia, Norfolk Island and New Zealand (Paxton *et al*., 1989). The species is distributed around the temperate waters of Australia in depths up to 100 m (Hutchins, 2002). As a strong, pelagic fish the species has become renowned as a catch and release sports fish.

In Western Australian waters *S. hippos* migrate to Rottnest Island spawning sites (32° S) from as far south as Hopetoun (34° S) on the south coast (Rowland, *in prep.*). Reproductive growth variables have assigned the *S. hippos* spawning period as late spring to early summer, peaking in November/December and continuing through January (Mackie *et al*., 2009). During this period *S. hippos* form relatively stationary pelagic aggregations of fish numbering in the thousands. However, smaller numbers of schooling *S. hippos* have been observed in prior and subsequent months (M. Mackie, Department of Fisheries WA, *pers. comm.*).

Rowland (*in prep.*) showed that the length at first maturity, $L_{50,}$ for female *S. hippos* is 831 mm FL (fork length) corresponding to an age of *ca* 4 years. Length distributions from a recent study revealed a range of 550 to 1600 mm FL with a median of 1070 mm during 2004/5 and 2005/6 summer seasons off the Perth coast (Rowland, *in prep.*). This suggests that most aggregation members are of spawning maturity.

A recent tag and release study has shown that *S. hippos* exhibit high post release survival rate (Rowland, *in prep.*). *S. hippos* is a physoclist (Fishbase, www.fishbase.org/Summary/SpeciesSummary.php?ID=71) and possesses a swimbladder of large relative size, which extends the length of the body cavity, as shown in Figure 2.4.1. Although, on occasion, subjects may exhibit a swollen swimbladder upon capture, the species possess the ability to vent gas from the swimbladder when rising to the surface, through an opening under the operculum (Rowland, 2010). Voluntary, rapid venting of significant volumes of gas has been observed in free swimming individuals as they followed a captured fish to the surface (Mackie *et al*., 2009). As a result, the volume of the swimbladder at capture has been assumed to be representative of the volume at greater depths (~50-100 m depth). Additionally, even though the species is physoclistous, variations in swimbladder volume due to upwards migration, would not be expected, hence variation in target strength with depth may be low. However, the speed with which the species may secrete gas to maintain volume during a downwards migration is unknown. Neither is the effect of tilt angle on target strength during vertical migration.

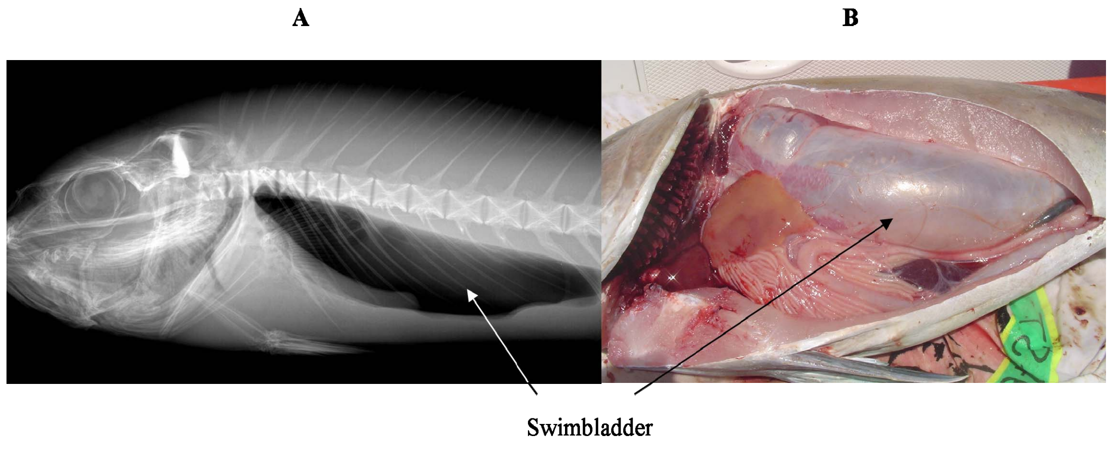

Figure 2.4.1. *X-ray (A) and photo (B) of Seriola hippos swimbladder from a fish of approximately 1 m fork length. Photo and X-ray courtesy of and A. Rowland, Murdoch University.*

The sagittae of *S. hippos* are small and fragile (Mackie *et al*., 2009) and atypical of those usually associated with vocalising species. No apparent musculature has been observed that would commonly be associated with swimbladder generated vocalisation (author, *pers. obs.*). Although other carangids have been shown to produce sound (Fish and Mowbray, 1970) the *S. hippos* biology, combined with typical spawning behaviour in clear open water where visual cues are effective suggested that *S. hippos* would not be suited to passive acoustic monitoring. The stationary, pelagic and non vocal nature of *S. hippos* aggregations led to acoustic targeting predominantly using single-beam echosounding and MBS.

2.4.2. Mulloway *(Argyrosomus japonicus*, Sciaenidae*)*

Globally *Argyrosomus japonicus* is a commercially important species reported along the coastlines of South Africa, India and northwest Pakistan, Taiwan and China through to South Korea and Japan. (Trewavas, 1977, Kailola *et al*., 1993, Griffiths and Heemstra, 1995). Therefore the identification of an effective means to monitor such a soniferous species of fish has international benefits.

In Australia *A. japonicus* is distributed in temperate and sub-tropical waters across the southern coastline, bound approximately by Carnavon on the west coast and Bundaberg on the east coast at roughly 25° latitude. Here *A. japonicus* is a recreationally and commercially important species which aggregates to spawn in near shore coastal waters or estuaries (Farmer, 2008). In Western Australia the species reportedly reaches spawning maturity at approximately 75 cm while the largest captured fish on record was 75 kg and 1.81 m (Silberschneider *et al*., 2009, Fishbase, http://www.fishbase.org/Summary/speciesSummary.php?ID=11979&genusname=Argyrosomus&speciesname=japonicus).

In recent years total commercial catch in Western Australia has been typically 50 to 100 tonnes per annum with the West Coast Bio-region contributing the greatest figures (Mackie *et al*., 2009). Estimates of recreational line fishing are such that in *A. japonicus*, it is greater than that of commercial fishing (Farmer, 2008). The decline in annual presence of *A. japonicus* in the Swan River, a prime Western Australia spawning site, is shown by the average annual commercial catch between 1912 and 1974 at 1.5 tonnes dropping to 173 kg between 1995 and 2004 (Riggert, 1978, Farmer, 2008).

Spawning of *A. japonicus* occurs on the lower west coast between November and April, when the mean monthly water temperatures typically exceed 19°C, in contrast to the upper west coast, where the mean monthly water temperatures do not fall below 19°C and spawning occurs all year round (Farmer, 2008). Seasonal movement in Perth metropolitan waters has been shown in adult *A. japonicus,* where near shore encounters were more common during the summer months with fish moving offshore to *ca.* 100 m depth from May through to winter (Farmer, 2008).

A comparatively small number of large individual *A. japonicus* migrate into the Swan River, Western Australia to spawn, reportedly linked with variations in salinity (Loneragan *et al.*, 1989, Farmer, 2008). In a recent study all samples captured in this area during the spawning period were above the L_{50}, the size of first maturity. The opportunistic biological sampling conducted during spawning months showed a male: female *A. japonicus* ratio of approximately 1.3:1 (n = 62) in the Swan River and 1.15:1 (n = 31) across the lower west coast of Western Australia (Farmer, 2008). Spawning typically occurs around dusk or at times of darkness, inferred by the capture of females between 21:00 and 23:30 all of which possessed stage VI ovaries (Farmer, 2008). As many of the females caught immediately prior to the peak of high tide had ovaries containing hydrated oocytes a further link between spawning and high tide was inferred (Farmer, 2008), though this behaviour was not reported in other studies (Ueng *et al.*, 1998). *A. japonicus* is an indeterminate spawner, *i.e.* fecundity is not determined prior to the onset of spawning (Hunter and Macewicz, 1985). In Western Australia the species exhibits batch spawning, often releasing and fertilising eggs on a daily basis in a cyclic pattern peaking every few days (Farmer, 2008, Challenger Institute of Technology, unpublished data, author, *pers. obs*.). Although feasible, whether female *A. japonicus* return every night to the same location in the Swan River to spawn is unknown.

A. japonicus possess large otoliths, bi-lateral sonic muscles surrounding a large swimbladder (as observed by specimens captured in shallow waters) and have been reported as exhibiting vocal behaviour around the time of spawning (Griffiths and Heemstra, 1995, Ueng *et al.*, 1998, 1999, 2007, Parsons *et al.*, 2006, Farmer, 2008). Although group spawning has been reported in captivity (Ueng *et al*, 2007), individual *A. japonicus* in the Swan River are thought to spawn in comparatively small numbers (Farmer, 2008). Vocalisations audible above water are also frequently reported (anecdotal reports by fishers, author, *pers. obs*.). At other locations, around the world, sound production has been reported at times of spawning groups of the same species (Ueng *et al*., 1998, 1999, 2007).

As a large member of the Sciaenidae family *A. japonicus* is particularly prone to exploitation (Farmer, 2008) similar to that of other family members (Griffiths, 1996, Sadovy and Cheung, 2003). Juvenile *A. japonicus* are considered as a poor quality food by many fishers and subsequently released (Cusack and Roennfeldt, 2002), however, the species is sensitive to handling and stress resulting in high catch and release mortality (Gray, 2002, Farmer, 2008, Mackie, 2009). The proximity and ease of access to the spawning aggregations resulting in recreational fisher targeting of mature *A. japonicus* within the lower Swan River combined with the acoustic conditions of Mosman Bay provides an ideal case study to observe the capabilities of monitoring spawning aggregations of soniferous fish and their reaction to anthropogenic impacts.

The vocal behaviour exhibited by *A. japonicus* aggregations in sparsely populated, shallow water, led to the study of this species primarily using passive acoustic techniques, with consideration for active acoustics. As the species is prone to capture and handling stress catch and release techniques were thought to be inappropriate for ground truthing.

2.4.3. Pink snapper (*Pagrus auratus*, Sparidae)

Distributed across Australia's southern coastline, *P. auratus* is a highly valued catch within Western Australian waters, which reaches maturity at approximately 57 cm and a maximum reported length in the Perth metropolitan area of 105 cm (Wakefield, 2006). Catches of *P. auratus* are the greatest of any demersal finfish species in many coastal regions of Western Australia (Wakefield, 2002). *P. auratus* are found from shallow coastal lagoons and embayments to depths greater than 200 m on the continental slope, forming dense spawning aggregations in shallow bays such as Cockburn Sound (Moran *et al*., 1998, Wakefield, 2006).

In the Perth region *P. auratus* spawn between October and December. Spawning peaks have been observed at new, and to a lesser extent, full moons when tidal ranges are at their greatest (Wakefield, 2006). This tendency to spawn at the same location on predictable nights, within the first three hours of slack high tide leaves the species susceptible to exploitation. Within Cockburn Sound, the egg concentrations have shown that an aggregation forms firstly in the northeast area of Sound, moving to the middle and ending in the northwest across the spawning season (Wakefield, 2006), suggesting that the aggregations respond to changing flow dynamics. During spawning, the fish themselves are particularly mobile and form aggregations in shallow waters where they are prone to wary behaviour in the presence of vessels (Mackie *et al.*, 2009).

Although sound production is unreported in *P. auratus* some members of the Sparidae family are soniferous (Tavolga, 1974, Cruz and Lombarte, 2004). Overall the relative sagittal otolith size of Sparidae is between Labridae and Sciaenidae with the smaller members displaying distinct male/female colour contrasts and no vocal ability (Cruz and Lombarte, 2004). Paxton (2000) hypothesised that members of the Sparidae family with a larger relative sagitta, such as *P. auratus*, ought to be soniferous, similar to other species of the family.

The aggregative spawning by large numbers of fish at a predictable location suggests that *P. auratus* will be suited to a form of active acoustic monitoring. Biological characteristics suggest that soniferous behaviour is possible; however, this hypothesis requires further investigation.

2.4.4. West Australian dhufish (*Glaucosoma hebraicum*, Glaucosomatidae)

Endemic to coastal waters of western and south western Australia the dhufish (*Glaucosoma hebraicum*) is a slow growing, sedentary, demersal species inhabiting reefs and caves to depths of 200 m (Kailola, 1993, McKay, 1997, St John and Syers, 2005, Mackie *et al.*, 2009). The maximum reported *G. hebraicum* was 1.22 m long, weighing approximately 26 kg (Fishbase, http://www.fishbase.org/Summary/speciesSummary.php?ID=14956&genusname=Glaucosoma&speciesname=hebraicum).

Although 100 by 10 m deep "ghost patches" of thousands of *G. hebraicum* have been historically reported in the Capes region of Western Australia, the species is now typically found in groups of three and to a lesser extent up to ten (Mackie *et al*., 2009). Occasionally groups numbering in the tens of *G. hebraicum* have been observed along the West Coast Bio-region (A. Grochowski; G. Shedrawi, University of Western Australia, *pers. comm*.). *G. hebraicum* exhibits low levels of migration (up to tens of kilometres onshore-offshore), possibly for spawning and the species is known to vary spawning locations from year to year (Mackie *et al*., 2009). Histological ovary examination showed the species is capable of spawning over several days (Mackie *et al*., 2009), an observation of possible behaviour which is problematic to confirm in the wild.

Lack of variation in seasonal reproductive timing across the west coast bioregion suggests that factors other than environmental variables, such as social cues influence spawning (Mackie *et al*., 2009). Male co-habituation of an area, indicative of lekking behaviour, has been observed and the relationship of lower spawning frequency with size corroborates a social structure whereby the largest males sire the greater number of juveniles with the largest female (Mackie *et al*., 2009). The influence of social cues is an important trait when considering the possibility of passive acoustic monitoring because it suggests a higher order of communication between individuals.

X-rays and dissection of *G. hebraicum* have revealed specialised red fibre muscles attached the anterior end of the swimbladder (Vu, 2007), typical of muscles used in sound production. It is therefore anticipated that passive acoustics may be an appropriate method for monitoring *G. hebraicum* at known spawning locations and that larger groups may be observed via single-beam and MBS active acoustic techniques.

2.4.5. Bight redfish (*Centroberyx gerrardi*, Berycidae)

Bight redfish (*Centroberyx gerrardi*) mainly inhabit deep waters along the edge of the continental shelf and can live to at least 64 years and 66 cm (Mackie *et al*., 2009). *C. gerrardi* abundance in WA waters has led to consideration of the species as a key indicator for environmental health and as such the need for biological data has been acknowledged (Penn, 2005, Mackie *et al*., 2009). Inshore migration has been reported in this species in the Cape Naturaliste region to form spawning aggregations between February and April (Mackie *et al*., 2009). *C. gerrardi* develop spawning testes greater than 3 % of total body weight indicating multi-male, multi-female spawning and therefore aggregations of large numbers of fish. The high numbers of spawning fish throughout the spawning season and their comparatively low batch fecundity indicates individual fish participate in multiple spawning events throughout the season (Mackie *et al*., 2009). Similar species of the Beryciforme order exhibit nocturnal behaviour (Cruz and Lombarte, 2004), possibly for spawning purposes.

Preliminary dissection of *C. gerrardi* revealed a swimbladder extending the length of the body cavity. The swimbladder walls were connected to several felixible pairs of surrounding ribs. A minimum volume for the swimbladder was therefore inferred as it was considered unlikely that effects of swimbladder expansion with pressure would have affected the rib cage. The species also possess proportionally large otoliths to body size, and a unique set of 'horns' at the swimbladder posterior end (Vu, 2007). A possible method of sound production was deemed to be contraction of extrinsic epaxial muscle over flattened ribs which were attached to the swimbladder wall (Vu, 2007), bearing striking resemblance to the Carapidae mechanism of sound production (Parmentier *et al*., 2006). Considering the positive relationship between hearing sensitivity and swimbladder size with acoustic communication (Ladich and Popper, 2001), combined with the species' nocturnal behaviour and the presence of swimbladder adaptations suggestive of producing sound, it is probable that *C. gerrardi* is a soniferous species. The propensity to aggregate in large numbers and possible vocal behaviour suggest the *C. gerrardi* is a candidate for both active and passive acoustic monitoring.

CHAPTER 3

3. Active acoustic techniques for monitoring fish aggregations

3.1. Overview

The aim of Section 3.1 is to introduce each stage of active acoustic study conducted on aggregations of demersal finfish species within the West Coast Bio-region of Western Australia. This section also outlines a brief summary of the fishery and acoustic characteristics of the aggregation sites for the primary study species *Seriola hippos*. The subsequent sections of Chapter 3 attempt to address the active acoustic analysis of spawning aggregations using echosounding and multi-beam sonar (MBS) systems. The assessment includes three individual sections, in the form of stand alone papers, concentrating on *S. hippos*. These sections are:

Section 3.2 reports the findings from single-beam echosounder surveys of *S. hippos* aggregations conducted over three consecutive spawning seasons. This section attempts to address some of the advantages and limitations of echosounding techniques available to most fisheries researchers, by evaluating relative biomass and spatial distribution, measured for *S. hippos* aggregations over differing sites and seasons. This section is an adaptation of a peer reviewed paper submitted and published in the conference proceedings of the Australian Acoustical Society, Busselton, WA, November, 9-11th, 2005 (Parsons *et al*., 2005). For brevity much of the paper's introduction has been removed as it been presented previously.

Section 3.3 describes surveys of *S. hippos* conducted with a RESON 8125 MBS and its ability to image the aggregation and in doing so highlights issues with current MBS for routine monitoring of fish schools and aggregations.

Few MBS systems maintain the capability of acquiring high resolution backscatter from the seafloor and water column at sufficient sampling resolution to map the seafloor habitat and fish school. The RESON 7125 is a recently designed MBS with combined processing and sampling capability greater than previous systems. Section 3.4 attempts to evaluate remaining issues with MBS systems in this acquisition by the evaluation of all acoustic backscatter data acquired in a RESON 7125 Seabat MBS survey of *S. hippos* aggregations.

Findings of acoustic backscatter collected from other targeted species are reported in Section 3.5, with conclusions drawn on the performance of active acoustics as a tool to study spawning aggregations discussed in Section 3.6.

3.1.1. Acoustic characteristics of the study sites

Rottnest Island lies approximately 12 nautical miles off the Perth coastline (Figure 3.1.2). In waters 5 - 10 nautical miles west of Rottnest Island is an area where several decommissioned vessels have been deliberately sunk over the past few decades. The wrecks lie in ~110 m of water, partially buried in a flat, sandy, seabed. Wreck protrusion into the water column and relief varies from site to site, with some wrecks all but covered by the sand. Seven sites were surveyed in all, with three primary sites “Secret Spot” (Site 1), “North Barge” (Site 2) and “Outer Patch” (Site 3) the focus of single-beam and MBS surveys (Figure 3.1.1). The location of Site 1 (relatively close to Sites 2 and 3) is withheld on behalf of local fishermen.

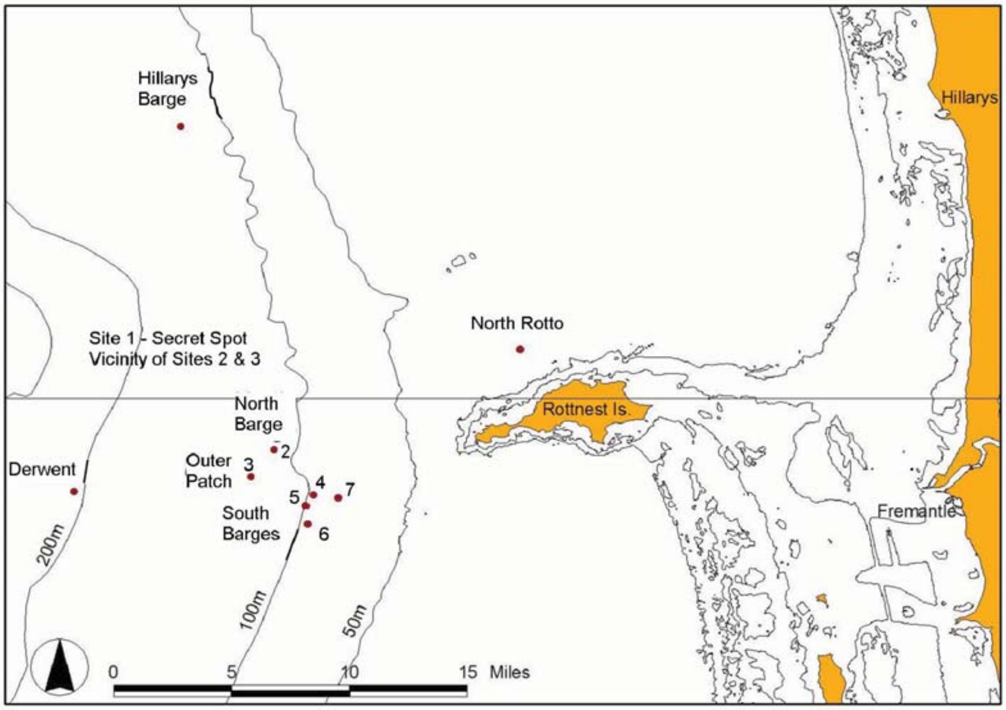

Figure 3.1.1. *Aggregation sites of Seriola hippos west of Rottnest Island. Primary sites used in this study included the North Barge, Outer Patch and Secret Spot (not shown). South Barges (Sites 4-7) are shown, together with other examples wreck sites from which aggregations have been reported (Derwent, North Rotto, Hillarys Barge). Map taken from FRDC report 2004/051 (Mackie et al., 2009).*

3.1.2. *Seriola hippos* fishery

Although *S. hippos* is considered as a poor quality table fish it is often found in the bycatch of fishers targeting other species. Aggregating *S. hippos* have been targeted at Rottnest Island sites by sport fishers since the 1990s. In more recent years the fishery, once only attainable to knowledgeable skippers, is now a popular tag and release sport fish area to local recreational fishers (Mackie *et al*., 2009). Information from fishers suggested that the Rottnest Island aggregations remain stationary for several months (M. Mackie, Department of Fisheries WA, *pers. comm.*), positioned above the wrecks. The combination of such stationary behaviour and the expectation of strong acoustic TS suggest that the *S. hippos* aggregations are an excellent subject to test assumptions and performance of single-beam and MBS systems.

Ground truthing of species presence and mean length were taken from 2004/5 and 2005/6 tag and release programs, towed underwater mono and stereo video cameras, and biological sampling conducted as part of the "Samson Science' Section of the FRDC project "Management and Monitoring of fish spawning aggregations within the West Coast Bio-region of Western Australia", No. 2004/051.

3.2. Seasonal spatial and temporal patterns of *S. hippos* aggregations, west of Rottnest Island using single-beam acoustics

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ABSTRACT

Coastal waters of Western Australia are home to many species of demersal fish that migrate to form short-lived aggregations in order to spawn. These spawning aggregations form at the same sites over successive, predictable spawning seasons. Due to the exploitation of demersal finfish spawning aggregations within the West Coast Bio-region, recent attention has been paid to using acoustic techniques to assess these aggregations. A well established tool for biomass estimates, used in these evaluations, is single-beam echosounding. As part of the Management and Monitoring of Fish Spawning Aggregations project of the Department of Fisheries, acoustic data on fish aggregations have been recorded between November 2004 and March 2007, using a SIMRAD EQ60, single-beam echosounder, operating at frequencies of 38 kHz and 200 kHz. These recordings were made at various locations around Rottnest Island, where recurring spawning aggregations have been reported. At selected sites, towed video and physical sampling techniques were employed to ground-truth acoustic results. Analysis of acoustic backscatter measurements from selected sites is presented. Relative abundance of each aggregation was calculated, based on acquired in situ target strength data and a target strength model of Yellowfin tuna. Preliminary conclusions are drawn in respect to the use of a single-beam echosounder for estimating fish stocks within dense and sparsely populated aggregations.

Keywords: *Single-beam, aggregation, biomass, target strength.*

3.2.1. Introduction

Detection of a received echo provides little more knowledge than the presence of an unidentified object within the field of the transducer; however, if viewed over time, characteristics of the object can be identified (Simmonds and MacLennan, 2005). The mapping fish schools using a single-beam echosounder was pioneered by Olsen (1969). It is considered a cost effective and simple method of managing an aggregation of fish, with the advantage that most commercial and charter fishing vessels possess the technology to add further data during opportunistic tracks over a given group of fish.

It is the evaluation of *S. hippos* aggregations near Rottnest Island, Western Australia, by means of single-beam acoustic surveys that this section and its associated research within the Department of Fisheries project are concerned with. The purpose of the study was to determine relative estimates of *S. hippos* abundance within the aggregations, to monitor the aggregations over consecutive spawning seasons and to identify any characteristic complexities associated with the single-beam acoustic observation of the *S. hippos* west of Rottnest Island.

3.2.2. Methods

3.2.2.1. Study areas

Rottnest Island lies 12 n. mi. off the Perth coast. In the waters to the west of the island is an area in which several decommissioned vessels have been deliberately sunk over the past few decades (Figure 3.1.1). The results presented below were compiled from data acquired from seven such sites, the location of one of which remains confidential on behalf of the fishermen and local bodies who supplied it. Acoustic characteristics of the sites can be found in Section 3.1.1.

3.2.2.2. Data Acquisition

Single-beam acoustic backscatter data were collected west of Rottnest Island between 23rd November 2004 and 23rd March 2007, from a 21ft Fisheries research vessel, the *RV Snipe*. A Simrad EQ60 echosounder was mounted on the port side of the vessel (as shown in Figure 3.2.1), at a depth of 2.7 m, when the pole was extended. System specifications of the Simrad EQ60 are given in Table 3.2.1, and the settings applied during the surveys in Table 3.2.2. GPS data was acquired with a Garmin 76 hand held system and recorded live on the Simrad EQ60 hard drive. Roll, pitch, heave and heading data were not recorded.

Figure 3.2.1. *Pole mounting of the Simrad EQ60 single-beam echosounder on port side of the <u>RV Snipe</u>. Mounting is shown with pole retracted.*

Table 3.2.1. *SIMRAD EQ60 Echosounder Specifications.*

Operating Frequency (kHz)	38.08	198.864
Beam width (°)	6.5 (fore/aft) 10.5 (lateral)	7
Two-way Beam Angle (dB re 1 Steradian)	-14.00	-20.50
Absorption Coefficient (dB/m)	0.0098	0.0523

As reports from fishers suggested that aggregations were relatively stationary each site was surveyed by conducting grid transects (Kloser *et al*., 2000, Doonan *et al*., 2003), rather than 'star' surveys, to gain the greatest available coverage of the aggregation. Survey speeds were kept to below 3 knots in order to acquire maximum backscatter from single targets. The greatest coverage was given to Sites 1 ('Secret Spot') and 2 ('North Barge'), each of them surveyed on at least ten occasions throughout the project, providing the majority of the results. The vessel speed and ping rate was equivalent to an approximate inter-ping surface distance of 0.8 m (excluding effects of heave).

Table 3.2.2. *Settings of the SIMRAD EQ60 Echosounder during Rottnest Island surveys.*

Operating Frequency (kHz)	38.08	198.864
Transducer Draft (m)	1.6	1.6
Transmit Power (W)	500	1000
Pulse Length (ms)	0.512	1.024
Ping Rate (s^{-1})	1.9	1.9
Receiver Gain (dB)	19.5	26.3

Video tows were conducted immediately after several acoustic transects. This video data (C. Delacy 2005, University of Western Australia, *pers. comm.*), along with direct sampling from an ongoing 'Tag and Release' project (M. Mackie 2005, Department of Fisheries WA, *pers. comm.*) were employed to estimate *S. hippos* characteristics at the surveyed sites.

Where possible, the surveys of each site were conducted at the same time of day. Due to aggregation location, vessel availability and the fact that in this region seasonal seas caused by afternoon winds are often unsuitable for small vessels, the surveys were conducted between approximately 9 and 11 am. Additionally, a significant number of cray pot lines in the surrounding area reduce the ability to travel during hours of darkness. Variations in behaviour and target strength between day and night were therefore not investigated during this research.

3.2.2.3. System calibration

Calibration of the Simrad EQ60 was conducted as per Appendix 3.1. However, it was felt that the results of calibration were insufficiently accurate, due to issues with the mounting of the target sphere. Logistical and temporal constraints inhibited further calibrations and determination of a TS model specific to *S. hippos*, and therefore biomass estimates detailed below are considered as relative.

3.2.2.4. Data Processing

Acoustic data were imported into Echoview v4.1 software for processing. Echoview creates a two dimensional echogram (vessel distance and depth) from the raw data, allowing various acoustic characteristics to be exported. Background noise was removed using an Echoview template courtesy of Myriax software. The steps involved in noise removal were essentially: the removal of time varied gain from the entire echogram; identification of a section of the echogram spatially located near the aggregation, but without evidence of any biota; averaging of noise along the water column for the identified section; linear subtraction of the noise from the entire echogram; replacement of time varied gain to the entire echogram. The 1999 ICES study group on echo-trace classification suggested that threshold levels of approximately -60 dB leave only backscatter from pelagic schooling fishes (Anonymous, 1998). A conservative threshold of -65 dB was set on the 38 kHz echogram, to remove remaining noise and/or acoustic backscatter from small scatterers.

Although the 200 kHz beam was used whilst conducting surveys, Simrad EQ60 200 kHz transducers have shown non-linear effects with power levels exceeding 100 W (Simrad, 2002). At depths of 100 m the Simrad EQ60 required significantly more than 100 W to acquire sufficient *S. hippos* backscatter. Therefore, the 200 kHz beam data was used only to aid mapping of spatial extents of the aggregation and identification of individual targets.

Aggregation mapping

Live viewing of the Simrad EQ60 echograms highlighted dense areas of fish with peripheral individuals scattered around the aggregation. The Echoview 'schools detection' algorithms were applied to the echogram to detect two levels of structure. Dense 'schools' were detected linking any acoustic targets with maximum lateral and vertical distances of 10 m and a minimum dimension, in any direction, of 20 cm. Detection of sparse 'schools' incorporated a maximum distance of 25 m and the same minimum 20 cm dimensions. 'Schools' on the two-dimensional echogram, detected by the second algorithm were deemed as the total aggregation and annotation of any characteristics were preceded by A_t, to denote 'total aggregation'. Similarly, dense areas of the aggregation, detected by the first algorithm were given A_d. A minimum threshold of -60 dB was set for school detection, but no maximum threshold was used.

To discriminate acoustic backscatter derived solely from the aggregations, transects were truncated to the echogram sparse 'schools' in which only fish deemed members of an associated aggregation were present (Doonan *et al*., 2003). The cruisetrack GPS and echograms were compared to mark the extents of the aggregation in each transect. Due to the finite size of the acoustic beam, the image location of the first and last acoustic contact with the aggregation exaggerates the true size (Simmonds and MacLennan, 2005). Targets are first detected nearer the edge of the acoustic beam than the acoustic axis, at an 'attack' angle, when the number of fishes within the beam is sufficient to generate signal amplitude above the processing threshold (Diner, 1999, Reid *et al*., 1999). Diner (1999) noted that the angle between the acoustic axis and half power (φ_0) and that of between the axis and the 'attack angle' (φ_a) were different as shown by Figure 3.2.2A. Misund *et al*. (1995) reported that in schools of herring the difference between attack and half power angles could be as much as 50%. However, as the attack angle is determined by the fish density, which requires *a priori* knowledge of school structure, calculations were based on half-power beam angle, as a crude approximation (Reid *et al*., 1999, Simmonds and MacLennan, 2005). At depths of 80, 90 and 100 m the acoustic footprint of the Simrad EQ60 extends 8.2, 9.2, and 10.2 m respectively, both fore and aft of the acoustic axis. Corrections for beam width effects used by Echoview (Diner, 1999) have been shown to generally perform best at processing thresholds between -60 and -65 dB. Therefore, the detection threshold of -60 dB was compared to the positions of targets in the -65 dB thresholded echogram. This comparison ensured that the true fish locations were not masked by the detection threshold to a significant distance (Diner, 1999).

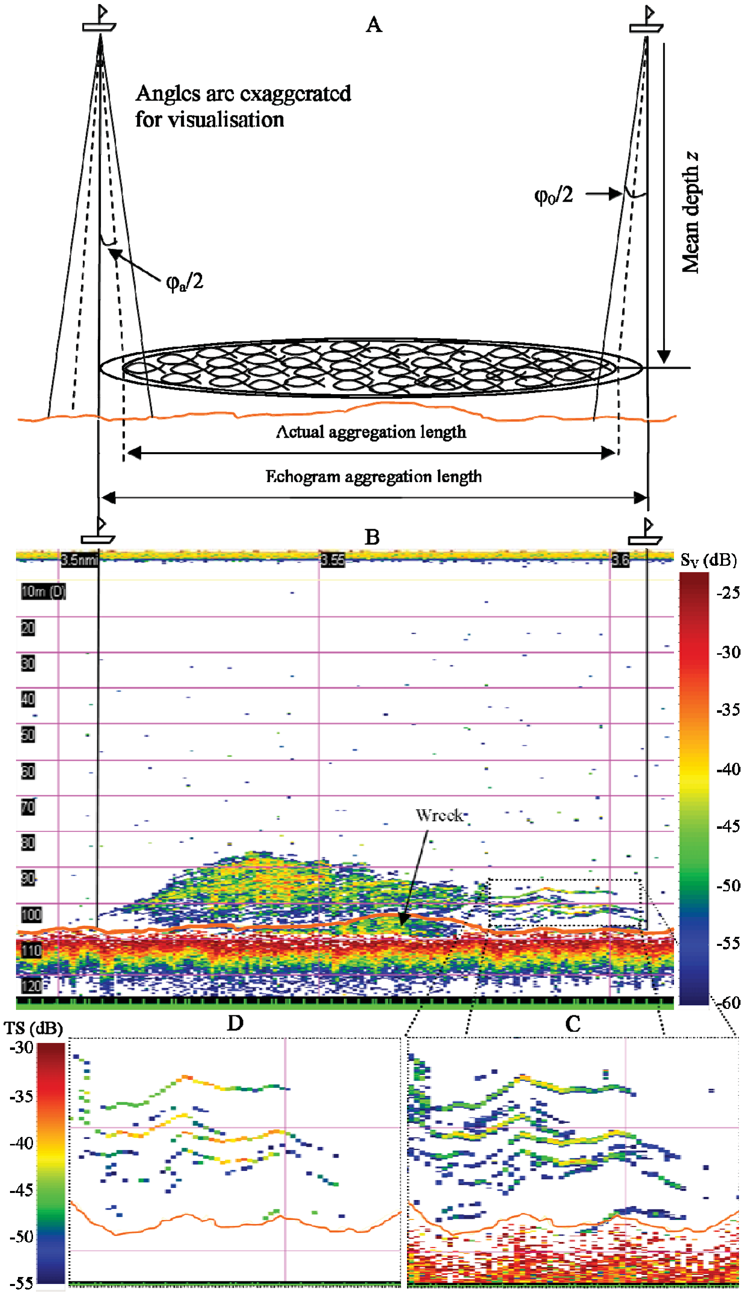

Figure 3.2.2. *(Top) Schematic of the effects of beam footprint on the echogram and actual locations of the edge of a* *S. hippos* *aggregation (A). Echogram of* *S. hippos* *aggregation thresholded at -60 dB, with backscatter from the wreck highlighted (B). Determined bottom used in analysis is shown by the continuous red line. An expanded area of single targets with associated* S_V *values (C) and TS values (D) is shown.*

As no motion sensor was available for the surveys the effect of roll and pitch on the location of detected aggregation edges, and therefore the aggregation spatial extents, was not evaluated. Morphometric and energetic characteristics of each echogram 'school' were corrected using Echoview algorithms and exported for cataloguing (further detail on school echo trace corrections can be found in Echoview help files).

To separate the backscatter from the seabed an Echoview line pick was applied to create a detected bottom. The line pick algorithm employed a minimum good pick S_V (volume scattering coefficient) of -70 dB, and a discrimination level of -50 dB, followed by a backstep of 1 m. The entire echograms were subject to scrutiny to ensure the correct application of the line picking and adjusted where required. Backscatter from the wreck, which protruded from the seabed, was often of similar intensity to that of a dense school of fish, as shown by the S_V values retrieved from the wreck and *S. hippos* aggregation in Figure 3.2.2B. Therefore, in several places manual scrutiny discriminated the seabed from biota based on three factors; the distance the region rose above the seafloor; how well the region was connected to the seafloor; and a comparison of GPS position to the bathymetry of the wreck obtained by bathymetric surveys (example bathymetric images can be found in Section 3.3).

Acoustic levels of data extraction can be split into three levels; school, Elementary Distance Sampling Units (EDSU), and region levels (Reid *et al.*, 1999). Analysis of *S. hippos* aggregation acoustic data was conducted at the 'school' level, split into three general categories; positional (temporal, geographic and vertical), morphometric (shape on echogram) and energetic (relative acoustic energy reflected) as outlined by Reid *et al.* (1999). Data on the further two categories of environmental and biological analysis was derived by ground truth data.

Relative biomass estimation

Without calibration coefficients or an accurate *S. hippos* TS – fish length relationship it was not possible to conduct accurate, absolute biomass estimates. This lack of relationship was compounded by the wide beam pattern of the echosounder and the inability to identify fish position within the beam. However, two methods were used to provide relative biomass estimates.

Firstly, calculations of *in situ,* relative mean TS were conducted using the Echoview single-beam method for target detection. To ensure that only single *S. hippos* targets were present within the sample volume, regions were defined around the tracks of numerous single fish in each aggregation (Figure 3.2.2C) and values of TS calculated (Figure 3.2.2D). The mean TS for each transect was then calculated in Echoview and exported. Mean TS is calculated in the linear domain (Ona, 1990). Therefore the mean target backscattering area $\overline{\sigma_{bs}}$ from each transect was calculated from Eq. 2.10. Averaging $\overline{\sigma_{bs}}$ from all transects provided $\overline{\sigma_{bs}}$ for the overall aggregation, which when substituted back into Eq. 3.1 gave the mean aggregation TS.

Secondly, to provide comparative figures, a known TS-length relationship was substituted for fish that are similar, and therefore acoustically comparable to *S. hippos*. Yellowfin (*Thunnus albacares*) and Bigeye (*Thunnus obesus*) tuna are physoclists of comparatively similar size and morphology to *S. hippos* (Fishbase, http://www.fishbase.org/Summary/SpeciesSummary.php?ID=71). From the ground truth data the mean fork length of *S. hippos* is 107 cm in the studied aggregations (Section 3.1). Using tuna TS at 38 kHz, as given by Bertrand and Josse (2000), with the *S. hippos* 107 cm Fork Length FL, the TS of each model is:

$$TS_{Ta} = 25.26 \log FL - 80.62 = -29.36 \text{ dB} \qquad (3.3)$$

$$TS_{To} = 24.29 \log FL - 73.31 = -24.02 \text{ dB} \qquad (3.4)$$

where TS_{Ta} and TS_{To} are TS values of *T. albacares* and *T. obesus* tuna respectively.

Individual *S. hippos* weight, W (kg) was estimated from Mackie *et al*. (2009) by:

$$W = 1.497e^{-4} FL^{2.982} \tag{3.5}$$

Integration of the Echoview detected 'schools' provided Nautical Area Scattering Coefficients (NASCs), which were averaged in the linear domain to give an equivalent NASC for separate, whole aggregations. Derived from the area backscattering coefficient (MacLennan *et al.,* 2002) the relationship between NASC and the biomass within the aggregation is as follows;

$$B = \frac{NASC}{4\pi 10^{\frac{TS}{10}}} \times W \tag{3.6}$$

where B is the biomass (tonnes/n.mi^2) and W is the weight of an individual fish (kg).

3.2.3. Results

The results obtained from the Rottnest Island sites broadly illustrated the advantages and disadvantages of using a single-beam echosounder to study the spawning aggregations.

Figure 3.2.3 shows a sample echogram from transects at Site 2, including some of the outlined school parameters. Many of the echogram images obtained, however, were not conducive to the image analysis protocols set out by Reid *et al*. (1999). Those techniques are more amenable to discrete, densely populated schools. By contrast, the morphological characteristics of the *S. hippos* aggregations contain not only a dense school, but also significant areas sparsely populated by dissociated members of the aggregation. These sparsely populated areas could not be easily dismissed, as they often contributed significant proportions of the biomass. In addition, the relationship between dense and sparsely populated areas may be correlated to behavioural patterns characteristic to a particular aggregation or its spawning maturity.

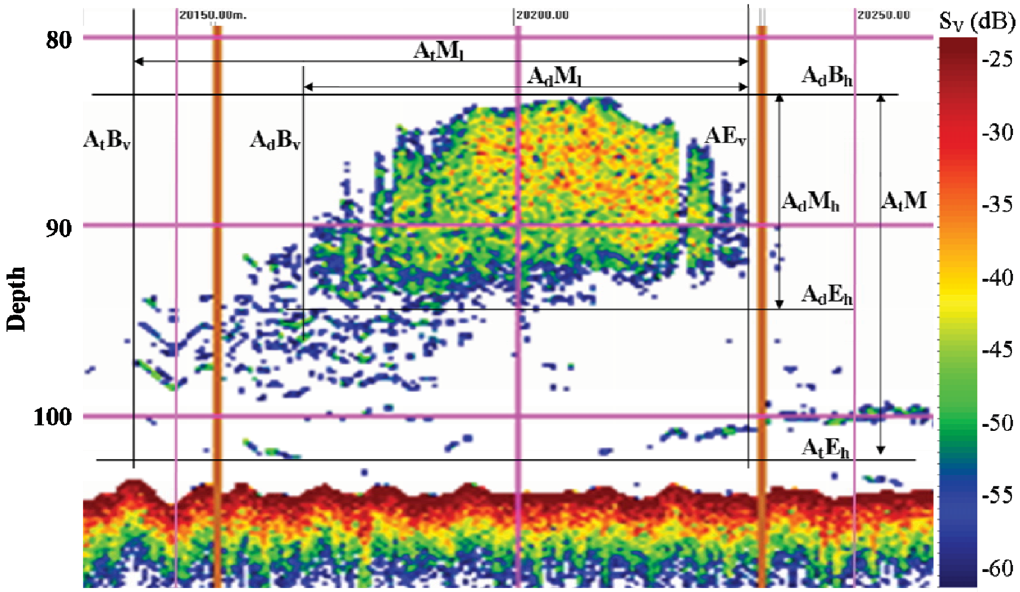

***Figure 3.2.3**. Echoview echogram of a S. hippos aggregation, including some morphological parameters. A_tB_v and A_dB_v are the vertical beginning of the total and dense area of the aggregation respectively. A_tE_v is the end of the total aggregation. A_dB_h and A_dE_h are the horizontal dense area beginning and end while A_tE_h is the horizontal total aggregation end. A_dM_h and A_dM_l are the maximum dense areal height and length respectively, while A_tM_h and A_tM_l are the total aggregation equivalent.*

Some of the catalogued school size parameters from echograms along multiple transects of the Site 1, 2 and 3 aggregations are shown in Table 3.2.3. Values measured from transects conducted at the edge of the aggregation were considerably lower due to the shape of the aggregation and the pattern of the acoustic beam, therefore maximum values are displayed to better illustrate the aggregation size.

Surveys showed that *S. hippos* aggregation depths ranged between 40 and 100 m, the lower limit being approximately level with the top of the wrecks, approximately 5-10 m above seafloor. Aggregations of increasing size expanded laterally and vertically, thus the height above the seafloor to which the aggregation rose was often dependent on aggregation size (Table 3.2.3). Sparsely populated areas contributed between approximately one and two thirds of the overall area, confirming that the dissociated individual contribute a significant proportion of populated area.

Table 3.2.3. *Example echogram school parameters of Sites 1, 2 and 3 from data acquired on 20/01/2005 and 02/02/2005. Values in metres relate maximum school size parameters along a transect, while the areas represent the aggregation horizontal area.*

	20/01/2005			**02/02/2005**		
Site	1	2	3	1	2	3
Survey Start Time	10:19:08	9:23:04	08:40:35	10:31:14	09:23:47	08:21:07
$A_dB_{v\ Max}$ (m)	96.17	87.55	97.21	85.52	100.40	93.45
$A_dB_{v\ Min}$ (m)	76.81	66.62	71.78	75.56	58.40	75.48
$A_dE_{v\ Max}$ (m)	101.93	106.17	102.36	99.10	105.66	101.84
$A_dE_{v\ Min}$ (m)	97.93	99.97	98.54	93.56	94.41	93.84
$A_tB_{v\ Max}$ (m)	90.77	83.85	84.45	81.01	85.40	90.54
$A_tB_{v\ Min}$ (m)	84.45	66.95	71.29	75.56	72.78	74.84
$A_tE_{v\ Max}$ (m)	98.56	93.61	94.51	93.56	105.17	107.26
$A_tE_{v\ Min}$ (m)	92.17	83.73	88.98	89.04	93.92	92.71
$A_tM_{h\ Max}$ (m)	13.67	38.63	34.21	22.80	47.79	16.58
$A_tM_{h\ Min}$ (m)	7.38	12.37	11.25	11.49	7.67	4.52
$A_dM_{h\ Max}$ (m)	9.22	28.43	26.15	12.71	21.48	13.29
$A_dM_{h\ Min}$ (m)	5.86	7.59	3.54	12.51	14.32	4.12
Mean altitude A_dH (m)	14.45	19.76	18.75	16.56	27.89	17.51
Maximum corrected length A_tL (m)	208.9	291.8	362.5	224.2	563.8	230.4
Echogram perimeter A_tP (m)	444	608.5	721	461.9	1141	489.4
Total Aggregation Area A_ta (m^2)	21,821	47,422	38,472	16,431	35,400	23,390
Dense Area A_da (m^2)	13,204	18,917	17,010	5,744	12,863	15,156

Aggregation areas were mapped out in the horizontal plane as per Figure 3.2.4, which provided long term relative size and movement of the aggregation. An example of variation in the aggregation horizontal structure during many surveys at Site 3, over multiple seasons, is shown in Figure 3.2.5. Throughout the season aggregations were shown to exhibit little movement around the wrecks, however, variation in area was considerable as standard deviation was approximately half the mean area in all three sites. For Site 1 the mean horizontal area was 18, 472 (s.d. = 9, 394) m^2, while Sites 2 and 3 were 36,092 (s.d. = 17, 216) m^2 and 41,196 (s.d. = 21, 085) m^2, respectively, highlighting that Sites 2 and 3 hosted larger aggregations than Site 1.

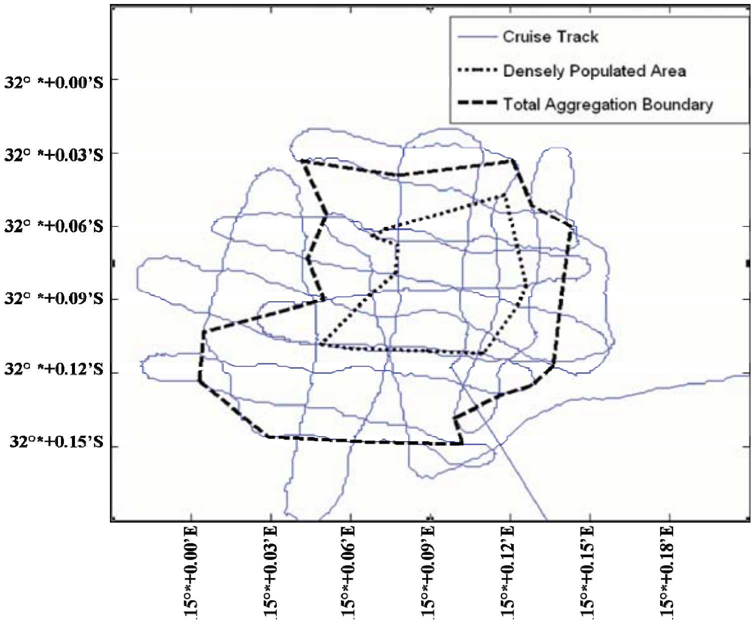

Figure 3.2.4. *Horizontal area plot of the aggregation surveyed at Site 2 20/01/2005. Dashed and dotted lines display the boundary of the total and dense aggregations respectively.*

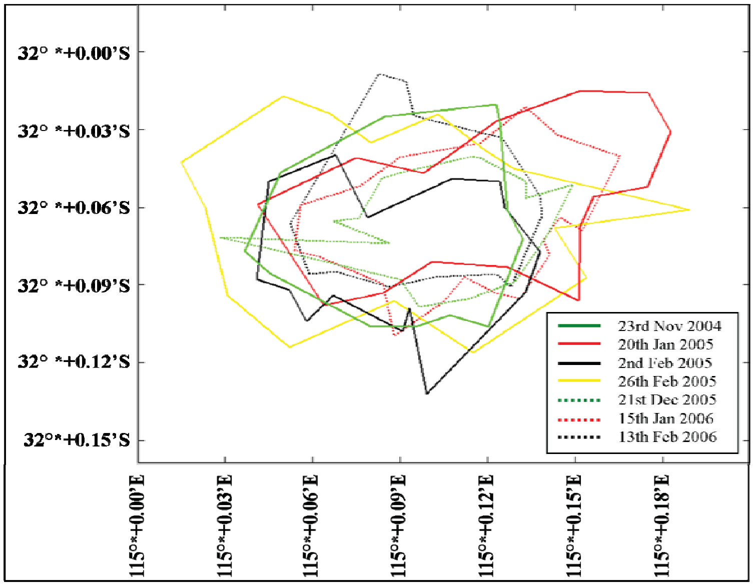

Figure 3.2.5. *Horizontal total aggregation boundary areas at Site 3 for selected 2004-5 spawning season (continuous lines) and the 2005-6 season (dotted lines) surveys.*

On the 15th January 2006 a survey was conducted at Site 3 the morning after a full moon. During the survey two charter vessels were fishing the aggregation at Site 2. The charter vessels were of the opinion that the aggregation was more mobile than usual. This speculation was confirmed by the varying GPS positions of the extent of the echogram detected 'school' between transects, and resulted in spikes on the plan area of the aggregation. Examples of such movement were also found (though to lesser extents) in other surveys, such as the eastern boundary of the Site 3 aggregation during the 20th February 2005 survey (Figure 3.2.5, yellow line). No attempt has, as yet been made to quantify this aggregation mobility although multi-beam sonar (MBS) surveys have shown aggregation movement, believed to be due to vessel avoidance (see Section 3.4).

Once the regions which were attributed to the aggregation had been geo-referenced three-dimensional visualisations of the acoustic backscatter of the school were created in Echoview (Figure 3.2.6). In Figure 3.2.6 three-dimensional images of the fish aggregations sitting above each site are shown, often with the associated wreck visible (light blue). The level of backscatter intensity is given by the colour bar and the green line on the water surface shows the vessel track.

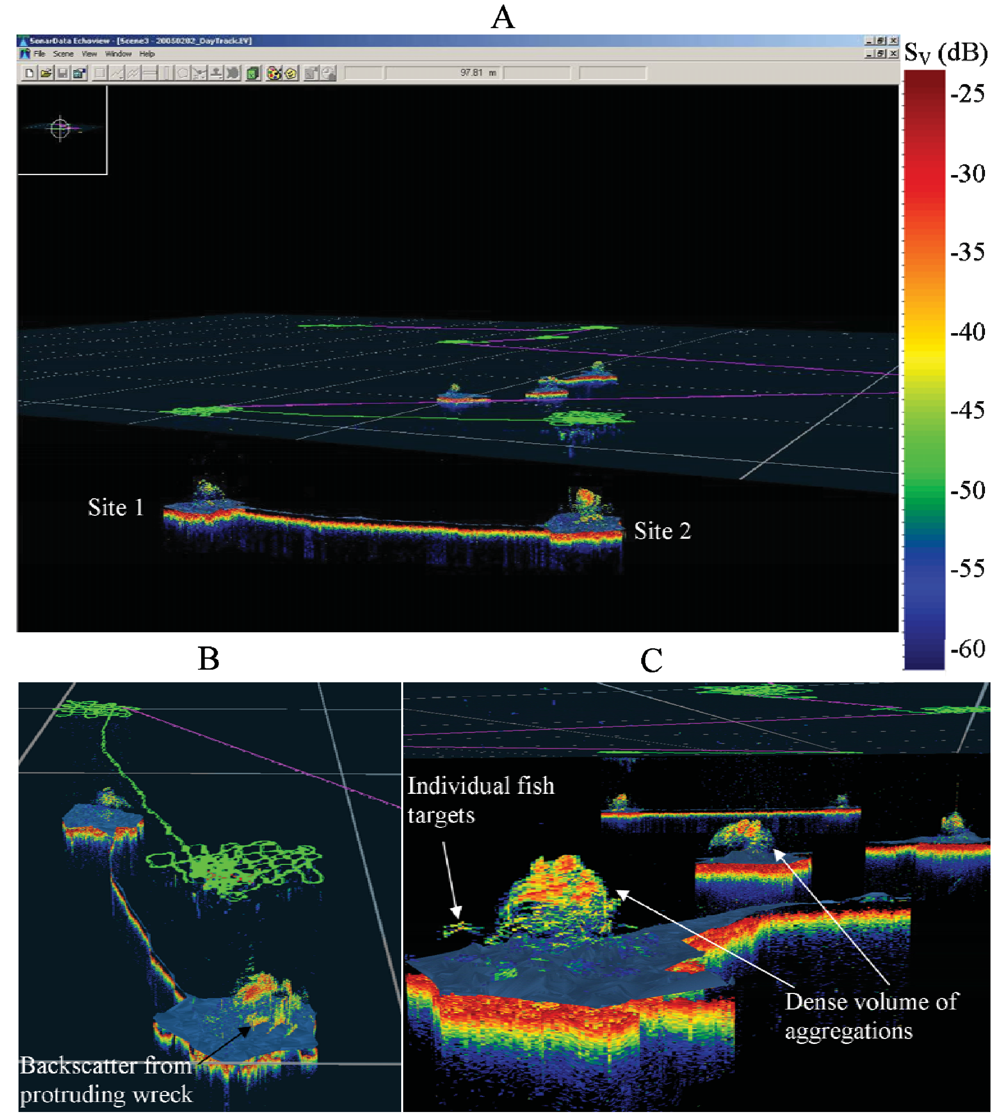

Figure 3.2.6. *3-D acoustic visualisation of five aggregations produced in Echoview (A). Aggregation backscatter intensity is shown as per the colour bar. Cruisetrack (green line) seabed (blue surface) and water surface (transparent grey surface) are also shown. B and C are different views of the Site 1 and 2, with backscatter attributable to the protruding wreck highlighted (B) and the three aggregations in the background of A with single targets and dense volumes of fish highlighted (C). Site 1 and 2 are approximately one nautical mile apart.*

Figure 3.2.7 displays two 3-D images, produced from the echograms of Sites 1 and 2, together with their respective cruisetracks. This visually illustrates the variations in inter- and intra-aggregation backscatter strength, structure and size throughout the spawning period. Densely populated areas of the aggregations can be seen at the centre of each while sparser areas lie closer to the seabed.

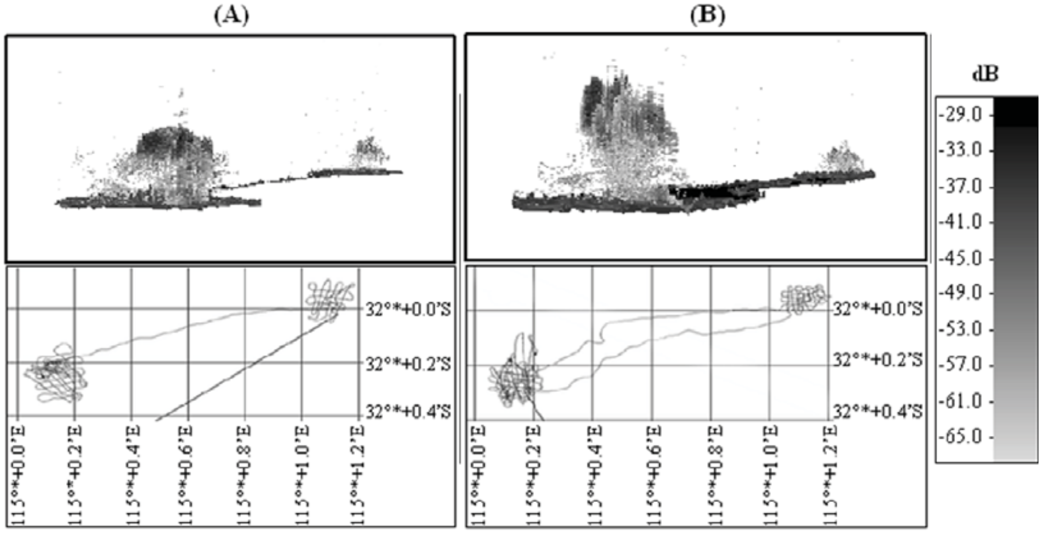

***Figure 3.2.7**. 3-D echogram plots of two S. hippos aggregations and bottom picked seabed, with associated cruisetracks conducted at Sites 1 (right aggregations) and 2 (left aggregations) at (A) 20/01/2005 and (B) 02/02/2005.*

Biomass estimates relating to Sites 1, 2 and 3, formed using the *T. albacares* and *T. obesus* tuna TS models and *in situ* acquired TS data are displayed in Table 3.2.4. An estimated number of fish calculated on an EDSU and aggregation level has been included to give an approximation of the population, however, it should be noted that these are comparative numbers only. The *in situ S. hippos* TS data was often similar to the *T. albacares* model value of -29.36 dB for a 107 cm fish (Table 3.2.4). Both of these models produced estimates of *S. hippos* numbers of the same order of magnitude to coarse estimates from sampling and catch effort records in the concurrent study (Rowland, *in prep.*). The *T. obesus* model, by contrast, predicted stronger TS, and as a consequence, lower numbers of fish (Table 3.2.4). Whether corrected calibration coefficients of the Simrad EQ60 would change these relationships is unknown. However, as the *in situ* TS data varied with each survey, so did the difference between *in situ* and *T. albacares* model abundance estimates (Table 3.2.4). The 15th January, 2006 survey at Sites 1 and 2, for example, displayed unusually low *in situ* TS values.

The estimates of relative abundance across the three seasons can be seen in Figure 3.2.8. Error bars bounding the estimated numbers were derived only from the standard error in aggregation *in situ* TS and the unknown position within the acoustic beam and do not consider the variation in NASC. Site 2 was found to have the highest abundance in the 2004-5 season, using the *in situ* TS model. *S. hippos* site preference appeared to vary slightly, changing to Site 3 in the 2005-6 season, in agreement with recreational catch data (A. Rowland, Murdoch University, *pers. comm*.). Site 1 displayed the lowest abundance, with minimal variation throughout the season, while maximum abundance levels were observed at Sites 2 and 3 between January and February, in agreement with catch and release and video data (Mackie *et al*., 2009). However, the *T. albacares* model showed considerable difference, with the highest estimates at Site 3 in January 2006, in contrast with the equivalent *in situ* TS model estimation. In all seasons, sites and models the *S. hippos* numbers were minimal by the end of March.

Table 3.2.4. *Aggregations areas and relative biomass estimates from Sites 1, 2 and 3 based on TS models of T. albacares and T. obesus tuna and the mean in situ target strengths. Estimates of the EDSU units in kg/n.mi^2 and relative numbers of fish are based on the three models.*

Survey Date	$TS_{in\ situ}$ Mean (dB) (no. samples)	S_v Mean (max) (dB)	Average Aggregation NASC (m^2/n.mi.-^{2})	EDSU Density weight (tonnes/n.mi^2)			Aggregation Area (m^2)	Estimated number of fish using		
				TS_{Ta}	TS_{To}	$TS_{in\ situ}$		TS_{Ta}	TS_{To}	$TS_{in\ situ}$
Site 1										
23/11/2004	-30.94 (253)	-46.03 (-41.75)	4,317	3,282	1,375	6,789	20,213	1,078	452	2,229
20/01/2005	-32.35 (448)	-43.82 (-26.51)	1,334	4,047	425	2,504	21,821	1,434	151	1027
2/02/2005	-31.71 (107)	-46.76 (-27.7)	2,287	2,886	729	4,292	16,431	1,660	194	1,146
26/02/2005	-26.86 (229)	-44.8 (-23.07)	2,072	3,630	660	1,272	32,085	1,892	344	663
18/10/2005	-31.74 (637)	-45.25 (-33.21)	5,475	5,531	1,067	10,341	10000*	950	182	1,680
20/10/2005am	-29.24 (278)	-45.43 (-20.59)	4,035	5,918	1,285	4,286	15000*	1,100	209	1,044
20/10/2005pm	-28.18 (436)	-49.43 (-29.56)	3,217	4,390	932	2,677	12000*	900	191	522
21/12/2006	-36.57 (965)	-43.69 (-31.2)	5,234	6,546	1,667	6,336	10,572	1,124	286	5167
15/01/2006	-35.09 (2254)	-43.09 (-26.9)	4,575	4,686	1,457	6,132	16,776	1,277	397	1,671
13/02/2006	-29.50 (3240)	-52.18 (-34.01)	391	939	125	387	15,708	240	32	99
13/09/2006	-30.43 (586)	-43.56 (-25.85)	7508	8,198	2,392	10,498	15,471	2,326	610	2,678
15/11/2006	-32.03 (308)	-48.65 (-38.62)	489	534	156	987	17,168	168	40	252
Site 2										
23/11/2004	-33.15 (306)	-34.61 (-29.6)	7,475	8,448	2,381	19,552	50,010	6,862	1,934	15,883
20/01/2005	-34.58 (232)	-44.29 (-36.08)	7,005	13,846	2,231	25,486	47,422	10,665	1,719	19,631
2/02/2005	-30.36 (515)	-41.46 (-16.2)	10,358	9,161	3,300	14,234	35,400	5,268	1,897	8,185
27/02/2005	-26.76 (1760)	-46.36 (-40.67)	1,752	2,128	558	1,050	23,389	808	212	399
21/12/2005	-30.56 (531)	-41.73 (-11.76)	5,559	13,725	1,771	6,730	70,820	15,789	2,037	9,204
15/01/2006	-36.09 (450)	-41.31 (-20.62)	7,354	18,734	2,343	18,968	35,597	10,833	1,355	21,867
13/02/2006	-28.93 (3502)	-40.78 (-18.32)	16,419	28,499	5,230	18,532	32,418	15,007	2,754	9,759
21/03/2006	-30.41 (1209)	-52.29 (-30.6)	672	673	214	935	20,273	222	70	308
13/09/2006	-34.75 (201)	-47.86 (-35.65)	1,842	2,011	587	6,958	9,378	346	89	1,060
15/11/2006	-37.29 (350)	-38.89 (-31.36)	10,596	11,570	3,375	22,712	36,211	7,682	1,985	21,167
Site 3										
23/11/2004	-30.93 (609)	-33.87 (-29.11)	1,976	2,158	629	3,096	66,576	2,634	681	3,348
20/01/2005	-31.78 (469)	-35.21 (-24.06)	8,041	8,781	2,562	17,477	38,472	6,194	1,601	10,922
29/01/2005	-37.52 (2474)	-37.85 (-24.61)	2,696	2,944	859	7,456	43,857	2,368	612	13,712
2/02/2005	-25.50 (239)	-30.96 (-16.28)	9,362	10,223	2,982	4,203	23,390	4,385	1,133	1,597
26/02/2005	-25.89 (168)	-47.87 (-33.01)	900	983	287	442	21,660	390	101	156
21/12/2005	-27.91 (319)	-32.76 (-17.35)	15,165	16,559	1,645	6,253	75,880	23,040	2,028	12,818
15/01/2006	-33.41 (181)	-31.64 (-9.44)	35,872	39,169	1,871	9,556	52,098	37,418	1,583	24,913
13/02/2006	-30.56 (1498)	-37.98 (-25.11)	4,535	4,952	1,445	5,118	54,747	4,971	1,285	4,552
13/09/2006	-35.71 (275)	-38.37 (-12.46)	4,587	5,009	1,461	6,837	21,419	1,967	508	2,379
15/11/2006	-33.41 (281)	-39.26 (-26.27)	5,846	6,383	1,862	16,233	13,863	1,623	419	3,655

* Area acquired from the plan view of multi-beam acoustic backscatter during concurrent survey. For details, see section 3.3.

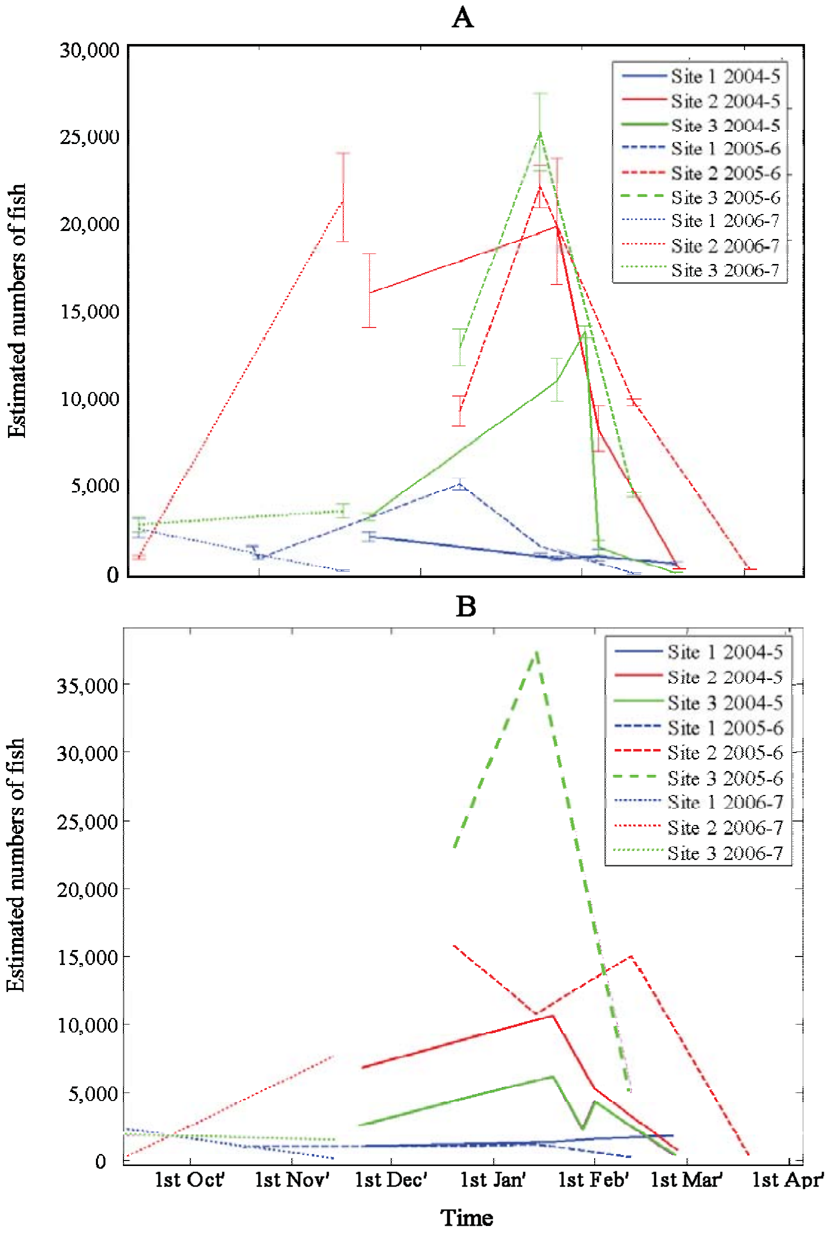

Figure 3.2.8. *Relative estimates of fish numbers at Sites 1 (blue), 2 (red) and 3 (purple) during the course of three spawning seasons. The 2004-5, 2005-6 and 2006-7 seasons are shown by continuous, dashed and dotted lines respectively for the in situ mean target strength (A) and T. albacares model target strength (B). Error bars in A are based on standard error of in situ TS values only.*

3.2.4. Discussion

Single-beam acoustics is a useful tool for providing long-term monitoring of fish aggregations. The data have shown levels of estimated *S. hippos* numbers at individual sites and distinguishable differences between biomass and aggregation structure at separate sites throughout the spawning period. It also illustrated that while logistically simple, providing high coverage of local aggregations, this technique is hindered by accuracy-related limitations and assumptions of temporal and spatial uniformity in the fish distribution, many of which are reconcilable.

The figures and times of aggregation arrival (October), abundance maximums (January-February) and *S. hippos* departure (March) are in agreement with coarse estimates of associated *S. hippos* spawning levels from similar, concomitant studies and reports from fishers (Mackie *et al*., 2009, Rowland, *in prep.*). Evidence from fishers that Site 2 (North Barge) generally hosted the greater number of *S. hippos* in the 2004-5 season, changing to Site 3 (Outer Patch) in the 2005-6 season, corroborated the results from acoustic backscatter, although the reasons behind site preference for the aggregations is unknown.

Mapping of the aggregations revealed a relatively consistent outline of fish presence. However, there were notable variations to the aggregation boundaries between transects. Such differences became easier to view on the three-dimensional visualisation where the images allow the viewer to visualise changes in aggregation structure over time and compare different aggregations throughout the spawning season. Some surveys displayed near spherical aggregations, while others extended further upwards into the water column, thus aggregations of small plan area may contained large numbers of fish (Figure 3.2.7 and Table 3.2.4). The results displayed that a significant portion of the aggregations comprised 'dissociated' individuals (Table 3.2.4, Figure 3.2.3). Whether the varying structures changed the swimming pattern of the fish and therefore the TS, and resulting biomass estimates, is unknown.

However, the protocols set out by Reid *et al.* (1999) to analyse echogram images were often not amenable to the *S. hippos* aggregations, due to the large number of dissociated members Figure 3.2.3). It may be prudent for future studies of aggregations to consider decision rules to analyse linking distances for dissociated members. More comprehensive datasets are required to discern patterns in an aggregation structure throughout the spawning season. Once relationships have been developed between differing structures and biomass it may be possible to achieve coarse biomass estimates from the volume of the overall aggregation. This would result in a catalogue of parameters of both dense and sparsely populated areas of the aggregations and more detail of variations throughout the season.

Uncertainties in the *S. hippos* biomass estimates originated from a number of sources. Behaviour and movement of targets is a significant source of error. It cannot be assumed here that large systematic errors, such as fish behaviour, remain constant throughout the year (Simmonds and MacLennan, 2005). Simmonds and Fryer (1996) demonstrated that confidence limits in biomass estimates may be improved with structured survey designs. However, the length of time for the *S. hippos* grid surveys above allowed significant aggregation movement. To counter this movement it may be possible to separate each survey of *S. hippos* into two surveys from the north-south and east-west transects to create two biomass estimates and provide an estimate of coverage induced sample error, similar to that of Aglen (1983) and Foote & Stefansson (1993). Such division of survey data has been deemed work for future analysis. Duration of the described surveys varied due to the adaptation of transect length and survey speed to suit survey conditions, however, this would also created comparative variation in movement by the aggregation. In the future it will be necessary to standardise the time of survey for a given area and therefore standardise likelihood of bias due to aggregation movement over time. Changes in the acoustic environment may affect received acoustic backscatter, however, errors due to the variation in assumed and true temperature or sound speed at *S. hippos* ranges were expected to be in the order of 1-2.5% (Foote, 1981).

The disparity in estimates of abundance using TS from *T. obesus* model and those of the *T. albacares* model and *in situ* data were, on occasion, an order of magnitude different emphasising the necessity for an accurate TS model of the surveyed species. This conclusion is corroborated by a study of blue grenadier (*Macruronus novaezelandiae*), using two independently derived TS models for that species, which resulted in approximately a fivefold difference in biomass (Kloser *et al*., 2005). Uncertainty in TS may be mitigated by an estimation of the length distribution of the surveyed fish, however, due to the limited sample size (Simmonds & MacLennan, 2005) and possible bias in hook-and-line sampling (Hetrick & Bromaglin, 2006, Zeller and Russ, 1998, Rowland, *in prep*.) a length distribution has not been estimated for the individual *S. hippos* aggregations. Species TS models may be determined using *ex situ* techniques or theoretical target strength modelling, however, in the acoustic study of a new species, comprising a comparatively small number of offshore aggregations, the ability to acquire subjects or samples may be logistically and/or technically inappropriate. By comparison, the *in situ* TS values, which varied throughout the season, highlighted the additional constraint that a simple regression model may not be sufficient to monitor an aggregating species where behaviour can vary through time, affecting biomass estimates. Anecdotal evidence suggests that the fish are more disperse at night. It is possible that this behaviour would lead to a higher number of single targets detected. However, the variation in behaviour would mean that TS derived from these counts would not be applicable to the biomass estimates made during the morning surveys (Section 2.3.1.2).

The results shown here have assumed all acoustic backscatter has been generated by *S. hippos*. However, ground truth data collected from the various sites illustrated two further issues relating to the effective estimation of *S. hippos* present. Examination of the video data confirmed that the aggregations were not completely homogenous and comprised at least two other species, skipjack trevally (*Pseudocaranx dentex*), and pink snapper (*Pagrus auratus*). Although their presence was small it could not be quantified. Discrimination between species in the aggregation may have been observed by employing multi-frequency techniques to analyse the backscatter (Kloser *et al*., 2000);

however, due to the non-linear effects of the 200 kHz transducer at high power settings this could not be tested. Addtionally, limitations of such discrimination methods would be driven by the similarities between the morphology of the species around *S. hippos* and the low numbers of fish present to identify difference in TS. Therefore the aggregations were considered homogeneous. Schools of *P. dentex*, a much smaller species, were often observed around the spawning season and may be partially responsible for the unusually low *in situ* TS observed during the 15th January surveys (Table 3.2.4), however, video data and sampling gave no reason to suggest a majority species other than *S. hippos*.

Secondly, *S. hippos* aggregations formed close to the protruding wrecks and towed video data confirmed that several fish swim around the wrecks, at a similar depth, complicating the bottom picking process. Although advanced algorithms are capable of accurately identifying seabed depth, the combination of bottom 'dead zone' and the beam footprint in this case (Urick 1983, Johanesson & Mitson 1983, Simmonds and MacLennan 2005) suggested a requirement to manually distinguish between projecting bodies and mid water organisms around them. In some cases it is unfeasible to accurately differentiate between the two. In previous studies the possible inclusion of wreck backscatter as part of the aggregation has led to overestimates of abundance (Parsons *et al*., 2005).

S. hippos do not feed whilst aggregating to spawn (Mackie *et al*., 2009) and thus fat content is expected to reduce throughout the season. To an extent fat content contributes to TS variation, in that as a swimbladder's purpose is for buoyancy control, it follows that a fish with higher fat content will have a relatively smaller swimbladder and resulting TS (Ona, 1990). This has been estimated at 0.2 dB per 1% increase in fat content (Reynisson, 1993, Ona, 2003) and that fat content can be verified experimentally (Kent, 1990). Thus TS throughout the spawning season should be expected to change. *In situ* TS from *S. hippos* across the season was shown to vary considerably, though no distinct trend was observed. However, TS measurements are inherently stochastic, thus more in situ data in each survey would be required to evaluate any trends in TS. Indeed, variations in behaviour and orientation would affect TS to a greater extent than biological changes.

3.2.5. Future work

Two areas of significant interest to confirm the accurate biomass estimation of *S. hippos* aggregations by single-beam echosounding involve the relationship between overall biomass and the densely/sparsely populated areas, and the effects of aggregation mobility during the survey. It was felt that further surveys of individual aggregations at a given time during the spawning season were needed to confirm these relationships.

An estimate of aggregation mobility could be extended from the overall roughness of the detected aggregation plan area. Such roughness would be defined similarly to that of the echogram image i.e.

$$Roughness = \frac{Perimeter}{Area} \tag{3.7}$$

If there is a correlation between aggregation horizontal area roughness and abundance estimates it may lead to improving confidence limits in single-beam echosounding. For example, a higher roughness, due to a greater perimeter, suggests that the aggregation is more mobile. Biomass estimates would then require adjustment to account for multiple detections of fish or fish which remain unsampled, as a result of the aggregation movement.

Variation was also observed in the shape and orientation of the aggregation areas, with some aggregations elongated or orientated towards a specific direction. Video data indicated that *S. hippos* orientated themselves directly towards the current, and therefore not exhibiting the typical circling behaviour of a school. Such behaviour may account for aggregation plans, i.e. elongated areas may be tilted towards the current. Further study of the plan areas, together with video data offers the opportunity to better understand the orientation of fish and therefore TS if the direction of the prevailing current is known.

The surveys showed that while single-beam techniques are inexpensive and logistically simple to deploy, data can be unreliable unless effective preparatory work is undertaken and sufficient data is acquired for the determination of true mean values for *in situ* TS at the time of survey.

3.2.6. Acknowledgements

Funding for this study was received from the Fisheries Research and Development Corporation (FRDC) and a PhD top-up scholarship from the Western Australian Marine Science Insitutue (WAMSI). Significant logistical support was supplied by the department of Fisheries WA and thanks also go to Toby Jarvis at Myriax for Echoview noise removal templates and Matt Wilson for technical support in data processing.

3.3. Evaluation of acoustic backscatter data collected from Samson fish *(Seriola hippos)* spawning aggregations in Western Australia Using a Reson 8125 multi-beam sonar

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ABSTRACT

Single-beam echosounders have been a well established tool in the estimation of fish stocks for several decades. However, a consequence of single-beam geometry is the ensonification, by individual pings, of a comparatively small volume of the water column. Extrapolation of acoustic backscatter mean values received from this volume is then required to calculate an estimation of abundance within a survey area. With recent advances in data processing technology, multi-beam sonar, originally employed to collect detailed bathymetric data, have been developed to include acoustic backscatter data from the water column. Surveys of Seriola hippos aggregations above sunken wrecks, west of Rottnest Island, Western Australia were conducted with multi-beam RESON 8125 Seabat sonar (455 kHz) and single-beam Simrad EQ60 echosounder (38 and 200 kHz). Acquired acoustic backscatter data from each survey were analysed for relative S. hippos abundance, their spatial distribution and vessel avoidance behaviour. Limitations and benefits were evaluated for monitoring comparatively stationary, uniform fish aggregations using single- and multi-beam techniques. Effects attributed to assumptions of uniformity, required when applying single-beam technology, were assessed and conclusions drawn on consequences of variable in situ parameters. Implications for monitoring and biomass estimation of aggregations using single-beam techniques are discussed.

***Keywords**: Multi-beam sonar, fisheries, targets, aggregation*

3.3.1. Introduction

Vertical echosounding results in two-dimensional resolution of the water column, along track distance and depth. As a consequence, acoustic surveys of fish schools or aggregations typically assume that geometric properties of the aggregation are isotropic, with negligible overall movement and an even distribution of fish within the aggregation (Petitgas, 1993). Additionally, surveys conducted with vertical echosounders only observe the region directly beneath the vessel. This region is most affected by noise and visual disturbances from the sampling vessel and is therefore where fish are most likely to exhibit avoidance behaviour (Gerlotto *et al*., 2000). Movement by fish during a survey may affect the estimates of the area within which the fish are distributed (Simmonds and MacLennan, 2005) and there have been many reported cases of pelagic fishes exhibiting vessel and/or noise avoidance (Mayer *et al*., 2002).

As a consequence of increases in data storage and computer processing speeds, multi-beam sonar (MBS) systems, have become capable of acquiring acoustic data from the entire water column. Geometric characteristics of MBS acoustic swaths result in a large and highly resolved sample volume offering enhanced spatial resolution and behavioural information on biomass present, compared with that from traditional single-beam echosounders. As a result the use of MBS in fisheries acoustics is increasing (Freon *et al*., 1992, Petitgas, 1993, Soria *et al*., 1996, Gerlotto *et al*., 2000). However, the increase of sampled volume available from the MBS swath involves a vast increase in data. Processing tools are required capable of dealing in four dimensions (x, y, z and time) with such complicated datasets (Wilson *et al*., 2005).

This paper focuses on evaluating backscatter data simultaneously acquired with a RESON Seabat 8125 MBS system and Simrad EQ60 single-beam echosounder. The objective of this research is to assess biomass estimates from counted single targets and spatial analysis using the acquired single-beam and MBS data. Conclusions will help evaluate previous and future surveys conducted with a single-beam echosounder alone.

3.3.2. Methodology

The acoustic survey was conducted between the 18th and 21st October, 2005 at sites of *S. hippos* spawning aggregations, west of Rottnest Island, Western Australia. Three sites were surveyed, labelled as Sites 1, 2 and 3, respectively (Figure 3.1.1). At these locations the wrecks lie in approximately 100 m of water in flat sand.

Results detailed below primarily concern one site known as the 'Secret Spot' (Site 1) where fish aggregate above a wreck that is orientated approximately north-south. Individual north-south transects along the length of the wreck were conducted to achieve the highest coverage of the aggregation. Seven transects were conducted between 8:35am and 9:45am on the 20th October, each lasting a maximum of five minutes. During the following two hours the aggregation was fished by three boats using rod and line, and a towed video camera was deployed to confirm that the aggregation comprised *S. hippos*. A further seven transects were conducted between 11:58am and 13:00pm after cessation of fishing and video tow transects. Vessel speed was minimised at 2.5 to 3.2 kn to give maximum coverage with the MBS and increase along track ping coverage.

The RESON 8125 Seabat MBS, originally designed for high resolution acquisition of bathymetric and seafloor backscatter ('snippets') can also be modified to acquire water column backscatter data (snapshot). It is a 455 kHz system where a transmit array ensonifies a swath of 120° across track by 1° along track. An orthogonal receive array forms 240 equidistantly spaced beams across track. Maximum along beam resolution is achieved at 2.5 cm (RESON Inc., 2002). Simrad EQ60 specifications and settings were as stated in Section 3.2.

Surveys were conducted aboard the *RV Naturaliste,* a 21.6 m Department of Fisheries vessel. The RESON 8125 and Simrad EQ60 were mounted off the port and starboard sides of the boat at depths below sea surface of 2.77 and 2.00 m respectively and distances of 6.95 and 0.47 m apart in athwartships and alongships directions respectively (Figure 3.3.1). The RESON 8125 head was mounted with nadir beams directed vertically down and swath positioned athwartships. The Simrad EQ60 was also directed vertically to the seabed. Ships positions were recorded using a differential GPS system. TSS Meridian Surveyor gyrocompass and DMS2-05 motion sensors recorded vessel roll, pitch and yaw.

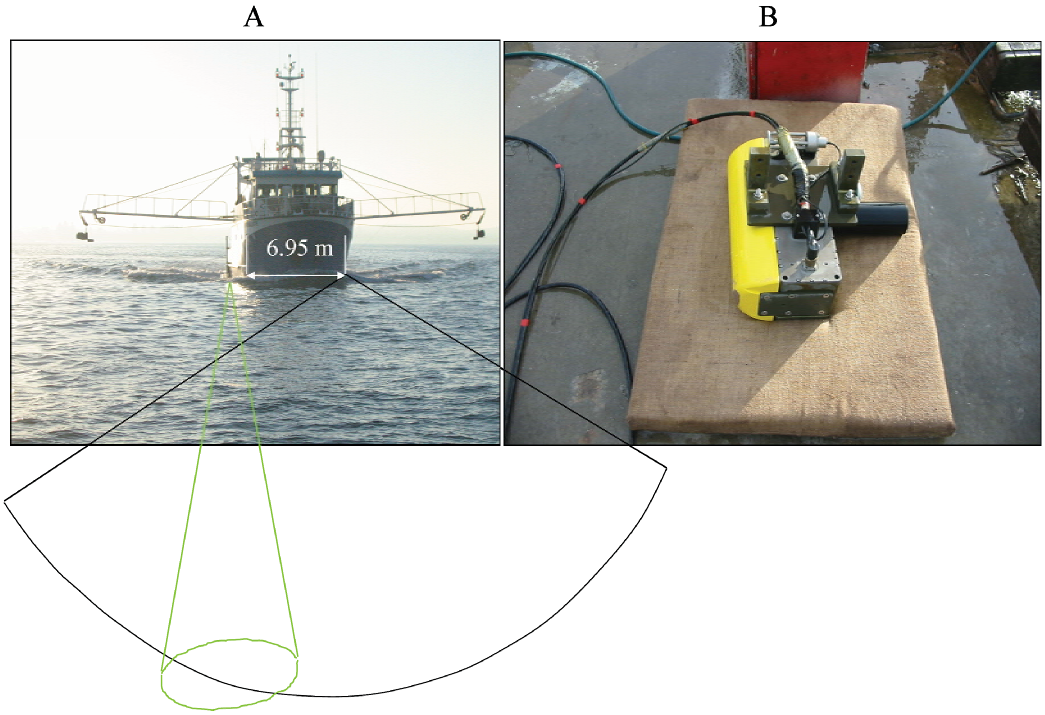

Figure 3.3.1. *A) Photo of the RV Naturaliste with a schematic of the beam pattern positions and directions for the Simrad EQ60 single-beam (green) and the RESON 8125 Seabat multi-beam (black). B) Photo of RESON 8125 prior to mounting. The yellow fairing protects the transmit array behind it (with long edge positioned perpendicular to ships line) and the orthogonal receive array towards the aft.*

Initial transects over the study area were conducted using the RESON 8125 to describe the wreck and associated bathymetry. Software modifications were then made to the MBS system, as per RESON Inc. (2002) guidelines, to allow collection of acoustic backscatter from the entire water column as snapshot data files. Due to the increase in acquired data the maximum achieved ping rate at the required depths was restricted to approximately one ping every 4.5 seconds. When combined with the vessel speed this ping rate equates to approximately 1 ping every 5.8-7.4 m (excluding effects from pitch and yaw). System guidelines for the RESON 8125 suggest surveying a maximum depth of 60 m with range setting of 120 m, however, previous experiments had shown the ability of the RESON 8125 to collect water column backscatter at depths close to 100 m (Trevorrow, 2005), similar to the maximum expected depth of *S. hippos* in this study.

Initial aggregation transects were conducted on the 18th and 19th October with varying settings of system power, gain and pulse length. Preliminary analysis of data acquired from transects showed that at several settings the volume backscatter strength from strong targets at *S. hippos* depth were saturated, in agreement with previous experiments (Trevorrow, 2005). Initial settings for acquiring unsaturated backscatter from *S. hippos* aggregations with minimal interference were established to be a power rating of 10, receiver gain 30 dB and pulse length 292 µs. During subsequent transects, live analysis of received amplitudes for individual beams was conducted to confirm that target backscatter remained unsaturated.

The 38 kHz beam of the Simrad EQ60 was positioned with 13° and 21° beam angles athwartships and alongships respectively. During surveys it was operated at a maximum ping rate of 2 Hz. A power setting of 500 W and pulse length 0.512 ms were found to generate unsaturated backscatter of sufficient resolution. The receiver gain was set to 19.5 dB. The minor axis beam angle equates to approximately 26 beams of the MBS swath at 100 m depth (roughly beams 114 – 140), however, the single-beam footprint extends considerably fore and aft of the RESON beams as well. The EQ60 200 kHz beam was left in passive mode due to interference with the MBS snapshots observed on the live feed echograms.

Bathymetry of the three sites were produced by importing RESON 8125 XTF format data into a suite of Matlab programs developed by the CMST (Siwabessy *et al*., 2005) to compare the level of protrusion of each wreck into the water column. Single-beam and MBS backscatter files were imported into Myriax, Echoview software for processing.

Echogram curtains from single-beam data were produced using a noise removal variable template in Echoview and acoustic backscatter was thresholded at -60 S_V as per previous studies (Section 3.2). Echoview single-beam school detection was employed to detect any remaining S_V values above threshold at distances less than 15 m.

Multi-beam 'school detection' algorithms were applied in Echoview to individual pings to extract any targets above a -21 dB threshold. This detection was separated into two definitions, one detection for schools of minimum dimensions 1 m width and height, with a linking distance no greater than 2 m (to eliminate *S. hippos* sparse targets) and a second detection of minimum dimensions of 4 cm width and height to include all sparse targets greater than one sample dimension. For visualisation purposes the detected regions were then extended a distance of one metre in the direction of travel to represent the acoustic fish.

Region variables were created such that any values spatially outside the detected targets were masked to become regions of no data. To calculate aggregation volumes the remaining swath S_V data, containing only detected sample positions, were exported into an adapted suite of Matlab programs originally developed by Myriax (B. Buelens, Myriax, *pers. comm.*). GPS and motion sensor data were imported into Matlab and swath target positions were adjusted for roll and heading before being geo-referenced in Cartesian coordinates. Acoustic targets were then linked to their three nearest neighbours and a number of tetrahedrons were formed, each containing four targets. Each of the tetrahedrons were then linked together to produce an overall object representing the aggregation. The length of tetrahedron edges were thresholded to create a maximum target linking distance by which an individual target could be either excluded or included from the aggregation, based on linking distance. The threshold length was incrementally increased by 1 m until approximately 90 % of all acoustic targets were contained within the object. The total volume of the aggregations was given by the sum of the volume of all tetrahedrons, based on the threshold nearest neighbour distance. The locations of targets and the overall object volume were referenced back to WGS84 format to be imported into Echoview for visualisation. The comparison of target numbers and densities within the volume provided the equivalent acoustic target packing density.

As calibration of the single-beam echosounder remains to be accurately conducted a comparison of biota presence between volumetric and echo-integration techniques was not performed.

3.3.3. Results

Bathymetric descriptions of the three wreck sites surveyed in this study are shown in Figure 3.3.2 of which Site 1 displayed relatively low relief. Live observations of MBS and single-beam acoustic backscatter confirmed that at the time of survey Site 1 hosted the greater number of fish, in contrast with findings from other surveys (Section 3.2).

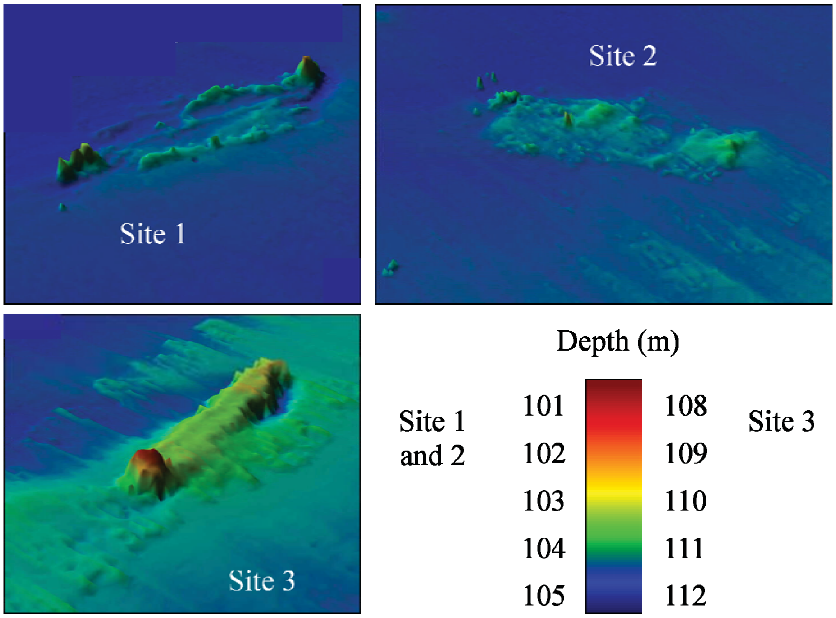

Figure 3.3.2. *Bathymetry of three primary S. hippos study sites produced from RESON 8125 acquired bathymetric data. Examples in this paper concern Site 1 of comparatively low relief.*

During transects conducted at the site, acoustic backscatter from the aggregation was visible with both single-beam and MBS systems, such as the example ping in Figure 3.3.3A. *S. hippos*, determined from catch data to range in length from 0.55 to 1.60 m with a 1.07 m mean fork length (Mackie, 2009) can be seen distinctly as targets ranging from roughly 65 to 95 m depth with defined separation between the targets (Figure 3.3.3A). Above the sparse area is a school of smaller fish, likely skipjack trevally (*Pseudocaranx dentex*) from video data, ranging from 60 to 70 m depth and 15 m width. Interference into the sidelobes, at approximately 60-70 m and 110 m range (rings, Figure 3.3.3A), caused by backscatter from the dense area and seafloor respectively (possibly due to saturation), was observed in raw images. Evidence of radial "spoking" interference (faint green lines radiating from the MBS source, Figure 3.3.3A) was present in many pings.

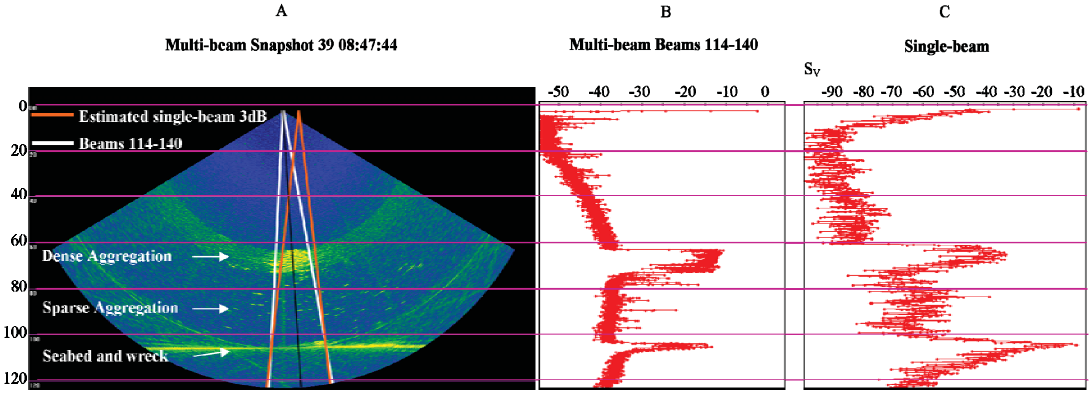

Figure 3.3.3. *A) Received swath of multi-beam snapshot ping 39 with areas of dense and sparse aggregation and seabed highlighted. Estimation of sample volume enclosed by the single-beam half angle (orange) and equivalent Reson 8125 beams (white) are shown.* S_V *values against depth for the equivalent Reson 8125 beams (B) and single-beam (C) for the equivalent ping.*

In the example above (Figure 3.3.3A) the half power beam of the single-beam (orange) is shown schematically to encompass the majority of the dense region and a considerable number of sparse targets (Figure 3.3.3 C between 60 and 100 m depth).

These findings are comparable to summed backscatter at similar depths from the RESON 8125 beams of the same area (area between white beams 114-140, Figure 3.3.3B). The single-beam data detected the dense school higher in the water column than the MBS. The depth difference was due, in part, to the offset of 6.95 m between the two transducers, combined with a minor degree of ships roll and position of the aggregation relative to MBS nadir beams and single-beam acoustic axis. Backscatter of the sparse region of fish (depths between 80 and 100 m), acquired by the single-beam, was less consistent than the MBS, most likely due to the absence of targets in the volume fore or aft of the MBS swath, which were still within the single-beam pattern.

A small number of artefacts, possibly created in the near-field of the 8125 at the top of the swath, were also observed in the nearest 30 m to the transducer head. These were similar to those described by Malzone *et al*. (2008). Such artefacts were visibly discernible only at the highest power and gain settings and fade into noise with reduced transmission power.

MBS data acquired from Sites 2 and 3 identified structure and movement of mobile fish schools (other than *S. hippos*) that were located outside the acoustic beam of the Simrad EQ60 (Figure 3.3.4). These were not present at either site prior to or in subsequent transects and would have remained undetected by a single-beam survey. Comparison of EQ60 echograms with single MBS pings in Figure 3.3.4 shows how the EQ60 footprint collected backscatter from targets up to two MBS pings before or after the concurrent one (approximately 10 m fore or aft the MBS ping). The high along track resolution of the EQ60 echogram displayed single fish tracks (Figure 3.3.4.B at 80 m depth) which confirmed that acoustic targets detected by consecutive pings of the MBS (Figure 3.3.4.C, all pings at 80 m depth) were actually multiple detections of the same fish. Comparison between the single-beam and MBS data also displayed evidence of two targets at approximately the same depth, but laterally separated by several metres appearing as the track of a single fish in the single-beam echogram. Multiple fish appearing as one in the single-beam echogram was a rare occurrence and attributed to the wide beam pattern of the EQ60 at depths populated by *S. hippos*. The impact of multiple fish detections is an overestimate of abundance; however, this effect is yet to be quantified and has significant implications for estimating biomass via target counting.

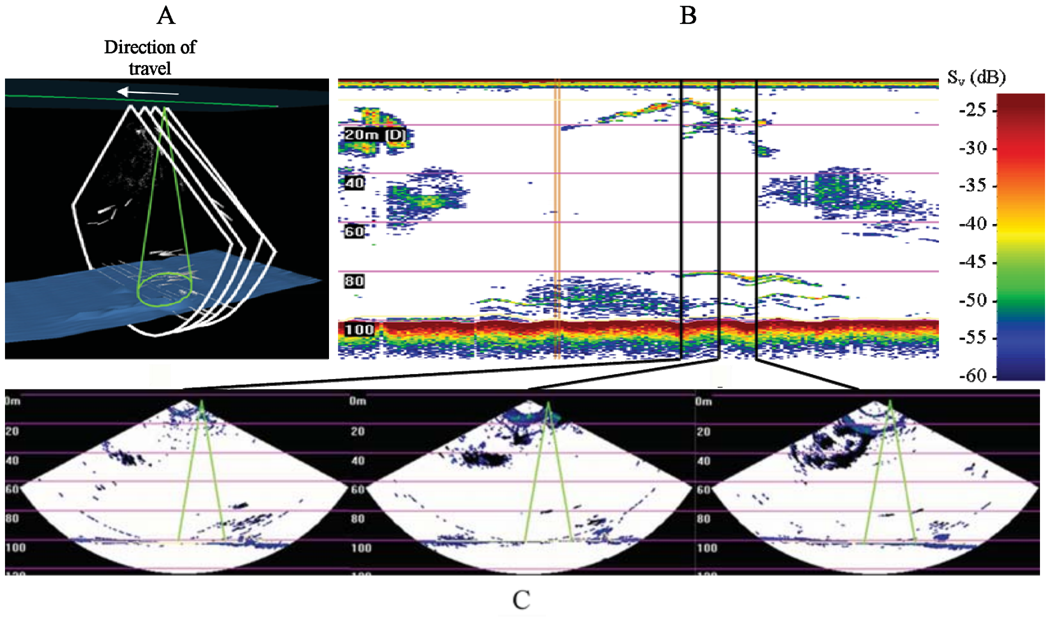

Figure 3.3.4. *Schematic of single-beam footprint (green cone), simultaneously taken with the second multi-beam ping, overlapping four multi-beam snapshots corrected for roll (white wedge) and the S. hippos detected by the multi-beam (rectangular grey regions) from a transect conducted on the 18th October at Site 2 (A). Corresponding section from the single-beam echogram (B) and three snapshots (C) taken at 11:52:04, 09, and 14 am (left to right) with the ideal single-beam minor axis shown.*

Once targets had been determined and attributed to dense or sparse areas of the aggregation, acoustic backscatter from single-beam and MBS surveys could be visually compared in 3-dimensions. In Figure 3.3.5 the MBS acquired backscatter (green and yellow) and single-beam backscatter (red) have been overlaid, above the MBS detected seafloor (blue) in the left hand image. Other images show the MBS and single-beam data separately (top) and together (bottom). Dense areas of the aggregation (yellow and opaque red) were easily discernible on both single- and MBS systems when transects ran above the centre of the aggregation. Sparsely populated areas were also detected even when single targets could not be counted (Figure 3.3.5 top right above the wreck). Figure 3.3.5 highlights the ability of MBS systems to visualise aggregation structure in three dimensions from the acoustic reflectance.

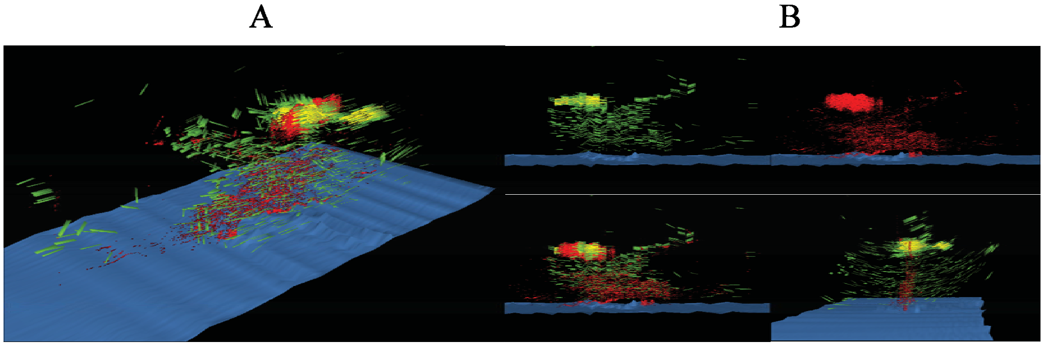

Figure 3.3.5. *Different views of an Echoview 3-D image of acquired single- and multi-beam acoustic backscatter from a S. hippos aggregation. Data from each system was simultaneously acquired during a single transect. Multi-beam detected sparse (green) and dense (yellow) regions overlaid with single-beam (red) acoustic data (A). Multi- and single-beam data separately and together, viewed from the east and south (B).*

Due to the distance travelled between pings and the effects of yaw, the distances between targets in consecutive pings were often greater than the distance between targets in individual swaths (as shown in Figure 3.3.6). Thus when targets were linked to calculate an aggregation volume for each transect, a minimum threshold linking distance of 12 and 15 m for dense and sparse regions respectively was required to encompass 90% of the targets although it was recognised that this distance was greater than the nearest neighbour distance expressed within a single swath.

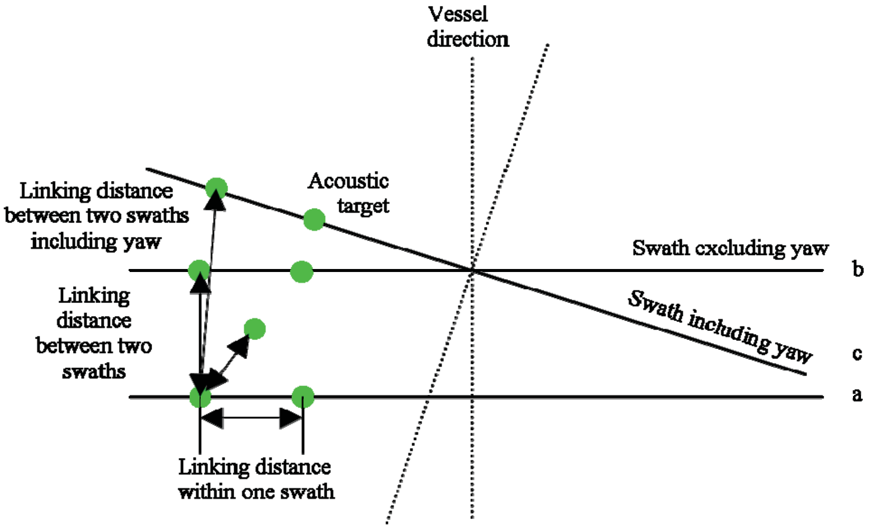

Figure 3.3.6. *Plan view of two acoustic swaths with example detected acoustic targets (green circles). The linking distance between two targets in the first swath (a) is less than that of two targets in successive swaths due to distance travelled (a and b), which is exacerbated once effects of heading/yaw are included (c).*

The process of linking individual targets and groups of targets together allowed the visualisation of the entire volume of the aggregation. A sample visualisation of aggregation volume has been shown in Figure 3.3.7 including two example MBS swaths as slices through the aggregation (white wedges). It was observed that the aggregation predominantly sat directly above the wreck, with the densely populated area of speculated *P. dentex* (yellow) located above the broader, more sparsely populated area (red). Backscatter from the dense and sparse volumes typically spanned alongships distances equivalent to between 3 and 6 pings (~15 to 42 m linear distance between the sample volumes at 80 m depth) and 10 to 20 pings (~50 to 150 m) of the MBS system respectively.

Figure 3.3.7 also highlights some of the more loosely linked targets which were not encompassed by the aggregation volume (for example, green regions, bottom right). Such targets are likely to be *S. hippos,* because of their size and distance from the aggregation, although the species was unconfirmed. In all transects (including other survey sites) the MBS swath covered the entire width of an aggregation of this size, typically 100 to 200 m wide at mean depths of 60 to 85 m. Thus a MBS system can survey an aggregation of this size in a single two-three minute transect.

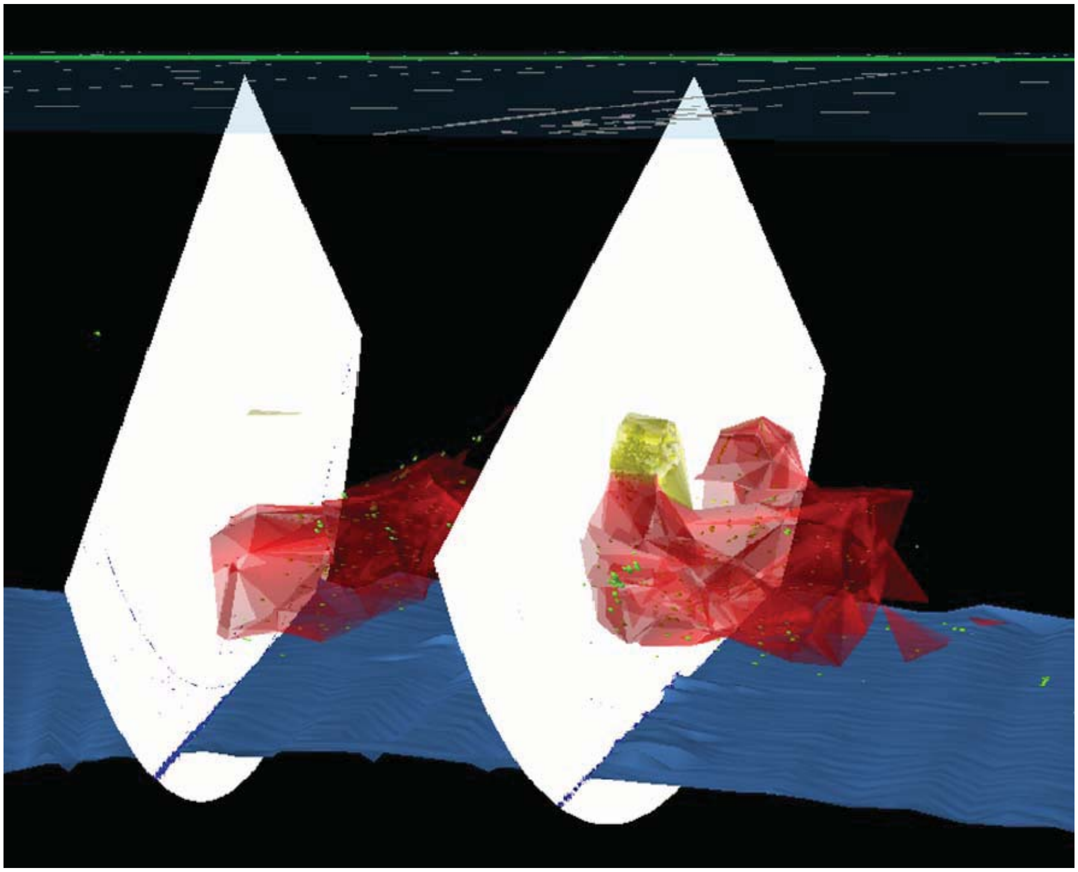

Figure 3.3.7. *Echoview 3-D visualisation looking westward towards a S. hippos aggregation (red volume surrounding detected individual green acoustic targets) with an associated dense volume of fish (yellow volume around yellow targets) speculated to be P. dentex. Cruisetrack (green line), seabed (blue surface) and example RESON 8125 single ping swath beams (white) also shown.*

In this dataset acoustic backscatter observed in MBS transects of the *S. hippos* aggregations displayed little evidence of lateral vessel avoidance behaviour even as the survey vessel passed directly above. Between individual morning transects the areal centroid of the aggregation moved a maximum of 22 m and the overhead surface area changed a maximum of 26.4%.

There was distinct backscatter variation in school density and structure between morning and afternoon transects, illustrated by the red and yellow volumes in Figure 3.3.8. During morning transects the aggregation appeared more widely spread and variable in movement, when compared with the afternoon school structure. In one day's MBS operation, transects were conducted before and after the aggregation was fished by three charter vessels, after which a video was towed through the aggregation. Immediately post fishing and video tows the aggregation structure in both dense and sparse regions were of smaller volume and more centralised (Figure 3.3.8 and Table 3.3.1) until approximately 30 minutes had passed when sparse targets become more dispersed. Such variation was possibly attributable to the time of day and/or a reaction to the fishing and video tows.

Post fishing, transects were conducted where no dense regions were detected in the MBS school detection algorithm, such as the 12:38:11 transect. It is conceivable that the dense regions were small enough to be located between consecutive MBS pings, as in other transects the region was present in only three pings. It is also possible that the individuals were, for some reason, linked by greater distances than that employed in the Echoview schools detection algorithm thus from the initial criteria they may have been detected as sparse targets. The actual reason for the difference in structure was not determined.

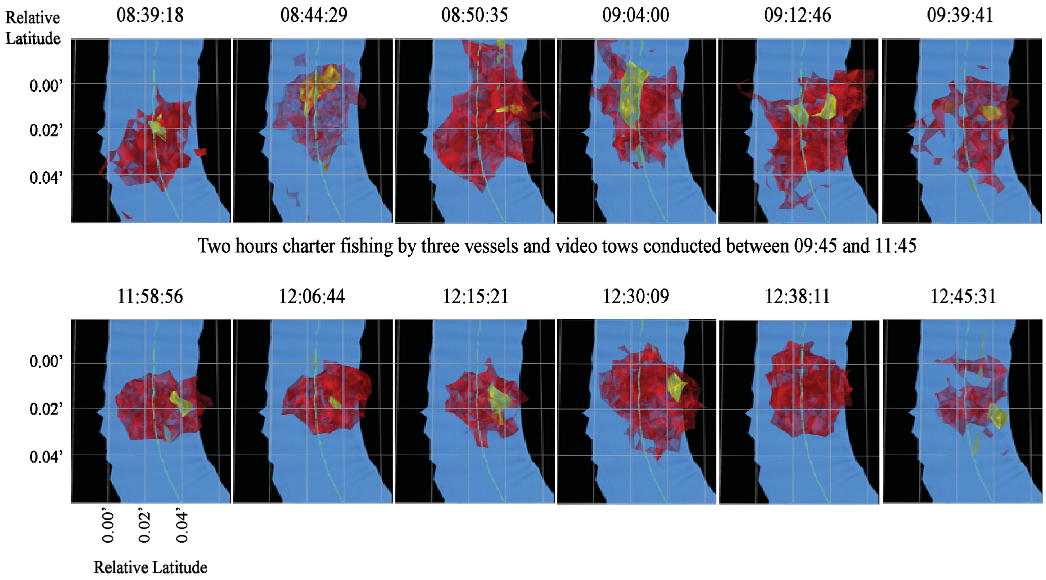

Figure 3.3.8. *Echoview formed plan view of sparse (red) and dense (yellow) volumes of the S. hippos aggregation at Site 2 as time passes. Views taken from six acoustic transects before (08:39 – 09:39) and after (11:58 – 12:45) two hours of fishing at the site by three charter vessels (transect start time shown above).*

In Table 3.3.1 various parameters of aggregation structure have been listed. During the final transect before fishing and the final two transects of the survey the aggregation appeared to split into smaller sections (Figure 3.3.8, transects 09:39:41 and 12:45:31). Depth ranges for both dense and sparse regions remained similar throughout all transects at approximately 60 to 70 m and 65 to 95 m, respectively.

Table 3.3.1. *Geometrics of S. hippos aggregation as determined by the detected targets from RESON 8125 Seabat acoustic backscatter. Volumes and number of targets are shown for the sparse aggregation of S. hippos and the dense volume of speculated skipjack trevally (Pseudocaranx dentex). Variations of plan S. hippos aggregation centroid are also shown.*

Transect Start	No. Targets Detected		Aggregation volume (m^3)		Overall plan area (m^2)	Variation of areal backscatter centroid from initial transect (m)
	Dense area at 12 m linking distance	Sparse area at 15 m linking distance	Dense volume	Sparse volume		
8:39:18	215	613	1297	57308	8963	0.0
8:44:29	1471	961	4444	84435	8975	22.3
8:50:35	768	2383	1308	148160	15135	15.7
9:04:00	1304	1143	4852	105966	12959	20.7
9:12:46	1109	1993	3208	143150	9533	11.7
9:21:12	0	731	0	44121	10343	10.8
9:39:41	384	789	962	52011	8786	11.1
Post fishing and towed video period						
11:58:56	444	760	1069	56163	7890	9.3
12:06:44	201	958	311	63193	6050	10.0
12:15:21	433	657	1914	51018	6181	3.7
12:30:09	544	2338	1103	139840	9583	18.9
12:38:11	0	1601	0	101975	9896	3.7
12:45:31	426	458	1089	33952	8618	11.0
12:54:01	0	601	0	35784	8956	10.8

Measurements from the dense and sparse aggregations were compared to give the acoustic packing density of each aggregation (Figure 3.3.9). As volume increased in both dense and sparse regions the number of detected targets also increased at an approximately linear rate. Initial comparison showed that a significant difference in acoustic packing between dense and sparse areas was observed. In addition, although there was a small data sample, sparse targets were more loosely linked during the morning transects than in the afternoon after fishing and video tows.

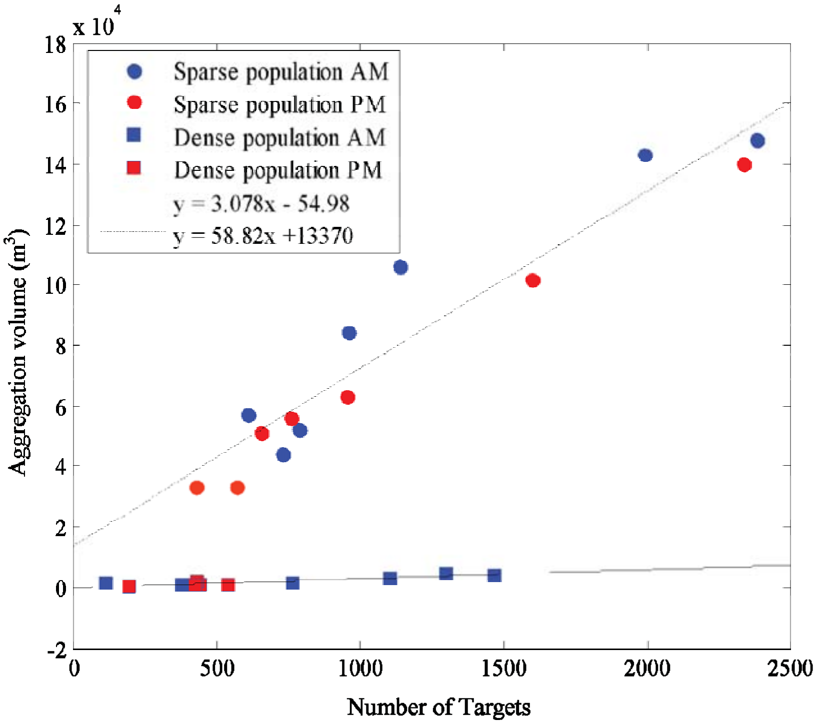

Figure 3.3.9. *Acoustic density packing of sparse (○) and dense (□) areas of fish as recorded from thirteen transects (seven AM and six PM) of the same aggregation.*

From least squares linear regression the acoustic target packing densities with correlation and confidence limits (c.l.) were:

i) dense volumes $= 3.078x - 54.98$ $R^2 = 0.840$, c.l. of (2.07, 4.09) and (-847, 738)

ii) sparse volumes $= 58.82x - 13370$ $R^2 = 0.926$, c.l. of (47.8, 69.8) and (-1426, 28200)

where x is the number of targets. Both sets of 95% confidence limits encompassed zero volume for zero targets. The density packing equates to approximately 3.08 and 58.8 m^3 per acoustic target for dense and sparse regions, respectively. If a mean body length of 30 cm for mature *P. dentex* is assumed throughout the aggregation (Fishbase, http://www.fishbase.org/Summary/SpeciesSummary.php?id=1002) and 1.07 m for *S.*

hippos (Rowland*, in prep.,* C. Delacy, UWA, *pers. comm.*) this gives an estimate of mean nearest neighbour distance of approximately 4.4 and 3.1 body lengths, respectively (allowing for body width and height). This acoustic target density packing has not considered the possibility of multiple detections of the same target, or missed targets between pings as although both phenomena were known to have occurred, neither were quantified.

3.3.4. Discussion

This survey has successfully acquired detailed acoustic backscatter from mid-water fish schools using MBS, including that of individual fish targets. Backscatter collected by the RESON 8125 MBS system provided unsaturated, highly resolved athwartships spatial data of the *S. hippos* aggregation at a high signal to noise ratio, allowing target counting to estimate numbers of fish present. Densely populated areas of the aggregation also displayed volumetric backscatter from schools of smaller fish (likely *P. dentex*), sitting above the *S. hippos*. Individual transects encompassed entire aggregations ranging 100-200 m in length and breadth. Data processing in Echoview and Matlab have facilitated the generation of 3-D visualisation of the aggregation, both as individual targets and the associated encompassing volume with minimal effects of fish movement.

Comparison of single-beam and MBS system data aided confirmation of whether targets in consecutive MBS pings were different fish or multiple detections of the same fish. Although the RESON 8125 offered sufficiently high resolution to discriminate targets within a swath, the repetitive occurrence of multiple detections in consecutive pings has significant implications in monitoring fish numbers. The ability of MBS to observe tracks of two fish which appear as a single target on the single-beam will have a limited effect on single-beam biomass estimates. Such multiple detections by a single-beam system would not occur with a split-beam echosounder. Therefore the combination of split-beam and MBS systems would greatly improve the accuracy of target counting individual MBS detected fish.

In these surveys dense and sparse fish aggregation regions displayed little sign of lateral avoidance from the survey vessel. It is possible that at slows speeds such as those employed in the survey, lateral vessel avoidance is a limited issue when studying fishes of sizes and depths such as those of the *S. hippos* and speculated *P. dentex* aggregations. Throughout previous surveys *S. hippos* have appeared unconcerned by vessel presence and during video tows did not appear to avoid the towed camera (Parsons *et al*., 2005). Although the aggregation remained above the wreck in a relatively stable position throughout the day, morning transects displayed a more mobile school of greater volume which condensed and became near stationary, immediately after being fished and exposed to the towed video (Figure 3.3.8 and Table 3.3.1). As time passed the aggregation volume increased, and in the final transects the aggregation appeared more dispersed (Figure 3.3.8 and Table 3.3.1). However, the number of MBS detected targets decreased at the same rate as the observed volume reductions, illustrated by the consistent acoustic packing density (Figure 3.3.9).

It is suggested that the higher estimates of target numbers observed in the morning transects were due to multiple detections of single fish and that the more condensed volume in the early afternoon transects is more representative of the number of *S. hippos* in the aggregation. It is thought that the behavioural change was a reaction to either fishing or video tow presence, though this is speculation. Similar observations were made of the dense regions of the aggregation. It is possible that a large number of fish were departing and rejoining the aggregation, during the minutes between transects, without detection by the MBS. For such movement to pass unnoticed is unlikely, and one would expect to see backscatter from a group of fish breaking away from the aggregation, possibly as observed in Figure 3.3.4.

A comparison of the fish aggregations in the single-beam and MBS data suggested that while lateral avoidance of fish to the survey vessel was not apparent, herding of fish along the vessel track may have occurred. Olsen *et al*., (1983b) suggested that fish react to noise stimuli by increasing speed and swimming radially and downwards. However, due to acoustic shadowing created by the vessel hull, lobes of maximum noise intensity (either side of the vessel) create a minimum in front of the vessel (Urick, 1983, Misund *et al*., 1996, Misund, 1997). This may result in herding by the vessel, similar to that reported by Misund and Aglen (1992) as the fish were guided in front (Misund *et al.,* 1996). Such potential herding would explain the variations in perceived *S.hippos* aggregation volume and number of acoustic targets. In contrast to the morning transects, the condensed acoustic nature observed in the early afternoon was due to lack of along track movement by the fish. Thus although the aggregation appeared relatively immobile the fish may have been moving along the line of vessel travel and did so less when the aggregation volume appeared smaller. The instances of multiple detections of a single target in consecutive MBS pings corroborate along track movement.

That the RESON 8125 and Simrad EQ60 combined datasets were capable of observing such effects illustrates the advantages of MBS in fisheries monitoring. Quantification of multiple detections due to along track movement by the aggregation or school is required to improve confidence in biomass estimates based on the volume encompassed by the fish.

The estimates of fish numbers shown in Table 3.3.1 do not consider those members positioned in-between pings. The RESON 8125 exhibited an inter-ping distance 5.4 to 7.2 m at the surface, which at 80 m depth is equivalent to approximately 4.1 to 5.9 m between the edges of the MBS swaths, leaving a high probability of fish falling between pings. As yet this level of missed fish remains unquantified. The effect will be more prominent in estimates of *P. dentex* as the smaller species will be more compact in the alongships direction than *S. hippos*. There is a need to determine an optimum ping rate which minimises inter-ping sample volume spacing, whilst maintaining manageable data levels. Alternatively, it is possible to quantify the targets lost between pings, by assuming an isotropic packing density.

Studies of nearest neighbour spacing in social aggregations suggest that social groups exhibit species-specific individual distances (Mogilner *et al*., 2003). Individuals in saithe (*Pollachius virens*) schools have been observed at less than one body length apart

(Cullen *et al*., 1965) and Niwa (2005) reported that school volumes in tanks approximated to Nb^3, where N is the number of fish and b the nearest neighbour distance, equivalent to the mean fish body-length. However, Misund (1993) reported herring (*Clupea harengus*) school packing density in the field at an order of magnitude lower than that in captivity, thus fish under differing circumstances exhibit different packing densities. The observed results of 58.9 and 3.1 m^3 per fish are equivalent to average nearest neighbour distance of approximately 3.1 and 4.4 body lengths (assuming *S. hippos* of length 1.07 m and *P. dentex* of approximate length 30 cm and adding the length of the fish in the appropriate dimension to estimated fish length, height and width). Although acoustic targets within a single MBS ping were not confirmed as individual fish the lateral and vertical spacing of targets in the sparsely populated areas suggest individual *S. hippos*. Therefore the number of targets detected in a single ping would be analogous to the number of fish in the equivalent MBS sample volume. Targets in the densely populated regions, however, were not always separated and could easily have been multiple fish. Therefore the volume per fish calculated in the dense region would likely be an overestimate compared to the sparse region. Furthermore, the inter-ping distance will result in a greater underestimate of dense area targets as the speculated species are smaller, exhibiting smaller nearest neighbour distances and so likely contain more individuals within the unsampled area between pings.

The calculated target densities have shown the ability of MBS technology to discern between species, or to discriminate between two schools of differing sized fish based on their acoustic packing density. Future surveys of *S. hippos* conducted with MBS may reveal packing densities of different order of magnitude given different fish behaviour. The fish can be expected to behave differently across a season, for example the end of the spawning season as opposed to the beginning.

The densely packed area of *P. dentex* has shown the possibility of MBS systems to survey schools of small, densely packed species using echo-integration. However, an accurate biomass estimate of any fish using this technology requires a species-specific, length dependent, three-dimensional TS model, due to anisotropic backscattering. These models are complicated, take time to develop and were outside the scope of this study.

Near field effects of the RESON 8125 were observed at full power and gain, extending into the tens of metres, with artefacts similar to those reported by Trevorrow (2005) and Malzone *et al*. (2008). These artefacts would have significant implications for surveys of fish swimming near the transducer, for example in shallow water. Therefore consideration must be given to the relative positioning of the MBS swath to the school or aggregation such as that reported by Gerlotto *et al.* (1998).

Although the sampling volume of the single-beam system did not provide accurate spatial resolution of targets, the high alongships resolution offered invaluable data for biomass estimates. When combined with the athwartships spatial coverage of the MBS, single-beam backscatter should provide accurate biomass estimates as well as structural and behavioural information. It should be noted that this survey was conducted early in the spawning season over a comparatively small aggregation. Surveys later in the season often report aggregations of far greater number (Section 3.5.3). It is the author's opinion that due to variations in TS and blurring of the aggregation area by fish mobility, only coarse biomass estimates may be made using single-beam techniques alone. In such a low value fishery as *S. hippos,* the large scale changes in abundance shown by aggregations at the various Rottnest Island sites may be all that is required.

3.3.5. Acknowledgements

The authors would like to thank the Australian Coastal CRC for supplying the RESON 8125 echosounder and additional support in conducting the surveys, Myriax for ongoing software and support, and the Department of Fisheries WA for vessel time. Myriax provided the use and support for processing using Echoview software and particular thanks go to Matt Wilson, Bart Buelens and Myounghee Kang. Funding was gratefully received from the Fisheries Research and Development Corporation (FRDC) and a PhD top-up scholarship was supplied by the Western Australian Marine Science Institute (WAMSI).

3.4. Multi-beam visualisation of Samson Fish *(Seriola hippos)* aggregations and spawning habitats in Western Australia using a RESON 7125 Seabat multi-beam sonar

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ABSTRACT:

The advent of multi-beam technology as a technique to acquire acoustic backscatter data from the entire water column now allows the surveying of extensive fish schools in a single vessel transect. Data processing capabilities which previously restricted maximum ping rates and consequently created alongships spatial aliasing, due to unsampled volumes between pings, have advanced sufficiently to allow the acquisition of water column backscatter whilst simultaneously collecting seafloor backscatter to establish and classify areas of Essential Fish Habitat (EFH). In February 2007 successive transects, ensonifying Seriola hippos aggregations west of Rottnest Island, Western Australia, were conducted with a Reson 7125 Seabat multi-beam sonar (400 kHz) during their spawning season. Detailed 3-D visualisations of the aggregations from acoustic backscatter were produced at sufficient resolution to identify individual targets. Bathymetric and backscatter data acquired from seafloor beneath aggregating fish displayed examples of EFH preferred as spawning locations for S. hippos. Short term temporal characteristics of school structure were investigated illustrating aggregation mobility as the fish moved 91 m during a fifteen minute period and exhibited lateral vessel avoidance behaviour in the form of alongships herding. Aggregation acoustic density packing at the survey site was observed at 12.1 m^3 per fish (s.d. = 2.53), equivalent to approximately 1.6 body lengths nearest neighbour distance.

Keywords: *Multi-beam, fisheries, habitat classification, essential fish habitat, backscatter*

3.4.1. Introduction

Concerns over climatic and anthropogenic impacts, combined with increasing fishing pressures on fish stocks have made the evolution from monitoring species fisheries to Ecosystem Based Fisheries Management (EBFM) an intrinsic component in maintaining sustainable fisheries worldwide (Anonymous, 1999, Pikitch *et al.,* 2004). To facilitate such a holistic approach to fisheries acoustic monitoring the simultaneous, rapid, accurate and replicable quantification of classified fish schools together with their associated Essential Fish Habitats (EFH) have become prime objectives of fisheries acoustics.

Several fisheries designed sonar systems have been developed with water column sampling in mind, including the Simrad SM2000 and MS 70, sometimes with the option to collect bathymetric data (Simrad ME 70 www.simrad.com; Andersen *et al*., 2006). However, the ability to simultaneously acquire and store adequately sampled water column and seafloor backscatter (rather than bathymetry alone) often inhibits the ping rate and hence along-track resolution of several MBS systems.

Previous studies on *Seriola hippos* aggregations, using a RESON Seabat 8125, illustrated that while sample resolution of an individual swath was sufficient to identify individual *S. hippos* targets and contain significant backscatter data from the seafloor, there were limitations in alongships resolution, due to the ping rate (Parsons *et al*., 2006b, Section 3.3). Such findings were in agreement with previous studies of fish at similar depths (Trevorrow, 2005). For accurate quantification of fish numbers and behaviour it is essential that ping rate is sufficient to remove spatial aliasing. One such MBS sonar, capable of simultaneously acquiring and logging water column and seafloor acoustic backscatter at up to 34.5 kHz along beam sampling and 40 Hz ping rate, is the RESON 7125 Seabat. Operating at 400 kHz the RESON 7125 generates a transmit beam nominally of width 1° alongships and 155° athwartships. An orthogonal line array forms an effective beam pattern of 512 equidistant beams across a 128° by 1° swath with samples numbering up to 456 per metre at a maximum resolution of 2.5 cm (Reson Inc. 2006). The sonar acquires data at a rate of approximately 155 GB/hour (at a range of 100 m with a 5 Hz ping rate) thus requiring 2 Terabytes of storage per survey day (Malzone *et al*., 2008). Recent advances in post processing techniques allow mining of considerable data volumes from entire surveys to provide the observation of detected schools in time and space (Wilson *et al*., 2005, Buelens *et al.,* 2007).

It was the purpose of this research to assess the performance of the RESON 7125, simultaneously acquiring backscatter from aggregations of *S. hippos* and the habitat over which they spawn. The primary objective was to evaluate biomass estimates and spatial analysis of the aggregations in comparison with previous studies.

3.4.2. Methodology

The RESON 7125 was mounted aboard the 21.6 m length Fisheries vessel, the *RV Naturaliste*, over a six day survey period. Positioned on the port side, 3.95 m from the vessel centre line and 2.77 m below the water surface, nadir beams were directed vertically downwards and the swath athwartships.

Examples of acoustic backscatter in this paper were acquired from a study site known as the 'Outer Patch' (Site 3, Figure 3.1.1), comprising a wreck lying partially buried 110 m deep on flat, sandy seafloor, running approximately north-south. During surveys the vessel speed was maintained, as consistently as possible, between 4 and 5 knots, in approximately 10 knot winds and 1 m seas. Ships positions were recorded using a Furuno Differential GPS system. Octopus F180 and Applanix POSMV motion sensors supplied pitch, roll and yaw data, which were logged in PDS2000 software together with sound velocity profile (SVP) data (Seabird) whilst on site. Towed underwater video transects were conducted before and after acoustic surveys to verify site species presence and confirm school structure.

Acoustic transects were conducted running north-south along the length of the wreck during the mornings of the 2nd and 3rd February, 2007, each transect lasting a maximum of three minutes. Initial transects were conducted beforehand to estimate optimal settings of power, gain and pulse length for acquiring sufficient backscatter responses from aggregation and seafloor, without saturation. Individual beam responses from densely populated areas of the aggregation were monitored visually using RESON 7125 software to ensure received backscatter remained unsaturated. System settings from preliminary transects were maintained at a pulse rate of 150 µs, power of 220 dB, gain of 25 dB, and range of 175 m. *S. hippos* aggregations were known to aggregate at preferred depths between 60 and 100 m (Parsons *et al.*, 2005) a depth at which the MBS swath covered approximately 345 m width, capable of encompassing the entire aggregation within the swath. At the above range and pulse rate the maximum RESON 7125 ping interval was approximately 1.2 seconds between consecutive pings. The combined effect of ping rate and vessel speed, resulted in inter-ping surface distance of approximately 2.3 to 2.9 m (excluding effects of pitch and yaw). At the typical *S. hippos* aggregation depths of between approximately 60 and 90 m the fore-aft beam widths of the 7125 were 1.05 and 1.57 m respectively. Combining inter-ping distance with acoustic beam widths resulted in approximate ranges of 1.25 to 1.85 m and 0.73 to 1.33 m between consecutive acoustic swaths at 60 and 90 m depth (excluding pitch and yaw).

Each transect collected acoustic backscatter files of between 3 and 5 GB which were split into manageable sizes (500 Mb) by a RESON 7kFileSplit program before being imported into Echoview 4.2 (Myriax©) for processing. Roll data was imported into each file, including an initial system tilt angle of 3.9° to port. A Kalman filter was applied to smooth GPS and heading data in order to limit effects of yaw on overlapping pings.

Preliminary findings displayed that although seabed and *S. hippos* acoustic targets were detected, significant interference occurred. This interference was attributed to transmit beam side lobes (radial multi-pathing noise) and coherent electrical noise (‘Spoking’) probably due to external electrical motors (Malzone *et al.,* 2008), shown in Figure 3.4.1. Noise removal was conducted in Echoview and involved subtraction in the linear domain of mean backscatter from a 10 ping subset within the transect which displayed no evidence of fish presence. A minimum S_V threshold of 12 dB was set to remove remaining noise and false targets. At a range of 100 m, where targets were predominantly detected, noise levels removed were typically in the region of 16 dB. After noise subtraction remaining backscatter was predominantly attributable to seabed and fish, however, not all noise was removed (Figure 3.4.1). The interference displayed in Figure 3.4.1 was typical of backscatter acquired in all transects, although in some instances pings of irreconcilable noise (nominally due to vessel slap) were discarded (typically 1 ping per transect). At other surveyed sites the effects of electrical interferences were exacerbated when surveying aggregations at greater depths, often restricting analysis of backscatter data.

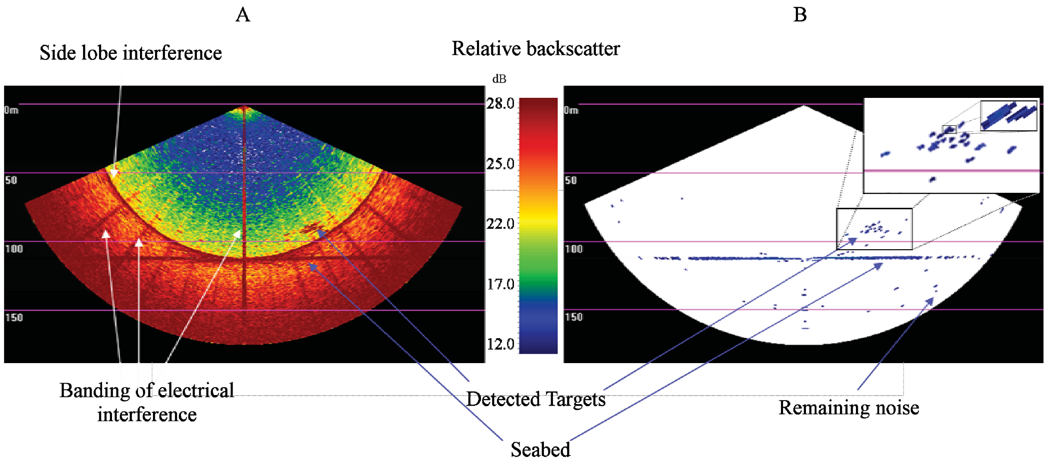

Figure 3.4.1. *Example RESON 7125 Seabat acoustic swath of aggregating Seriola hippos displaying echogram before (A) and after (B) acoustic noise removal. Sv values were thresholded at 12 and 28 dB, graduated as per the colour bar. Coherent electrical noise, side lobe interference ("spoking") together with detected targets and seabed are highlighted. Insets show expansions of detected targets highlighting one target comprising several samples (top right).*

Echoview school detection algorithms were run to identify *S. hippos* targets within individual pings at height, width and length dimensions of more than 0.02 m, i.e. a size smaller than one sample volume, thus detecting all targets above the seabed. Pings were visually scrutinised and regions attributed to noise were manually identified and removed. Samples were considered to be noise based on the same equivalent sample position in neighbouring pings (i.e. the same beam and range) and target position relative to known noise artefacts (side lobes and electrical interference). Each remaining target was then extended through one metre for visualisation purposes within Echoview.

The swimbladder size of *S. hippos* is considerably larger, dorsoventrally, than each Reson sample volume size, thus a single fish target comprised several samples of varying S_V values (Figure 3.4.1B, top right). Targets were separated into constituent samples by S_V values in 2 dB bins. Swath positions and S_V values were exported into Matlab to calculate aggregation volumes using Myriax developed Matlab programs (B. Buelens, Myriax, *pers. comm.*). The methods outlining the generation of aggregation volumes from target nearest neighbour linking distances can be found in Section 3.3.2. Aggregation volume was calculated by linking acoustic targets separated by less than a threshold nearest neighbour distance. Threshold distances between targets were tested at metre intervals and final thresholds set once 90 % of all detected targets were encompassed within the calculated aggregation volume. For coherence the same linking distance was applied to all transects.

Seafloor acoustic backscatter was corrected for angular dependence using Matlab programs developed by the CMST. The algorithm works by removing the mean and standard deviation angular trends before restoring local mean levels to acoustic backscatter (for detail see Parnum, 2008). The algorithm can be expressed in the following form:

$$BScor(\theta) = \frac{BS(\theta) - \overline{BS}(\theta)}{BS_{std}(\theta)} + \overline{BS}(30°) \qquad (3.8)$$

where $BS(\theta)$, $BScor(\theta)$, $BS_{std}(\theta)$ and $\overline{BS}(\theta)$ are the uncorrected, corrected, standard deviation and mean backscatter for all data at angle θ, respectively. The mean backscatter for all data at 30° is added to provide comparable absolute values derived after corrections. Corrected backscatter was then compared to bathymetric data for seafloor classification.

3.4.3. Results

The *S. hippos* aggregation was observed over several transects and in each case individual targets were detected by the RESON 7125 Seabat, processed using Echoview MBS school detection modules. Acoustic targets rarely comprised single samples, but clumps of samples containing S_V values above the threshold level (see Figure 3.4.1). Such targets resembled the nature of acoustic backscatter expected from individual *S. hippos*. The clusters of samples were separated by a distance comparable to the expected distance between individual *S. hippos*, implying that a cluster of samples represented an individual fish.

Figure 3.4.2 displays a three-dimensional visualisation of a transect started at 10:14 am, viewed from the north. Each image illustrates the detected target backscatter samples of varying intensities separated into S_V bands of 2 dB, as per the colour bar. These samples sit above the seafloor and wreck (shown as the blue surface). The angle of incidence with which the acoustic beam ensonifies a target is a function of the fish depth and lateral distance from the cruisetrack. Therefore the combination of front (Figure 3.4.2, top panels) and plan (Figure 3.4.2, bottom panels) views of the aggregation display how the S_V values vary with increased angle of incidence of the acoustic beam to the swimbladder. Due to the extent of electrical interference it was felt that during the noise removal stages of data processing an unknown portion of backscatter from the acoustic targets was also removed. Therefore the S_V values should be considered on a relative, rather than absolute basis. If the vessel is assumed to exhibit little or no roll then the green line (cruisetrack) represents near zero angle of incidence. Initial impressions suggested a relatively even distribution across the swath, however, a single transect does not contain enough sample data for statistical evaluation. Such analysis has been deemed for future investigation.

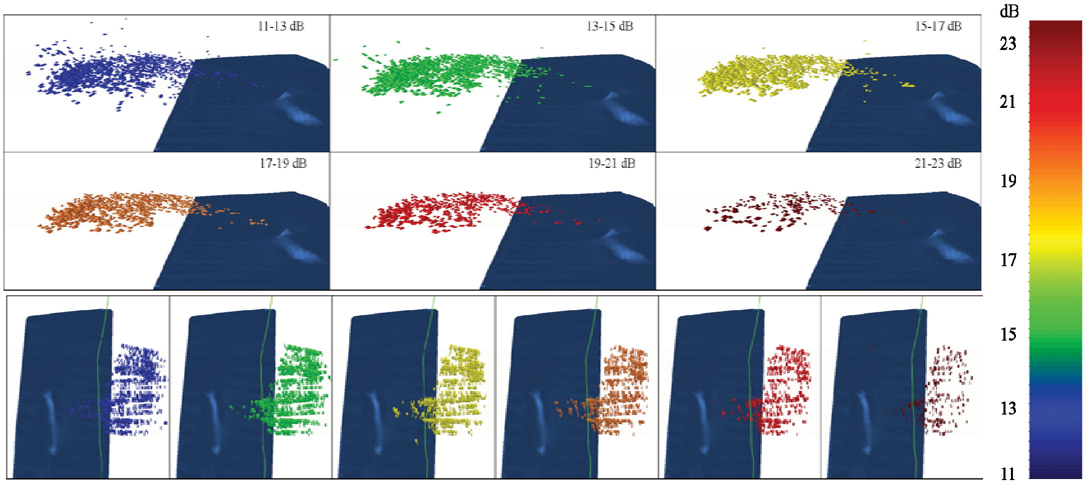

Figure 3.4.2. *3-D visualisation of regions generated from varying Sv bands of the acoustic backscatter acquired from S. hippos aggregation during the 10:14am transect 3rd February, 2007. Top panels are an oblique view of the aggregation, lower panels a plan view. The sub panels display different* S_V *bands as indicated on the colourbar.*

Viewed as a whole aggregation, comprising the samples of varying backscatter, the overall distribution of acoustic targets and their respective sample groups illustrated the anisotropic nature of fish TS in three dimensions (Figure 3.4.3). The samples ranged in backscatter by 15 dB, even at the same angle of incidence. Such backscatter variation is greater than attributable to that created by a TS/length relationship and the estimated size distribution of *S. hippos*.

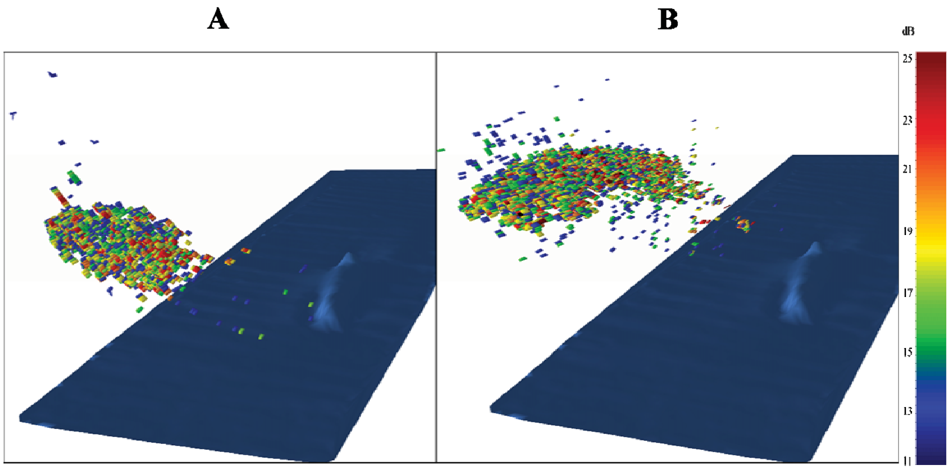

Figure 3.4.3. *3-D visualisation of a* *S. hippos* *aggregation at 10:11:44 (A) and 10:14:18 (B). Detected individual samples coloured as per the colour bar to their respective acoustic backscatter (S_V).*

In previous studies the aggregations of *S. hippos* were thought to remain relatively stationary over long periods of time (Parsons *et al.,* 2005). However, a subsequent survey has shown that this assumption is not always correct and in some circumstances the school is mobile, above the wreck on which it is located (Section 3.2). Transects conducted between 10:05:30 and 10:17:45 3rd February, 2007 displayed evidence of the mobility of the aggregation over short periods, as shown in Figure 3.4.4. The aggregation was observed to move from close to the eastern side of the wreck to the north end and then down to the far southeast side over approximately 91 m (substantial mobility by comparison with the general fishers' opinion). In each transect the school was observed to be at the side of the cruisetrack and not directly beneath (Figure 3.4.4, green lines), similar to behaviour observed during transects at other sites during this survey. This is in contrast to previous surveys, where the *S. hippos* have been positioned directly below the survey vessel (Section 3.2). In some cases it appeared as though targets moved in conjunction with vessel tracks, altering direction concurrently with the cruisetrack (Figure 3.4.4, 10:05:30 transect).

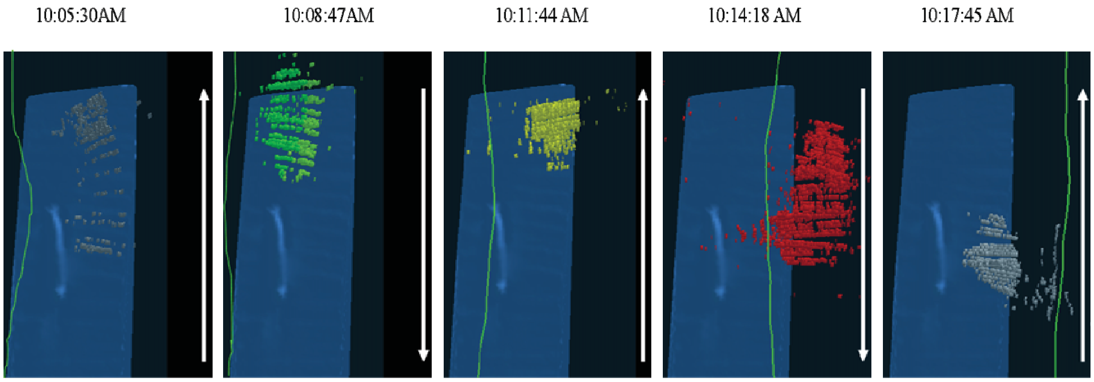

Figure 3.4.4. *Plan views of five consecutive acoustic transects displaying aggregation movement during a fifteen minute period, with transect start times noted above. Seafloor is shown in blue and fish regions generated from backscatter are shown in various colours. Cruisetracks are shown by green lines and vessel direction by the white arrows.*

The period of time between the start of consecutive transects was typically three minutes (Figure 3.4.4). Between the 10:11am and 10:14am transects there was a noticeable change in the number of detected targets, plan area and volume (Table 3.4.1); though no apparent variation in vertical distribution or nearest neighbour distances between targets within a single ping. In the 10:11am transect, conducted south to north, 740 targets were detected at the northern end of the wreck, as opposed to 2426 in the successive southerly transect where the aggregation spread along the eastern length of the wreck.

Previous studies have shown that *S. hippos* aggregations comprise a significant number of individuals separated from the main group (Parsons *et al.,* 2005, Section 3.2). Therefore, aggregation volumes were calculated based on a 12 m nearest neighbour linking distance which was sufficient to encompass over 90% of the aggregation targets and allow for effects of vessel yaw (Figure 3.3.7). Remaining targets were considered external to the aggregation. The numbers of acoustic targets were then compared to aggregation volumes to produce the effective acoustic target packing density (see Table 3.4.1). Mean acoustic volume per target for the 7125 survey was found to be relatively consistent throughout transects of the site, at 12.1 m^3 per target (s.d. = 2.3 m^3), equivalent to approximately 1.6 body lengths nearest neighbour distance. This does not, however, consider targets between pings which were not sampled.

Table 3.4.1. *Density packing of acoustic targets observed in five transects of the S. hippos aggregation at the Site 3 on the 3rd February, 2007.*

Transect start time	Aggregation volume at 12 m linking (m^3)	Number of acoustic targets	Acoustic target density packing (m^3 per target)
10:05:30	6 248	446	14.01
10:08:47	3 865	289	13.37
10:11:44	6 537	740	8.83
10:14:18	33 387	2 426	13.76
10:17:45	9 809	933	10.51

Seafloor acoustic backscatter produced considerable data from which it was possible to classify the habitat beneath the aggregation. Bathymetric data identified flat sand at 112.5 m depth surrounding a semi protruding wreck rising to depths of approximately 109.5 m (Figure 3.4.5A). To the east of the wreck a rise in bathymetry (111 m) and to the southwest a longitudinal depression (to approximately 113.5 m) were also observed. Areas of no data were present to the east and west (white areas Figure 3.4.5). Specular backscatter acquired from nadir and neighbouring beams in MBS often display higher standard deviation trends (Parnum, 2008). Acoustic transects on the 3rd February were conducted directly above the wreck, a less than optimal angle for acquiring acoustic backscatter to compare wreck data with that of surrounding habitat. Uncorrected backscatter highlighted the issue of near nadir effects (north-south line of high backscatter, Figure 3.4.5B). The corrected backscatter confirmed the wreck as a rough surface and the surrounding sand of low backscatter (Figure 3.4.5C). Elevated bathymetry east of the wreck produced the lowest area of backscatter, and the longitudinal depression to the southwest relatively high backscatter.

The most likely explanation for the rise of low backscatter sediment to the east and depression of high backscatter to the southwest is the sediment transfer of particles due to a current from the northeast, i.e. scouring. The accretion of fines of low backscatter on the eastern, 'updrift' side of the wreck is similar to that of other sites in areas of low current (DeAlteris, 1975), compared to sites in high current where a scour hollow would be expected. The width of this scour pit, compared to the length of the vessel, is indicative of scour in fine substrate where coarser debris remains in the scour pit (Caston, 1979, Quinn et al., 1997). By constrast, scour where coarser sediment is removed would leave a thinner scour pit. Typical twin, longitudinal scour pits may be in the form of one mark because of the orientation of the wreck to predominant current direction, or a varying current which alters the scour pit dimensions (Caston, 1972).

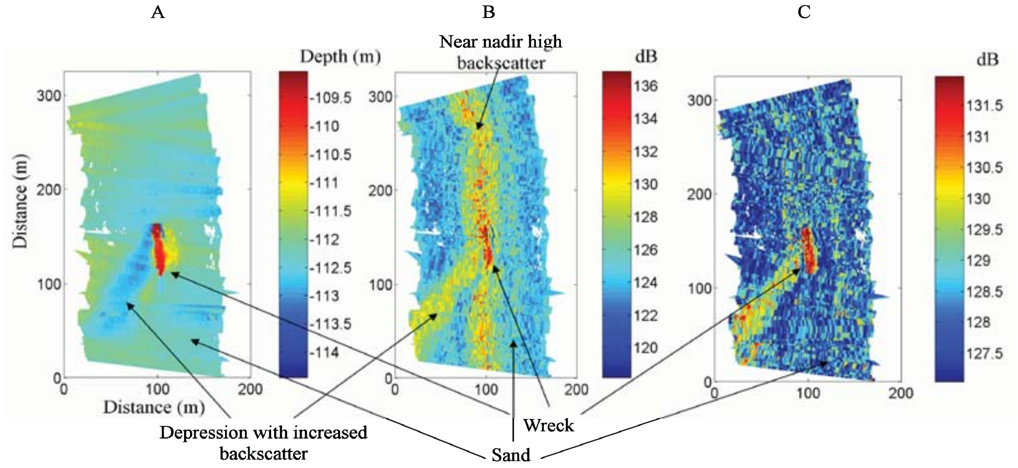

Figure 3.4.5. *Bathymetry (A), uncorrected backscatter (B), corrected backscatter (C), from left to right respectively acquired from a single acoustic transect of the Outer Patch site. Axes display relative distance in metres while depth and relative backscatter are shown as per the respective colour bars.*

Once combined with water column acoustic data more comprehensive images of the aggregating *S. hippos* and the habitat around which they spawn were produced (Figure 3.4.6). Figure 3.4.6 illustrates the ability to observe the entire aggregation as a group of individually detected samples separated by S_V bins (A) and/or encompassing aggregation volumes relative to the wreck (B) (volume opacity represents target density within the aggregation). Aggregation movement during multiple transects was observed in the differing positions of the aggregation volume around the wreck (Figure 3.4.6C).

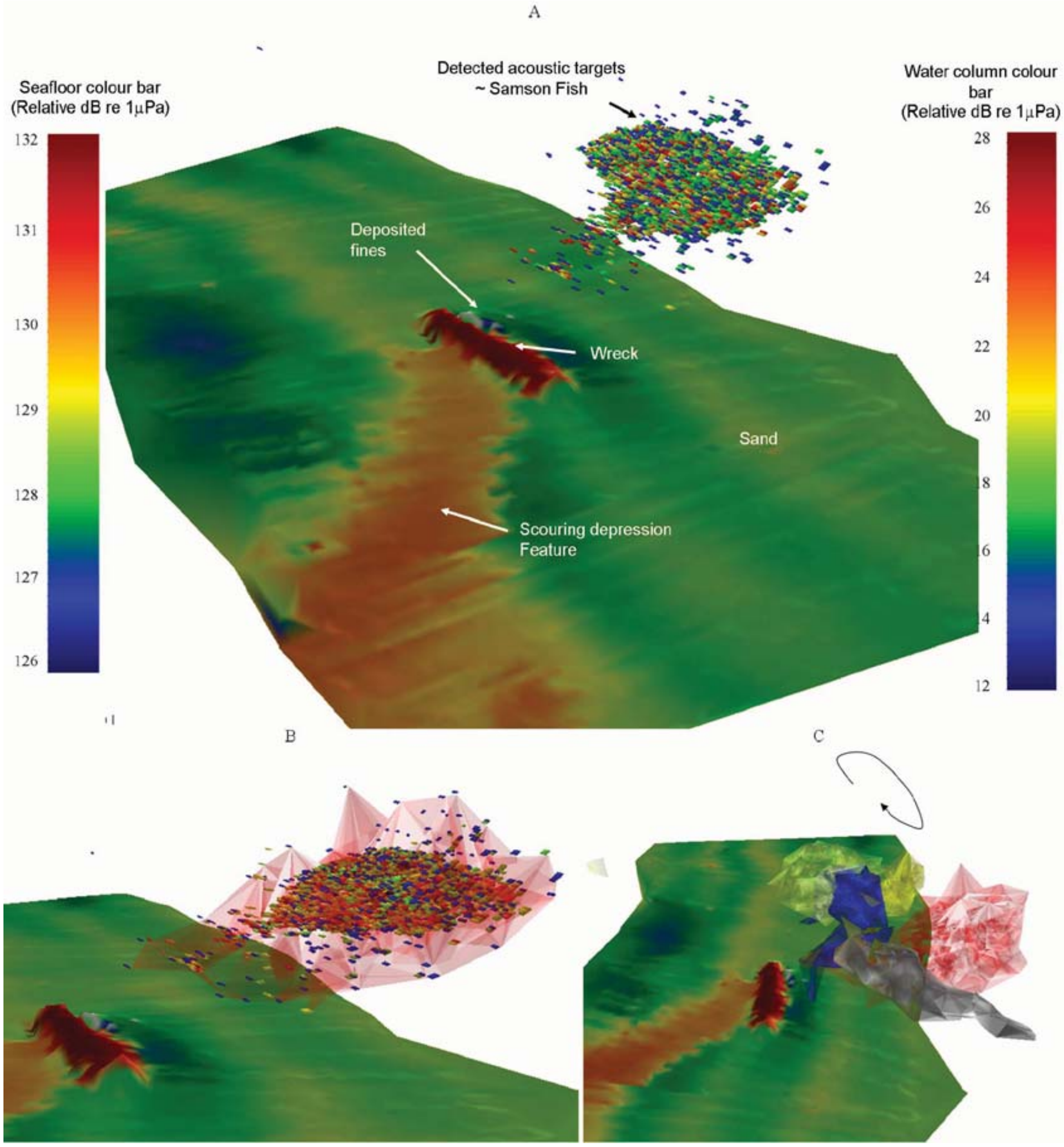

Figure 3.4.6. *A) 3-D visualisation of the seafloor and* *S. hippos* *aggregation at the Outer Patch with colouring of surface and volume backscatter as per the respective colour bars. B) Acoustic targets separated by less than 12 m enclosed within an aggregation volume (red). C) Aggregation movement during five transects, each separated by approximately three minutes, running order; blue, green, yellow, red and finally grey (motion highlighted by grey arrow).*

3.4.4. Discussion

Despite significant electrical interference the RESON 7125 detected individuals and groups of fish at high resolution, in depths of up to 100 m, covering the aggregation in a single transect. The data acquisition and processing techniques have displayed the ability to produce high resolution images of entire aggregations together with their associated habitat from individual transects of a few minutes duration. Acoustic backscatter from *S. hippos*, a species of significant size and nearest neighbour distances, were imaged as acoustic reflectance of individual samples, acoustic targets representing individual *S. hippos* (comprising several samples), and as the volume encompassed by the aggregation. The combined inter-ping distance and beam width, acquired acoustic *S. hippos* backscatter data from sample volumes separated by 0.73 to 1.83 m (excluding pitch and yaw) taken every 1.2 s (the maximum rate available with the Reson 7125 using 175 m range). This was sufficient sampling to alleviate spatial aliasing due to undetected targets falling between the pings of previous surveys (Section 3.3.4).

The data acquired with the RESON 7125 Seabat provided significant behavioural information on *S. hippos* aggregations and confirmed previous suggestions that the aggregations do not always remain stationary. The rapid coverage of the entire school allowed recording of this movement, previously only estimated from data acquired with single-beam techniques. Plane distance of the aggregation from vessel cruisetracks implied that the fish may sometimes exhibit lateral vessel avoidance. The transect conducted at 10:05 in particular highlighted such movement.

A lack of change in acoustic target density between transects suggested two possible explanations for the variation in target numbers between consecutive transects. The first possibility is that a large influx and exodus of fish occurred prior and subsequently to the 10:14 transect. Such an explanation is unlikely, given the short space of time between transects, and would most likely be detected on the MBS data. However, the second possibility was that the aggregation moved in a uniform group in the direction of vessel travel. This suggestion was in part corroborated by the lack of vertical migration shown as the transect was conducted. The herding described would result in the perceived elongation of the aggregation as it attempted to move away from the vessel (Figure 3.4.4, Transect 10:08 and 10:14). By comparison with the previous transect, the vessel the volume would be shortened if the aggregation stopped or moved towards, as shown in Figure 3.4.4 (Transect 10:11). This would explain the variation in target numbers, due to multiple detections of individual fish as they swam along with the vessel, while maintaining the same nearest neighbour distances. However, confirmation of whether this movement was influenced by the vessel presence is undetermined. The extent of this movement is in contrast to that exhibited in previous studies. Previously, the larger *S. hippos* aggregations were surveyed towards the beginning of the spawning season and, although possible reaction to fishing and video tows could be inferred by a condensing of the aggregation (Section 3.3), little vessel avoidance was observed. This suggests that the aggregations become more volatile in nature near the end of the spawning period, which is when this survey was undertaken.

Balchen (1984) suggested that vessel avoidance is a result of sound and visual stimuli, and natural behaviour. Vessel avoidance variation within a species has been proposed to be a function of fish depth (Misund, 1997), range (Diner & Massé, 1987, Olsen *et al*., 1983b, Soria *et al*., 1996, Fréon *et al*., 1992) vessel speed (Olsen *et al*., 1983b), vessel size (Olsen *et al*., 1983a), noise propagation (Engås *et al*., 1995, Gerlotto *et al.,* 2004) and ontogenetic stage (Neproshin, 1979, Misund, 1992). It is therefore likely that vessel avoidance will vary significantly throughout the spawning season as environmental, acoustic and behavioural conditions change. These causes of avoidance could contribute to the difference in *S. hippos* packing density behaviour between the October and February surveys.

Although many fish species exhibit avoidance prior to a visually detectable survey vessel (Olsen *et al*., 1983b, Diner and Masse, 1987, Soria *et al*., 1996, Gerlotto *et al.,* 2004), others avoid the vessel once within visual distance and/or the vessel passes above (Soria *et al*., 1996) and some only avoid if in the path of the vessel (Diner & Masse, 1987). However, on several occasions no vessel avoidance has been observed at all (Ona and Toresen, 1988, Ona and Godø, 1990). Vessel avoidance prior to visual stimuli will be partially dependent on the hearing frequency and dynamic ranges of the species. For example, polar cod (*Boreogadus saida*) have been reported to exhibit directional hearing and avoid a survey vessel at 150 m in front of the vessel (Olsen *et al.,* 1983b). The otolith to body size of *S. hippos* combined with a lack of recorded vocal behaviour (see Section 4.8) could be considered to suggest that the species possess a limited hearing range. This may contribute to the limited avoidance behaviour has been observed in *S. hippos* at vessel ranges where noise levels may be significant, but visual stimuli is comparatively lower.

Quantified avoidance in fish schools has suggested that effects predominantly occur at close range, between 25 and 50 m from the vessel (Soria *et al*., 1996). However, Soria *et al*. (1996) also noted slow movement in the opposite direction of the vessel track at distances >50 m, similar to some of the behaviour of *S. hippos* in this study (shown in Figure 3.4.4). Such herding is possibly due to the acoustic lobes created by the hull of the survey vessel, as previously mentioned in Section 3.3. However, *S. hippos* was minimal was minimal, a possible reason for which is the acclimation to the presence of charter fishing vessels, combined with limited hearing at ranges of >50 m due to the aggregation depth.

Herding reactions assume that the fish are always swimming away from the vessel, however, this may not always be the case. Simmonds and MacLennan (2005) described avoidance behaviour as resulting in a lower fish density within the acoustic beam, compared to an undisturbed value. Such movement would explain the elongation of some transects of the *S. hippos* aggregations, but not transects where the backscatter volume from the aggregation was lower. *S. hippos* aggregations demonstrated the tendency to alter movement direction between transects. It is possible that an attraction with a habitat related structure from which the fish maintain a maximum distance could result in apparent variations in movement with differing vessel position and direction. Thus the aggregation may avoid the survey vessel up to a maximum distance from the wreck before stopping. Where the aggregation stops there would be an increase in density, which could be the reason for smaller detected volumes, such as the 10:11

transect (Figure 3.4.4). However, such postulation requires quantification. Simmonds and MacLennan (2005) suggested that movements of fish could be conceived as having two components, random motion (swimming at a particular speed in directions which change randomly with time) and migration (swimming consistently in the same direction). On small scales it is suggested that this could also include a further variable whereby the fish respond to their habitat, such as a wreck around which the fish spawn and are disinclined to leave.

Regardless of reason, the relatively low movement of the aggregation produced significant variation in the abundance estimates. This highlights the need for replication in MBS surveys, to determine the level of movement of a species at the time of survey, and quantify the effect on biomass. It is the author's opinion that the lower numbers of target estimates represent less movement and are more analogous to the number of individuals present.

An alternative explanation for the aggregation movement is a lack of stability as fish prepare to migrate. Evidence from charter and recreational fishermen suggested that during the 2006-7 spawning season the aggregations of *S. hippos* dispersed earlier than previous years thus by early February fewer fish were aggregating above the wrecks than expected. Prevailing current direction in waters around the Rottnest Island region of Western Australia is predominantly determined by the interaction of the Leeuwin current (running north-south), the Leeuwin undercurrent (running south-north) and the wind driven Capes current (running south-north) which vary in magnitude and resultant direction over the spawning season (for further detail see Rennie, 2005). Fertilised *S. hippos* eggs are carried south with the Leeuwin current (Mackie *et al.,* 2009). It has been suggested that the early departure of the *S. hippos* was due to a weak Leeuwin current affecting the southwards dispersal and transport of the eggs and larvae, leaving no reason for the aggregation to remain spawning. As a result this survey occurred towards the end of the spawning season by comparison to previous years when it would have been near the height of the season. Although overall the movement was not a significant distance it highlights the need to accurately quantify aggregation mobility to assess confidence levels in acoustic surveys conducted with single-beam echosounders. Single-beam surveys require assumptions of uniformity of fish distribution, complicated by the duration time of survey, compared to transects of MBS surveys.

Acoustic density packing has been shown to discriminate between different sized species of fish (Section 3.4.3), believed to be due to a size relationship rather than a species specific trend. However, packing density observed during this survey (12.1 m^3 per fish) is in contrast to that previously reported for *S. hippos* of 58 m^3 (Section 3.3). The observed contrast has shown that the same species can form schools of varying packing density. Similarly to the vessel avoidance behaviour this disparity may be due to difference in seasonal time of survey implying additional behavioural monitoring benefits of MBS systems, examining aggregation behaviour and stability throughout the spawning season. The results reported above, however, more closely resemble the often reported nearest neighbour distance of approximately one body length (Misund, 1993, Niwa, 2005), than the previous study (Section 3.3). This could be due to increased preferred nearest neighbour distances in newly formed aggregations such as the October, 2005 RESON 8125 survey, interaction behaviour associated with the presence of a second species as in the previous study (Section 3.4.4) or spatial aliasing in previous survey due to the distance travelled between pings.

A significant limitation of echo-integration from MBS data is the anisotropic nature of acoustic backscatter from fish swimbladders. Distribution of Sv values across the swath illustrated the complications of using a 3-Dimensional model of fish TS with MBS systems. Variation in target backscatter was found to be greater than expected from a typical TS/length relationship across all beams in the swath where detection occurred (including near nadir). Due to the stochastic nature of TS, significant volumes of data are required when determining mean TS of a school using split beam techniques and therefore finer angles of incidence (Korneliussen *et al.*, 2007). This quantity is greatly increased when observing fish at angles acquired in the MBS swath. The advantage of monitoring such a relatively stationary aggregation of known species and typical length distribution is the ease of acquiring significant quantities of backscatter from several beams across the swath to assess the *in situ* effects of acoustic backscatter at varying angles to the MBS system and the fish.

Video data suggested that *S. hippos* were typically orientated near stationary, into the current. It is therefore feasible that video data could be used, not only to ground truth species, but also orientation, providing significant information for the derivation of fish reflectance directivity pattern. In addition, stereo video data could aid in determining nearest neighbour distances and therefore packing densities. Relative packing densities would help in confirming behavioural responses to vessel presence and/or towed body presence.

Despite the below optimal transect incident angles with respect to the wreck data, processing of the RESON 7125 data was able to produce habitat images identifying features previously unrecognised such as the scouring and fines build up around the wreck. Although in this case such features, together with the wreck and surrounding sand, could be discerned with high resolution bathymetry the additional backscatter information highlighted the difference in roughness between what were likely to be fines and sand with fines removed.

The scouring direction observed around the wreck was similar to bathymetry acquired in previous studies, reported earlier in the spawning season (Section 3.3.4, Figure 3.3.1). This scouring direction suggests that at such depths either the current remains in a similar direction throughout the season, or that current variation is only serves to widen scouring. Interaction between the cooler and warmer waters of the Capes and Leeuwin currents at the location of the wreck graveyard suggest the likelihood of a south-westerly current at seafloor (Rennie, 2007). The length of time to create such scouring and its variation throughout the season at this location is undetermined. Variation in the current may create a change in orientation of the *S. hippos* aggregation. Transects at this site therefore suggest that the aggregation prefers to sit up-current of the protruding wreck. Aggregation positions at other sites in the earlier season studies, however, were directly above the wreck (Section 3.3.4, Figure 3.3.4). Further study may reveal a response to changing currents with respect to protruding objects and illustrates the advantage of MBS capabilities to produce high resolution imagery of habitat and water column information, together with the response to environmental changes.

The number of along beam samples observed for each water column target highlights the over sampling which can be obtained in the water column data when producing similar sampling resolutions to those employed in habitat mapping. However, in systems where resolution has been sacrificed to obtain water column data for fisheries acoustics before extending to the seafloor, sampling is often not sufficient to acquire the necessary seafloor resolution. The RESON 7125 has provided high resolution of seafloor and water column acoustic backscatter while retaining sufficient ping intervals required for fisheries surveys, at the expense of storage capacity. Previously, complex post processing has resampled along-beam water column data to reduce data volumes while retaining sufficient resolution (Weber *et al.*, 2007). More recently (and since the undertaking of this survey) MBS systems have addressed such excessive data volumes using internal sonar system processing to provide high quality, clean acoustic data of sufficient resolution and ping rate for both seafloor and water column classification, while maintaining manageable data volumes (Malzone *et al.*, 2008). Both water column and seafloor datasets can now be recorded in a single file with the same accurate (25 ns) time stamp, allowing for more accurate co-location of datasets (Malzone *et al.*, 2008).

3.4.5. Acknowledgements

The authors would like to acknowledge: RESON for the supply of equipment and technical expertise; Fisheries Research and Development Corporation (FRDC) for funding; Western Australia Department of Fisheries for the availability and use of the *RV Naturaliste*; Myriax for Echoview software and technical support with which water column data was processed (in particular Matt Wilson, Bart Buelens and Myounghee Kang); and the Centre for Marine Science and Technology for their expertise in acoustic processing techniques. A PhD top-up was gratefully received from the Western Australian Marine Science Institute (WAMSI).

3.5. Preliminary findings of active acoustics applicability to alternate species.

3.5.1. Glaucosoma hebraicum

Descriptions by fishers indicate that single-beam acoustic techniques may be appropriate for surveys of medium to large *G. hebraicum* aggregations. The large size of the swimbladder in this species makes it likely that individuals could be easily discerned with this method. However, topography of *G. hebraicum* habitat is often complex and the fish is often known to reside near the seabed, potentially making it difficult to discriminate the fish from the seabed. During the current study limited single-beam acoustic data where acoustic targets were confirmed as *G. hebraicum* were acquired. More research of the application of single-beam acoustics to this species is therefore required to determine the full potential in monitoring fish stocks.

The application of single-beam acoustics to study habitats is well documented (Jordan *et al*., 2004, Parsons *et al.,* 2007a, Colquhoun and Heyward, 2008). Although outside the scope of this study, it is feasible to consider the use of single-beam acoustics in the ongoing habitat monitoring of known *G. hebraicum* spawning sites, to research inferred population health based on variation in habitat.

During the February 2007 multi-beam sonar (MBS) survey of a suspected spawning site in Geographe Bay a school numbering in the tens of *G. hebraicum* was observed on towed video. The video GPS stamp confirmed the location of the tight *G. hebraicum* school with five larger *G. hebraicum* separated to the north and a school of baitfish to its southwest. A MBS acoustic transect was conducted five minutes after the video tow (see Figure 3.5.1 for the acoustic transect line and fish locations). Acoustic backscatter suggested two schools of fish, one at each of the locations identified by the video tow. Data from the two acoustically derived groups revealed differences in aggregation features that suggested *G. hebraicum*, sparsely populating an area to the north west of a seabed lump, and a school of baitfish hovering above the seabed lump. Target counting and aggregation volume calculation (see section 3.3.4 for methods) of the *G. hebraicum* revealed 129 acoustic targets encompassed by a volume of 2, 381 m^3 based on a threshold 9 m nearest neighbour linking distance providing an estimate of 18.45 m^3 per acoustic target (mean nearest neighbour distance based on body length was not calculated due to lack of biological sampling and therefore no accurate known mean length). Video data displayed tens of *G. hebraicum*, an order of magnitude less than the number of acoustic targets, reiterating the issue of multiple target detections in MBS surveys of fish schools and aggregations in biomass estimation.

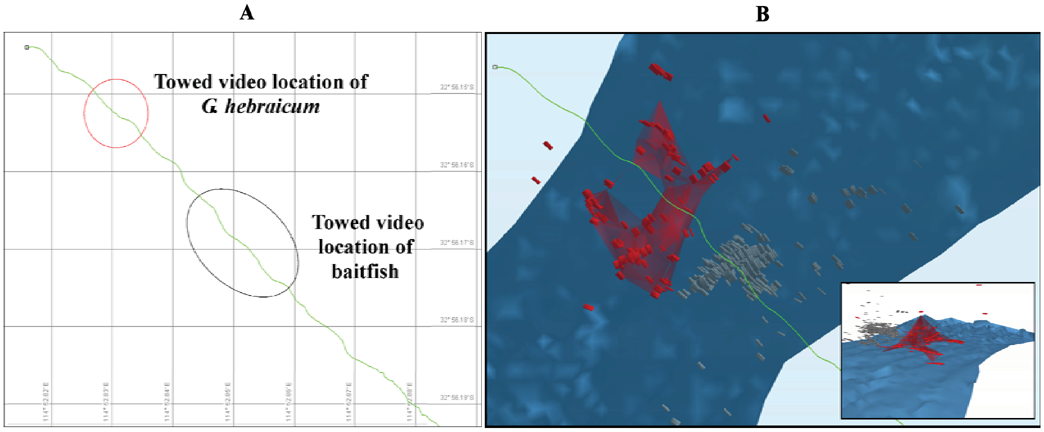

Figure 3.5.1. *Map outlining locations of G. hebraicum and baitfish confirmed by towed video (A). Plan and aerial view (inset) of 3-D visualisation of detected targets speculated to be G. hebraicum (red) and baitfish (grey) (B).*

3.5.2. *Centroberyx gerrardi*

Acoustic surveys of *C. gerrardi* were conducted with the Simrad EQ60 from the *RV Naturaliste* in December, 2006 and February, 2007 at sites close to Cape Naturaliste recommended by local fishermen. Given the weather conditions and vessel capabilities the most compact star pattern transects employable by the *RV Naturaliste* were conducted (Figure 3.5.2C). The acquired echogram (Figure 3.5.2B) displayed a tightly packed school of small fish hovering above a lump as well as scattered *C. gerrardi* around and above the school (as verified by video tows). 3-D visualisation illustrated some of the distances at which individual fish remained from the main group (Figure

3.5.2B). Discriminating the number and species using this method required towed video confirmation. The amount of acoustic data acquired at this site was small with little sampling and there is currently no TS/length relationship for *C. gerrardi,* thus echo-integration methods were not applied. However, once compared with future surveys the data will provide information on the particular school structure of *C. gerrardi*. In the case shown it was possible to count 47 individual targets to obtain an estimate of numbers present, although fish undetected or detected multiple times due to variation in fish distribution between transects has not been accounted for.

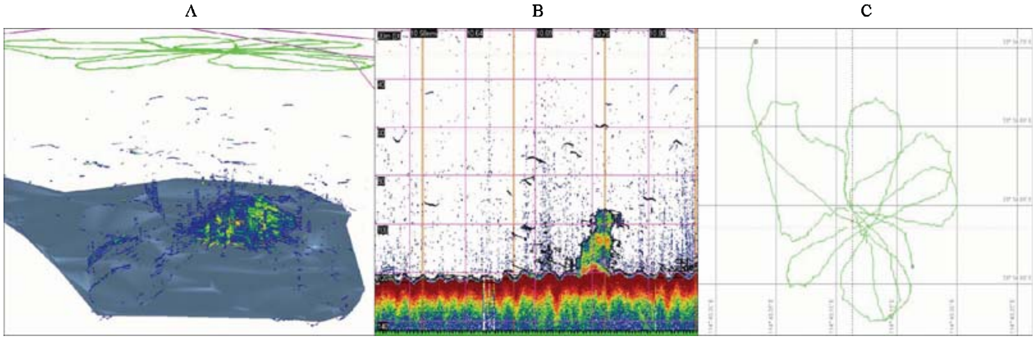

Figure 3.5.2. *3D visualisation of C. gerrardi (A) together with a segment of the associated echogram (B) and cruisetrack (C).*

During the February 2007 survey aboard the *RV Naturaliste* several small multi-species aggregations which included *C. gerrardi* were located and surveyed with the RESON 7125 MBS sonar. This survey highlighted the need to ground truth using video data, since the aggregations were initially thought to predominantly comprise *C. gerrardi* based on line fished biological sampling. By contrast, video evidence displayed few *C. gerrardi* scattered amongst individuals from at least two alternate species. However, this speculative survey was conducted outside the spawning season at a time when there were few *C. gerrardi* on the inshore lumps. Information from fishers suggests that at other times of year, particularly during the species' main spawning season period around April, aggregations would otherwise be dominated by *C. gerrardi*. Acoustic surveys at such time should therefore provide more information on stock abundance of the species.

An example of a RESON 7125 acoustic swath over a speculated *C. gerrardi* aggregation acquired in February, 2007 and the subsequent 3-D visualisation are shown in Figure 3.5.3. The detected targets displayed visible school structure and TS differences from those aggregations of *S. hippos* surveyed with the same system. Target counting and aggregation volume revealed 262 individual acoustic targets in a volume of 10, 739 m^3 based on a threshold 9 m nearest neighbour linking distance. At the centre of the aggregation *C. gerrardi* acoustic targets were more closely linked than those of *S. hippos* and comprised fewer individual samples with each target. Whether such acoustic characteristics are species specific or attributable to the size of aggregation, fish size or environmental conditions have yet to be determined (see Section 3.6).

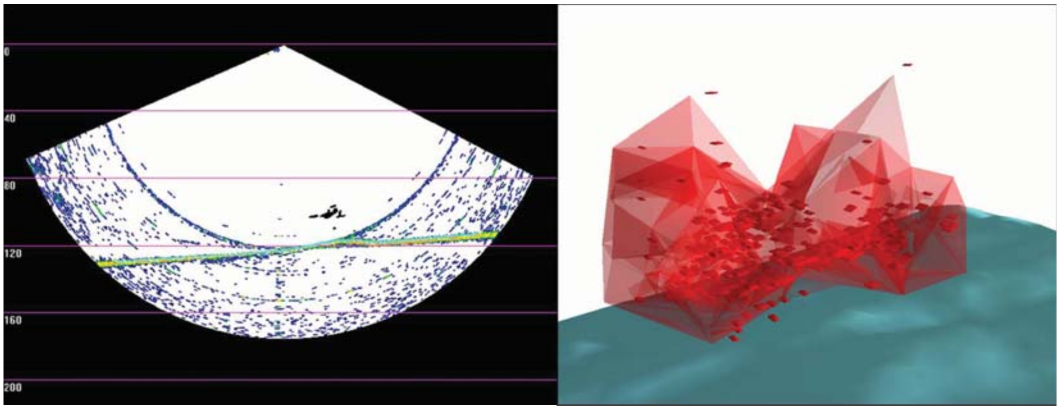

Figure 3.5.3. *Acoustic multi-beam swath of speculated C. gerrardi targets (left) and 3-D visualisation of the aggregation (right).*

3.5.3. *Pagrus auratus*

There are currently no acquired single-beam acoustic data where acoustic targets have been confirmed as *P. auratus* by video techniques or biological sampling.

During the RESON 8125 survey, on the 20th October, 2005, a survey to estimate fish in an aggregation of *P. auratus* was conducted in the Cockburn Sound. A second vessel was employed to locate the aggregation. Unfortunately, although the *P. auratus* were located on several occasions, due to the wary and mobile nature of the aggregation none remained when the *RV Naturaliste* arrived. This may have been simply the mobility of the aggregation or avoidance behaviour in such shallow water. A possible solution to this reaction is the mounting of the MBS system. During the October survey the MBS was mounted with principal beams directed vertically downwards, however, other studies (Gerlotto *et al.*, 1998) have employed principal beams directed to port or starboard sides. Such mounting facilitates the lateral observation of a school at a greater distance, thus reducing vessel avoidance effects (Figure 3.5.4).

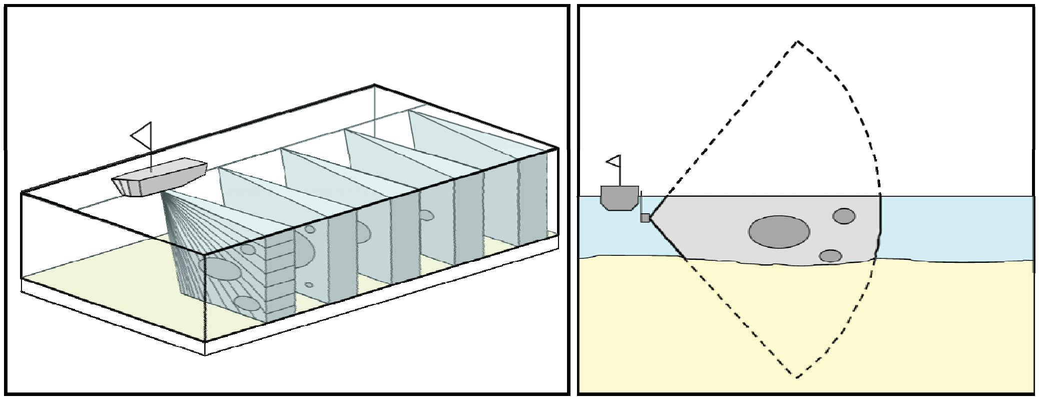

Figure 3.5.4. *Side mounting of a multi-beam sonar in shallow water. Figure adapted from Gerlotto et al. (1998).*

It has been recorded on video and from fishers that *P. auratus* aggregate in large numbers in deeper water and that the aggregations are mobile. Due to mobility and aggregation size it is anticipated that MBS would be an effective method for monitoring *P. auratus* aggregations in deeper water.

3.5.4. *Argyrosomus japonicus*

Single-beam surveys conducted in the Swan River revealed one possible instance of capturing *A. japonicus* on an echogram, though this was only backed by the presence of vocal *A. japonicus* in the area at the same time. Such lack of active acoustic success probably reflects the dispersed nature of the species within this spawning area (see Section 4), difficulties in discriminating fish within the acoustic dead zone (i.e. too close to the riverbed to be detected), and possible vessel avoidance behaviour. Future Mosman Bay surveys will be conducted based on estimated *A. japonicus* locations from localisation using passive acoustic techniques. It has also been reported that this species exhibits group spawning (Ueng *et al*., 2007), though that has not been observed in the Swan River. Group spawning behaviour would be more conducive to single-beam techniques and may provide greater success monitoring aggregations reported by fishers in deeper waters where vessel avoidance behaviour may be less prevalent.

Surveys of *A. japonicus* in Mosman Bay have not yet been conducted using MBS systems. It is anticipated that the high along beam resolution and fine beam pattern in MBS systems such as the RESON 7125 should alleviate issues of surveying sparse *A. japonicus* in shallow water. The fine size of each sample should reduce the size of the acoustic dead zone, and hence allow observation of fish on the riverbed. Swath dimensions would provide coverage of nearly the entire width of the river covering all *A. japonicus* in one pass.

3.6. Conclusions

3.6.1. Single-beam acoustics

3.6.1.1. Equipment feasibility

Single-, dual- and split-beam echosounders are easily mobilised via a variety of sized vessels, simple to run with limited training, and data processing has been standardised by several decades of employment in fisheries surveying. However, the spatial coverage is comparatively small resulting in additional hours conducting numerous transects in order to adequately map aggregations or schools, and gain statistically accurate levels of acoustic TS data. This is accompanied by the associated levels of personnel and vessel time.

3.6.1.2. Biomass estimation

Single-beam techniques are restricted by the relationship between the system sample volume directly beneath the vessel and the volume encompassed by the aggregation. As such, it must be assumed that fish distribution and aggregation movement remain more or less stationary when applying single-beam techniques to estimate biomass. However, surveys *of S. hippos* have shown that even in relatively stationary aggregations it is possible to observe movement of the aggregation, from the comparison of echogram detected 'school' positions between acoustic transects. It is hoped that future work using image analysis techniques of aggregation boundaries will correlate to biomass estimates to account for the fish movement.

While target counting is feasible in areas sparsely populated by fish, the size of the single-beam footprint results in few acoustically identifiable targets in more densely populated regions. When surveying aggregations comprising small numbers of fish it is not possible to determine whether a fish has been subject to multiple detections through the various transects, thus the number of targets may or may not reflect the number of individual fish (Section 3.5.2). Such multiple detections require quantification as it limits the use of MBS systems in abundance estimates.

The inability to identify location within the beam affects the accuracy of individual TS values and echo-integration S_V values in biomass estimation. These target locations can be easily addressed with dual- or split-beam techniques.

The issues of accurate TS-length relationship specific to an aggregation at differing times of the spawning season can be addressed if sufficient, calibrated, *in situ* TS data can be acquired during a single survey of the targeted aggregation. Such data can be related to species and length via stereo video techniques or catch sampling. The stereo video ground truth data adds considerable information on behaviour in relation to fish orientation effects on TS.

3.6.1.3. Habitat classification

In this study single-beam habitat classification was not conducted around the surveyed wrecks. At depths of ~100 m the footprint of the Simrad EQ60 covers an ellipse of major and minor axis diameters of approximately 37.1 and 22.8 m respectively (compared with the multi-beam equivalent of approximately 3.5 and 1.7 m respectively). For such a small survey area as that of the Rottnest wrecks (lengths and widths of <100 and <15 m respectively) it was felt that the EQ60 ensonified too broad an area. However, single-beam techniques are often used in the broad scale classification of habitats and delineation of marine park boundaries (Jordan *et al*., 2004, Parsons *et al.,* 2007b, Colquhoun and Heyward, 2008). A number of descriptors both energetic and morphometric (derived from the acoustic backscatter the bathymetry respectively) can be used to characterise the seafloor habitats using a number of methods. Further details on the Roxanne, QTC and acoustic ground discrimination methods (AGDM) of single-beam seafloor characterisation can be found in Hamilton (2001), Hamilton *et al*. (1999), Foster-Smith *et al*. (1999 and 2004) and Foster-Smith and Sotheran (2003). The ease of deployment of echosounders on research or commercial vessels means that large scale mapping can be conducted during any surveys or fishing cruises. Such mapping of coastal waters can be used to identify essential fish habitat, or monitoring of habitat at known spawning sites and their relationship with spawning productivity.

3.6.2. Multi-beam acoustics

3.6.2.1. Equipment feasibility

Before considering the deployment of MBS acoustic techniques it is necessary to ensure the logistical capability is available to conduct a survey accurately, effectively and efficiently. In many MBS systems the ability to acquire the increased resolution, large sample volume, and accurate data has come at the cost of requiring additional levels of space, skilled personnel, processing capabilities and funding. During the course of this study single-beam surveys were conducted aboard vessels unsuitable for the deployment of MBS systems. Additionally, in many circumstances where fisheries acoustic surveys are required the use of MBS systems is outside the financial budget. Therefore finance, logistics and support are three primary and initial considerations prior to conducting MBS surveys.

3.6.2.2. Spatial coverage

Investigations of *S. hippos* have shown the ability of MBS systems to survey aggregations up to 200 m wide at depths of 60 to 90m in one pass. Such spatial coverage is invaluable to alleviate temporal and spatial assumptions of school uniformity required when employing single-beam techniques, especially whilst surveying mobile school or species which exhibit significant lateral vessel avoidance behaviour. One limitation, however, in the spatial coverage of MBS surveying is the quantification of alongships fish movement which has significant impacts on biomass estimates through target counting, density packing and echo-integration (see below). Such constraints, however, are far from irreconcilable. Speculative surveys conducted in less than 20 m of water in Cockburn Sound to study *P. auratus* illustrated considerations required when surveying a mobile aggregation of fish in shallow water. Variations of system configurations have been suggested and reported previously to observe fish under such conditions at greater distances (Gerlotto *et al*., 1998).

3.6.2.3. Target counting

MBS surveys of *S. hippos, C. gerrardi* and *G. hebraicum* have shown the capability of identifying individual acoustic targets within the acoustic swath. High beam resolution such as that of the RESON 7125 implies that most sized fish could be predominantly detected as individual targets (excluding shadowing effects). Such beam resolution and fine beam pattern can be applied to more sparsely separated fish anticipated to be near the bottom at the time of survey, such as Mosman Bay *A. japonicus* aggregations which spawn near the riverbed. Issues encountered with single-beam techniques of undetected fish due to the acoustic dead zone and relatively small sample volumes could be alleviated with MBS survey. However, alongships resolution in MBS systems primarily designed to acquire seafloor backscatter is often inhibited by the system ping rate when adapted to sample the entire water column. Thus spatial aliasing of detected acoustic targets is created by the unsampled volumes between pings. Such restrictions have been alleviated by recent system developments such as the RESON 7125 (Malzone *et al.*, 2008), although in several other systems there is a trade off between the resolution of acoustic data collected and the storage and processing capacity required to acquire and analyse it.

The RESON Seabat MBS surveys conducted during the course of this study have displayed considerable variation in counted targets of both dense and sparsely populated areas of fish. Even in aggregations comprising large, well separated species of fish which exhibit limited vessel avoidance behaviour at the survey ranges (>50 m) provided results of fourfold variation in target counts likely due to multiple fish detections from aggregation movement. These variations were deemed to be due to avoidance in the direction of vessel travel. The level of vessel avoidance behaviour movement must therefore first be assessed before absolute estimates of abundance can be made via target counting with a high degree of confidence. During acoustic transects several instances occurred where video tow data of *C. gerrardi* and *G. hebraicum* schools and aggregations displayed numbers of fish and order of magnitude less than the number of acoustic targets detected by the MBS (see Sections 3.5.1 and 3.5.2). This disparity between acoustic and video data highlights the impacts of multiple detections of the same fish by successive acoustic pings on abundance estimates. Whether the extent of multiple detections of a single target is school, seasonally or species dependent requires investigation. A possible partial solution to this in future studies is the combined MBS/single-beam survey (see Section 3.7.3).

3.6.2.4. Density packing

RESON 8125 MBS surveys conducted in October 2005 have shown the ability to discern between two species of fish in the same survey using the density packing of acoustic targets. Whether the differing packing characteristics were due to species or fish size remains to be seen. Previous reports suggest that packing density is predominantly related primarily to body length, behaviour (Misund, 1993, Niwa, 2003) and to a smaller extent species (Mogliner *et al.,* 2003).

The comparison of acoustic packing densities of different species of fish from different surveys requires the pings per distance travelled to be equated, in terms of the number of targets detected. Reson 8125 data acquired during October surveys (Section 3.5.4) employed a distance travelled of 5.4 to 7.2 m per ping, compared with that of 2.3 to 2.9 m in the RESON 7125 survey. The number of targets in the 8125 study was increased by the difference in ping per distance between the two surveys (2.53 times). This increase compensated for the coverage disparity, reduced the number of targets potentially missed between pings in the 8125 survey and allowed comparison of the two survey results. The adjusted acoustic target densities of the two surveys are shown in Figure 3.6.1. Adjusted acoustic target density for dense areas of *P. dentex* from the 8125 survey produced an acoustic packing density of 1.27 m^3 per target (95% confidence limits of 0.89 and 1.64) with least squares regression correlation of $R^2 = 0.865$. By comparison the sparse area of *S. hippos* produced 23.8 m^3 (c.l. = 18.81, 28.78) with $R^2 = 0.909$ and 13.89 m^3 (c.l. = 9.79, 18.1) with $R^2 = 0.965$ for the 8125 October and 7125 February surveys respectively. These acoustic target densities equated to approximately 3 (*P. dentex*), 2 (*S. hippos,* 8125 survey) and 1.6 (*S. hippos*, 7125 survey) body lengths as nearest neighbour distances. Although this adjustment ratio is notably based on a small sample set the point is that the two acoustic densities of the same species (*S. hippos*) are significantly different, illustrating the variation caused by behaviour.

Acoustic packing densities of small aggregations such as those of *G. hebraicum* and *C. gerrardi* are at a stage where the convergence of linear regression based models based from other species were similar. Figure 3.5.1 displays all the acoustic packing density relationships obtained in this study. The two investigations of *S. hippos* aggregations providing contrasting acoustic packing results at different times of years illustrates the limitations of density packing techniques when a single species can exhibit such differing behaviours. Once a species has been identified, however, this technique offers significant information on absolute biomass present if a behaviourally related species target packing density can be developed.

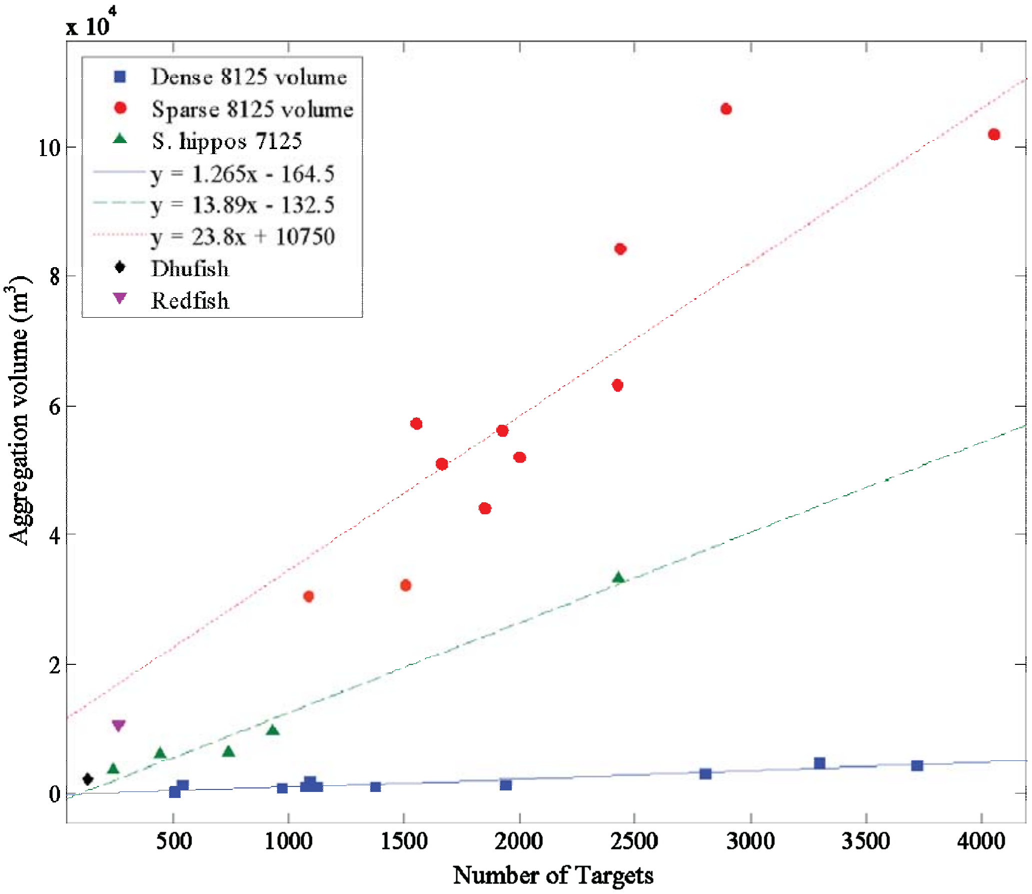

Figure 3.6.1. *Detected acoustic target to aggregation volume relationships for a dense volume of P. dentex (■), sparse S. hippos (●) (as detected by the RESON 8125 – not all points are shown) and sparse S. hippos (▲) as detected by the RESON 7125). Calculated single transect values for G. hebraicum (♦) and C. gerrardi (▼) are also shown.*

3.6.2.5. Echo-integration

The densely populated regions of speculated *P. dentex* in the October, 2005 RESON 8125 survey highlighted the possibilities of MBS biomass estimation from echo-integration. However, the considerable variation in S_V values found both across the swath and within individual beams illustrated the complexity of MBS echo-integration to estimate fish numbers. Several studies model the three-dimensional TS of fish species, such as Kirchhoff-ray mode (KRM) backscatter models (Clay and Horne, 1994), using determined swimbladder dimensions from swimbladder casts (McClatchie *et al*., 1996a, 1996b, Coombs and Barr, 2003) or magnetic resonance (MR) scans (Pena, 2007). These models illustrate the variation of reflectance with angle of incidence. As a result, without ground truthing fish orientation the increased volumes of data required to provide *in situ* confirmation of three-dimensional variations in TS are substantially greater than those required for single-beam echo-integration. Similarly with single-beam echosounders it is likely that more detailed multi-frequency techniques will offer a more comprehensive approach to discriminating between species and identifying orientation surveyed by MBS systems.

3.6.2.6. Habitat classification

Water column and seafloor acoustic backscatter acquired using RESON 7125 Seabat have shown the ability of MBS systems to monitor fish aggregations and their associated habitat. However, not all MBS are capable of data acquisition at such high qualities. Data considerations for the simultaneous collection of seafloor and water column acoustic backscatter to sufficient fisheries and habitat classification levels include: 1) along beam resolution sufficient for seafloor backscatter acquisition; 2) ping rate sufficient for fish and habitat classification; and 3) manageable data volumes requiring minimum storage and processing capacity. Several systems offer one or more of these requirements; however, to date few systems are capable of all three whilst maintaining highly accurate, clean data.

3.6.3. Combined techniques

The ability of MBS to image volumes of mobile schools combined with single-beam to obtain acoustic density for quantification purposes and where possible species identification is an invaluable combination for fisheries acoustics (Weber *et al.*, 2007). This combination is becoming increasingly widespread (Malzone *et al.*, 2008). Where targets are significantly separated in space and echo-integration of MBS data is less viable single- or split-beam acoustics can provide confirmation of same target multiple detections from the MBS target counting data. The elimination of multiple detections will mitigate the effects of alongships vessel avoidance behaviour and increase confidence in abundance estimates. Packing density and target counting through active acoustic and video data often show a level of disparity. Both techniques influence behaviour of the fish and thus although comparison is useful for estimating error and identifying species composition it is not feasible to use one method to ground truth abundance estimates of the other.

The future of replicable fisheries active acoustics lies in the accuracy and calibration of multi-frequency, MBS systems where the same fish aggregation (or individual), given the same behavioural state and orientation displays the same backscatter (or target) strength, regardless of depth, location or instrument used (Malzone *et al.*, 2008).

CHAPTER 4

4. Passive acoustic techniques for monitoring fish aggregations

4.1. Overview

The philosophy of the passive acoustic section of this study is a series of successive papers which aim to describe the steps taken in analysis of a soniferous aggregation of spawning fish from identifying the calls through to estimating biomass. Following an overview of each of these steps Section 4.1 also introduces the pertinent acoustic characteristics of the study site for the primary study species, *Argyrosomus japonicus*, and the mutual methods in data acquisition and processing utilised in each of the following sections. Further methodological detail will be outlined where appropriate.

In Section 4.2 the characteristics of *A. japonicus* calls are examined. Calls are classified into categories relating to differing acoustical characteristics and behavioural functions associated with spawning. The vocal repertoire amongst a species which spawns in dark, turbid waters, and exhibits no outward sexual dimorphism, forms important communication cues to optimise spawning success. It is therefore necessary to understand the function behind each call and the regularity with which it is used in order to base further inferences from the sound production.

Environmental variables have often been estimated to affect the timing and level of fish spawning behaviour (McKeown, 1984, Sturlaugsson and Thorisson, 1997, Farmer, 2008). Long term variations in sound production together with supplementary physical environmental data offer significant information on variables which affect spawning behaviour of a soniferous species. Section 4.3 assesses extended datasets of acoustic data retrieved from Mosman Bay across four spawning seasons, with respect to behaviourally influential variables such as temperature, light levels and salinity.

The observation of individual spawning behaviour gives a good indication of what cues drive spawning success and the interaction that goes on between fish at that time.

Localisation of individuals within an aggregation of spawning *A. japonicus* using a passive acoustic hydrophone array is the subject of Section 4.4. This section describes a method to accurately locate soniferous fish using the arrival time differences of calls to each hydrophone within the array and has been published in ICES Journal of Marine Science (Parsons *et al.*, 2009).

Determination of fish call source levels and their constituent contribution to overall sound pressure levels (SPLs) is an important step towards the estimation of absolute abundance via passive acoustic techniques (Sprague and Luczkovich, 2004). Hydrophone array localisation results given in Section 4.4 provide acoustic data on calls of known range. The investigation of SPL range relationships is presented in Section 4.5 with established call source levels for each category of *A. japonicus* call.

An ultimate goal of passive acoustic monitoring of fish is the calculation of absolute biomass estimates without survey effects biasing acquired data. The aim of Section 4.6 is to develop a robust method with which to estimate numbers of fish within a hydrophone detection range from a combination of call counting and call contributions to overall SPLs.

During the course of this study numerous biological, physical and environmental variables were observed to alter the perceived acoustic characteristics of fish calls as well as the characteristics of the biological mechanism producing the sound. Section 4.7 aims to address this and assess how the environment may influence the perceived acoustic features of fish sounds and the resulting conclusions drawn from them. The author suggests a set of standard protocols for the reporting of fish sounds to facilitate comparison between calls of differing spatial and/or temporal origin.

Results of the passive acoustic study of *Seriola hippos, Glaucosoma hebraicum, Pagrus auratus* and *Centroberyx gerrardi*, the four other targeted species, are presented in Section 4.8.

Conclusions drawn from this work on the efficacy of passive acoustic techniuqes can be found in Section 4.9.

4.1.1. Mosman Bay acoustic characteristics

In situ surveys were conducted in the waters of Mosman Bay, Swan River, Western Australia, 7 km upstream from the river mouth. Centred at a location of 32° 00.72' S, 115° 46.56' E the study site encompassed the width of the river running roughly southeast to northwest between Chidley Point and the Freshwater Bay yacht club (Figure 4.1.1). Steep banks on the western side of the river drop into an approximately uniform silt substrate riverbed 16 to 20 m deep (low tide) with sporadic, mild depressions to 22 m before rising steadily to an inter-tidal spit on the east. Shelter from low cliffs to the south and the western banks of river results in typically calm water through most of the regions spring and summer winds (typically ranging from westerly around to south-easterly). Significant wave motion is observed only in strong (>15kn) easterly winds and/or generated by passing vessels (author, *pers. obs.*).

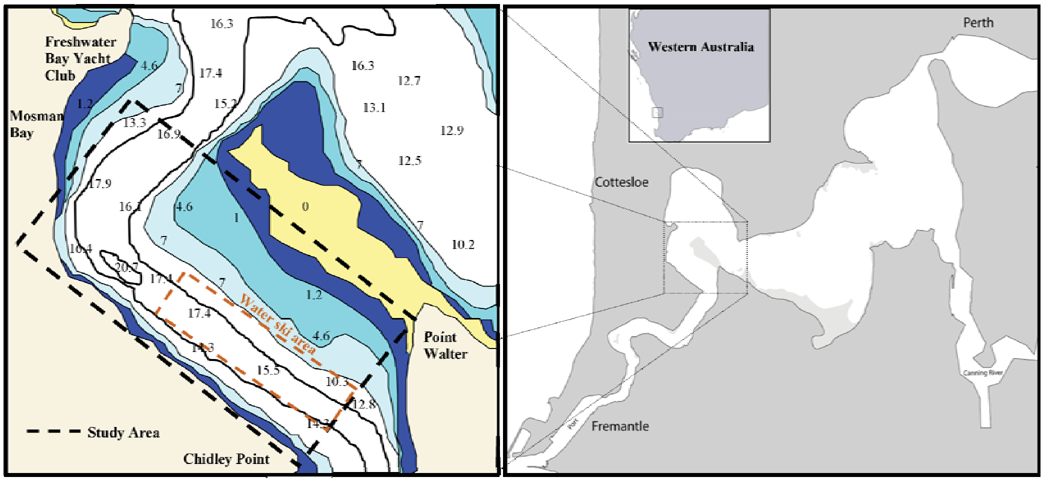

Figure 4.1.1. *Map of Western Australia, Swan River and Mosman Bay (inset) highlighting the passive acoustic study area of* *<u>A. japonicus</u>* *surveys. Depths at various locations are shown in metres.*

The study area is subject to significant though sporadic anthropogenic noise, both aquatic and terrestrial. Ferries run at regular intervals throughout the day between Perth and Fremantle, and motorised vessels from several upstream yacht clubs have to pass through Mosman Bay to exit and re-enter the river. These vessels generate consistent levels of noise, typically varying in character as they pass. The study area also includes a water ski area which, during the summer months is used by wake boarders and water-skiers in the hours before sunset. An area within this section of the river contains a number of moorings for local recreational vessels. In periods of high wave motion the underwater mooring chains produce significant noise levels as the moorings rise and fall. During the *A. japonicus* spawning period these moorings are often frequented by recreational fishermen until the early hours of the morning (author *pers. obs.*). Sporadic evening noise has also been observed from recreational scuba divers most likely fishing for prawns after sunset, during likely hours of spawning (author, *pers. obs.*). Biological noise in the river is most prevalent in the form of snapping shrimp (Parsons *et al.,* 2006a). Shrimp 'snaps' occur over frequencies ranging from 500 to >2 500 Hz and while outside most of the frequency analysis for pertinent fish calls do impinge on the analysis of waveforms (see Section 4.2). The Swan River is host to a number of resident dolphins during the summer months (Finn, *et al. in prep.*) which are often heard underwater before and after sunset (author *pers. obs*.) Terrestrial based noise has been observed from previous studies on spectrograms (see Section 4.3) most likely due to automobiles and trains (author, *pers. obs.*).

4.1.2. Data acquisition

Hydrophone deployments to acquire underwater noise were conducted using differing configurations and a variety of combinations of associated acoustic recording equipment. Surveys employed HTI-90U and/or -96min omni-directional hydrophones (Hi-Tech Industries Inc., MS, USA; specifications found in Table 4.1.1) located either in mid-water (HTI-90U or -96min) or on the riverbed/seafloor (HTI-90U only).

Mid-water river based recordings were conducted from moored or drifting vessels with hydrophones lowered to one of 4, 5 or 10 m depth with an attached fishing 'sinker' to ensure negative buoyancy. One or more of the following measures were taken to reduce low frequency noise induced by current, vessel movement or surge; cables were enclosed in netting to lessen vortex shedding from current flow; weighted lines from the riverbed to a sub-surface buoy were attached to hydrophone cables to limit vertical movement due to surge; where accurate hydrophone GPS locations were required the sub-surface buoy and aerial were periodically checked to ensure hydrophone position directly below GPS aerial (Figure 4.1.1); and sufficient distance was maintained from moorings to limit the recording of noise from mooring chains. Mid-water hydrophones were attached to Sony TCD D8 or D100 Digital Audio Tape (DAT) recorders at maximum gain settings, sampling at 32 kHz. The mid-water HTI-90U hydrophones were attached to CMST designed pre-amplifiers at maximum gain settings of 40 dB re 1µPa. HTI-90min hydrophones possess an in-built pre-amplifier.

Riverbed, bottomed recordings were acquired by attaching hydrophones to sea-noise loggers developed by the CMST and Defence Science and Technology Organisation (DSTO) in steel housings (Section 4.1.3.1). During short term deployments (single evening) housings were connected to a surface buoy and retrieved by pulling up all equipment (Figure 4.1.1C). Over long-term deployments (up to 4 months) additional dump weights where added to the housing. A 5 m chain, followed by a 30 m rope connected the housing to a secondary dump weight, to which was attached a Sonardyne 7986 Lightweight Release Transponder with release canister containing a length of rope (nominally 3 times water depth) and small sub-surface buoy. Equipment retrieval was conducted by acoustic message sent to the transponder which released the sub-surface buoy and attached rope to the surface, from which all equipment was retrieved. Unfortunately, heavy fouling caused the release to commonly fail and equipment was recovered by diving or grappling.

Passive acoustic surveys were conducted in open-water along the Western Australian coastline to record possible *S. hippos, G, hebraicum and C. gerrardi* vocalisations. Housings were deployed from the *RV Naturaliste* for periods between 2 and 24 hours configured as one of: drifting mid-water, attached to a GPS beacon; seafloor, positioned on a Baited Remote Underwater Video (BRUV) frame (Mackie *et al.*, 2009); or moored mid-water (Figure 4.1.1E, F or G respectively).

Specifications of hydrophones, noise loggers and Sony DAT recorders can be found in Table 4.1.1. The resulting calibration coefficients for each equipment combination together with the respective survey can be found in Table 4.1.2. Calibration coefficients were calculated from:

$$C = |hyds| - gain \tag{4.1}$$

where *hyds* = hydrophone sensitivity (dB re V/μPa) and *gain* = total system recording gain in dB (McCauley, 2001). As V and Pa are root mean squared values the sensitivity is actually dB re 1 $V^2/\mu Pa^2$. Once the system response was added to correctly scaled FFT values the spectral level was given in dB re 1 $\mu Pa^2/Hz$ (McCauley, 2001).

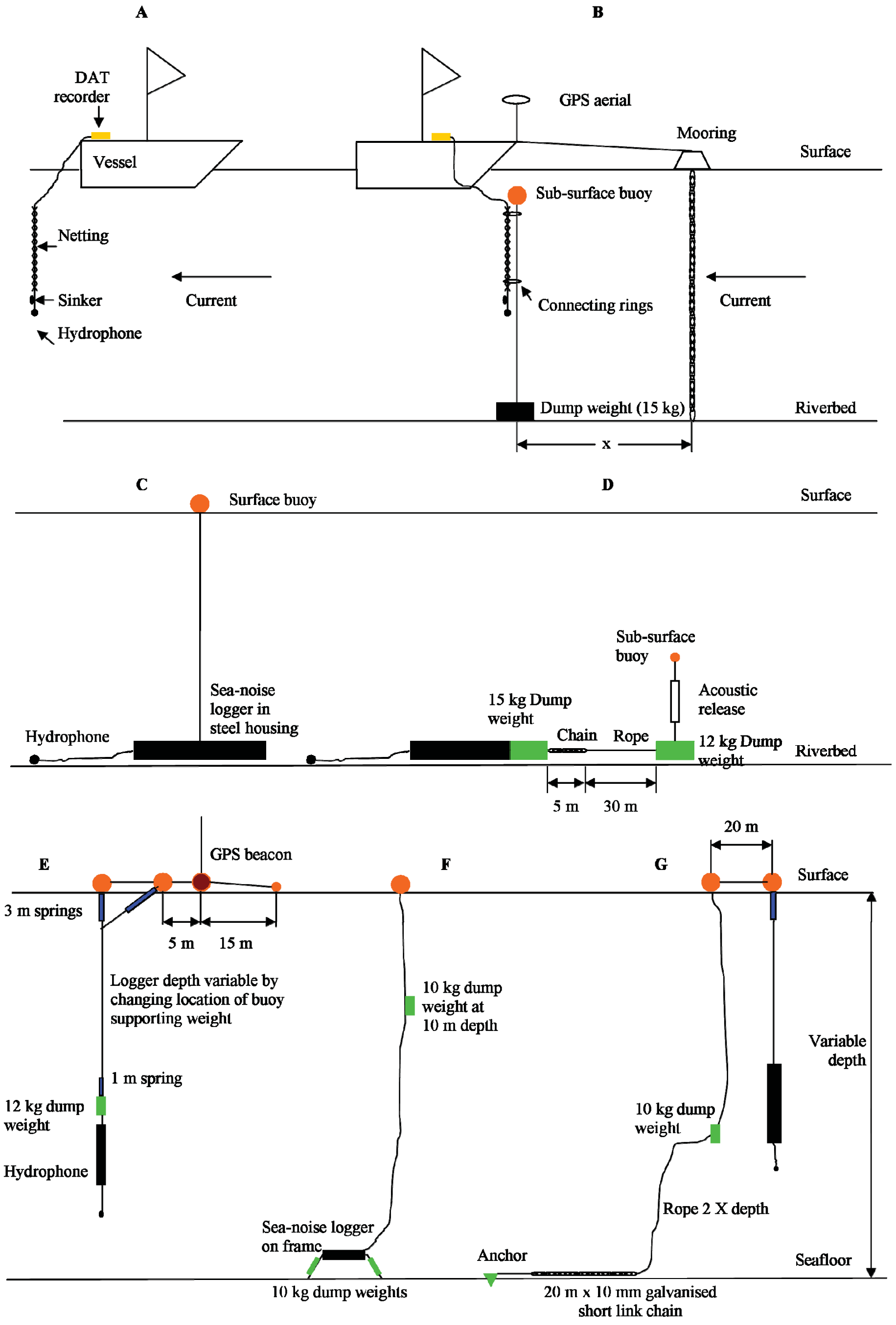

Figure 4.1.2. *Hydrophone deployment configurations from drifting vessel (A), moored vessel (B), short-term bottomed sea-noise logger (C), long-term bottomed sea-noise logger (D), drifting mid-water (E), frame attached (F) and mooring mid-water (G).*

Table 4.1.1. *Specifications and calibration coefficients for various combinations of hydrophones, noise loggers and Sony DAT recorders deployed in Mosman Bay.*

Item	Model/parts	Characteristics
Hydrophones	HTI-90U (3)	Cable length 10 m; sensitivity -197.7, -198.1, -198.1, dB re 1 V/µPa respectively; hydrophone capacitance 14.09, 14.31, 14.10 nF
	HTI-96min (2)	Cable length 10 m; sensitivity -164.1 dB re 1 V/µPa; pre- amplifier required
	GEC Marconi S11101X (1)	Cable length 45 m; sensitivity -203.5 dB re 1 V/µPa; hydrophone capacitance 9.4 nF
DAT Recorders	Sony D100 (2)	Digital Audio Tape deck, 16-bit; frequency response quoted as 20-14,500 Hz (± 1.0 dB) at Fs 32 kHz; dynamic range quoted as >87 dB
	Sony D8 (1)	Digital Audio Tape deck, 16-bit; frequency response quoted as 20-14,500 Hz (± 1.0 dB) at Fs 32 kHz; dynamic range quoted as >87 dB
Sea-noise loggers	Sir Gawain, Sir Tristan, Sir Galahad	Hydrophone pre-amplifier, 20 dB gain with 0-20 dB pre filter gain, giving maximum 40 dB gain, 16-bit; low frequency cut-off 8 Hz, high frequency cut-off ranging 1-15 kHz, survey dependent
Pre-amplifiers	CMST design	20 or 40 dB gain; 4 Hz ->20 kHz; impedance 1 MΩ
Noise generator	White noise	-70, -90, -110 dB re 1 V^2/Hz output

4.1.2.1. Sea noise loggers

The CMST acoustic Sea Noise Recorders are autonomous recording units designed for medium to long term deployment. During the term of this study the sea noise loggers were deployed in 30 kg stainless steel housing units of 114 mm outer diameter and 900 mm length powered by two 9 V battery packs (one to power the hydrophones pre amplifier and one to power the recorder). The loggers were configured to interface with one hydrophone (though two are possible) via a Subconn LBH3F connector. An integral hydrophone pre-amplifier (20 dB gain with user selectable lower frequency cutoff filter at 8 Hz or 160 Hz) and an integral anti-aliasing filter (0-20 dB pre filter gain with a 6th order Butterworth filter and high frequency cut off from 1 kHz to 15 kHz) provide a maximum 40 dB gain. The system employs 16 bit Analogue to Digital Conversion with

RMS noise of A/D at 3 quantisation levels (total recorded noise level depends on hydrophone capacitance, amplifier gain, cut-off frequency). Data was stored on one 2.5” hard disk drive using FAT32 format and standard IEEE file structure, buffered by a Type 1 Compact Flash Card. Recording intervals were set to bear in mind the time taken between recordings to download files from the flash card to hard disk drives. A RS232 interface allowed user configuration of sampling rate (up to 26 kHz), bandwidth, gain, sampling durations, record intervals) with support for multiple user configured sampling schedules. At maximum sampling rate the system draws 75 mA, in comparison with sleeping and dozing rates of 20 uA and 8 mA respectively.

4.1.3. Processing

System frequency response in each deployment configuration was confirmed with a white noise source of either -70 or -90 dB re 1 V^2/Hz (Table 4.1.1). Digital files acquired with sea noise loggers were read into Matlab® using programs written by the CMST. Acoustic data from DAT recorders were transferred from tapes to digital files by means of a 486 PC based FFT signal analysis package with a DP430 signal processing card (Data Physics Corporation) at 1 or more sample frequencies (rates) from 2 604 (38.4 ms), 5 208 (19.2 ms), 10 416 (9.6 ms) and 20 833 Hz (4.8 ms). The waveforms were stored on hard disk drive at the CMST, Curtin University.

Data were processed using Matlab® programs developed by the CMST, and passed through high (20 Hz) and low (2000 Hz) pass filters to limit noise effects such as hydrophone movement and shrimp clicks. Analyses of data were then conducted from spectrograms (produced with a 0.7 FFT overlap and typically a 1024 point ‘Hanning’ window) and waveform plots, produced in Matlab.

For analysis the start of each call (and each pulse) was taken as the first detected voltage amplitude peak in the waveform deemed associated with an individual mulloway vocalisation. This peak was referred to as the Call Initiation Peak (CIP). The end of a call was noted as the point at which the final pulse had decayed below background noise. The following characteristics of each call were recorded: call duration, pulse period, number of pulses in a call, modulation frequency and call carrier frequency or spectral peak frequency (frequency peak of the power spectrum of an entire call). Time between calls possibly originating from the same source, and variations in amplitude between different calls were also noted. Specific functions used in call analysis are described below where pertinent.

Passive acoustic units are specified as follows: dB re 1 μPa for broadband levels; dB re 1 $\mu Pa^2/Hz$ for spectral levels; dB re 1 $\mu Pa^2.s$ for sound exposure level (at specified frequency bandwidths, bins and bandwidths respectively). Statistics for results which are perceived on the logarithmic scale have been applied in the same scale for continuity, rather than after transformation into the linear domain.

4.2. Characterisation of mulloway (*Argyrosomus japonicus*) spawning sounds.

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ABSTRACT

Annual spawning aggregations of mulloway (Argyrosomus japonicus) are found in Mosman Bay, Swan River, Western Australia during the months of the southern hemisphere summer. As a soniferous member of the Sciaenidae family A. japonicus produce various vocalisations during spawning. In situ recorded calls and vocal behaviour of Swan River A. japonicus have been observed and compared with that of control callers habituated in aquaria for periods of several years. Calls are produced via repetitive contractions of paired muscles along the posterior two thirds of the swimbladder in the form of forced excitation of a heavily damped resonating body. Resulting A. japonicus calls from this region exhibit call carrier frequencies varying between 175 and 350 Hz and pulse repetition frequency of approximately 59 Hz. Acoustic features of call signals have been characterised by spectral and detected amplitude variation parameters and analysed for discrimination between callers. It is suggested that A. japonicus spawning related vocalisations can be generalised into three categories; a short grunt of 1-6 pulses ('Bup') employed in the formation of the aggregation; long grunts comprising 11-32 pulses ('Baarp') as a call of attraction between spawning males and females; and a short call of 1-5 swimbladder pulses ('Thup') possibly made when a male and female interact directly. The second category is classified further into several types of call where a single audible tone can also be broken into two or more parts, often preceded by one or more short 'Bups' (for example, 'Ba-bup-baarp').

***Keywords**: soniferous, passive acoustics, vocalisation, spawning*

4.2.1. Introduction

Passive listening to fish calls can greatly improve a biologist's ability to delimit spawning areas for conservation of essential fish habitat (Luczkovich *et al.*, 1999a, 2000). Despite considerable literature on Sciaenidae sound production, we know little about the biological significance of their sounds. Tavolga (1971) stated that it is no longer sufficient to present another instance of a fish making sounds and such information needs to be correlated with the behavioural context of the sound, which is still warranted because our understanding of the importance of acoustic signalling to the ecology of fish has not improved (Ramcharitar *et al.*, 2006b). For example, Ueng *et al.*, (2007) observed evidence of pair and group spawning together with vocal behaviour in aquaculture ponds by *A. japonicus,* though no apparent call function distinguished either type of behaviour.

In most Sciaenidae species the swimbladder is surrounded by bilateral muscles often called 'sonic' muscles (Tavolga, 1971). These sonic muscles determine the magnitude of the force exerted on the swimbladder. Fish have four predominant muscle fibre types, defined by their mechanical and metabolic properties; red 'slow-twitch'; white 'fast twitch'; pink (an intermediary of red and white); and 'superfast-twitch' muscle (Rome, 2005). Sciaenidae sonic muscles are dark red in colour due to high vascularisation, though they cannot be classified as typical red muscle (Ono and Poss, 1982). The muscle fibres are typically orientated perpendicularly to the long axis of the swimbladder and are uniformly thick (around 30 µm thick, Ramcharitar *et al.*, 2006). The fibres become highly convoluted at endplates (Ono and Press, 1982), giving rapid contractile properties (Rome *et al.*, 1993, Rome, 2005). Due to CA^{2+} transfer rates, muscles contract faster than they relax, thus maximum repetition frequency of muscle contraction is determined by the relaxation rate of the muscle (Rome, 2005). In addition to calcium transfer limitations Sciaenidae swimbladders lack antagonistic muscles, thus lateral body musculature elasticity and internal swimbladder pressure return muscles to their original shape (Rome, 2005), contributing to the highly damped waveforms seen in many sciaenid calls, similar to those found by Lagadere and Mariani (2006).

Classically, the production of sound via a swimbladder has been treated as a resonating gas bubble where acoustic features, such as spectral peak frequency (often called the 'dominant', 'carrier' or 'peak' frequency) are dictated by swimbladder volume, wall stiffness, applied damping and pressure differential between internal swimbladder gas and that of the water surrounding it (Hall, 1981). More recently this sound production model has been adapted to include the magnitude of an excitation force on the swimbladder (Sprague, 2000). The relationship is such that for a fish at the same water depth a larger sized swimbladder would be expected to emit sounds of lower spectral peak frequency (McCauley, 2001).

Given time to adapt to variations in depth fish may work to keep their swimbladders at a constant volume through secretion/reabsorpion of gas and maintain a similar pressure inside and outside the swimbladder (Sand & Hawkins, 1972, Sundnes & Sand, 1975). Constant swimbladder volume may also be maintained during short-term vertical movements, as shown by the diel migrations of the Chinese sturgeon (*Acipenser sinensis;* Watanabe *et al*., 2008). However, during fast vertical migration, such as a spawning rush, the rate of gas exchange may not be rapid enough to keep up with the pressure change in water and the swimbladder volume would vary with water depth at a simlar rate to Boyle's Law (Alexander, 1959). This effect would be exacerbated if the migration occurred in shallow waters where a depth change of a few metres would experience considerable change in relative pressure when compared with the equivalent distance at greater depths. Although swimbladder resonance may be heavily damped in shallow waters, possibly due to surrounding tissues (Alexander, 1966, McCartney & Stubbs, 1771, Lewis & Rogers, 1996), the resulting changes in volume and pressure differential between swimbladder gas and external water, due to rapid depth change, will affect the spectral content of a fish call.

Within a species, sonic muscles increase with size and consequently so do the maximum sound pressure levels (SPLs) (Connaughton *et al*., 2000). An increase in muscle size increases the amount of time taken for a single sonic muscle contraction and increases the force exerted on the swimbladder, which affects the acoustic characteristics of the call (Sprague, 2000). Thus for similar sized fish, at the same depth in the water column, those with larger sonic muscles will exhibit the lower frequency call as the larger muscles take longer to return to their original shape in each twitch (Sprague, 2000, Connaughton *et al.,* 2002b, Rome, 2005). For prolonged calls produced by consecutive sonic muscle contractions, the spectral composition differs in that calls contain a carrier frequency, but also several local spectral frequency peaks of uniform spacing, related to the time between consecutive sonic muscle contractions. Known as the modulation frequency (and also 'fundamental' or 'pulse repetition' frequency), the spectral peak spacing is dependent on the repetition rate of swimbladder pulses, (Watkins, 1967, Nilsson, 2004).

Many fish sounds contain species-specific spectral peak frequencies, waveforms, pulse rates and signal repetition (Lobel and Macchi, 1995, Mann and Lobel, 1998), allowing the identification of a sound by simple parameters (Mann, 2002). Thus from a conspecific call, a female and male fish can determine physical information about a caller (McCauley 2001). In species such as bicolour damselfish (*Pomacentrus partitus*), for example, females discriminate between males based on call frequencies and court with larger males (Myrberg and Spires, 1972, Myrberg *et al*., 1993). Size may reflect genetic quality and fertilization potential, since a large male has more sperm (Nilsson, 2004). However, in broadcast spawning, with urogenital openings opposite each other, males much larger than spawning females often sire a smaller proportion of the larvae than expected, because size difference is not conducive to the courtship behaviour (Nilsson, 2004).

Aggregations of *A. japonicus* are known to form in Mosman Bay, Swan River, Western Australia, during summer months (Section 4.1). The location of spawning grounds is nominally inferred either from catches of mature fish or from the distribution of eggs (Hawkins, 2002). Although previous extensive sampling of the Mosman Bay region of the Swan River did not reveal *A. japonicus* eggs and larvae (Gaughan *et al*., 1990), individuals at stage V and VI reproductive maturity (mature and spawning stages respectively; Blackburn and Gartner, 1954) were caught between October and May (peaking in December) during a recent study (Farmer, 2008). Samples included females with ovaries containing hydrated oocytes, confirming spawning activity within Mosman Bay (B. Farmer, Murdoch University*, pers. comm*.). Mosman Bay catch data for the 2004-5 and 2005-6 seasons suggested a mean fish length of 101 cm (Farmer, 2008).

Several seasons of vocalisations have been recorded in Mosman Bay during periods in which spawning is known to have occurred since 1997. No other species, expected to make such vocalisations, have been reported in the river. The aims of this study are to describe *in situ* vocalisations recorded in Mosman Bay, examine the possible sound generation mechanism of *A. japonicus,* investigate the possibility of call discrimination and associate those calls with a particular behaviour during the spawning cycle.

4.2.2. *A. japonicus* sound production related anatomy base on dissection

Griffiths and Heemstra (1995) described the biology of *A. japonicus*, including the following details related to the species sound production. *A. japonicus* possess a uniform, carrot shaped swimbladder spanning the length of the abdominal cavity. Lipid deposits ran the length of the swimbladder, on either side, approximately half way up the height of the swimbladder. Between 21 and 31 aborescent appendages were attached to the deposits bi-laterally along the swimbladder. The limbs of these appendages were orientated in a dorsoposterior (up and backwards) and lateroanterior (forward and to the side) direction. However, in the appendages of the posterior half the lateroanterior directed limbs became ventroanteriorly (down and forwards) orientated with branches lying down and forwards, against the swimbladder. The presence of bi-lateral red blocks of sonic muscles around the swimbladder was noted.

Sonic muscles have been reported in both sexes of *A. japonicus* (Griffiths and Heemstra, 1995), and Ueng *et al.* (2007) recorded vocalisations from males and females in aquaculture ponds. On dissection, Ueng *et al*. (2007) noted that sonic muscles in *A. japonicus* females are thinner, lighter and shorter than those of the male.

Dissection of a purchased, 836 mm total length, *A. japonicus* at the Centre for Marine Science and Technology (CMST) revealed bi-lateral, dark red, sonic muscles comprising dorsoventral fibres, lining the posterior two thirds of the swimbladder. These muscles are shown in Figure 4.2.1 (dotted lines), comparable with those reported in *A. regius* (Lagadere and Mariani, 2006). The front of the muscle block in Figure 4.2.1 was located 5 cm from the posterior of the 33 cm body cavity and extended forward 19 cm finishing 9 cm from the body cavity anterior. The block was positioned at the same cavity height as the lipid deposits surrounding the swimbladder. White muscle fibres (orientated in an anterior-posterior direction, Figure 4.2.1 mark i) surrounded the body cavity aponeurotic lining (Figure 4.2.1, marks ii and iv). The sonic muscle fibres (Figure 4.2.1A, mark iii) appear to have developed under the body cavity lining around Figure 4.2.1A mark v, splitting the lining in two. Similar to other Sciaenidae (Ona and Poss, 1982), it is thought that the sonic muscle fibres extended ventrally, such that the sonic muscles were partially bounded by the body cavity lining (Figure 4.2.1A inset, and B). Figure 4.2.1B inset shows the orientation of the sonic muscle fibres in relation to the body cavity. These sonic muscles were not observed in any previously dissected specimens, ranging up to 54 cm total length.

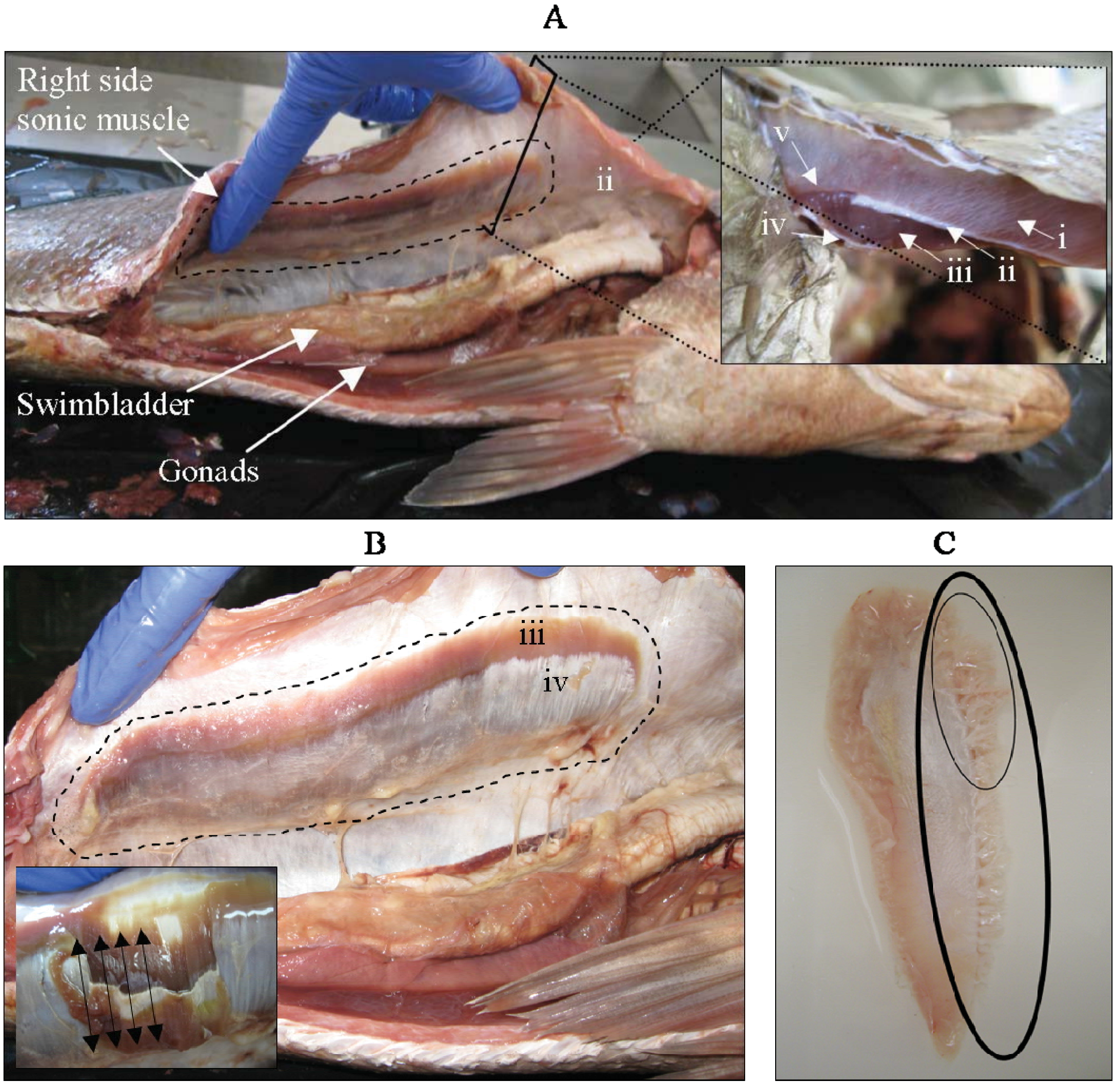

Figure 4.2.1. *Photos of dissected 86 cm A. japonicus. (A) Body cavity, highlighting positions of swimbladder, gonads and right side sonic muscles. Inset shows a cross section of the sonic muscle area with: white body muscle fibres (i); aponeurotic lining (ii); dark red sonic muscle (iii); aponeurotic lining outside sonic muscle (iv). (B) Expansion of sonic muscle area with inset showing sonic muscle fibre direction. (C) Separate swimbladder plan view image with all aborescent appendages on one side (thick black line) and enlarged anterior appendages (thin black line) highlighted.*

The 32 cm long and 6.2 cm wide (recorded from a flattened swimbladder, due to perforation on dissection) swimbladder shown in Figure 4.2.1C highlighted the enlargement of the anterior appendages (Figure 4.2.1C thin black line). The swimbladder material was of 1.71, 2.54 and 2.32 mm thickness for the dorsal, middle and ventral of the anterior section, thinning to 1.09, 1.59 and 1.37 mm for the same dimensions at the posterior. Internally, an enclosed, 0.39 mm thick membrane was connected to the inside wall of the swimbladder from posterior to approximately 5 cm from the anterior, where it separated from the wall. The swimbladder was connected to the vertebral column at the anterior, the posterior was loosely attached at the anus, and two lines of fine tendons loosely attached the top of the swimbladder to the aponeurotic lining at the top of the body cavity.

The lining was cut at the spinal column and peeled back to approximately the centreline of the sonic muscle block, shown by the dotted line in Figure 4.2.2A. This revealed what were speculated to be nerves running from the spinal column, between ribs to the sonic muscle block and along the centreline of the muscle block, perpendicular to the direction of the sonic muscle fibres (Figure 4.2.2B).

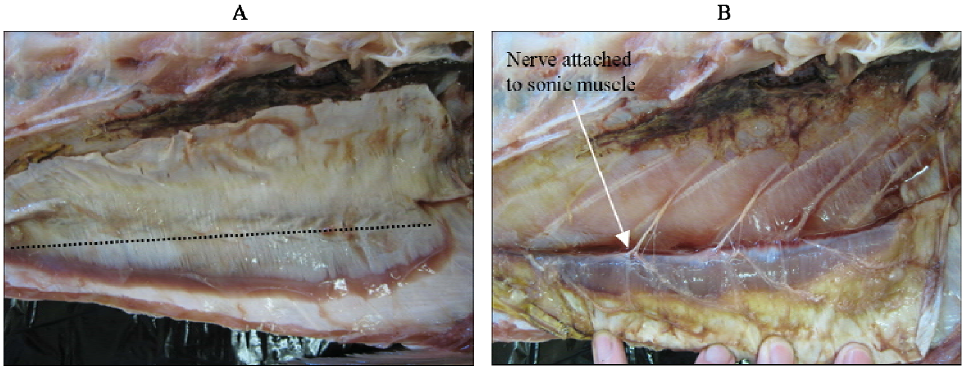

Figure 4.2.2. *Photos of left hand side of an 86 cm A. japonicus with swimbladder and internal organs removed (spinal column at top of images, cavity lining has been cut away at the spinal column). (A) Aponeurotic lining and sonic muscles in place, dotted line represents the centreline of the sonic muscle. (B) Musculature after aponeurotic lining has been peeled back at dotted line in A to reveal nerve endings running along the centreline of the muscle block, perpendicular to the direction of the sonic muscle fibres.*

4.2.3. Acoustic Data acquisition and processing

Passive acoustic recordings were acquired from Mosman Bay, Swan River over several evenings between November and March during the 2006-7 and 2007-8 spawning seasons ranging from 17:00 (prior sunset) to 01:00 (Western Australian Standard time, WST). Water temperature during recordings ranged between 18 and 26° C. Recordings were conducted either by suspending an omni-directional HTI (-90U or -96min) hydrophone from a moored or drifting 4 m vessel, or from an HTI-90U hydrophone attached to a Centre for Marine Science and Technology (CMST) developed sea-noise logger (Figure 4.1.2A, B, C and D) set on the riverbed. General data acquisition and processing techniques are detailed in Sections 4.1.3 and 4.1.4. The HTI 90U hydrophone was connected to a CMST pre-amplifier, while the 96min possesses an inbuilt pre-amplifier.

Ground truth recordings of mulloway in aquaria where conducted at various times of day at the Technical and Further Education (TAFE) college aquaculture facilities in Fremantle, Western Australia. Recordings were acquired by positioning the HTI 96min hydrophone in a tank with a pair of isolated fish and logging signals onto a DAT recorder. A covered, concrete, broodstock tank of approximately 40, 000 L (maintained at 18 to 22°C) holds one male and one female *A. japonicus*, each in excess of 25 kg and >1 m length (exact measurements not taken to avoid causing distress). The fish have been habituated in aquaria for over four years and are well acclimated as the only pair in the tank, thus calls of distress were not expected to occur. As broodstock, both fish are considered larger than expected in the wild.

The captive TAFE male and female fish were each injected with approximately 1250 International Units of Human Chorionic Gonadotropin (HCG) on the 16th October 2007 to induce spawning. On the morning of Thursday 18th aquaculture researchers heard the first signs of spawning as the fish began calling. The following analysis of ground truth data is of calls recorded between 10:30 and 19:25 of Thursday 18th October.

4.2.4. Results

4.2.4.1. Vocalisations in the Swan River

During several evening choruses, at times of maximum call rates, discrimination of signals was viable in spectrograms and waveforms. Figure 4.2.3 shows the spectrogram (A) and waveforms (B, C and D) for 17 seconds of *A. japonicus* calling. A number of variations were determined in *A. japonicus* calls. Calls exhibited spectral peak frequencies between approximately 175 and 350 Hz with sidebands of amplitude modulation at regular intervals (56.21 Hz, s.d. = 9.87) resulting from repetitive swimbladder pulses. The recorded signals were divided into three predominant categories, defined by the acoustic features and apparent associated behaviour. Each call type comprised trains of swimbladder pulses of varying characteristics, some of which are shown in Figure 4.2.3C and Table 4.2.1. Table 4.2.1 shows the type of calls and acoustic characteristics recorded for the first minute of every half hour between 16:30 and 23:31 on the 5th March, 2007. Due to interference, overlapping calls or signal-to-noise, not all characteristics of all audible calls could be discerned.

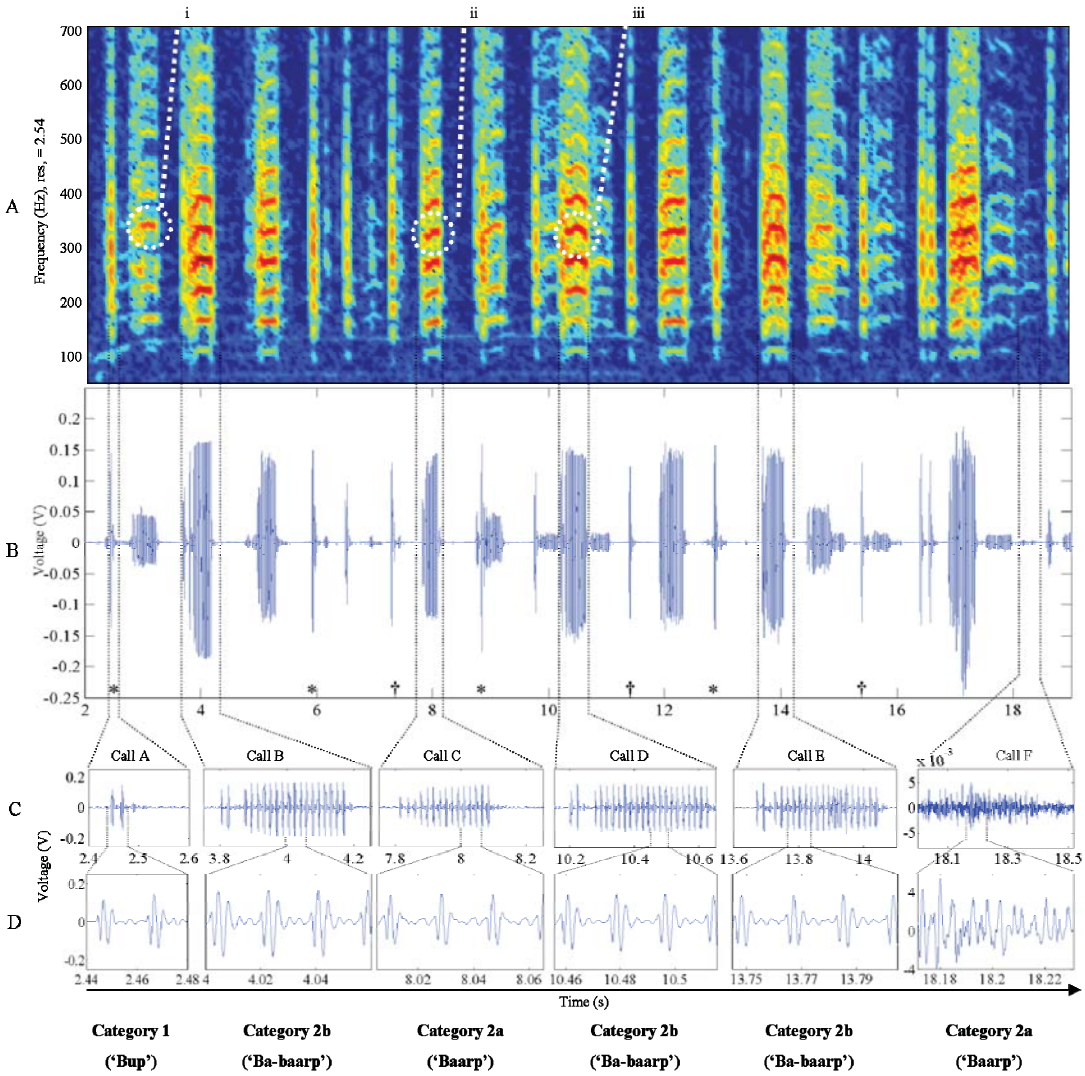

Figure 4.2.3. *Spectrogram (A) and waveforms (B) from 17 seconds of Mosman Bay A. japonicus calling, recorded at 4 m depth in 19 m of flat water at 19:35, 17th January, 2007. Spectrogram frequency bandwidth and waveform sampling frequency were 2.54 Hz and 10, 416 Hz, respectively. Expansions of six selected call waveforms highlighting the entire calls (C) and sets of swimbladder pulses (D) are shown. Call F highlights an audible call of low signal-to-noise where waveform structure is distorted by noise. * and † denote examples of suspected repetitive Category 1 calls from individual fish. Marks i, ii, and iii denote calls of interest.*

Table 4.2.1. *Example acoustic characteristics of A. japonicus calls on the 5th March 2008 for the first minute of each hour between 17:30 and 23:31. Sunset occurred at 19:43.*

Time	Call Type	Number calls (no. analysed)	Call duration (s) x10 (max, min, s.d.)	Pulse number (max, min, s.d.)	Modulation frequency (Hz) (max, min, s.d.)	Median spectral peak frequencies (Hz)
17:30	1	12 (0)	0	0	0	0
	2a	2 (0)	0	0	0	0
18:00	1	15 (0)	0	0	0	0
	2a	3 (0)	0	0	0	0
19:00	1	27 (11)	0.757 (1.58,0.274, 0.414)	3 (5, 2, 1.16)	43.97 (72.84, 25.28, 13.73)	275.4
	2a	27 (5)	3.31 (4.33,2.09, 0.56)	0	0	0
	2b	5 (2)	4.40 (5.15, 3.65, 1.06)	0	0	0
19:30*	1	23 (11)	0.42 (0.52, 0.34, 0.055)	2 (2, 2, 0)	48.86 (58.85, 38.36, 6.29)	250.5
	2a	51 (24)	2.72 (3.20, 1.73, 0.39)	16.65 (20, 11, 2.32)	61.30 (63.82, 56.15, 2.35)	250.5
	2b	5 (2)	2.87 (3.15, 2.58, 0.40)	16.00 (17, 15, 1)	56.04 (58.06, 54.01, 2.86)	243.1
	3	1 series (31)	0.22 (0.044, 0.087, 0.12)	2.11 (4,1,1.09)	91.3 (114.2, 74.3, 10.34) (22 measured)	259.6
20:00	1	21 (21)	0.51 (0.83, 0.31, 0.14)	2.71 (5, 2, 0.90)	53.25 (65.51, 34.66, 9.9)	228.9
	2a	75 (23)	4.01 (4.63, 2.43, 0.58)	24.35 (29, 15, 3.71)	60.69 (62.62, 54.38, 1.91)	306.5
	2b	2 (0)	0	0	0	0
	2c	1 (1)	4.54	26	57.27	315.7
20:30	1	53 (23)	0.38 (0.46, 0.29, 0.06)	2.5 (3, 2, 0.53)	64.83 (78.51, 50.44, 9.32)	263.7
	2a	87 (17)	4.14 (5.17, 3.37, 0.62)	25.44 (32, 20, 3.75)	61.42 (63.57, 59.33, 1.48)	250.5
	2b	15 (7)	4.49 (5.27, 3.90, 0.70)	25.3 (30, 22, 4.16)	56.32 (56.92, 55.73, 0.60)	251.8
	2c	8 (1)	4.13	24	53.15	285.6
	2d	2 (1)	3.47	21	47.98	276.5
21:00	1	13 (4)	0.370 (0.42, 0.29, 0.064)	2 (2, 2, 0)	55.46 (70.14, 47.78, 10.55)	285.3
	2a	97 (27)	3.83 (0.5, 2.42, 0.08)	22 (29, 14, 6.78)	58.69 (62.67, 56.27, 2.77)	250.9
	2b	22 (1)	0.406	22	54.21	248.0
	2c	4 (1)	0.415	26	62.62	254.3
21:30	1	14 (6)	0.44 (0.76, 0.26, 0.27)	2.67 (4, 2, 1.15)	64 (77.7, 52.47, 12.77)	283.6
	2a	87 (33)	4.15 (5.11, 3.52, 0.60)	23.5 (29, 20, 4.04)	57.73 (59.58, 52.47, 1.50)	300.1
	2b	9 (4)	3.96 (4.42,3.62,0.42)	19.66 (21, 19, 1.15)	50.11 (54.84, 52.52, 6.31)	241.6
	2c	4 (2)	4.59 (4.76, 4.42, 0.24)	23 (27, 19, 5.66)	49.82 (56.7, 42.9, 9.72)	276.58
22:00	1	39 (13)	0.39 (0.54, 0.31,0.068)	2.1 (3, 2, 0.32)	54.22 (63.51, 45.78, 5.92)	261.5
	2a	73 (17)	4.20 (4.50, 3.26, 0.99)	20 (26, 16, 5.29)	51.26 (52.69, 49.04, 1.95)	297.6
	2b	8 (5)	5.24 (6.97, 4.23, 1.50)	24.33 (31, 20, 5.86)	46.80 (48.63, 44.49, 2.11)	295.3
	2c	3 (3)	3.87 (4.66, 3.17, 0.48)	22.5 (27, 18, 2.71)	58.24 (60.03, 56.33, 1.14)	189.5
	2d	2 (0)	0	0	0	
22:30†	1	99 (31)	0.58 (1.18, 0.36, 0.19)	3.2 (6,2, 0.87)	56.12 (75.30, 42.24, 6.55)	246.7
	2a	77 (24)	3.96 (5.09, 3.17, 0.65)	23.83 (30, 19, 3.82)	60.22 (61.58, 58.95, 0.94)	189.5
	2b	15 (7)	4.01 (4.83, 3.08, 0.58)	20.44 (25, 16, 2.79)	51.23 (57.40, 45.53, 3.46)	245.4
	2c	4 (4)	3.86 (4.40, 3.42, 0.48)	19.75 (24, 16, 3.5)	51.05 (58.33, 46.76, 5.25)	275.3
23:00	1	177 (23)	0.577 (1.41, 0.15, 0.31)	2.75 (5, 1, 1.13)	51.02 (79.66, 31.42, 13.41)	279.6
	2a	8 (0)	0	0	0	
23:30	1	16 (7)	1.00 (1.08, 0.96, 0.056)	4 (4, 4, 0)	38.98 (41.79, 36.84, 2.17)	204.7

Table 4.2.1. continued

Totals	1	509 (140)	0.56 (1.58, 0.26, 0.25)	2.79 (6, 2, 0.92)	52.58 (79.66, 36.24, 10.92)	250.5
	2a	498 (170)	3.66 (5.17, 1.73,0.76)	21.6 (32, 11, 4.49)	59.99 (63.83, 48.63, 2.62)	250.1
	2b	81 (28)	3.94 (5.27, 2.68, 0.68)	20.62 (30, 15, 3.69)	52.40 (58.06, 42.95, 3.87)	245.4
	2c	24 (12)	4.15 (4.54, 3.50, 0.46)	22.75 (26, 18, 3.95)	54.75 (62.62, 47.71, 6.55)	275.3
	2d	4 (1)	3.47	21	47.98	261.8

Between * and † an unquantified number of calls were not counted, due to overlap between calls and masking of distant calls by close calls. During this period only calls of high signal-to-noise ratio such that the background calls can be considered to contribute little relative energy to the call spectral content, have been reported.

The majority of calls recorded were classified into two categories, dependent on the number of pulses employed in the call. Category 1 calls, ‘Bup’ (Figure 4.2.3C, Call A), comprised 2.79 (s.d. = 0.92) pulses at an average spectral peak frequency of 52.58 Hz (Table 4.2.1). These signals were classed as an individual call if no further associated call, deemed to be from the same individual, followed within 1 second.

Category 2 calls were significantly longer than Category 1, and comprised a greater number of pulses, ranging between 11 and 32 (Figure 4.2.3C, Calls B, C, D and E, and Figure 4.2.4). Acoustic characteristics of this category can be found in Table 4.2.1. This category of calls comprised successive swimbladder pulses at sufficient repetition frequency to create a long audible tone. The tone could, however, be broken into constituent parts by a short cessation of pulses within the train, (Figure 4.2.4). The gap in the acoustic tone, created by the cessation of pulses, most commonly occurred after the initial two swimbladder pulses and lasted between one and three pulse periods (Figure 4.2.4B). However, the position of this gap was found to vary in different calls. The Category 2 calls were therefore classified into five different types, as shown in Figure 4.2.4 (although it was thought that the classification of calls could continue *ad nausea*). Category 2a was a single audible tone, unbroken by pulse cessation (‘Baarp’) (Figure 4.2.3 C, Call C and Figure 4.2.A). If the tone was preceded by one or more of the two pulse ‘Bups’ it was classed as Category 2b (‘Ba-baarp’ or ‘Ba-bup-baarp’) (Figure 4.2.3C Calls B, D and E, and Figure 4.2.4B and C). Category 2c calls contained a break later in the call (Figure 4.2.4D) (‘Baa-aarp’). Finally, Category 2d calls contained a number of different parts characterised by two of more points of cessation within the call ('Bup-baa-baarp') (Figure 4.2.4E).

In general, recorded mean peak to peak amplitudes of the first cycle in the waveforms of Category 2 calls were greater than those of Category 1 calls (0.078 V ± 0.035 s.d., and 0.052 V ± 0.028 s.d., respectively, using the same recording/analysis system). This observation did not account for caller position and therefore signal propagation to the hydrophone, although a random distribution of Category 1 and Category 2 caller ranges was assumed. In comparison, it was observed that in many cases the first one, and often two pulses of the long calls were of lower detected amplitude than the successive pulses (Figure 4.2.4C).

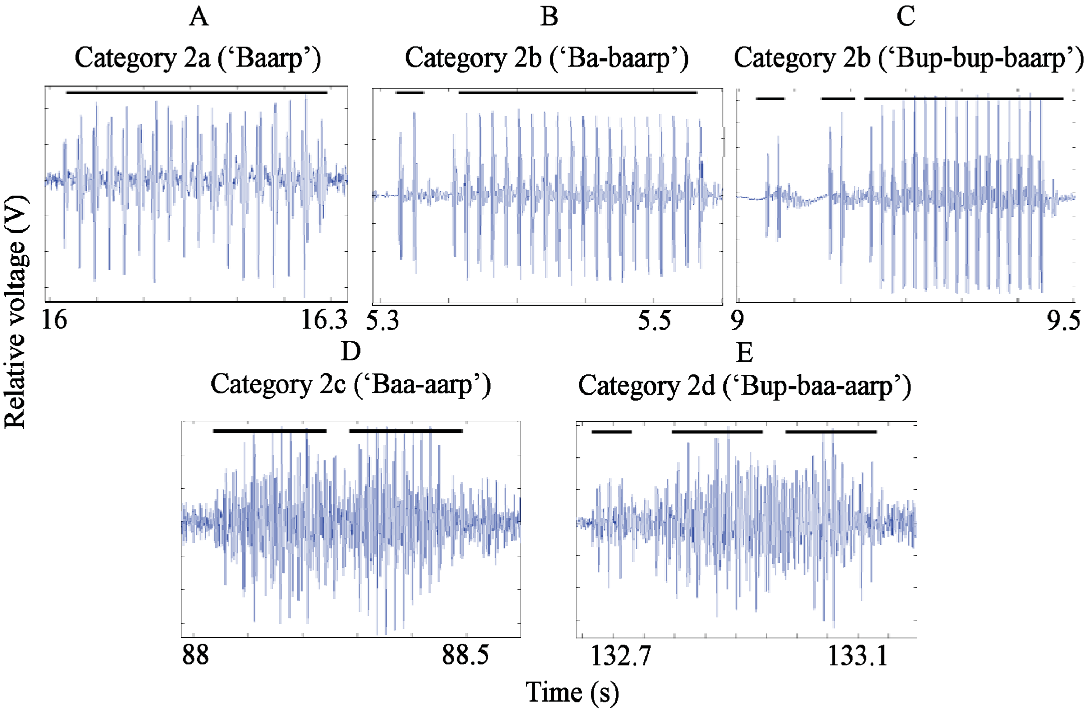

Figure 4.2.4. *Waveforms of various detected Category 2 calls at times and location similar to that of Figure 4.2.3. Black lines shown above each waveform provide an impression of the audible periods of tone structure for each call type.*

An evening spawning cycle typically began with few Category 1 calls from a small number of individuals at distances greater than 500 m (Table 4.2.1, author *pers. obs.*). As callers approached the hydrophone, calls became of sufficient signal-to-noise ratio to analyse acoustic characteristics. By comparing call amplitude, waveform shape and spectral peak frequency it was possible to discriminate between callers and note individual repetitive calling (Figure 4.2.3, marks * and †). At times of low caller density this discrimination allowed a mean estimate of repetitive calling rates of 3.72 s between Category 2 calls, though this did not account for periods of cessation of calling by individuals. Category 1 calls were recorded up to four hours prior to sunset on some evenings, but more typically began approximately 2 hours before sunset. As time passed the number of Category 1 calls increased, along with the number of callers (Table 4.2.1). Where repetitive calling was detected, early evening Category 1 calls displayed rates of 3.6 s (s.d. = 0.85, n = 174) between calls.

The number of Category 2 calls increased as sunset approached, with types 2a, 2b, 2c and 2d in order of occurrence (Table 4.2.1). The peak in call numbers occurred approximately an hour after sunset and during this period predominantly Category 2 calls were observed. Category 1 calls were detected in greatly reduced numbers during the peak of Category 2 calling; however, whether they were not emitted or were masked by the greater number and intensity of Category 2 calls could not be confirmed. After peak calling, Category 2 calls became less frequent. At this point the Category 1 calls were heard again, in greater numbers than prior to peak calling (Table 4.2.1). Category 1 calling intervals at this time were between approximately 1.8 and 3.1 s (call rates became slower as the late evening progressed). Typically several hours after sunset the Category 2 calls had all but disappeared leaving a few callers emitting quiet Category 1 calls, typically between the hours of 22:00 and 00:00, until all calls ceased.

During the hour prior and post sunset, periods of calls were recorded which could not be classed as Category 1 or 2 calls (Figure 4.2.5) and so were deemed of a third category. This category of calls was less frequent than the others, observed only once or twice in an evening. Figure 4.2.5 illustrates a typical series of Category 3 calls, as recorded by a bottomed hydrophone in 18.5 m of flat water. These Category 3 calls each comprised 1-5 pulses at elevated pulse repetition frequency in comparison with Category 2 and 3 calls (Table 4.2.1 and Figure 4.2.5C and D). However, in Category 3 calls comprising three or more pulses the pulse repetition frequency declined with each pulse (Figure 4.2.5D). In one instance, a slow moving, individual fish emitted Category 3 calls as it approached the hydrophone. Caller range was calculated using surface reflection techniques (Cato, 1998; described in detail in Section 4.4). During the nearest call the time difference between the direct path and the surface reflected path was such that for the fish to be positioned in the water column (and not beneath the riverbed) the caller must have been located within 1.6 m of the hydrophone (Figure, 4.2.5, 40.33 s).

In this call series the calls initially began as single and occasional double pulse calls separated by seconds (Figure 4.2.5). As the fish passed the hydrophone and moved further away, the calls increased in rate and became predominantly double, tripl or quadruple pulse calls. Concurrent Category 3 calls of different amplitude were observed during the later stages of this period. Analysis of call surface reflections from these concurrent calls suggested a similar range and thus, likely a second caller, of differing size (though without localisation it cannot be confirmed if they were in the same location). The call rate increased to a maximum with several multiple pulse calls a second (around 55 s on Figure 4.2.5). A Category 2 call of similar amplitude was observed at 58.9 s, before the rate of Category 3 calls declined sharply. During the call series other Category 2 calls were audible, but were of considerably lower intensity. Series of these Category 3 calls, such as the example in Figure 4.2.5 were observed only once or twice an evening throughout the spawning season.

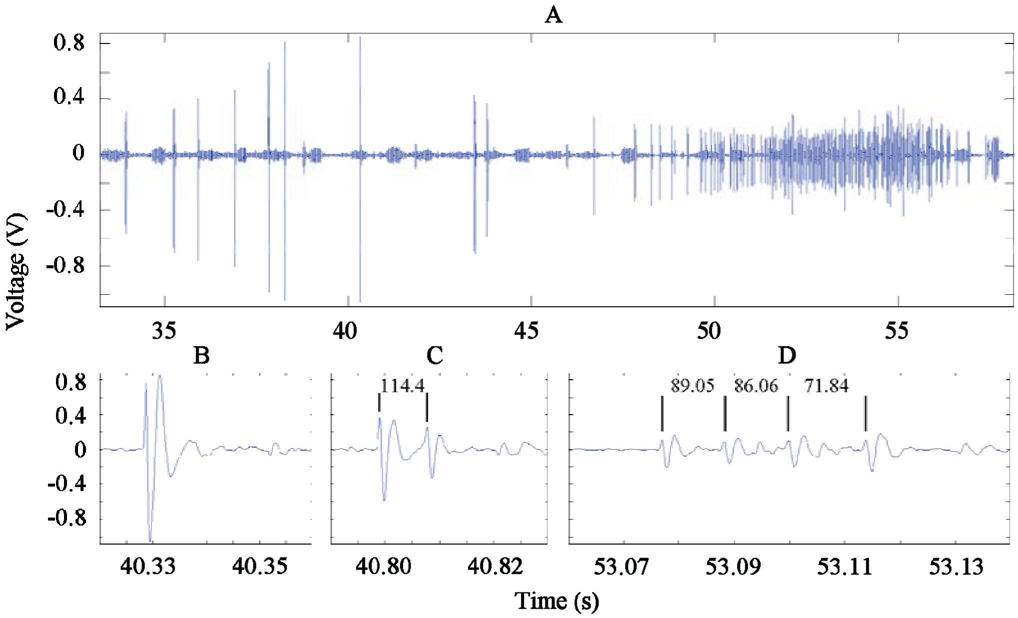

Figure 4.2.5. *Waveforms of a series of Category 3 calls (A) recorded by a bottomed hydrophone in 18.5 m of flat water on the 5th March, 2008 at 19:57, post sunset. Expansions of single (B), double (C) and quadruple (D) pulse calls within this category are also shown with pulse repetition frequencies highlighted above (Hz). The repetition frequency of the multiple pulses in D decreased through the call (i.e. the spacing between pulses increased).*

Where calls displayed sufficient signal to noise ratio, the waveforms of pulses within individual and separate calls could be compared. In many individual calls successive pulse waveforms exhibited distinct similarities, as can be seen in Figure 4.2.3D, suggesting the vocalising fish was relatively stationary for the duration of the call. Other calls displayed pulse waveforms differing significantly throughout the call, likely due to variations in the ray multi-paths received. Such received waveform variations imply movement of the fish during the call. Although aural examination distinguished many signals of low voltage amplitude (due likely to distance from the hydrophone) as *A. japonicus* calls, multi-paths and signal-noise ratio restricted discrimination as the calls displayed indistinguishable waveform patterns. For example, the waveforms shown in Figure 4.2.3C and D, Call F could be audibly discerned as a Category 2a long call, however, the signal-to-noise was too low to display an accurate waveform.

For some calls, the peak spectral density and the pulse repetition frequencies of different calls displayed distinct similarities suggesting the same fish was responsible for the calls (Figure 4.2.6, Calls B, D and E). Conversely, calls of differing frequency content suggested a different fish (Figure 4.2.6, Call C). For example, Call C displayed spectral peak and pulse repetition frequencies of 264.3 and 52.1 Hz respectively, while Calls B, D and E were 279.5 and 55.6 Hz. Repetitive calling was often indicated by similar or identical waveforms and voltage amplitudes of separate calls. Similarities in such acoustic features of calls B, D and E suggested a single fish, at a similar range, as the source (Figure 4.2.3A and D) when compared to other calls (Figure 4.2.3D, Call C).

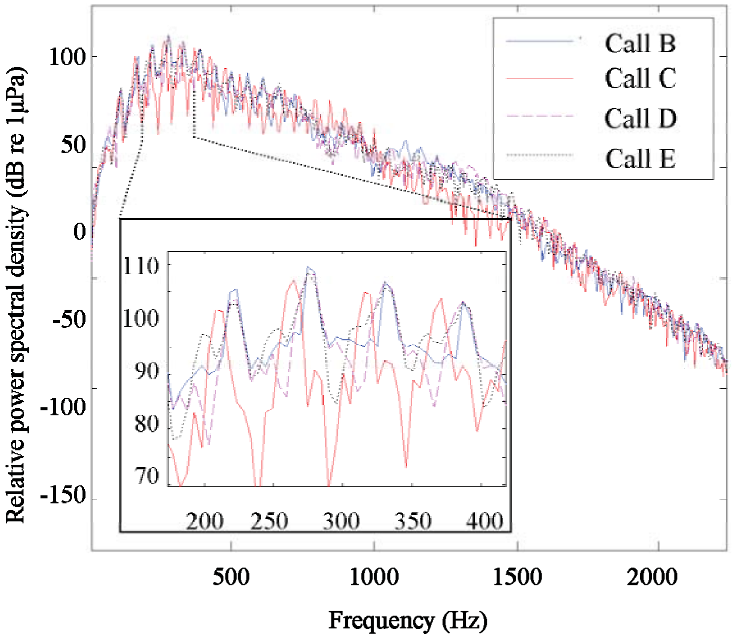

Figure 4.2.6. *Power Spectral Density of four A. japonicus Category 2 long calls (Calls B, C, D, E from Figure 4.2.2) with peak frequencies magnified.*

Calls thought to originate from a single fish often varied in number of pulses and therefore duration. Calls B, D and E, in Figure 4.2.3C, for example, thought to originate from one individual, comprised 19, 22 and 19 pulses lasting 380, 433 and 385 ms, respectively. Therefore the number of pulses and the resulting call duration could not be used to discriminate between callers.

Linear relationships for pulse number and call duration were determined for *in situ* Category 1 and 2 calls (shown in Figure 4.2.7). The lower R^2 value this relationship in Category 1 calls (0.59) in comparison with the Category 2 calls (0.81, 0.81 and 0.78 for Categories 2a, 2b and 2c respectively) was due to the small number of pulses of which the call category spans (2-6 pulses, compared to 11-32 pulses). The offset in duration visible between the single tone Category 2a calls (red line), and the multiple tone 2b (black) and 2c (green) calls is accounted for by the gap between initial pulses and main pulse train in the different call types (Figure 4.2.4B).

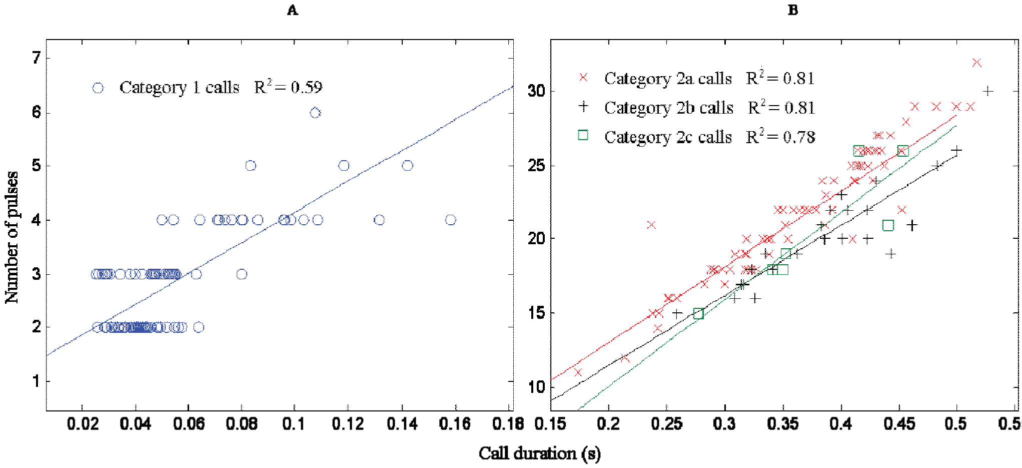

Figure 4.2.7. *Pulse number to call duration relationship in Category 1 calls (A) and Category 2a, 2b and 2c calls (B) of A. japonicus.*

In Category 2, calls free from significant surface reflections, pulses displayed 2-3 cycles before decaying below background noise. The second of these cycles typically displayed the greatest detected amplitude (Figure 4.2.3D). Cycle periods increased through each pulse, in agreement with data on *A. regius* calls (Lagadere and Mariani, 2006), suggesting a damped response to swimbladder excitation in *A. japonicus*.

Sciaenidae calls are reported as of constant frequency (Gilmore, 2002). However, in several Mosman Bay *A. japonicus* calls distinct variations were observed in pulse duration and repetition frequencies, within a call. Both characteristics were observed not only remaining constant, but increasing and decreasing throughout an individual call. These changing features resulted in several spectral variations (Figure 4.2.3A, Marks i, ii and iii). Where pulse duration and repetition rate remained constant, so to did the respective spectral peak and repetition frequencies (Figure 4.2.3A, i). However, varying pulse durations and amplitudes varied the spectral peak frequencies throughout the call (Figure 4.2.3A, ii and iii). The most notable of pulse repetition variations came from the initial pulses of Category 2 calls. Two examples of such variation were shown by calls C and D in Figure 4.2.3. Expansions of calls C and D are shown in Figure 4.2.8 and 4.2.9. In these figures panels A and B display the waveform and spectral content of the whole call respectively. In panel C the expanded waveform of each pulse within the call has been synchronised to the initial amplitude peak and overlaid for comparison. The amplitude and timing of each waveform peak, within a pulse, can then compared over the duration of the call. As the call progressed, the differences in the detected voltage amplitude between successive waveform peaks within each pulse are shown in panel D.

In Call C the duration and amplitude of the first two waveform cycles increased significantly after the first three pulses and then continued to increase throughout the call (Figure 4.2.8C and D). The spectral peak frequencies and amplitude peaks all increased significantly after the first three pulses (panel B, approximately 7.85 seconds) and continued to increase throughout the call (Figure 4.2.8B). By comparison, in Call D the first pulse amplitudes of each section of the call were lower than the succeeding pulses, accounting for the differing spectral content at approximately 10.2 s on the spectrogram (Figure 4.2.9B and C). As the call progressed the amplitudes increase and then decrease (panel D) and the peak frequency values rise and fall (panel B).

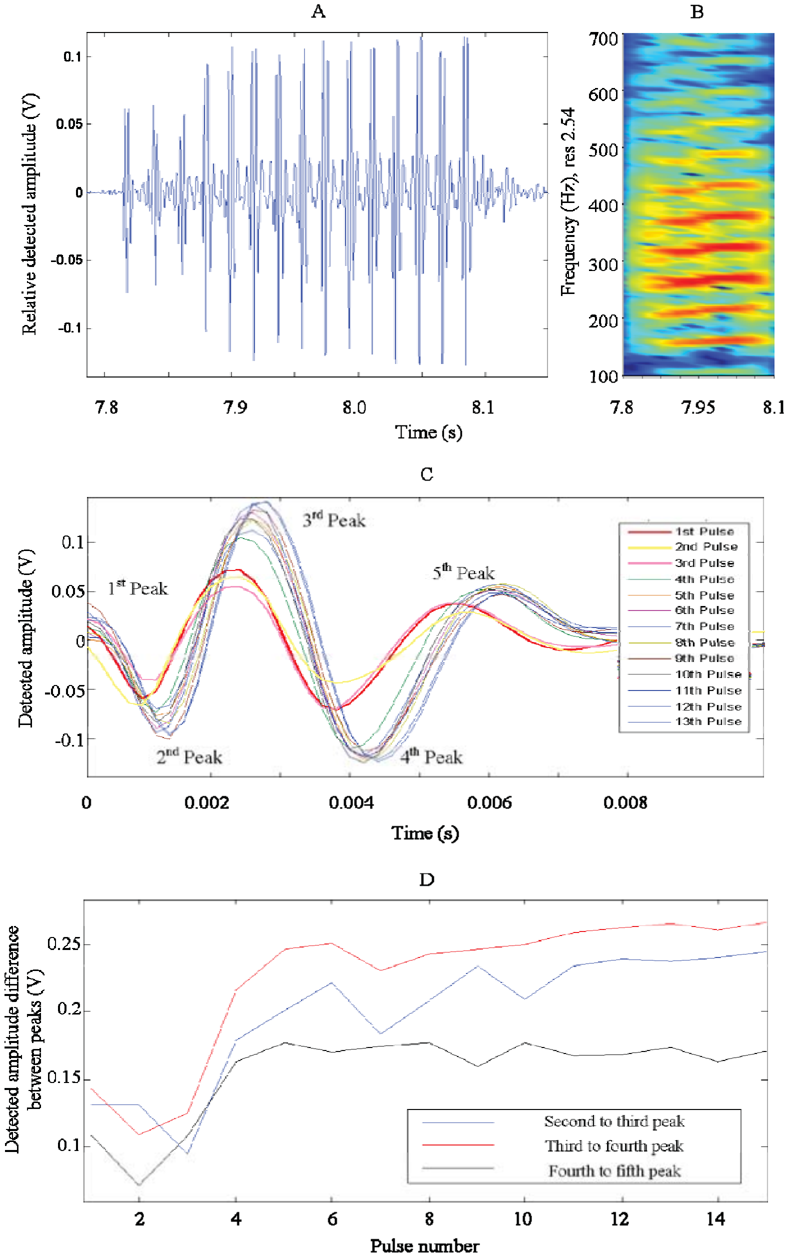

Figure 4.2.8. *Expanded waveform (A) and, spectrogram (B) of Call C from Figure 4.2.2 together with the waveform of each pulse, synchronised to the first pressure peak of the pulse (C) and the progressive amplitude differences between three sets of peaks throughout the call (D, blue, red and black lines). Spectrogram frequency resolution was 2.54 Hz.*

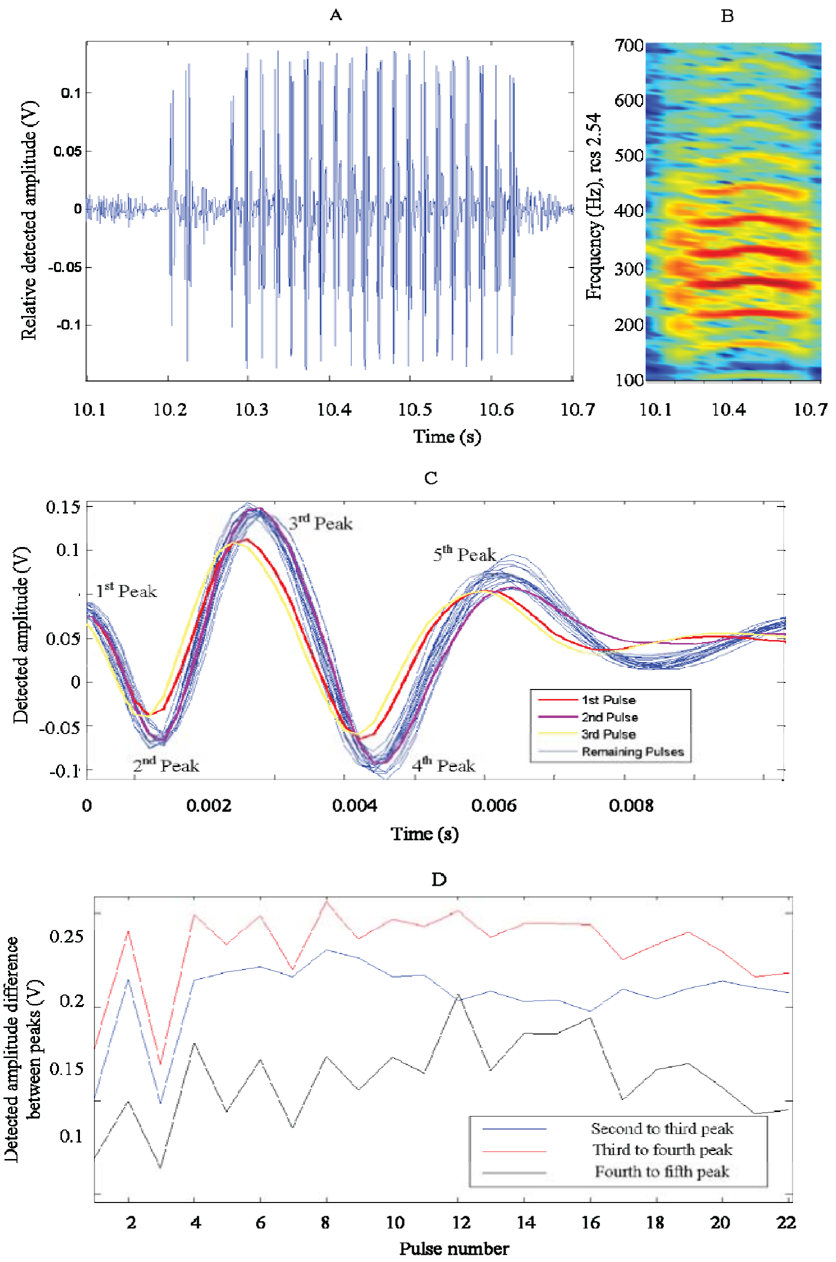

Figure 4.2.9. *Expanded waveform (A) and, spectrogram (B) of Call D from Figure 4.2.2 with the waveform of each pulse, synchronised to the first pressure peak of the pulse (C) and the progressive amplitude differences between three sets of peaks throughout the call (D, blue, red and black lines). Spectrogram frequency resolution was 2.54 Hz.*

Whether the variations between the waveforms of each pulse within individual calls are due to differences in call emission by the fish or sound transmission factors is undetermined. Such waveform variations could be attributable to potential micro variations in the sound speed profile, during the course of the call, affecting multi-path interference/refraction as the call propagates to the hydrophone.

4.2.4.2. Ground truth vocalisations in aquaria

Aural and temporal characteristics, and pulse structure, of calls from captive fish displayed similarities to calls from Mosman Bay. However, there were distinct differences in other call characteristics. Internal tank reflections affected signals such that analysis of pulse duration, decay, spectral peak frequencies and SPLs of *aquaria* calls were not considered. Signals where interference rendered call characteristics inconclusive were omitted.

Calls produced by mulloway at the TAFE aquaculture facilities began at approximately 08:30 on the 18th October. Category 1 calls were recorded lasting for approximately 30 seconds to a minute at a call interval of 3.81 s. Examples of the acoustic characteristics of calls *in aquaria* can be found in Table 4.2.2. Bursts of calls occurred intermittently until approximately 10:45. At 11:05 two sets of Category 3 calls were recorded, lasting <2 seconds each. Beginning at 11:30 Category 2 calls occurred intermittently until 12:30. Two hours later Category 1 calls were heard again; however, in this period calls would repeat a cycle of beginning several seconds apart and increasing in call rate to approximately 2 seconds between calls, before ceasing for an arbitrary period of time (ranging from seconds to minutes). The cycle would then restart with calls several seconds apart (Figure 4.2.10A). These cycles continued until 16:30 when calls became less frequent, until the final call at 17:51. Records from the following day confirm that the 18th was the first day of spawning for the broodstock mulloway. No calls were heard following the 18th or throughout the spawning period. It was not confirmed whether the male or female emitted calls during the study.

Table 4.2.2. *Example characteristics of captive A. japonicus calls on the 18th October 2007. Call repetition of Category 2 and 3 calls was sporadic and not reported.*

Time Period	Category	Number of calls (analysed)	Pulse number (s.d., max, min)	Pulse repetition frequency (Hz) (s.d., max, min)	Call repetition interval (s)
08:30 - 12:00	1	137 (75)	2 (0, 2, 2)	39.63 (4.9, 63.51, 27.89)	3.81 (0.8, 5.6, 3.2)
	2	11 (11)	11.23 (2.3, 8, 14)	41.25 (3.2, 47.56, 33.78)	N/A
	3	2 (2 series)	1.3 (0.3, 2, 1)	75.43 (6.54, 84.32, 64.70)	N/A
12:00 - 15:00	1	189 (81)	2.02 (0.27, 3, 1)	38.75 (3.89, 55.69, 31.77)	3.4 (0.65, 7.5, 2.1)
	2	6 (6)	12.71 (3.6, 8, 15)	42.65 (3.45, 49.56, 35.76)	N/A
15:00 - 18:00	1	96 (34)	2.07 (0.31, 3, 1)	40.68 (2.67, 46.50, 28.46)	6.75 (2.2, 11.2, 3.8)

Category 1 short calls predominantly comprised 2 pulses (Table 4.2.2 and Figure 4.2.10) at a mean pulse repetition frequency of 39.89 (s.d. = 4.25) Hz. During the morning individual calls were observed at a mean call interval of 3.97 s with periods of cessation for tens of seconds and often minutes. At approximately 15:00 calls became more frequent with a 3.4 s call interval, and periods of cessation reduced to seconds.

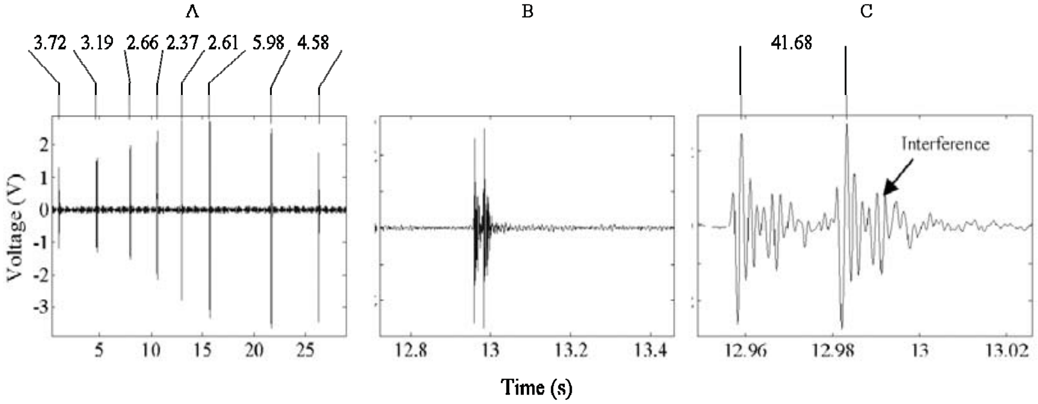

Figure 4.2.10. *Waveforms of 30 seconds of captive A. japonicus Category 1 short calls in aquaria recorded at 14:57 18th October. Call repetition rates (s) are shown (A). Expanded time series illustrate the waveform at varying lengths (B and C) with the call pulse repetition frequency shown (Hz) and interference from tank reflections highlighted.*

Several types of Category 2 calls were recorded and classed as equivalent *in situ* Category 2a, b, c and d calls (Figure 4.2.11D, B, A and C respectively). These calls displayed similar structural characteristics as *in situ* calls, but contained less pulses (Table 4.2.1). Recordings illustrated that a number of different types of Category 2 calls, with varying numbers of pulses, could be produced by an individual within a short period of time. Pulse repetition frequency, however, was considerably lower than equivalent *in situ* calls (41.74 Hz *in aquaria* versus 58.68 Hz *in situ*, s.d. = 3.3, Tables 4.2.1 and 4.2.2), and as a result calls were audible as a series of knocks, rather than a single tone.

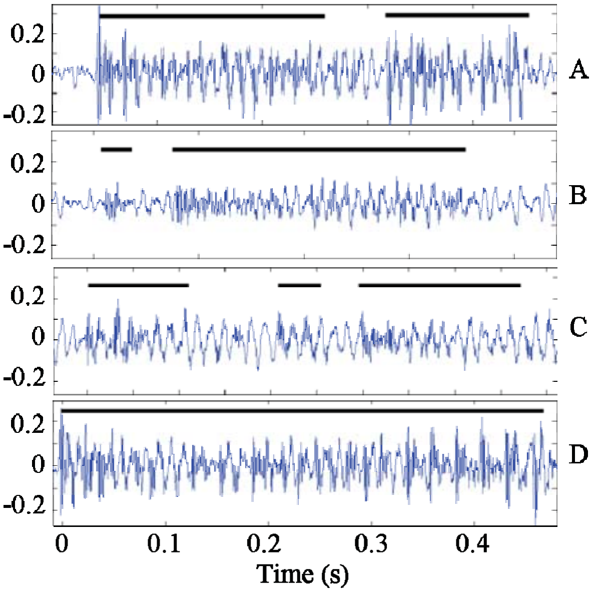

Figure 4.2.11. *Waveforms of Category 2c 'Baar-aarp' (A), 2b 'Bup-baarp' (B), 2d 'Baa-ba-baarp' (C) and 2a 'Baarp' (D) calls in order of occurrence during a 30 second period in aquaria. Black lines above waveforms represent estimates of periods of audible sound thus illustrating the type of Category 2 call.*

Few Category 3 calls were observed *in aquaria*. Those that were detected were deemed as Category 3 due to the similarities in single pulse nature, the pulse repetition frequency of double pulse calls and call repetition rate (as shown by Figure 4.2.12 and Table 4.1.2). The recorded calls mostly resembled short excerpts from the peak of the *in situ* Category 3 call series (compare the signals shown in Figures 4.2.12 and 4.2.5), although some appeared without prior calls. The elevated pulse repetition frequency observed in some of the Category 3 double pulses, as shown in Figure 4.2.12B (lower), was comparable to those of the *in situ* Category 3 calls (Figure 4.2.5C and D). The call repetition rate of the *in aquaria* Category 3 calls has been shown on Figure 4.2.12 in Hertz rather than seconds, due to the quick succession of calls, similar to that at the peak of the *in situ* Category 3 calls.

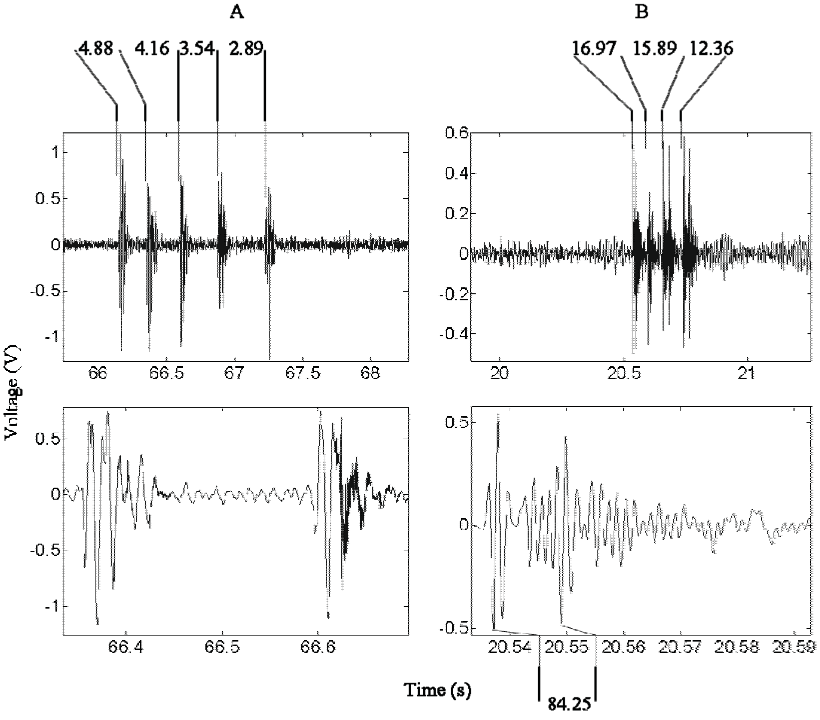

Figure 4.2.12. *Waveforms from Category 3 calls of single (A) and double (B) pulses recorded in aquaria. Expansions of pulses are shown in lower waveforms. Repetition rates of pulses sets (Hz) are shown above and the pulse repetition frequency of a double pulse shown below (Hz).*

4.2.5. Discussion

Vocalisations were recorded at locations and times known for *A. japonicus* spawning activity (Farmer *et al*., 2005, Farmer, 2008), with evidence from captive fish and corroborating data (Ueng *et al*., 2007, TAFE, Fremantle, *pers. obs*.) to associate calls with spawning behaviour of the species. *In situ* and *aquaria* recordings demonstrated that *A. japonicus* have a greater variety of vocalisation linked to spawning behaviour than previously thought (Ueng *et al*., 1998, Parsons *et al*., 2006a).

Like the majority of Sciaenidae, *A. japonicus* produce sounds by multiple contractions of sonic muscles, exciting the swimbladder in a train of pulses (Connaughton *et al*., 2002). However, in contrast to many soniferous species, such as the weakfish (*Cynoscion regalis*, Luczkovich *et al*., 2008), *A. japonicus* pulse repetition frequencies are of a higher frequency, such that the produced sound is a singular tone much like those produced by *A. regius* (Lagadere and Mariani, 2006), rather than a series of knocks. The spectral and waveform structures observed in *A. japonicus* calls are characteristic of amplitude modulation (Watkins, 1967) and typical of muscular vibration of a swimbladder.

A. japonicus calls in Mosman Bay satisfied the criteria for discontinuous and continuous fish chorus (McCauley, 2001, Cato, 1978). A continuous chorus is defined by Cato (1978) as "when the noise from many individuals is continuous above background for an extended period (usually an hour or more) using an equipment averaging time of 1 second", i.e. where calls overlap and may or may not be discerned, with a significant increase above background (>3 dB re 1 μPa). A discontinuous chorus here being considered that which does not create a significant increase when averaged over 1 second, but does over 1 minute (McCauley, 2001). Thus a discontinuous chorus accounts for calls which do not overlap, but are frequent enough to raise one minute time averaged noise levels. However, with Mosman Bay *A. japonicus*, a population of vocalisation fish with significant separation between relatively stationary callers, which definition criteria the chorus achieves may vary depending on which members of the aggregation are within the hydrophone detection range. More importantly, which calling members are close enough to warrant a >3 dB re 1 μPa increase above background noise. A continuous chorus in one location of the river may be considered discontinuous less than a few hundred metres away and may vary with time and propagation properties.

4.2.5.1. Call mechanism

Recent studies of *C. regalis* (Connaughton *et al*., 2000) and *O. tau* (Fine *et al*., 2001) have provided details on the relationship between swimbladder displacement, pressure and electromyogram (EMG) generated muscular action potential during sound production. Combining these findings with the anatomical structure and received call waveforms of *A. japonicus* has led to the proposal of a mechanism of sound production detailed below. Figure 4.2.13 displays a schematic diagram of a side (A) and cross section (B) view of the *A. japonicus* swimbladder. Blue arrows in Figure 4.2.13 represent the direction of muscle movement and the resulting motion of the body cavity wall during sonic muscle contraction and relaxation. The black arrows display the swimbladder motion as the body cavity constricts.

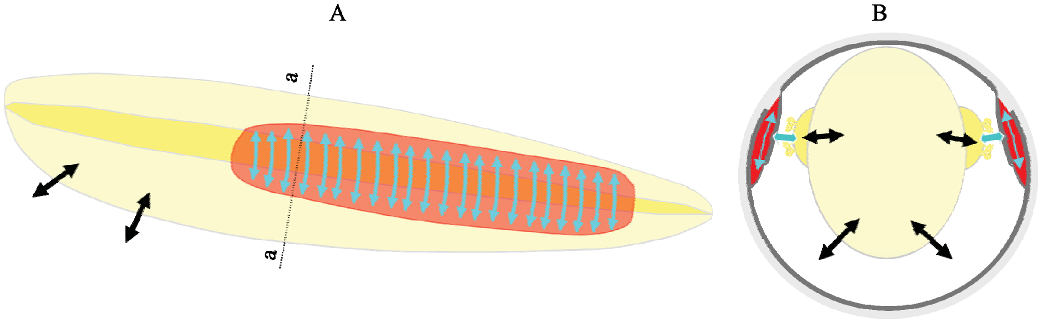

Figure 4.2.13. *Schematic of A. japonicus swimbladder (cream) with dorsoventral sonic muscles (dark red) and position of lipid deposits and aborescent appendages (yellow), showing the proposed mechanism for muscular twitching of swimbladder (A and B). B is a section view of a—a, where the dark grey line represents the aponeurotic lining and light grey the surrounding tissue or muscle. Adapted and redrawn from Vu (2008).*

Figure 4.2.14 displays the received waveform from two swimbladder pulses of an *A. japonicus* call. As the waveform progresses through time points associated with anatomical or acoustic events have been highlighted to describe the mechanism involved with the production of sound.

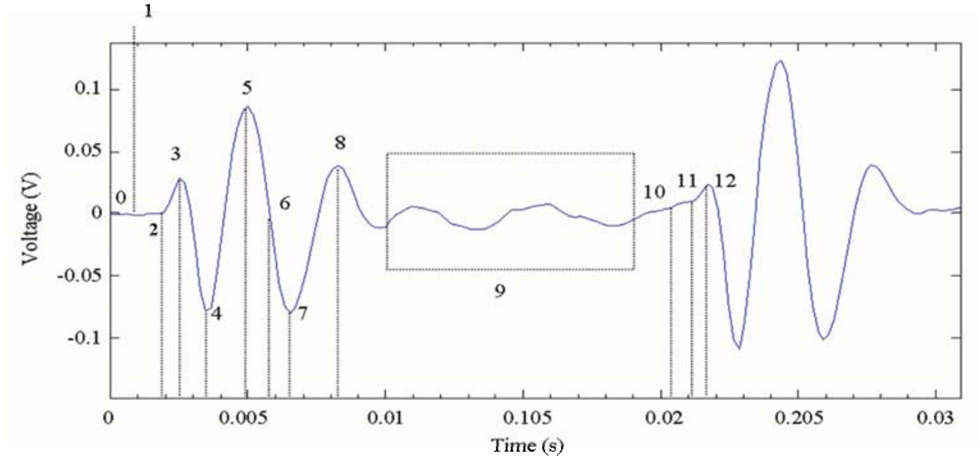

Figure 4.2.14*. Hydrophone detected waveform as a result of a swimbladder sonic muscle contraction. Numbers highlight waveform features associated with differing anatomical or acoustical events during sound production and are explained in the text.*

Previous studies of Sciaenidae (Connaughton *et al*., 2000) have shown that a latency of approximately 1 ms occurs between an EMG spike and maximum muscle action potential. In the waveform shown on Figure 4.2.14 the EMG spike is presumed to occur at time label 0) and the maximum muscle action potential achieved at 1), after 1 ms latency. Following maximum action potential the bi-lateral sonic muscles contract dorsoventrally pulling the body cavity wall inwards, around the aborescent appendages, either side of the swimbladder (Figure 4.2.13B, blue arrows), between times 1) and 2). In *O. tau* vocalisations Fine *et al.* (2001) reported 1 ms latency between commencement of sonic muscle contraction and the detected pressure change by the hydrophone, equivalent to the period between 1) and 2). The author proposes that as the muscles contract they reduce the volume around the appendages before the contraction affects the swimbladder volume. The appendages are thought to provide cushioning for the swimbladder from the repetitive muscle contractions. *A. japonicus* appendage orientation varies between areas where sonic muscle is present and absent (Griffiths and Heemstra, 1995), corroborating the proposal that the two are correlated.

It is proposed that as the body cavity wall constricts around the appendages (before reaching the swimbladder) the reduced cavity volume allows a slight expansion of the swimbladder, producing the positive detected pressure peak outside the swimbladder after approximately 0.5 ms, shown at 3) on Figure 4.2.14. It is proposed that this positive amplitude peak is a consequence of de-coupled, extrinsic sonic muscles because the equivalent peak was not present in studies of the intrinsic sonic muscles of *O. tau* (Fine *et al*., 2001). Further muscle contraction then pushes the sides of the swimbladder inwards increasing pressure within the swimbladder and producing negative pressure outside the swimbladder, corresponding to the minimum at 4). Internal pressure pushes the bottom surface of the swimbladder downwards and when this surface is at maximum velocity, maximum pressure is exerted outside the swimbladder, 4-5), similar to that of *O. tau* (Fine *et al*., 2001). At 6) the bottom surface would reach maximum downward displacement and therefore velocity is zero and swimbladder surface acceleration is at a maximum (Fine *et al.,* 2001). The bottom surface moves upwards increasing pressure within the swimbladder and producing negative pressure outside, 6-7). As the muscles relax the swimbladder sides move outwards creating a small positive pressure outside, at 8).

Fine *et al*., (2001) observed remnant damped swimbladder surface oscillation, without an associated variation in the waveform and noted that only rapid motion generated sound. It is therefore possible that the peaks in region 9 are due to residual oscillation of the action potential and resultant minor contractions of the sonic muscles, but most likely they are due to surface reflections from the water surface and riverbed. At approximately 10) the subsequent pulse from the sonic nerve occurs and the next swimbladder pulse begins (11, 12).

4.2.5.2. Call functions and behaviour

The three call categories and numerous Category 2 variations illustrated that *A. japonicus* possess a large vocal repertoire, in comparison with that reported of other species (Connaughton *et al*., 2000, Luczkovich *et al*., 1999, Nilsson, 2004). Calls of different categories are thought to be associated with specific functions. Corroboration of suggested call functions is found in previous studies of *A. japonicus* (Ueng *et al*., 1999, 2007), the recorded calls of captive *A. japonicus* at the TAFE facilities, and studies of similar species, such as *A. regius* (Lagadere and Mariani, 2006) and *C. regalis*, (Connaughton, 1996).

In Mosman Bay, evening spawning cycles began in sunlight with Category 1 short calls, when visual cues were viable at close range. These are thought to be preparatory calls which aid aggregation formation similar to other reported species (Mok and Gilmore, 1983, McCauley 2001) and the leks of frogs and birds (Hauser, 1997). The early Category 1 calls recorded in aquaria occurred several hours before spawning (Section 4.2.4) corroborating that while not directly linked with courtship behaviour they are indicative of a spawning cycle. Leks can be loosely defined as an aggregation of males which females visit for the purpose of mating (Hoglund and Alatalo, 1995). However, Bradbury (1981) suggested the following four criteria by which a 'classical' lek may be distinguished from other types of mating systems:

- The males contribute only gametes to the next generation and there is no parental care;

- There is an arena where many males aggregate (in greater numbers of that species than would normally be found at the site) and to which females visit and the majority of mating occurs.

- Males display in individual sites which contain no significant resource required by the females (for example, food, nest sites, egg deposit sites) except the males themselves.

- Females retain the prospect of selecting any male they wish from the arena.

In classical leks these sites are usually in close quarters where males are in view of each other. However, display sites can also be more diffuse, encompassing larger territories and classed as an ‘exploded’ lek (Emlen & Oring, 1977). Within these arenas lekking behaviour is characterised by males aggregating and establishing dominance hierarchies, followed by mating visits from females who choose males based on dominance rank and sexually selected characters (Hoglund and Alatalo, 1995). In Norway male cod arrive earlier than females to form leks, defending small territories and forming sized based hierarchies (Nordeide, 1998, Nilsson, 2004). It is possible that the Category 1 calls in Mosman Bay are made, not only in association with adult males congregating in anticipation of spawning, but also the formation of a hierarchy combining visual and acoustic cues, during hours of light. The fact that individuals call from distinctly separate locations supports the hypothesis that the fish are not together in a large group.

The Category 2 calls are proposed as calls of attraction. Ueng *et al*., (2007) reported that long calls were predominantly emitted by males. In Mosman Bay it is suggested that once a male has begun to affirm its position he emits the Category 2 calls in an attempt to attract a ripe female. In Mosman Bay calls occur prior to and peak typically around an hour after sunset, similar to the crepuscular spawning behaviour of other populations of *A. japonicu*s and other Sciaenidae (Ueng *et al*., 1998, 2007, Farmer, 2008). The reason for the differing call variations in this category and whether they are voluntary or involuntary is unknown. Calls recorded at the TAFE aquaculture facilities confirmed that each fish is capable of producing more then one type of Category 2 call within a short space of time.

After the time of peak calling the Category 2 calls decline in numbers. Lagadere and Mariani (2006) reported cessation of calling by *A. regius* when a short call was heard and suggested this as evidence of a female selecting a male for courtship. This cessation was not evident in Mosman Bay, possibly because the male fish appear to be separated by greater distances thus a female selecting a male in one area, does not preclude a male attracting another female in another area. Instead callers appeared to cease calling of their own volition, often without acoustic cues, and remained silent for periods of tens of seconds. Whether the individuals vacate the vicinity to return later, or remain silently in position is unknown. Category 1 short calls of lower intensity were observed during and after the time of peak Category 2 calling, but it is thought that this was range related rather than behavioural.

The increasing call rate, combined with the range from the hydrophone in the example shown in Figure 4.2.5 suggests that these calls involve interaction between fish possibly as a male dominance interaction (as it was observed during hours of sunlight, close to the beginning of the evening spawning cycle) or more likely the interaction of a male and female. This is corroborated by the apparent addition of another fish to the calls emitting similar calls. These calls were suggeseted to have originated from a second caller because of the difference in amplitude and energy. The increasing call rate and then cessation suggest that the fish had been travelling towards a desired point, whether that was another fish, or a location. The acoustic characteristics of Category 3 calls are also indicative of a call reserved for specific circumstances (Section 4.2.5.3). There is the possibility that the Category 3 call is a courtship related call, however, like the Category 1 call this requires visual confirmation.

4.2.5.3. Call acoustic characteristics

Pulse repetition frequencies exhibited by captive *A. japonicus* were significantly lower than those observed in Mosman Bay recordings. Connaughton *et al*. (2000) reported that pulse repetition frequencies in weakfish (*Cynoscion regalis*) are not dependent on fish length. However, fish of significant size variation should exhibit sonic muscle size variation. Nilsson (2004) observed a lower pulse repetition frequency (at a given temperature) for larger fish, in agreement with the hypothesis that sonic muscles of larger individuals take longer to relax, resulting in lower rates of muscle contractions. Ueng *et al*. (1999) reported a distinct correlation between fish age (and therefore muscle length) and pulse period of *A. japonicus* in aquaculture. Thus the increased size of the fish and associated muscle length is likely to result in longer contraction/relaxation times and therefore lower pulse repetition frequencies. The low pulse repetition frequencies exhibited by *A. japonicus in aquaria* may be due to an adaptation to the aquaculture environment or a degradation of sonic muscle. Increased habituation to aquaculture and an environment of reduced calling may lead to relative lack of sonic muscle use and atrophy. Captive *A. japonicus* displayed the ability to increase pulse repetition frequency on a short term basis during Category 3 calls, although the maximum observed repetition frequency in captive *A. japonicus* (84 Hz) was significantly lower than that in Mosman Bay (114.4 Hz).

A further inference from the data regarding muscle condition and efficiency is the length of call employed. Long calls of *A. japonicus* are significantly shorter than those of *A. regius* (Lagadere and Mariani, 2006). By comparison, the oyster toadfish (*Opsanus tau*) possesses efficient, intrinsic sonic muscles and emits tonal calls much longer than *A. regius* (Fine *et al*., 2001). Alternative possibilities are the length of calling cycle (this is unreported in *A. regius*) and that *A. japonicus* produce shorter calls than other species to conserve energy to enable a longer period of calling over an evening.

The pulse repetition frequency of Category 1 and 2 calls remains relatively consistent and it is suggested that this is by design for maximising efficiency of repetitive calling in the wild. Minor pulse repetition frequency variations in these calls are currently attributed to natural variation, although voluntary variation cannot be ruled out without further investigation. Rome (2005) speculated that the repetition rate of sonic muscle contractions is governed by the relaxation rate of the sonic muscle, rather than voluntary variation. In comparison, Nilsson (2004) observed pulse repetition frequency variations by Atlantic cod producing different calls with distinctive characteristics where there was little variation within calls of the same type at a given temperature. The author proposes that similarly *A. japonicus* retains the voluntary ability to ‘twitch’ a swimbladder at more than one defined rate.

The greatly increased pulse repetition frequency of multiple pulse Category 3 calls is suggested to be due to opportunistic interaction with other individuals reserved for short term calling. Mitchell *et al.* (2008) observed that sound production in *O. tau* is fatigue limited, suggesting that the faster pulse repetition frequency of Category 3 calls is limited to short term sound production as a result of fatigue. The extra exertion taken by *A. japonicus* to emit Category 3 calls is shown in the declining pulse repetition rate of the multiple pulse calls (Figure 4.2.5D and 4.2.12D). A decrease in pulse repetition frequency during a call could be explained by tiring of the sonic muscle due to build up of lactic acid and has been noted in calls by other fish such as the long 'hum' of the Atlantic cod which exhibits declining modulation frequency not present in its shorter calls (Nilsson, 2004). The *in situ* repetition rate of 114.4 Hz is higher than that of most species and indeed quicker than the typical 100 Hz exhibited by the *O. tau* (Fine *et al*., 2001) adding credence to the proposal that Sciaenidae sonic muscles are amongst the fastest vertebrate muscle types (Connaughton *et al.*, 2002b).

Lagadere and Mariani (2006) observed that *A. regius* short calls are of lower intensity than the long calls. Similar observations were made of *A. japonicus*, however, initial pulses of long calls were also often of lower amplitude. It is therefore possible that the sonic muscles take time to attain the tension required to generate amplitudes exhibited by later pulses of the long calls.

This change in tension may also be responsible for variations in spectral peak frequencies of individual calls. The spectral peak frequency variation is due predominantly to the changing peak to peak amplitude and duration of the second cycle of each pulse. In similar Sciaenidae it is the second cycle which dictates the spectral peak frequency and the amplitude peak is related to sonic muscle tension (Connaughton *et al*., 2000). As the second amplitude peak contributes a large portion of the spectral energy, and is at a high frequency, the increased amplitude (relative to that remaining within the pulse) results in an increased spectral peak frequency.

Amplitude variation within a single call could be due to a change in water depth, and therefore pressure differential between swimbladder gas and the surrounding water, however, the differences were recorded over such a short time (<0.1 s) that this was deemed unfeasible. As SPLs are related to tension (Connaughton *et al*., 2000), it is feasible that extra tension is responsible for the increased amplitude. Connaughton *et al*. (2000) suggested that increased spectral peak frequency was related to increased tension during the contraction of sonic muscles and therefore the force applied to the swimbladder. This corroborated the hypothesis of Ueng (2007) that female Japanese croaker produced lower dominant frequency calls than similar sized males due to the thinner, weaker sonic muscles. Thus by varying the muscle tension the caller is able to manipulate the call spectral peak frequency throughout in individual call. Whether this is voluntary or involuntary has not been confirmed, although the inclination to vary pulse repetition frequency for different call categories suggests limited voluntary variation.

Variations in spectral peak frequencies have shown the ability of passive acoustics to discriminate between individual fish. This was confirmed by observation of similarities/differences in pulse waveforms (Figure 4.2.3C, Calls B, C, D and E), as well as the detected voltage amplitude and therefore estimated caller range (allowing for interference). However, the mechanism of sound production is simple, producing similar waveforms, and spectral peak frequency is related to size (Connaughton *et al*., 2000). Therefore discrimination between callers is limited to fish of differing sized swimbladders and/or sonic muscles, and assumes that the fish are at the same depth. The minimum size difference which can be determined by passive acoustics, or by female *A. japonicus* employing frequency as a discrimination feature between males, remains to be determined.

Calls thought to originate from a single fish often varied in number of pulses and therefore duration (Figure 4.2.3C, Calls B, D and E) while the control recordings of captive fish, at the TAFE facilities, confirmed that although spectral peak frequencies may remain the same, an individual fish is capable of using different call structures within a short space of time. Thus it is not always possible to discriminate between individuals by the call duration or type.

Assuming call source levels of different, but similar sized fish are comparable, then calls of differing detected SPLs which occur within space of time (i.e. the fish is not expected to travel the required distance in the time allowed) show that individual fish are separated by a minimum distance (Figure 4.2.3B). Consistency in this separation highlights the low density of calling fish in the recording area and corroborates the suggestion of individual calling territories for the fish. The separation also supports a proposal of pair spawning in Mosman Bay, rather than group spawning where an indistinguishable (dense) chorus would be more prominent, similar to that of other species (Sprague and Luczkovich, 2002, Lagadere and Mariani, 2006). Thus while callers are exhibiting repetitive calling behaviour from the same locations it is possible to observe the different fish within the detection range of the hydrophone. It has not been possible for this study to confirm whether other non-calling *A. japonicus* are associated with these areas.

4.2.5.4. Management

It would be advantageous as a fisheries tool for passive acoustics to monitor a fish throughout the spawning cycle and observe individual spawning success. This would provide significant information on the behaviour and mate selection in the spawning process. However, the cessation of calls by an individual for extended periods of seconds or minutes such as those in the afternoon of the TAFE recordings has significant impact on the ability to follow an individual's calling in the wild.

4.2.6. Acknowledgements

The authors would like to acknowledge the Fisheries Research and Development Corporation (FRDC) for funding of this work. The Western Australia Marine Science Institute (WAMSI) provided a PhD top-up scholarship. Mal Perry receives grateful thanks for time and effort exercised during the course of the project's fieldwork. Department of Fisheries Western Australia has provided logistical support during the course of this study.

4.3. An assessment of temporal variations in mulloway (*Argyrosomus japonicus*) vocalisations in Mosman Bay, Swan River throughout successive spawning seasons from a single hydrophone.

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ABSTRACT

The long term monitoring of vocalisations by soniferous species of fish in environments not conducive to alternative observation methods offers an effective means of obtaining information on biomass, behaviour and any influences of environmental and anthropogenic factors. Passive acoustic datasets of vocalising mulloway (Argyrosomus japonicus) were recorded at Mosman Bay, Swan River during spawning seasons from 2004 to 2008 between October and May. At the height of spawning activity average daily sound production peaks occurred approximately one hour after sunset each day. Seasonal commencement and cessation of vocal behaviour in Mosman Bay was found to be driven by a water temperature threshold (18.5℃, morning temperature). Generalized Additive Models, using backwards stepwise regression and AIC selection criteria showed that levels and time of sound production were related to environmental drivers. Broad scale deviance in mean sound pressure levels of the evening chorus could be explained by temperature, salinity, sunset time, peak tide levels and tidal range (in order of explained deviance). Time of maximum A. japonicus sound production was related to sunset, temperature and salinity. Monthly trends in sound production occurred on a semi-lunar basis (R^2 = 0.67, p<0.005), while fine scale local maxima were found to occur on a 3.97 (s.d. = 1.78) day basis, similar to that found of egg collection in aquaria. Results highlighted the ability to observe behavioural patterns and relative abundance on daily, weekly to multi-seasonal scales.

Keywords: *passive acoustics, soniferous, environmental correlates, GAMs*

4.3.1. Introduction

The collection and evaluation of spawning related calls offers an alternative to more traditional means of determining the spawning area of soniferous fish species, particularly the labour intensive and often misleading sampling of eggs and larvae samples (Luczkovich *et al*., 1999a). Determination of egg species, numbers and age is a subjective observation, often dependent on the researcher (Luczkovich, *et al*., 1999a), while the collecting and sampling of mature fish is destructive. Luczkovich *et al*. (1999a) demonstrated the correlation between sound pressure levels (SPLs) and water column fertilised eggs of silver perch (*Bairdiella chrysoura*) and weakfish (*Cynoscion regalis*), illustrating the possibility of monitoring spawning aggregations of sound producing species by studying their vocal behaviour. As the technologies to record and analyse underwater sounds improve the ability to utilise fish vocalisation as a cost effective, low impact tool in the long term management and understanding of vocalising species is becoming increasingly evident (Rountree *et al.*, 2006).

As sound production can be related to calling fish and therefore aggregation numbers it is possible to monitor the relative population response to external factors (environmental and anthropogenic) such as variations in spawning commencement and cessation, mid-season spawning peaks and reactions to anthropogenic impacts such as vessel noise. Once the impacts of external dynamics are known it may be possible to estimate years of high or low spawning levels.

The Swan River spawning aggregations of *Argyrosomus japonicus* move appreciable distance into the river around October each year, near the onset of summer (Farmer, 2008) when salinities reach marine levels (Loneragan *et al*., 1989). Spawning occurs approximately between October and May, while mean water temperatures exceed 19° C, before many fish return to oceanic waters (Farmer, 2008). Similar behavioural patterns have also been reported of *A. japonicus* in South Africa (Smale, 1985). Continuous observation of natural behaviour over entire spawning seasons can offer significant information on how a species or local population responds to various environmental factors.

Circadian rhythm of sound production by fish has been shown to be species characteristic (Ueng *et al.*, 1998), indicating implications for fisheries management based on vocal behaviour. A number of environment related trends of *A. japonicus* vocal behaviour *in aquaria* have previously been reported (Ueng *et al*., 1998), however, in many species captivity has resulted in restricted vocal behaviour and as a result disparities between observed calling trends in captivity and the wild (Midling, 2002). The focus of this study was to observe *in situ A. japonicus* vocalisations and how they vary throughout four spawning seasons to establish or confirm major environmental factors contributing to variations in spawning rhythms.

4.3.2. Methods

Mosman Bay is subject to a tidal influence which ranges from 10 cm to 1.2 m. The water temperatures range between ~16 and 25° C and salinity between brackish and marine (~9 600 to 38 400 mg/L, respectively) between winter and summer months (L. Twomey, Swan River Trust, *unpublished data*). Local vessel related noise is present throughout the daytime reaching maximum levels in the late afternoon and reducing significantly after sunset (further detail can be found in Section 4.1).

Passive acoustic recordings were acquired over several spawning seasons between 2004 and 2008 (Table 4.3.1). Sea noise loggers designed at the Centre for Marine Science and Technology (CMST) and Defence Science and Technology Organisation (DSTO) were attached to various HTI-90U hydrophones (see Section 4.1.2 for specifications). The systems were calibrated using a white noise generator (-70 or -90 dB re 1 V^2/Hz). Each system recorded five of either every ten or fifteen minutes at sampling frequencies of 6 or 4 kHz, respectively. The loggers were returned each year to approximately the same location in the middle of the river, on the riverbed, in approximately 21 m of water (32.01°S, 115.776 °E, Figure 4.1.1 illustrates the study site).

Analysis was carried out in the Matlab® software environment using processing algorithms developed at the CMST. Since marine fauna respond on a daily basis to the sun elevation and not our clocks, much of the analysis has zeroed the daily clock time base to the time of sunset (upper limb hitting the horizon). The time of local sunset at the study site was retrieved from the Geoscience Australia website for each day and used as the local zero hour point. Thus time each day is often given as hours prior (-ve) or post (+ve) local sunset. As a measure of fish calling the intensity across the 250 Hz one third octave band, in spectral level units (dB re 1μPa^2/Hz), has been used throughout. This one third octave band spans the frequency range of most energy in *A. japonicus* calls and measured choruses (Section 4.2) and so has been used as indicative of their vocalisation behaviour.

Table 4.3.1. *Mosman Bay hydrophone specifications and deployments*

Season	No. of samples/days	Date / time of first sample	Date / time of last sample	Sample rate (Hz)	Sample schedule
2004-5	3608 / 25	11/01/2005 12:00	5/02/2005 12:50	6 kHz	300 s every 10 minutes
2005-6	9409 / 98	6/12/2005 18:00	14/03/2006 17:45	6 kHz	300 s every 10 minutes
2006-7	21597 / 223	11/10/2006 17:00	22/05/2007 14:00	4 kHz	300 s every 15 minutes
2007-8	14381 / 151	19/10/2007 18:00	13/03/2008 13:00	4 kHz	300 s every 15 minutes

Recording samples which were significantly affected by vessel noise required removal. During daily vocalisation the SPLs of each five minute sample were calculated and if the preceding and succeeding samples displayed a greater than 3 dB re 1 μPa drop (as in Figure 4.3.1, right hand evening SPLs) the sample was tested for vessel noise. Spectral analysis of the individual sample confirmed or rejected the presence of vessel noise. Figure 4.3.1 shows how SPLs within the 250 Hz centred, one third octave varied throughout the day, and which portions can be attributed to vessel noise or fish calls.

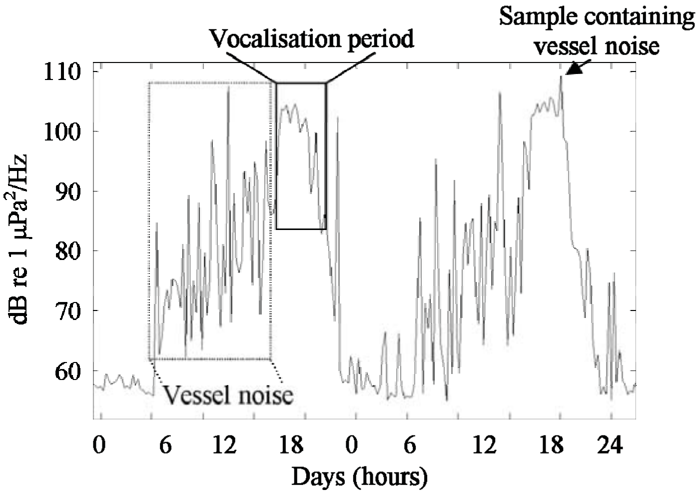

Figure 4.3.1. *Five minute averaged sample sound pressure levels of the 250 Hz, one third octave for two days of the spawning period. Areas of vessel noise and fish vocalisation are shown.*

Solar, lunar (Geoscience Australia), tidal (Department of Planning and Infrastructure - DPI), water temperature, salinity, and pH level (Swan River Trust) data were obtained for correlation against chorus levels and times. DPI tidal data were sampled every five minutes at the Barrack Street jetty, approximately 8.5 km upstream, and Swan River Trust data originated weekly at approximately 9 am from a sampling station in Blackwall Reach, 600 m downstream from the hydrophone location.

The environmental data was modelled against three characteristics of sound production to determine spawning drivers or correlates. These sound production variables were; mean SPLs of the 250 Hz centred one third octave, taken between one hour prior and three hours post local sunset; the peak SPL during the chorus period; and the time of the peak sound production. The environmental variables tested were as follows: time of sunset; water temperature at 14 m depth; salinity at 14 m depth; maximum level of peak high tide; time of high tide; tidal range; lunar phase; pH levels; and the time difference between high tide and sunset.

Generalized Additive Models (GAMs) were produced in S-Plus (v. 6.2, Insightful Corporation, Seattle) to model trends in response variables (sound production) to trends in the descriptor or predictor variables (environmental conditions). GAMs are a method of analysing data responses which may be non-normal distributed with non-linear smooths of the predictor variables (Embling, 2007).

A major issue with modelling responses to autocorrelated environmental variables is overfitting, as the underestimation of standard errors causes a variable to be incorrectly considered significant (Lennon, 2000). For example in summertime water temperatures increase, and lower rainfall brings less freshwater into the river, thus salinity also increases. As a result, pair tests were conducted between all of the environmental variables and the least significant of the pair discarded if an R^2 value greater than 0.7 was observed (Moore, *in prep.*). To minimise model overfitting the smooths for each variable were limited to five degrees of freedom and confidence in fit was conducted using five-fold cross validation (Hastie and Tibshirani, 1990, Chambers and Hastie, 1993).

As noise levels are normally distributed the GAMs were run using a Gaussian distribution with an identity link function. Variables were selected using Akaike's Information Criterion (AIC) method and backwards stepwise regression to detect the model exhibiting the least variance. The models were checked for efficacy within S-Plus. A significance value of $p<0.05$ was used. GAMs were produced for the 2005-6, 2006-7 and 2007-8 seasons, however, the recording of the 2004-5 spawning season was considered to contain too few samples to accurately model the data.

The correlation between sunset and temperature was higher than the 0.7 threshold in the 2004-5 and 2006-7 seasons (0.767, and 0.769, respectively), though lower in the remaining seasons (0.264 and 0.643 for 2005-6 and 2007-8). However, temperature and light levels have been shown separately to be significant in characteristics of sound production and spawning behaviour of some soniferous fishes (Ueng *et al.,* 1998, 1999, Connaughton *et al.*, 2000, Section 4.2). Therefore both temperature and sunset time were included in the GAMs.

The sound production datasets in this study were of varying sample sizes. When comparing similar models of differing sample size adjusted D^2 (also referred to as adjusted R^2) establishes the deviance in the response variables accounted for by the model. A model with no residual deviance has an adjusted D^2 of 1. As such, the adjusted D^2 was considered a statistically sound measure to compare models and was calculated as per Guisan and Zimmermann (2000).

It should be noted that the recording taken from the 2005-6 season requires a further calibration constant due to a recording system fault (water leaking down the wires changing the effective hydrophone capacitance). This fault was due to an insidious failure of the underwater connector. As a result illustrated SPLs of the season appeared lower than in reality. This fault was not expected to affect the recorded trends in sound production as the problem was constant.

4.3.3. Results

All Mosman Bay recordings displayed consistent evidence of *A. japonicus* vocalisations during the summer months. Figurative confirmations of calling were observed in stacked spectrograms, during periods of high SPLs, shown by the sidebands of amplitude modulation at frequencies typical of *A. japonicus* calls between 100 and 1000 Hz (Section 4.2). This can be seen in Figure 4.3.2. Varying sources of anthropogenic noise were distinguishable over a similar frequency band (Figure 4.3.2). Vessel noise occurred typically prior to, and overlapping with, fish vocalisation, such that *A. japonicus* calls were often masked. Acoustic characteristics of vessel noise features on spectrographic figures have been well documented (Ross, 1976). Vessel propeller cavitation and engine noise leave a signature horizontal line of noise on high temporal resolution spectrograms at the characteristic frequency of the engine (Parsons *et al.*, 2006a, Section 4.7.12). If time averaged, over a longer period (for example 5 minutes), the additional SPLs of the passing vessel leave tonals across the associated frequencies (see Figure 4.3.1 local pre-chorus peaks and Figure 4.3.2, thin vertical blue lines). In addition, noise was observed between 15 and 25 Hz (Figure 4.3.2), exhibiting similar characteristics to that observed in previous research, shown to correlate to peak hour traffic on a nearby highway and the local train timetable with this energy speculated to arrive in the river via coupling through the local limestone bed (R. McCauley, Curtin University, *pers. comm.*).

Figure 4.3.2 displays the spectral difference between fish calls and vessel noise, which on the temporal scale match the respective spikes and constant SPLs shown in Figure 4.3.1. Vessel noise was significantly more prevalent over the weekend, shown by the noise during the morning and afternoon hours of the Saturdays and Sundays in Figure 4.3.2.

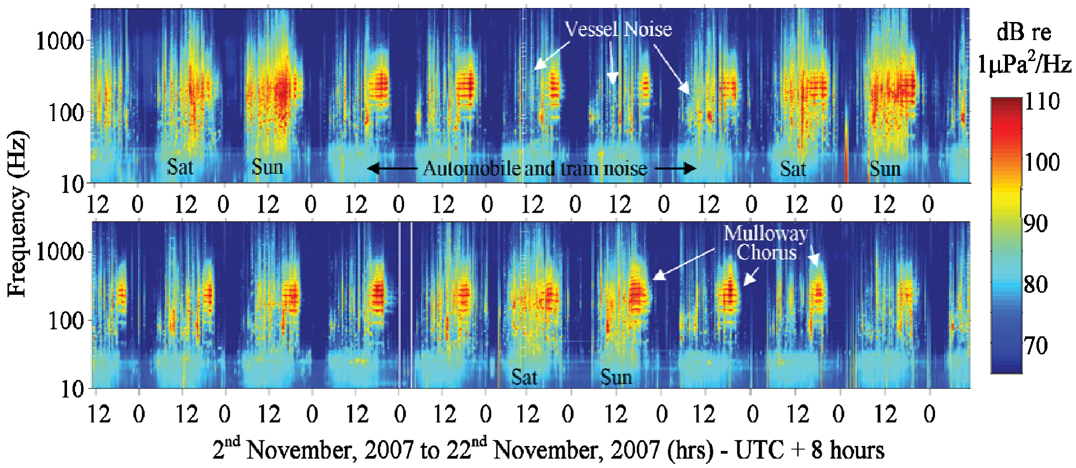

Figure 4.3.2. *Stacked spectrograms of five minute samples on logarithmic frequency scale from 2nd – 22nd November, 2007 with sound pressure levels as per the colour bar. Typical evidence of A. japonicus calls, vessel, automobile and train sounds are highlighted. Saturdays and Sundays exhibiting extensive vessel noise are marked.*

When averaged across the entire spawning season recordings the daily peak SPLs occurred predominantly approximately one hour after sunset, as shown by Figure 4.3.3A. The effect of weekend, chorus time vessel noise averaged over each season can be seen in the difference between Figure 4.3.3A and B. Weekend noise (dotted lines) can significantly increase SPLs in the hours before sunset, when compared to SPLs if weekdays (continuous lines). Although spectral and aural examination confirmed masking of early evening calls during periods of vessel noise there was no apparent effect on fish SPLs recorded later in the day, as measured by the 250 Hz centred, one third octave (Figure 4.3.3B). These responses highlighted the necessity to limit the tested period to one hour prior to sunset, while confirming that the four hour period (testing to three hours post sunset) encompasses greater than 95% of the total sound production.

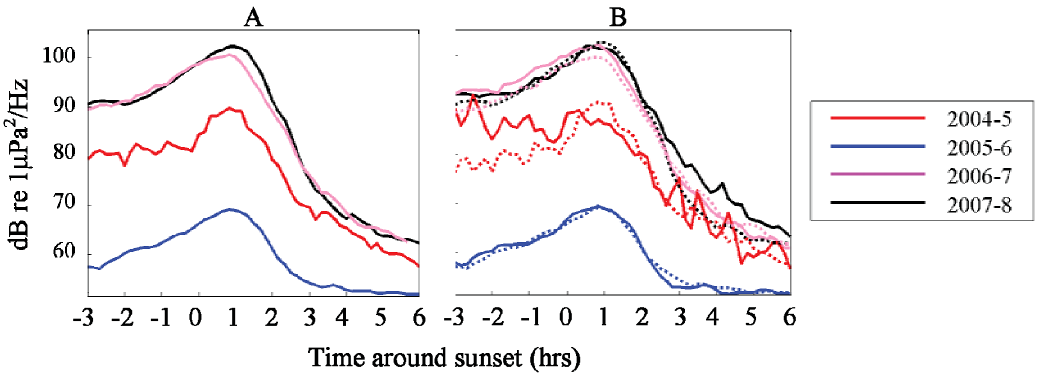

Figure 4.3.3. *Average sound pressure levels from seasonal acoustic recordings, zeroed around sunset. All sound production (A). Monday-Friday (dotted line) and weekend (solid line) sound production (B). Spawning seasons are identified by colour.*

Figure 4.3.4 illustrates the variation in time averaged sound production over each evening spawning cycle between one hour prior and three hours post sunset from four spawning seasons (C), and interpolated seasonal trends in salinity (A) and temperature (B). As fish are environmentally driven and not expected to follow our Gregorian calendar, which is based on day length, the datasets were synchronised to the October full moon for inter-season comparison.

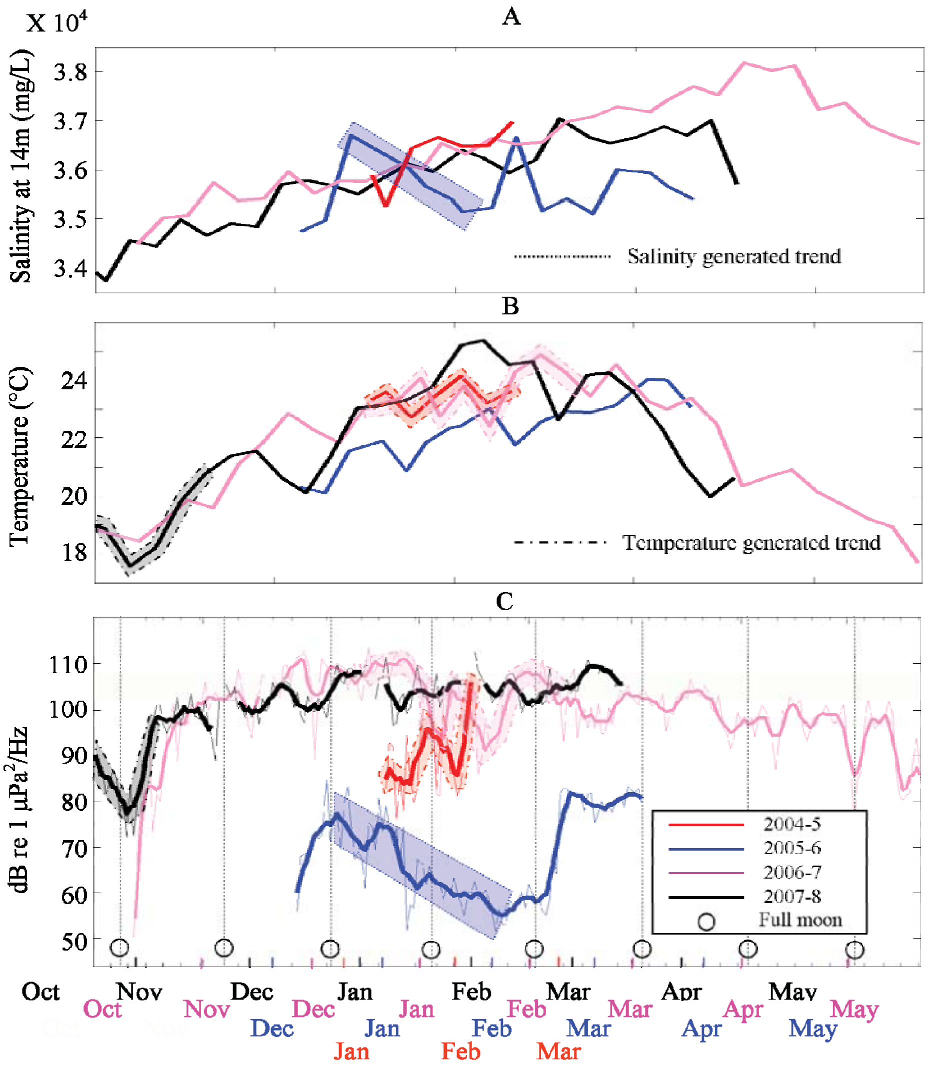

Figure 4.3.4. *Salinity (A) and temperature (B) readings taken approximately once a week at a depth of 14 m, 600 m downstream from the hydrophone location. C) Raw and two day averaged sound pressure levels for the 250 Hz centred one third octave taken across one hour prior and three hours post sunset across four spawning seasons (raw and smoothed data shown by thin and thick continuous lines respectively in colours representing the shown seasons). All datasets have been synchronised to the respective date of the October full moon. Examples of trends in sound production thought to be generated by salinity and temperature have been highlighted (dotted and dashed areas respectively).*

Single evening recordings taken in late September and early October of the 2006-7 and 2007-8 seasons displayed little or no aural/spectral evidence of fish vocalisations. Sound production increased rapidly (30 to 40 dB re 1μPa^2/Hz) in October/November, after the October full moon, and once water temperature had exceeded 18.5° C (Figure 4.3.4C). In the 2006-7 and 2007-8 seasons SPLs reached maximums between December and March, before reducing in April and May. Peak SPLs of 112 dB re 1 μPa^2/Hz (111 dB, based on two day smoothing) in December of the 2006-7 season were comparable with the 110 dB re 1 μPa^2/Hz (110 dB smoothed), during the same lunar period of the following year (Figure 4.3.4C, pink and black lines, respectively). Due to the underwater connector failure, SPL levels of the 2005-6 season are currently not comparable to recorded levels of other seasons.

High correlations between mean chorus levels around sunset and the peak chorus levels (R^2 = 0.934, 0.873, 0.769 and 0.813 for the consecutive spawning seasons) confirmed that the observed peak choruses were due to fish vocalisations, rather than vessel noise. SPL spikes due to vessel noise would skew the sound production curve towards the time of vessel noise, with unrealistically high values and therefore lead to a lower correlation.

Overall trends in *A. japonicus* sound production were seen in the two-day smoothed SPLs of the time averaged, 250-Hz centred, 1/3-octave around sunset. Examples of temperature-correlated trends can be seen in Figure 4.3.4 (dot-dash areas) where drops and increases in temperature correspond to similar variation in sound production. A speculated salinity driven drop in sound production is shown by the dotted areas between December and February of the 2005-6 season where no variation was observed in other variables.

The overall correlation and explained deviance displayed by the final chosen GAMs for each spawning season can be seen in Table 4.3.2. In each case a significant proportion of deviance was accounted for (all adjusted D^2 values were greater than 0.62 and 0.52 for mean chorus levels and peak chorus time respectively).

Table 4.3.2. *Overall deviance explained (D^2), adjusted D^2 and correlation values (cor) for each of the three tested spawning season GAMs run with mean chorus SPLs around sunset, peak chorus levels and the time of peak chorus.*

	2005-6 (n=98)			2006-7 (n=149)			2007-8 (n=222)		
	D^2	adj D^2	cor	D^2	adj D^2	cor	D^2	adj D^2	cor
Mean chorus SPL around sunset (dB re 1μPa^2/Hz)	0.785	0.765	0.798	0.642	0.629	0.803	0.849	0.641	0.884
Time of peak chorus	0.563	0.524	0.636	0.747	0.737	0.865	0.747	0.732	0.865
Maximum Chorus SPL (dB re 1μPa)	0.788	0.769	0.751	0.765	0.757	0.844	0.770	0.757	0.750

Over the 2006-7 and 2007-8 seasons the GAMs determined that five descriptors contributed significantly to explained deviance in sound production. Figure 4.3.5 shows the selected GAMs with the explained deviance by each descriptor alone and their respective contributions to the models overall explained deviance. Temperature, salinity and sunset were the dominant variables, but tidal range and levels also contributed significantly to overall explained deviance. Individually, temperature and salinity explained similar levels of deviance (Figure 4.3.5A potential contributions), however, the fact that both contributed significantly to the selected model shows that the descriptors were, to an extent, correlated with different aspects of the SPL variation.

The response curves for each selected variable in the 2006-7 model for mean SPLs around sunset are shown in Figure 4.3.6. The curves show that the model displayed best responses to temperatures above 20 °C, salinity between 34 500 and 36 800 mg/L, sunset earlier than 18:45, and high peak tides of low tidal range. The sunset time response curve appears counter intuitive in that later sunset (associated with summer) were expected to be positively correlated with sound production, however, this reponse curve shows that at times of later sunset the deviance is better explained by variations in temperature and salinity, while the sunset time explained a higher proportion of the deviance during early and later months.

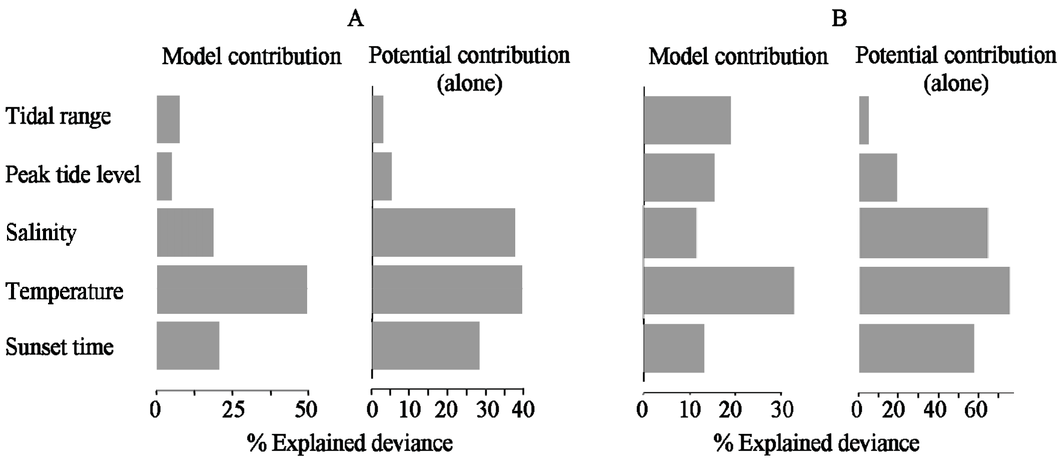

Figure 4.3.5. *Correlations of AIC selected descriptor variables to recorded mean sound pressure levels during the four hours around sunset as individual correlates and their relative contributions to explained deviance in the most parsimonious Generalised Additive Model for the 2006-7 (A) and 2007-8 (B) spawning seasons.*

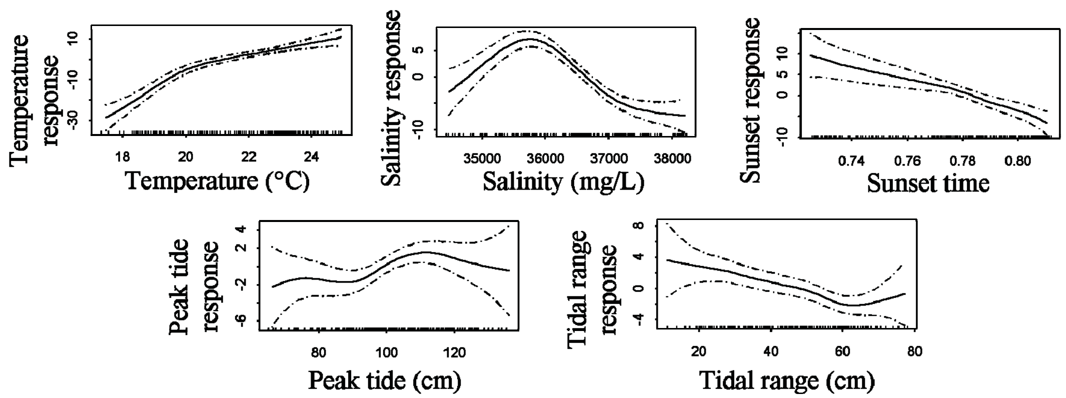

Figure 4.3.6. *Response curves (continuous lines) and 95% confidence limits for the AIC selected descriptor variables for the 2006-7 season for the Generalised Additive Model shown in Figure 4.3.4.*

Notably, the GAM for the 2005-6 season, which spanned a shorter period than the other two, also selected the lunar phase as explaining 9.65% of the deviance. The model illustrated semi-lunar spawning behaviour explaining deviance well at the new and full moons.

A similar, but simpler model was generated for the daily peak time of the chorus. During the 2006-7 spawning season the final model determined that only the time of sunset, temperature and salinity, in descending order of contribution, explained the deviance in the peak calling times, as shown in Figure 4.3.7A and B. The response curves for the maximum chorus times (Figure 4.3.7C) showed that later sunset, temperatures below 19 °C and above 21 °C, and low levels of salinity could explain nearly 75% of the deviance in time of maximum SPLs.

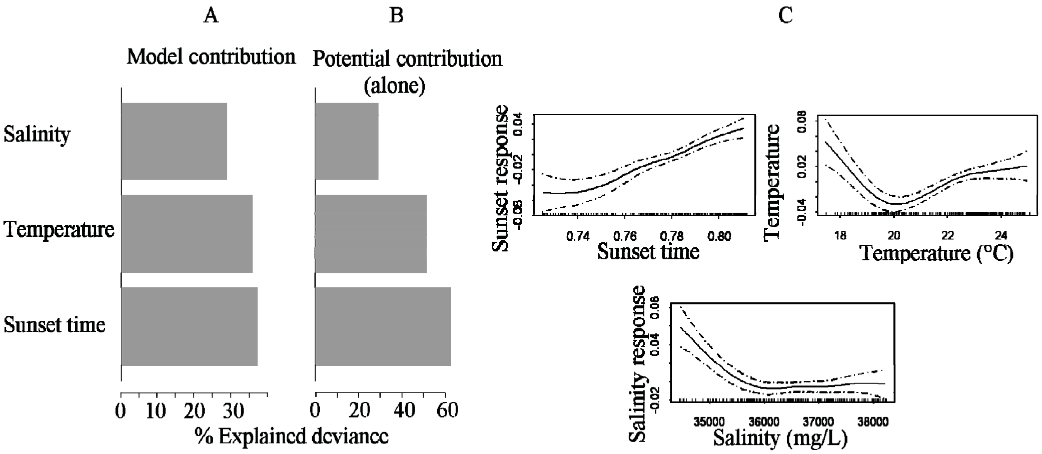

Figure 4.3.7. *Correlations of AIC selected descriptor variables with the time of peak chorus as individual correlates (A) and their relative contributions to explained deviance in the best Generalised Additive Model (B) together with the response curves for the 2006-7 spawning season.*

Similarly to the GAM describing mean sound production (Figure 4.3.5), not all of the deviance in peak chorus time was explained (Table 4.3.2). The models appeared to explain broad scale temporal trends. Shorter trends were also observed in the data, similar to the trend within the declining SPLs of the 2005-6 season, peaking at the full and new moon (Figure 4.3.4C, blue line). The GAM model trend was removed from month long sections of data in the 2006-7 and 2007-8 seasons. Standardised residuals displayed significant correlation with semi-lunar phases. Sound production was positively correlated with the new and full moons in both seasons ($R^2 = 0.652$ and 0.692, respectively). Evidence of these semi-lunar cycles can be seen in the SPLs zeroed around sunset, in Figure 4.3.8, when compared with the difference between high tide time and sunset (white line). On an approximately two week cycle the evening chorus occurred later and later, after sunset, often with increasing intensities, before returning to earlier peak chorusing times of day. The peak chorusing level did not always occur at high tide, but regularly up to several hours pre or post high tide. Notable anomalies occurred in each season where peak chorus time altered significantly, such as January 2007 and the end of February 2008 (Figure 4.3.8) often coinciding with marked drops in temperature (pink and black lines, Figure 4.3.3B). Currently, a complete model encompassing the trends on widely varying temporal scales for mean SPLs and time of peak chorus has not been developed. This has been set as a task for future work.

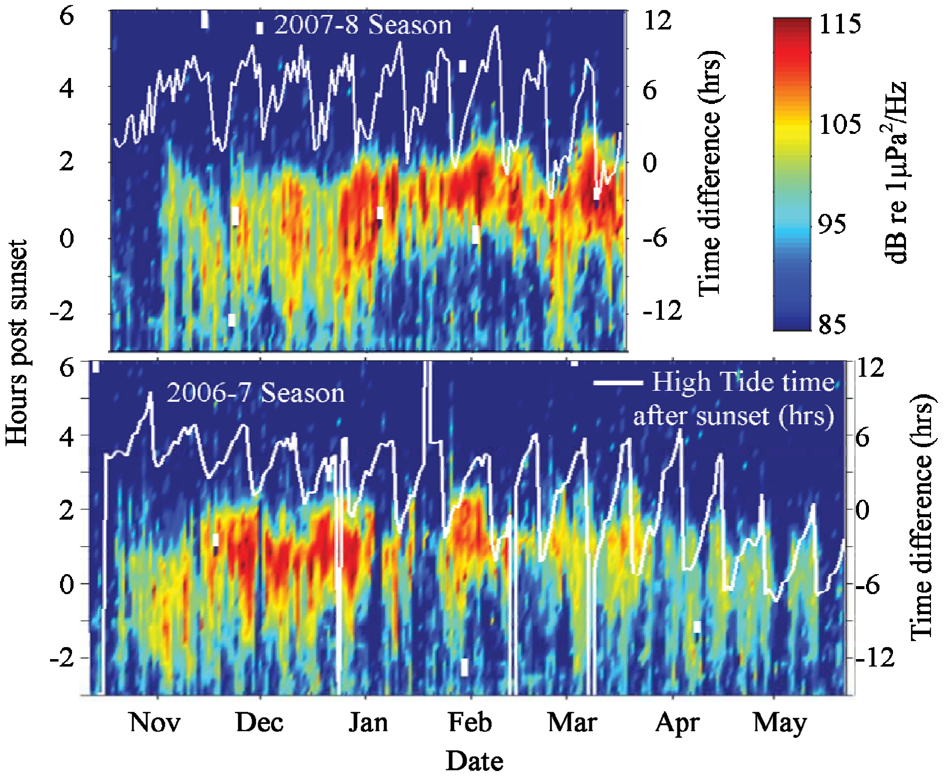

Figure 4.3.8. *Sound pressure levels zeroed around sunset times for the 2006-7 and 2007-8 datasets. Time difference between sunset and high tide in hours is displayed by continuous white line.*

Day-to-day variations in SPLs were as much 24 dB re 1µPa, and maximum time variation in peak chorus time, between two consecutive days was 2.2 hours (s.d. = 28 mins, or approximately 2 sample periods). These day-to-day variations did not necessarily coincide with a comparative change in any of the descriptor variables. Local SPL maxima occurred every 3.97 days (s.d. = 1.87, max = 9, min = 2) across all seasons (Figure 4.3.3, thin continuous lines).

SPLs and calling times were not the only characteristics to vary throughout the spawning season. Variations in spectral peak frequency were also observed, as shown in Figure 4.3.9. Although correlations were comparatively low (R^2 = 0.516 and 0.415 for 2006-7 and 2007-8 seasons, respectively) an increase in water temperature in both seasons occurred with in an increase in average call spectral peak frequency over the evening's calling, as shown by Figure 4.3.10.

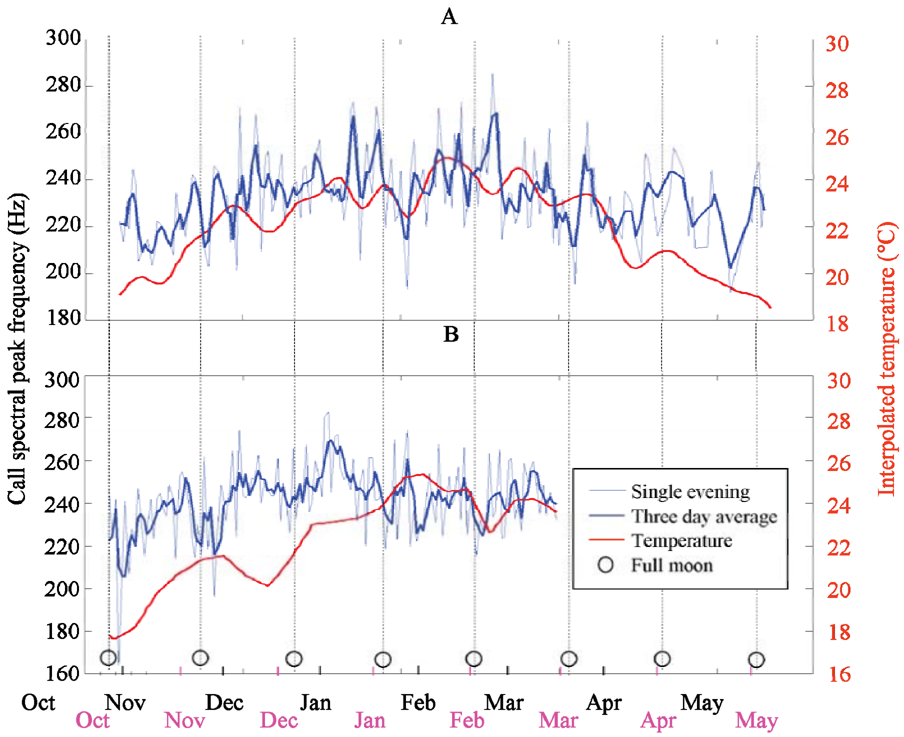

Figure 4.3.9. *Variation of spectral peak frequency throughout the 2006-7 (A, pinkg dates)) and 2007-8 (B, black dates) spawning seasons with the associated temperature trends.*

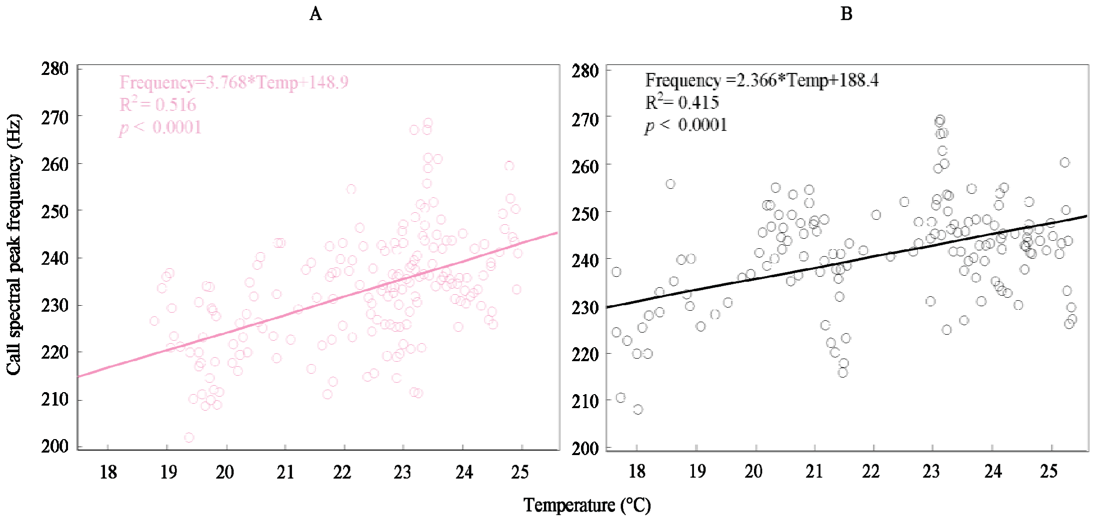

Figure 4.3.10. *Relationships between call spectral peak frequency and temperature during the 2006-7 (A) and 2007-8 (B) spawning seasons.*

4.3.4. Discussion

As a proxy to spawning behaviour, recordings of sound production have shown the Mosman Bay *A. japonicus* aggregation spawning season to begin around the October full moon, when morning water temperatures exceed 18.5 °C and salinity is in excess of 34 500 mg/L. Sound production increases through summer to maximums between December and February, declining with temperatures between March and April. Environmental variations throughout the year affect sound production to differing extents. These SPLs are in agreement with spawning maturity stages of *A. japonicus* sampling data in previous seasons (Farmer, 2008).

Sound production has been shown to be comparable between seasons, providing a proxy for relative levels of spawning related behaviour within the range of the hydrophone. Thus the observation of relative levels of sound production provides a means of monitoring aggregation relative abundance over multiple seasons.

Final GAMs illustrated *A. japonicus* SPLs environmental drivers, or correlates, on a broad temporal scale, to be temperature, salinity, time of sunset, peak tide levels and tidal range. Temperature explained the highest level of deviance in each model while salinity and sunset also explained a significant percentage of deviance, and tide related variables explained a minor portion of deviance. Time of peak calling was more simply explained by sunset time, temperature and salinity.

The correlates have previously been reported as affecting spawning behaviour, either in *A. japonicus*, or other species. Farmer (2008) observed that *A. japonicus* in southwest Australian coastal waters only spawned between October and May, while temperatures were in excess of approximately 19° C. In waters of lower latitude where temperatures do not fall below this suggested threshold spawning can occur all year round (Farmer, 2008). However, Ueng *et al*. (1998) did not observe, from *aquaria* recorded data, similar temperature correlated trends to those shown in this study. A possible explanation for lack of temperature correlation is that in previous studies captive *A. japonicus* have exhibited restricted vocal behaviour (Section 4.2), similar to other species (Midling *et al*., 2002). In addition, broodstock spawning at Fremantle TAFE aquaculture centre where individuals are habituated in near perpetual twilight have exhibited sound production and spawning behaviour at various times of day (Section 4.1, author *pers. obs.*).

Adult *A. japonicus* are marine fish (Griffiths *et al*., 2005) and, as such their presence/absence in the waterway and behavioural differences in response to salinity variations was not unexpected. The additional contribution of salinity levels to egg buoyancy and survival rate adds credence to positive salinity related correlation (Barrios, 2004).

Ueng *et al.* (1998) noted *A. japonicus* sound production and spawning at low light levels and compared the behaviour to that of other species, describing *A. japonicus* vocalisation after dusk and pre dawn as nocturnal. In contrast, sound production during the several hours prior to sunset in Mosman Bay, and the lack of dawn chorus showed that *A. japonicus* is not purely nocturnal, and it is the author's suggestion that the two populations have responded to different environmental conditions. Such behavioural disparity confirms the need for observation of species *in situ* and *in aquaria* to determine optimum natural spawning conditions.

At this stage the derived GAMs have been unable to simultaneously determine finer scale variation, such as trends within individual lunar months. This is in part due to the limiting of degrees of freedom to avoid overfitting. Analysis of residuals, around the model trend, displayed correlation with full and new moons. Lunar spawning in Sciaenidae has been regularly reported, occasionally using point data of sound production as confirmation (Holt *et al.*, 1985, Barrios, 2004, Aalbers, 2008, Lowerre-Barbieri *et al.*, 2008). Semi-lunar spawning, at new and full moons has been reported in spotted seatrout (*Cynoscion nebulosus*), as well as non-Sciaenidae marine and brackish fishes (Takemura, 2007, Manabe *et al.*, 2008), corroborating Mosman Bay data.

In this study, the time of peak calling was also shown to correlate with tidal influences. During sampling of a recent study (Farmer, 2008) individuals of spawning maturity were only caught between 21:00 and 23:30 leading to speculation that spawning occurs at night. Females containing hydrated oocytes were caught immediately prior to high tide and speculated that *A. japonicus* spawn at, or close to, high tide such that fertilised eggs would be transported downstream and out into open water on the ebb tide. Although on a seasonal scale *A. japonicus* have been shown to time spawning with sunset, Figure 4.3.8 highlights the additional correlation, with peak calling occurring later in the day when the difference between high tide time and sunset are greatest coincident with the lunar half cycle.

However, some of the environmental data (temperature, salinity and pH) were collected on a weekly basis. In future surveys it will be necessary to observe high resolution variables (temperatures, salinity, pycnocline, pH, and dissolved oxygen) at times and locations of *A. japonicus* sound production to develop an accurate SPL related model with all environmental data.

It is not possible for passive acoustic techniques to confirm the absence or presence of *A. japonicus,* prior to spawning commencement, as species vocalisation is typically only distress or spawning related (Ueng *et al*, 2006, Section 4.2). However, data observing relative catch levels of *A. japonicus* in deeper, offshore waters (Farmer, 2008) suggest that at the commencement and cessation of the spawning season fish migrate into the river from coastal waters in order to spawn, in agreement with the acoustic data.

Sound production in Mosman Bay displayed local maxima every 3.97 days in agreement with typical *A. japonicus* and other Sciaenidae behaviour in the wild (Farmer, 2008) and largely in agreement with egg collection data from Fremantle TAFE *A. japonicus* in *aquaria* (author, *pers. obs.*). This short-term variation in calling/spawning displayed no significant correlation with tested environmental variables suggesting it is a biologically driven phenomenon.

It is therefore suggested that maximum concentrated levels of sound production and by proxy spawning, rely on optimum levels of light, temperature, salinity and high tide time relative to sunset. The correlation of sound production with environmental drivers or correlates suggests that circadian rhythm for *A. japonicus* is environmentally set. Further investigation into species behaviour across different habitats and environmental conditions will offer great information on the adaptability and optimum conditions for spawning. Thus possible fisheries management strategies, such as temporary closures (for example dusk until dawn), require investigation of not only species behaviour, but also environmental factors.

Levels of low tidal influx inhibit deeper water mixing and contribute to the rise in temperature. The summer trend of little or no intake of freshwater into the river system allows salinity to increase to marine levels and therefore the river is brought closer to preferred adult *A. japonicus* conditions (salinity) with a temperature more conducive to expending energy on spawning. Therefore the environmental variables dictating spawning activity are, to an extent, correlated.

The question of vessel noise affecting spawning levels of *A. japonicus* has been raised, however, weekday sound production levels surrounding sunset displayed little variation to weekend levels, when vessel noise is more prevalent (Figure 4.3.3). Studies have shown that in some situations vessels can pass directly above calling *A. japonicus* with little or no affect on the occurrence of the individual's calls (Parsons *et al.,* 2009). However, although it can be shown that vessel noise limited effect on the calls produced the study has not tested the ability of recipients to detect the call. It is possible that the predisposition to call is a behavioural response to environmental conditions and continues without regard as to whether or not calls can be heard. Successful courtship in such conditions would be difficult to observe. A reduction in the ability of animals to hear spawning calls due to masking by vessel noise may reduce the number of fertilised eggs released, and so stock recruitment success.

A. japonicus vocalise over seasons typically encompassing a change in water temperature of more than eight degrees. Connaughton *et al*. (2000) reported an increase in weakfish *(Cynoscion regalis)* spectral peak frequency of approximately 40 Hz over this temperature range. Figure 4.3.10 illustrated that the trend in call spectral peak frequency increased by approximately 30 and 20 Hz in the 2006-7 and 2007-8 spawning seasons, over the eight degree range. Therefore, early season vocalisation, at temperatures just exceeding the spawning threshold should contribute to lower spectral peak frequencies. Females of some species discriminate between males by call spectral peak frequency, with preference to lower frequencies (Myrberg and Spires, 1972, Myrberg *et al*., 1993) thus early season calling, in lower temperatures could produce the impression of a larger caller. As a result, it is conceivable that early season calling could be more advantageous to *A. japonicus* males. However, the decline in calling during minor drops in temperature opposes this hypothesis and requires testing.

4.3.5. Future Work

The long term deployment of sea-noise loggers, sampling various points along the Swan River will provide a spawning map for *A. japonicus* throughout the river system. More high resolution environmental data is required to accurately develop the relationship between temperature, salinity and spawning time at the hydrophone location. Once loggers are fitted with appropriate environmental recording equipment, optimum habitat conditions for spawning can be determined. It is anticipated that high resolution environmental data will facilitate the determination of accurate models describing SPLs deviance on both broad (temperature, salinity, time of sunset) and fine (lunar phase, tidal variation) scales. Multi-seasonal passive acoustic monitoring of fish vocalisation will be able to observe changes in behaviour in all the *A. japonicus* aggregations and identify preferential spawning site characteristics.

4.3.6. Acknowledgements

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4.4. Localisation of individual mulloway (*A. japonicus*) within a spawning aggregation and their behaviour throughout a diel spawning period.

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ABSTRACT:

Mulloway (Argyrosomus japonicus) are a soniferous member of the Sciaenidae family. During summer in the Swan River of Western Australia, individuals of this species form spawning aggregations in turbid waters around high tide, during late afternoon and early evening. Mulloway produce pulsed vocalizations that are characteristic of the species and to an extent of individuals. Crepuscular passive acoustic recordings of vocalizing mulloway were collected from a four-hydrophone array during March 2008. Arrival-time differences proved the most robust technique for localization. Corroboration of fish position was observed in relative energy levels of calls, surface-reflected path differences and relative range of successive calls by individuals. Discrete vocal characteristics of the tone-burst frequency and sound-pressure levels assisted the determination of caller identification. Calibration signals were located within a mean distance of 3.4 m. Three-dimensional locations, together with error estimates, were produced for 213 calls during a sample four-minute period in which 495 calls were audible. Examples are given of the movement and related errors for several fish successfully tracked from their vocalizations. Localization confirmed variations in calling rates by individuals, calling altitudes and the propensity to vary call structure significantly over short periods, hitherto unreported in this species.

4.4.1. Introduction

Knowledge of where and when fish spawn is crucial to manage the affects of fishing activities on population levels (Luczkovich *et al.*, 1999a). The location of such spawning areas is typically inferred through the capture of fish with reproductively active gonads or by sampling recently spawned eggs and larvae (Holt, 2002). Such techniques can adversely affect the studied population and may only provide a limited 'snap-shot' of information unless additional sampling is undertaken (Luczkovich *et al.*, 1999b).

For soniferous fish species an alternative method for determining the location and timing of spawning is to listen for and identify the source of the sounds produced during spawning. Sound production by fish has been categorized into several functions, including reproduction (Winn, 1964). Many species, such as haddock (*Melanogrammus aeglefinus*; Hawkins and Amorim, 2000) and damselfish (*Dascyllus albisella;* Mann and Lobel, 1998) exhibit differing vocal behaviour at various stages of courtship. In an environment where visual confirmation is not easy, observation of *in situ* vocal behaviour and the inferred activity of individuals provide valuable information on spawning patterns. For example, weakfish (*Cynoscion regalis*) and red drum (*Sciaenops ocellatus*) may form leks where males attract females through repetitive calling (Gilmore, 2002), as opposed to the oyster toadfish (*Opsanus tau*; Schwartz, 1974), which exhibits pair spawning. The passive recording of sound-production facilitates the observation of fish without survey-induced behavioural bias. Behavioural knowledge provides information needed for the management of exploited species, for instance by permitting proper timing of seasonal fishery closures designed to protect spawning fish (Luczkovich and Sprague, 2002).

Recently, techniques have been employed to map spawning locations from vocal behaviour (Luczkovich *et al*, 1999b, Hawkins, 2002, Luczkovich and Sprague, 2002, Holt, 2002). However, little research has been done to locate and monitor individual fish within an aggregation. With a rigid array containing at least two hydrophones located in the horizontal plane and a third offset in the vertical plane it is possible to locate a sound in three dimensions from arrival-time differences (Watkins and Schevill, 1972). However, three-dimensional localization of fish using passive acoustic tracking of vocal behaviour has rarely been reported, largely because a sufficiently large vertical separation of the hydrophones is required (Watkins and Schevill, 1972). Many spawning aggregations form in areas with complicated topography that affect sound transmission and require a thorough assessment of the acoustic properties of the location. For this reason it is necessary to test fish localization techniques in a natural, acoustically simple environment before they are applied to fisheries where aggregations may form in more complex surroundings, such as coral reefs.

The individually characteristic sounds of *Argyromsomus japonicus* are discernible from other conspecific calls and biological or anthropogenic noise (Parsons *et al.,* 2006a), thereby facilitating detailed studies of their behaviour. Evidence of both pair- and group-spawning behaviour of mulloway living in aquaculture ponds has been reported (Ueng *et al.*, 2007). Neither behaviour has, as yet, been confirmed in the spawning aggregation in Mosman Bay in the Swan River.

The main objective of this research was to locate and discriminate individuals within an aggregation using three-dimensional localization of their calls. A further aim was to assess behaviour such as mobility, position in the water column and the separation of callers throughout the spawning cycle, and particularly at various stages of courtship. In this manner the deployment of hydrophones for passive recording of fish vocalizations provides non-invasive, behaviourally unbiased comprehensive coverage of an aggregation site, compared with the unnatural behaviours that can be induced by the presence of survey vessels or fishing gear.

4.4.2. Methodology

Mulloway vocalizations were recorded in the Swan River between 18:00 and 23:59 on 5 March 2008, approximately seven kilometres upstream from the coast (Figure 4.4.1A). In Mosman Bay, the river banks descend rapidly to a 21 m deep channel comprising a sand/silt substrate, a few artificial reefs and several depressions, some of which are 23 m deep at high tide. The uniform silt-substrate riverbed has low acoustic reflectance (Jensen *et al.*, 1997). During the survey, calm wind conditions resulted in a flat water surface, suggesting that under survey conditions Mosman Bay was an acoustically simple site, suitable for testing localization of individual callers.

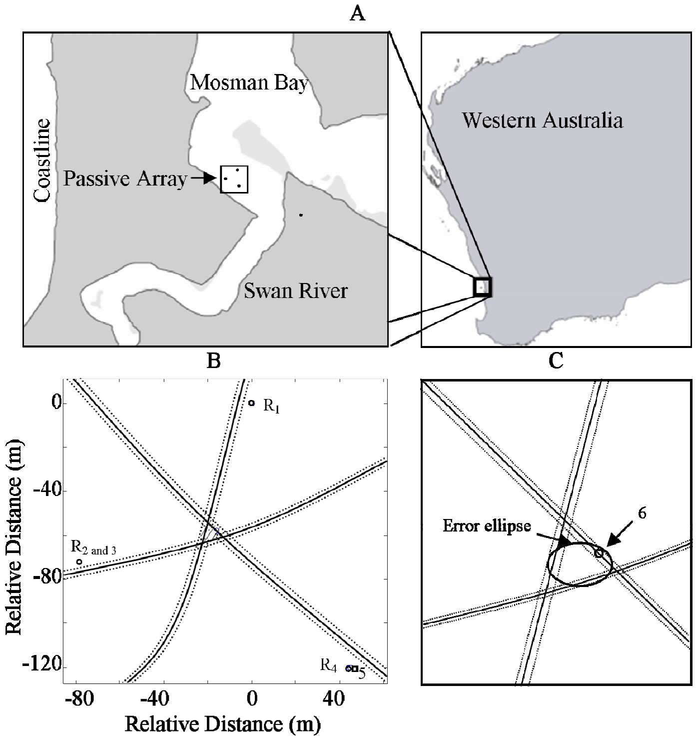

Figure 4.4.1. *Location of the passive hydrophone array within the Swan River, Western Australia (A). Two-dimensional location of an example signal with ellipses of confidence limits are illustrated (B). Four hydrophones ($R_{1\text{–}4}$) and implosion device (5) locations are displayed. Expansion of the error ellipse formed in localization and true position of speaker (C).*

Four omni-directional hydrophones were set in Mosman Bay, as an array bounding approximately 8000 m^2 (Figure 4.4.1B). One HTI–90U hydrophone (High Tech, Inc., MS, USA) was connected to a Centre for Marine Science and Technology (CMST, www.cmst.curtin.edu.au) – Defence Science and Technology Organisation (DSTO) sea-noise logger moored on the river bed (point R_1). Two hydrophones (HTI–90U and HTI–96min) were deployed from a moored vessel at depths of 5 and 10 m (points R_2 and R_3 respectively) and one (HTI–96min) from another moored vessel at 5 m depth (point R_4). Point 5 represents a time-synchronization device (a light bulb implosion every 30 minutes, at a depth of 5 m) and point 6 is an example of the location of an underwater speaker (Lubell Labs LL9162T). The speaker was deployed playing a pre-recorded, characteristically identifiable mulloway call at a maximum source level of 146 dB re 1 μPa at 1 m, at depths of 5 and 10 m, repeated approximately every six seconds. The speaker was deployed at various positions to aid calibration, depth-location accuracy and to estimate detection range under survey conditions. Locations of the hydrophones, speaker and implosion device were recorded with a Fugro Starfix HP8200 Differential GPS (±0.2 m) and throughout the evening DGPS fixes of the moored-vessel hydrophones were recorded to account for vessel movement. The sea-noise logger recorded for 25 minutes of every half hour, at a sample frequency of 10.417 kHz from 8 to 8000 Hz.

Signals from midwater hydrophones were recorded on Sony TCD–D100 DAT recorders. The two boats operated DAT recorders at maximum gain settings and sampled at 32 kHz. Recordings were digitized at a 92 μs sample interval (10.4166 kHz) before all datasets were processed in Matlab. Highpass (50 Hz) and lowpass (1500 Hz) filters were applied at various stages of processing to remove noise, then compared with unfiltered data to assure minimal sample offset. Post digitization datasets from the DAT recorders and CMST–DSTO logger displayed sampling-rate offsets and temporal drift; both inherent and thermally variable. Such variations were characterized in the laboratory by replicating the experimental ambient conditions and thermal variations during which a 1 kHz sine wave was continuously logged (as outlined in Appendix 4.1). Data from a fifth hydrophone, originally planned for redundancy in localisation calculations were rendered unusable by the presence of crosstalk with one of the vessel deployed hydrophones (Appendix 4.2).

The localization of call signals was conducted by calculating arrival-time differences in the first voltage-amplitude peak attributable to a call, referred to below as the Call Initiation Peak (CIP), as recorded by the separate hydrophones. The call was then localized in two dimensions using trigonometry, to produce hyperbolas that intersected at the call origin (Watkins and Schevill, 1972, Cato, 1998), using CMST designed Matlab functions. An example error ellipse for the location in two dimensions of a speaker signal is illustrated in Figure 4.4.1B together with estimated calculation errors (dotted lines) of CIP identification. A magnification of the error ellipse formed in this calculation is illustrated in Figure 4.4.1C. Standard errors of CIP sample choice were estimated based on signal-to-noise ratios, signal overlap with other calls and hydrophone position, the latter affecting expected surface reflections. Comparisons of peak-voltage amplitude, call energy (McCauley, 2001) and maximum power spectral densities (PSD) of a call, as recorded by the different hydrophones, helped confirm call locations and identify individuals (Parsons *et al*., 2006a).

In large-scale marine-signal localization, ray bending can create substantial path-length variations (Urick, 1983). However, when localizing whale calls at greater ranges relative to array dimensions than employed here, Wahlberg *et al.* (2000) determined that ray bending contributed errors an order of magnitude lower than those of receiver-position uncertainty. Sound-speed profiles taken at hydrophone locations on 8th March indicated a similarly negligible relative level of refraction at the ranges observed in this study.

Because of the sampling rates and array dimensions used, the optimal vertical offset of an individual hydrophone (corner of the array) required to provide vertical-caller position from arrival-time differences was at a greater depth than that available in Mosman Bay. For that reason a number of methods were evaluated for estimating the depth of a calling mulloway. These were: time difference between direct- and surface-reflected paths detected by one hydrophone (Cato, 1998, McCauley, 2001; Figure 4.4.2), as observed by the bottomed hydrophone and those at 5 and 10 m depth (R_1, R_2 and R_3, Figure 4.4.2 respectively); direct/surface-reflected signal levels detected by the bottomed hydrophone (R_1; McCauley, 2001); and signal-phase shift as detected by the two vertically separated hydrophones (R_2 and R_3). In the latter method, source range was assumed to be such that direct paths to each hydrophone were parallel. The elevation angle was estimated with $\theta = \cos^{-1} c\Delta t$, where c is the speed of sound and Δt is the detected time difference (Figure 4.4.2). Once calls had been localized and attributed to individual fish, monitoring of callers through time was undertaken to observe fish mobility and variation in call type.

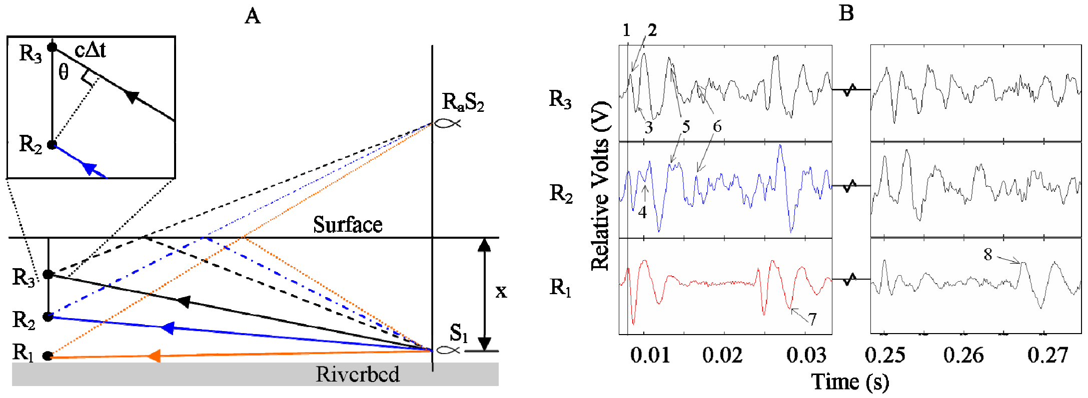

Figure 4.4.2. *Ray paths of a signal originating in a riverbed source propagating to three hydrophones at varying depths (A), with an illustration of the elevation-angle calculation (top left). Source* S_1 *at depth x and reflected source* (R_aS_2) *positions are illustrated. Waveforms of an initial (left) and final (right) swimbladder pulse with successive surface reflections as recorded by the three hydrophones (B). Points of interest are highlighted and explained in the text.*

Figure 4.4.2B illustrates the possible detection of surface reflections of a mulloway call by the two vertically offset hydrophones (R_2 and R_3). It also illustrates the complexity of surface reflection effects detected concurrently with swimbladder pulses. Point 1 highlights the CIP arrival-time used in localization: the signal from R_1 has been synchronized to that of R_2 for illustration purposes only. The equivalent direct-path peak in the top recorded hydrophone signal arrived at 2, corresponding to the additional path distance $c\Delta t$. At 3 the higher hydrophone detected the phase-inverted surface reflection

(Figure 4.4.2A), indicated by the change in waveform gradient. The corresponding reflection peak was detected by R_2 at 4. At 5 both hydrophones displayed constructive interference, increasing the waveform peak compared with R_1. The received surface reflections 6 continued and can be compared with the bottomed hydrophone, which exhibited no such variations in the direct-path signal until the first surface reflection arrived at approximately 7. In the right-hand waveforms the surface reflection of the final pulse is visible in R_1 at 8, when the other two waveforms only displayed residual reflections.

4.4.3. Results

4.4.3.1. Control Signal Localisation

Mulloway calls replayed while the Lubell speaker was positioned within the array (point 6 Figure 4.4.1A) were localized in two dimensions at a mean distance of 3.36 m (s.d. = 1.22, n = 16) from the actual source position and they appeared to be unaffected by speaker depth. Source depth was only determined by the phase-shift method as surface reflections were embedded within the call structure and not distinguishable from the effects of noise. The mean differences in the number of samples between the signal arrival at the upper and lower hydrophones were 0.8 samples (s.d. = 0.79, $n = 10$) at 5 m and 0.83 (s.d.= 0.75, $n = 6$) at 10 m, corresponding to depth estimates of 3.50 ±0.88 m and 7.4 ±0.93 m, respectively.

When the Lubell speaker was positioned outside the array, accuracy dropped and localization of the speaker signal was reduced to 5.53 m (s.d. = 2.79, n = 8) at 221 m from the farthest hydrophone. At greater distances (292 and 327 m) the speaker signal could not be localized and in several instances it was not audible above calling mulloway. Reduced performances in tests done outside the array were in part a result of increased numbers of calling mulloway at that time, increasing noise and overlapping fish calls.

Table 4.4.1 illustrates some standard deviations and error ranges for two-dimensional location and depths of sample speaker signals and mulloway long and short calls.

Coordinates are given relative to the bottomed hydrophone (Figure 4.4.1, R_1). Depth error was estimated only from the range-related elevation angle error, as a consequence of rounding sample difference to the nearest sample. The standard deviations and error ranges illustrate how the system accuracy reduced with range. Fish 3, for example, displays how depth-error ranges increased significantly when compared with the available depth.

4.4.3.2. A. japonicus call localisation

The results given below are taken from an example four-minute localization period at the beginning of the evening spawning cycle when the calling density was comparatively low. The reported results used the bottomed hydrophone as a reference point. During the four-minute period 495 audible calls of various types were recorded, 332 (67%) were determined to have identifiable CIPs on the three two-dimensional locating hydrophones (301 of which also produced depth estimates using the fourth hydrophone, R_3) and 213 (43%) could be localized in three dimensions to within 5% of the range (65 short and 148 long calls). Depth estimates revealed that short calls typically occurred higher in the water column (9.27 m above the riverbed, s.d. = 2.78) than long calls (4.38 m, s.d. = 2.58). Surface reflections of calls were often detected by all four hydrophones (Figure 4.4.2B, Points 3, 4 and 8); however, in midwater datasets they were embedded within the call structure and often only detectable as a gradient change in the waveform and not as an identifiable peak (Figure 4.4.2B Point 3). The bottomed hydrophone displayed the most distinct surface reflections (Figure 4.4.2B, Point 8) as these could often be observed after the call had ceased, comparable with other reports (McCauley, 2001). However, noise and density of calls often distorted estimation of the reflection-peak position and amplitude, reducing the accuracy of path-length difference and consequently that of depth estimates. For this reason, neither reflection arrival time nor energy levels were deemed suitable for primary estimates of depth and so were employed, where possible, to confirm the depths determined from R_2 and R_3 arrival differences.

Table 4.4.1. *Localisation characteristics of some speaker signals and A. japonicus short and long calls.*

Caller (call no.)	Two-dimensional Localised Coordinates (m)		Distance from Depth Estimate Hydrophones (m)	Depth Estimate (m) and error range (±m)	R_1 hydrophone recorded call intensity (dB re 1 µPa)
	x (s.d.)	y (s.d.)			
Speaker (1)	-15.96 (0.73)	-58.26 (1.21)	65.04	-3.13 (0.88)	115
Speaker (3)	-15.70 (0.75)	-57.41 (1.18)	65.41	-5 (0.89)	115
Fish 1 (1)	38.38 (2.29)	-41.28 (1.56)	120.81	-15.48 (3.10)	134
Fish 1 (67)	69.51 (4.07)	-63.74 (0.95)	148.21	-22.19 (4.74)	124
Fish 2 (1)	-18.18 (0.87)	-37.06 (1.20)	68.07	-8.93 (0.97)	113
Fish 2 (10)	-30.88 (1.1)	-18.30 (1.77)	71.74	-7.07 (1.07)	112
Fish 3 (2)	178.36 (3.87)	-154.13 (4.69)	224.76	-5 (10.48)	118
Fish 3 (6)	130.69 (4.58)	-191.91 (4.24)	283.50	-21.36 (16.84)	109

Repetitive calling was noted from several individuals, three examples of which are illustrated in Table 4.4.2. Bracketed values denote standard deviations and error ranges. Corroboration of the Fish 1 to 2 localizations can be seen in trends of call-energy levels with range and the comparative stability of successive call altitudes (Figure 4.4.3). The error ellipses of Fish 1 highlighted the proximity of emitted long calls to the riverbed (Figure 4.4.3B). In many cases, call overlap at the start or end for both points of the call rendered call-energy levels inaccurate. Consequently only 37 (48%) of Fish 1 calls and 12 (66%) of Fish 2 calls were analysed using energy levels. Energy losses were compared with spherical spreading (Cato, 1998) on a $20\log r$ basis where r is the slant-path distance. As such, over the calculated ranges Fish 1 and 2 were expected to display losses of 5.7 and 1.2 dB re 1 µPa. The best-fit curves displayed losses of 9.7 and 1.0 dB re 1 µPa, respectively (Figure 4.4.3A). Least-squares linear regressions for Fish 1 ($r^2 = 0.713$) and 2 ($r^2 = 0.31$) are illustrated in Figure 4.4.3A, together with the expected spherical-spreading curves. The oscillation of call-energy levels of Fish 1 around the spherical-spreading curve in Figure 4.4.3A indicated constructive or destructive interference with range. The Fish 1 calls exhibited near-spherical spreading losses whereas those of Fish 2 were less than that attributable to spherical spreading. The four analysed speaker signals, which originated in the same position, varied only by 0.4 dB re 1 µPa over the test period. Fish 1 varied its call in structure and length over the four minute period, ranging between one-part long calls (Parsons *et al.,* 2006) of minimum duration 0.276 s, comprising 11 swimbladder pulses and two-part long calls, of maximum duration 0.457 s, comprising 23 pulses.

Table 4.4.2. *Selected acoustic characteristics of three localised repetitive calling fish.*

Fish	Call type	No. localised / audible calls	Call interval (s) (s.d., max, min)	Behaviour		
				Swimming direction	Ranges (m)	Mean height (m)
1	Long	73/77	3.2 (0.9, 7.3, 1.8)	Downstream	56.4-108.8	2.65
2	Short	18/23	3.6 (0.7, 5.4, 2.1)	Upstream	45.4-25.7	8.54
3	Long	7/14	4.5 (1.2, 6.9, 3.6)	Downstream	158.1-270.5	5.35

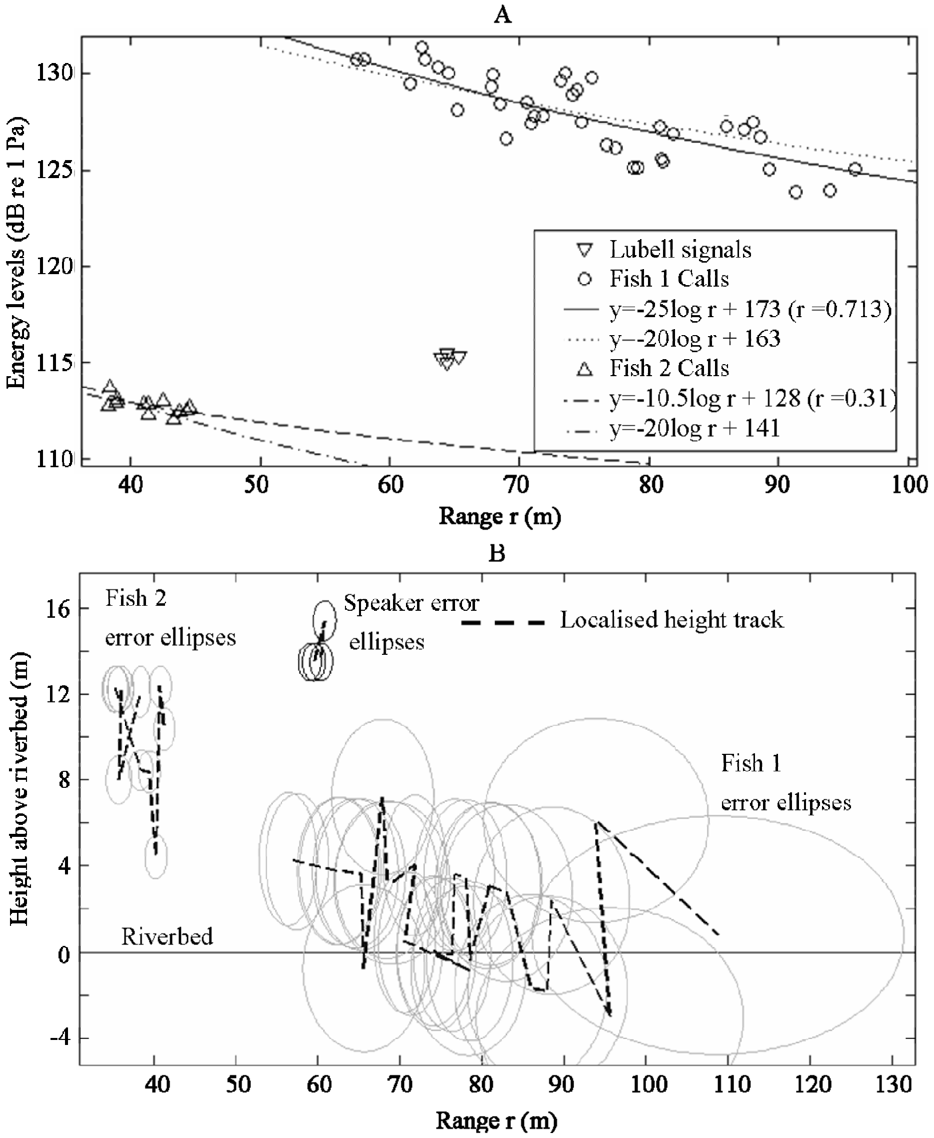

Figure 4.4.3. *Call energy levels of Speaker signals (▼), Fish 1 (○) and Fish 2 (▲) calls as recorded by the bottomed hydrophone plotted against range (A). Height of Speaker signals, Fish 1 and 2 calls with range from the bottomed hydrophone (B). Ellipses describe height error and maximum range variance.*

The localizations described above are comparable with other calls recorded within and around the array throughout the evening. Once calls had been attributed to individuals and location errors assessed, tracks were plotted of call-based fish movement (Figure 4.4.4). Selected calls have been chosen for ease of viewing. In Figure 4.4.4 the successive calls of three fish together with selected example calls from four other fish are presented to indicate the calling-population density. A plan view of Figure 4.4.4 is shown in Figure 4.4.5 which displays the horizontal locations, separation and movement of the fish. For each call the two-dimensional error ellipse was combined with the depth error range to produce a three-dimensional error ellipsoid within which the call originated. Shades of the ellipsoids vary with call-energy levels (cf the colour bar) as determined by the received intensity at the bottomed hydrophone. During the sample period a minimum range of approximately 35 m was observed between calling individuals.

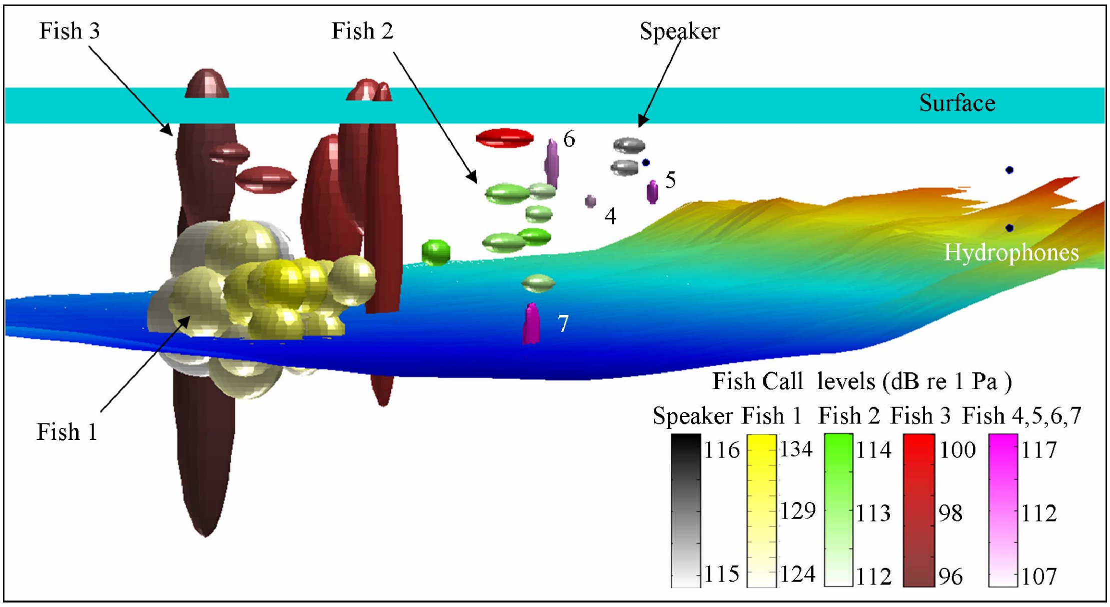

Figure 4.4.4. *View from the northwest of selected call locations of the speaker, Fish 1, 2, 3 positioned over the bathymetry of Mosman Bay (depth has been exaggerated by 10%). Single calls from Fish 4, 5, 6 and 7 are also shown to provide an impression of caller density. Dimensions of each ellipsoid are determined by localisation variance and error ranges. Colouring of each ellipse reflects the received sound pressure level and therefore relative range from hydrophone* R_1. *All four hydrophone positions are shown as black spheres.*

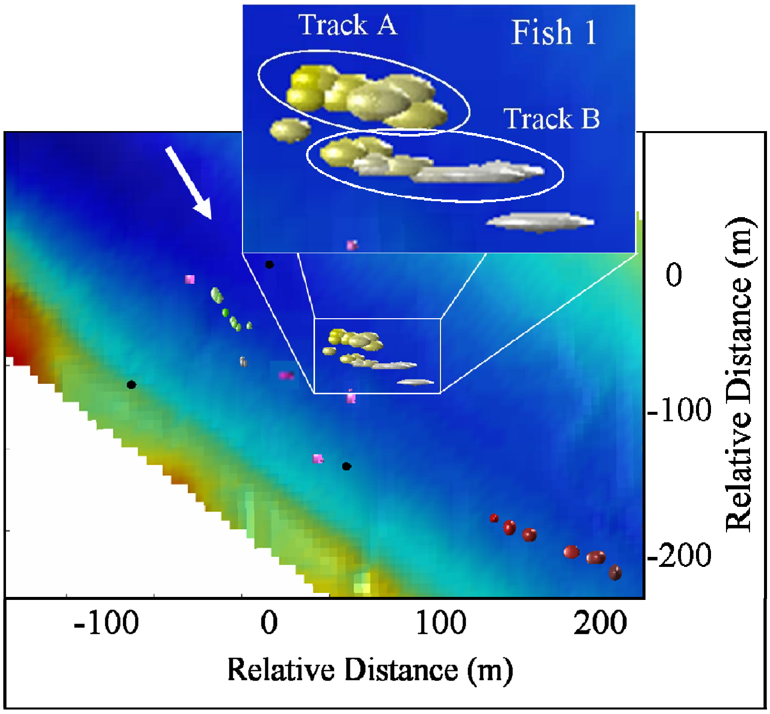

Figure 4.4.5. *Plan view of Figure 4.4.4 with a white arrow illustrating direction of view in Figure 4.4.4. Insert highlights selected localisation ellipsoids of Fish 1calls, highlighting the change in track pattern (A and B) which occurred as a vessel passed overhead.*

Fish 1 travelled downstream along the depth contours (localised calls began at the top left of the insert and ended at the bottom right), at an average rate of 0.36 m s^{-1} in the deeper part of the river. Fish 1 changed movement from the right hand end of Figure 4.4.5 Fish 1 Track A to Track B when a ski-boat, towing a water-skier, passed overhead. However, on two other occasions during the sample period, the same ski-boat passed twice above Fish 1 without inducing any visible changes in travel direction. On three occasions the vessel noise completely masked a period when Fish 1 expectedly would have called. Fish 2 and 3 travelled at 0.26 m s^{-1} upstream and 2.04 m s^{-1} downstream, respectively. Behavioural differences were evident in call altitudes (Figure 4.4.3B). Fish 1 remained consistently within a few metres of the riverbed whereas Fish 2 called from midwater. The remaining fish in the example period produced insufficient calls to determine travel direction.

During the evening spawning cycle, in general, long calls were initially located downstream of the hydrophone array. With time an increasing number of callers appeared around the array, then farther upstream. As caller density increased, the ability to discriminate callers was reduced due to similarities between fish, variability of individual-fish calls, background noise of other callers and increased numbers of overlapping calls. For that reason, once a fish ceased vocalizing it became increasingly difficult to re-identify the same caller's position.

Extrapolation of the Fish 1 calls based on spherical spreading and early evening ambient noise (~110 dB re 1 μPa) gave a maximum detection range of approximately 1000 m for a riverbed-positioned long call with a source level greater than 170 dB re 1 μPa. However, CIP identification could only be estimated at approximately 400 m based on the signal-to-noise ratios seen at the time of the Fish 1 calls. Variance of the estimated *x–y* coordinates increased significantly with range from the array (Table 4.4.1 and Figure 4.4.4, illustrated by error ellipsoid size vs. range). The maximum array-localization range could not be calculated because it was impossible to confirm positions.

The riverbed of the Mosman Bay area comprises silt substrate of estimated compressional sound speed ~1600 ms^{-1} (Jensen, 2000). When considering a calling fish and hydrophone, both positioned on or near the riverbed, the difference in sound speed in water and substrate would result in a head wave of the call arriving at the hydrophone prior to the waveform propagating through the water. Evidence of the head wave would then exist in the waveform as a pre-cursor to the call, similar to that of airgun signals in seismic surveys (Brekhovskikh and Lysanov, 2003). Calls of Fish 1 were localised moving from approximately 55 to 110 m range from the hydrophone and due to differing ranges the arrival-time difference between a head wave and waterborne wave would be expected to vary between calls. No evidence of head waves was found during analysis of these calls.

4.4.4. Discussion

Results from the Lubell speaker signals within and close to the array confirmed that calls could be localized from arrival-time difference to within a few metres, a range within allowable confidence limits when considering noise and vessel drift. The source level of the Lubell speaker was quiet compared with calling mulloway (146 and an estimated 170 dB re 1μPa at 1 m, respectively), reducing the relative performance of control tests. Characteristics of the replayed call, such as structure and dominant frequency, chosen to ease the identification process against other calls increased the bias in the accuracy of CIP detection of the signal start. The number of actual calls localized (43% of example audible calls, during early spawning) promoted the arrival-time difference technique above other energy-level ratio and surface-reflection methods, which were more affected by noise and call structure.

System performance within and close to the boundaries of the array was demonstrated by the Fish 1 calls. Signals easily discernible on all four hydrophones, were successively localized within metres of each other. However, even at close range, not all Fish 1 and 2 calls could be located accurately (4 and 5 calls respectively), as a result of overlapping calls distorting the CIPs. The success of this technique consequently depends on ambient noise and coherence of conspecific calls. Sprague and Luczkovich (2004), for example, estimated a greatly reduced maximum detection range of silver perch (*Bairdiella chrysoura*) calls given a lower signal-to-noise ratio. Further confirmation of the system performance can be seen in the energy levels of the speaker signals and Fish 1 and 2 calls as energy/range trends are similar to those of spherical spreading losses (Figure 4.4.3A). Minor variations can be attributed to localization errors, selection error of CIPs or natural variation in call intensity. The evidence of constructive/destructive interference displayed by Fish 1 calls suggests that the energy method of call localization is inappropriate for fine-scale locating of species that produce tone-burst calls, such as mulloway, as the interference alters the perceived call intensity depending on the range, depth and topography. That 44% of Fish 1 calls provided results from energy-level ratios re-affirms that the arrival-time difference technique (94% of Fish 1 calls identified) was more appropriate for localizing fish. The low best-fit confidence and the deviation from spherical spreading for Fish 2 can be attributed to the few data points collected within a confined range.

Localization performance decreased with increasing range in agreement with previous reports (Cato, 1998), as illustrated by the increased coordinate variance and decreasing Lubell localization with range. This is a consequence of smaller arrival-time differences relative to the array dimensions combined with decreased signal-to-noise ratio. The optimum situation is a trade-off between increasing array dimensions while maintaining detection of initial call peaks from within the array. Larger hydrophone spacing improves the localization accuracy and maximizes range, but decreases the coherence of signals between the receivers.

Techniques based on surface reflections and energy levels proved insufficiently consistent to determine source depth. Surface reflections were predominantly embedded within the call structure or disrupted by a following call. Although the reflections could be observed in waveforms, the accuracy required to determine the call depth was often unachievable. For that reason the method of arrival-time differences between vertically separated hydrophones is recommended as the primary technique to estimate depth. However, the ratio of hydrophone separation to call range was small, so that minor changes in arrival-time detection between hydrophones had a significant affect on the depth estimation.

Later recordings displayed increased densities of callers. The increased presence of overlapping calls inhibited CIP detection of many calls. This overlapping limits the possibility of biomass estimation by call counting because the number of callers is underestimated. A possible solution is to observe CIPs with an increased sampling rate, giving better temporal resolution, in this manner allowing gradient-change analysis to help detect CIPs within the structure of another call.

Results so far suggest that although fish may move while vocalizing, the spatial separation between callers is maintained (Figure 4.4.4). Individuals were localised while fish emitted long calls from, or near, the riverbed (Figure 4.4.3B), in the deeper areas of the channel. The callers appeared to move slowly along, rather than across, depth contours. While the fish are moving slowly they do appear to retain separation between the displaying males. Additionally, the fish movement may be a result of swimming against the tidal current present at the time of calling such that maintaining exact position is unfeasible. Although the aggregation occurs in dark, turbid waters and the callers may not visually observe each other, they can audibly compare displays, thus this aggregation fits the 'classical' lek description suggesting that Mosman Bay mulloway spawn in pairs after a female has been attracted to male calls. This behaviour is similar to the lekking behaviour of other species, such as Atlantic cod (*Gadhus morhua*) reported by Nordeide and Folstad (2000). Slow, demersal and along stream movement also agrees with the increasing or decreasing waveform amplitudes of single-caller sounds described in previous reports (Section 4.2), confirming this as a regular spawning behaviour.

The tracks of Fish 1 calls illustrated that vessel noise, even when directly above the fish, did not prevent calling, although there is evidence to suggest that such noise may, on occasion, cause minor relocation. It also confirmed the nearly complete masking of several calls. Although these calls did occur, possibly as a biological response to spawning conditions, it cannot be confirmed that they were audible to nearby fish. Call rates of individual fish and also their variation with time were observed. Such details are often recorded as a standard measure (Section 4.2), but Fish 1 exhibited substantial variation in the timing of calls. Fish 1 was able to vary the length of calls, number of pulses and the structure between Category 2 calls, as previously reported in Section 4.2,. This variation is neither a physiological phenomenon nor a means of discrimination between callers, but it does exemplify the variation of vocal repertoire of individual fish.

By comparison, short calls were located higher in the water column (Figure 4.4.3B) indicating fish in a mobile state. It is thought (Parsons *et al*., 2006a) that the short calls produced by mulloway at the time of the Fish 2 calls (early in the spawning cycle) are a preparatory signal to gather males before spawning and may involve a hierarchy of territorial dominance at the beginning of spawning. The higher position of the short calls within the water column and the mobility of the source fish agree with this behaviour.

Localization revealed that the aggregation formed downstream of the hydrophone array and either moved or expanded upstream as the evening progressed. This movement was concurrent with the increased density of callers. Further studies should reveal whether this behaviour is a selection of particular habitat features when spawning, or increasing caller density that compels late arrivals to call from locations farther upstream.

4.4.5. Conclusions

Research on the localization of marine animals is often reported. However, detailed, accurate monitoring of individuals and observation of their behaviour are less readily available. To our knowledge this paper is the first report of fine-scale localization of calling individuals within an aggregation of spawning, soniferous fish using a passive array of hydrophones. The techniques and example calls detailed above illustrate the ability of passive acoustic localization to provide behaviourally unbiased, *in situ* information on fish position, movement, conspecific interaction and response to anthropogenic affects, such as vessel presence. Long-term observations should reveal their reaction to environmental trends such as temperature, salinity and tidal variations. Once baseline knowledge of vocal and movement behaviour has been gained, this technique can be employed to observe natural response of individuals and species to ecosystem variations, anthropogenic or environmental, and therefore provide invaluable advice for management decisions from both fishery and aquaculture perspectives.

This survey has highlighted the effects of localizing a tone-burst signal with amplitude modulation in a shallow-water environment using arrival-time and energy-level differences. Arrival-time difference techniques have been more robust for localization because of the effects of multi-path interference, background noise and overlapping calls, often leaving the CIP as the only unaffected call feature. Further, hydrophone synchronization, sampling frequency, sensitivity and relative positions are all factors requiring further consideration before future surveys are done.

4.4.6. Acknowledgements

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4.5. *In situ* source levels of mulloway (*Argyrosomus japonicus*) calls.

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ABSTRACT

Mulloway (Argyrosomus japonicus) in Mosman Bay, Western Australia produce three call categories, divisible into several types of call associated with different spawning behaviours. The determination of call source levels provides significant information on the range at which a caller may be heard by the intended recipient. The contribution of call source levels to overall recorded sound pressure levels is a significant step towards estimating the number of calling fish within the detection range of a hydrophone. In March 2008 an array of four hydrophones was deployed to record and locate individual fish from call arrival-time differences. Successive calls by individual mobile fish produced samples at various source ranges. Calls used for source level measurement were located between 1 and 100 m from the hydrophone. The three dimensional localisation of calls together with the removal of ambient noise facilitated the determination of source levels for each call category from observed trends in propagation losses and interference. Calls of various individuals were compared to estimate a species range of source levels for each call category. Mean source levels (mean squared pressure) of the three call categories were calculated as: 163 dB re 1µPa for Category 1 calls (short call of 2-4 pulses); 172 dB re 1µPa Category 2 (long call of 11- 32 pulses); and 157 dB re 1µPa for Category 3 calls (1-5 pulse calls of decreasing pulse repetition frequency).

Keywords: *source level, soniferous, range, localization, passive acoustics*

4.5.1. Introduction

A method of estimating the number of fish vocalising within the detection range of a hydrophone is to measure the contribution of individual callers to the overall sound pressure levels (SPLs) (McCauley, 2001, Sprague and Luczkovich, 2002). *A priori* knowledge of species call source level ranges is therefore required in order to achieve caller density estimates (Sprague and Luczkovich, 2004). In addition to source level, the transmission of a call in its local environment requires quantification to assess the proportion of emitted energy which is received by the hydrophone (McCauley, 2001).

In order to quantify a signal source level it is necessary to isolate energy associated with the call and the range of the source from the receiver. Although *Argyrosomus japonicus* often spawn in groups (Ueng *et al*., 2007) and in the wild often produce high densities of overlapping calls (Section 4.4), during the commencement of an evening spawning in Mosman Bay cycle calls are of sufficiently low density to offer the opportunity to monitor individual fish and identify each call (Section 4.4).

The accurate measurement of source level requires knowledge of range with source ground truth data. Range can be easily observed *in aquaria*; however, internal tank reflections and reverberation contribute significant complications towards source level calculation. *In situ* source levels are rarely reported (Cato, 1998, McCauley, 2001), and to the author's knowledge only visually confirmed at a reference distance of 1 m once, for one individual Sciaenidae, a silver perch (*Bairdiella chrysoura*; Sprague and Luczkovich, 2004), fortuitously caught on camera at the time of vocalisation. For Sciaenidae, this is in part due to spawning, and therefore vocalisation in turbid, post sunset waters, inhibiting discrimination of source range by visual methods. Thus the identification of source position or range remains the restricting factor in the accurate determination of *in situ,* fish call source level.

Fish calls offer significant information about the caller to the intended recipient and inadvertently, the observer. The size of the individual can be related to both the call source level (McCauley, 2001) and spectral peak frequency (Connaughton *et al*., 2000).

However, calls emitted by the same fish are not all necessarily of the same source level. For example, Lagadere and Mariani (2006) proposed that the short grunts of *A. regius* are weaker than the long grunts. Similar results from *in situ A. japonicus* calls have been observed, but it was not confirmed whether intensity differences were due to range, weaker swimbladder twitches, or multiple ray-path interference (Section 4.2). Therefore a measured range of source levels may be required to categorise a fish call. It is the aim of this study to determine source level ranges for each category of call exhibited by *A. japonicus* in the Mosman Bay aggregation.

4.5.2. Methods

On 5th March, 2008 a passive acoustic hydrophone array was deployed in Mosman Bay, Swan River, Western Australia in order to localise individual fish within a spawning aggregation of *A. japonicus.* Calibrated, omni-directional, HTI 90-U and 96-min hydrophones were attached to Sony Digital Audio Tape (DAT) recorders or autonomous sea-noise loggers developed at the Centre for Marine Science and Technology (CMST), Curtin University of Technology, Western Australia and Defence Science and Technology Organisation (DSTO). The deployment encompassed a single evening's spawning period of *A. japonicus* in approximately 20 m of flat water above a relatively uniform silt substrate riverbed. Recordings were sampled at 10, 412 Hz and time synchronised on a half hourly basis. Calls were localised by the arrival time difference technique (Cato, 1998) and error ellipses provided for each call. The localisation of calls from the array determined source ranges (the details of which are outlined in Section 4.4), from which source levels were determined. To minimise noise error, calls analysed for source level in this study were recorded at a time of relatively little background noise (Figure 4.5.1).

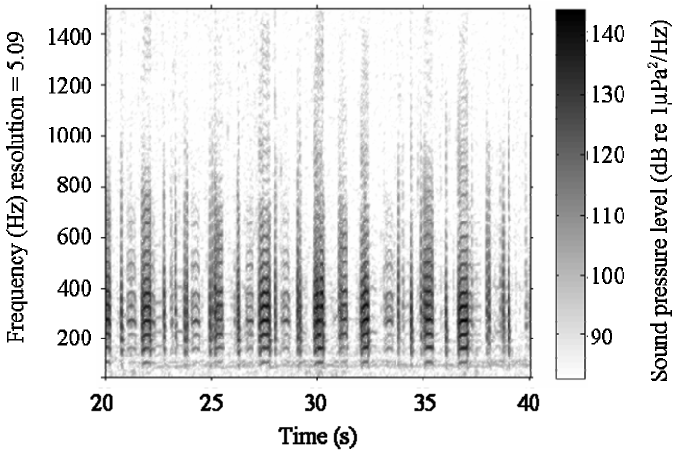

Figure 4.5.1. *Spectrogram of 20 seconds of A. japonicus call period from a hydrophone positioned on the riverbed in 18 m of flat water. Individual A. japonicus calls (grey-black areas) are clearly visible above minimum background noise (white areas). Spectrum produced using a 1024 point Hanning window with 0.5 overlap. Spectrogram frequency resolution was 5.09 Hz.*

Example waveforms of the three *A. japonicus* call categories are shown in Figure 4.5.2. Further details on characteristics of each category can been found in Section 4.2. Each of these call categories were analysed separately to observe variations in source level.

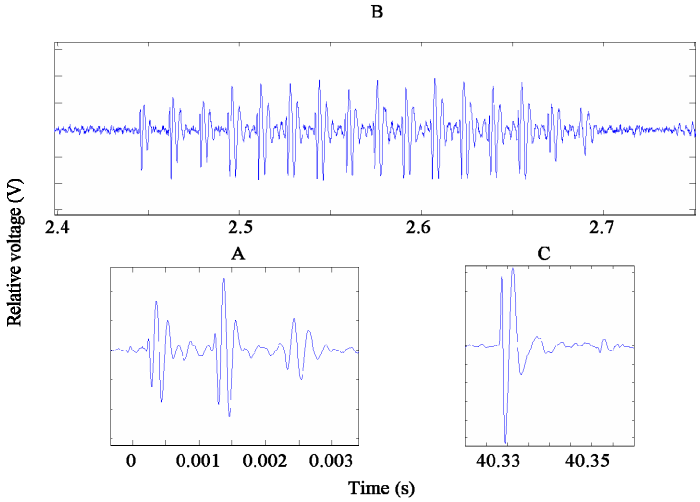

Figure 4.5.2. *Example waveforms of Category 1 (A), 2 (B) and single pulse Category 3 (C) calls as recorded by a bottomed hydrophone.*

To accurately determine fish call source level it is necessary to first remove the background noise. For this purpose *A. japonicus* calls and background noise were considered as incoherent signals. By Parseval's Theorem the time averaged squared total pressure recorded by the logger was equal to the sum of the time averaged squared partial pressure of each constituent signal in the form of:

$$p_{av}^2 = \left(p_c^2\right)_{av} + \left(p_{bg}^2\right)_{av} \tag{4.2}$$

where p_c is the partial pressure of the fish call, p_{bg} is the background partial pressure and subscript "av" indicates a time average (Sprague and Luczkovich, 2004). The level of fish call once background noise was removed (C_f) was given as:

$$C_f = 10\log_{10}(10^{\frac{L_{c+n}}{10}} - 10^{\frac{L_n}{10}}) \tag{4.3}$$

where L_{c+n} was the dB re 1 μPa of the overall signal and L_n was the background noise level (McCauley, 2001).

The method of call energy level analysis in this study employed standardised techniques and theory outlined by McCauley (2001).

For a plane wave, which is taken to be a good approximation for recordings in this thesis, the energy per unit area (or "energy flux"), over the duration of a signal where T_0 and T_e denote the signal start and end, is given by:

$$\frac{E}{A} = \int_{T_0}^{T_e} I dt = \int_{T_0}^{T_e} \frac{p_{s+n}^2(t)}{\rho c} dt \tag{4.4}$$

where E = signal energy; A = unit area; and I = intensity, defined by the combined signal and noise pressure p_{s+n}, and the acoustic impedance ρc. If the signal start and end points are the same, then the mean intensity or mean squared pressure values ($\overline{p^2}$) can be derived as:

$$\overline{p^2} = \frac{1}{T}\int_{T_0}^{T_e} p_{s+n}^2(t)dt \tag{4.5}$$

where T is the signal length, between T_0 and T_e. Thus, from equation 4.5.1 the 'equivalent signal energy', $E_s(t)$ is given by:

$$E_s(t) = \int_0^T p_{s+n}^2(t)dt - \int_{T_n}^{T_n+T} p_n^2(t)dt \tag{4.6}$$

and the peak to peak pressures (p_{pp}) are given as:

$$p_{pp} = \max\left(p(t)\right) + \left|\min\left(p(t)\right)\right| \tag{4.7}$$

where max($p(t)$) and min($p(t)$) were the respective maximum positive and minimum negative values from the pressure waveform (McCauley, 2001).

Figure 4.5.3 visually displays three of the steps involved in analysing the acoustic pressure attributable to a call and determining the frequency band over which the energy occurs. A digitised segment of the recording, including the call and encompassing a minimum of 500 sample points either side of the call, was converted to pressure waveform (Figure 4.5.3A). An estimate of the equivalent energy function E_s (t) was calculated as a vector of cumulative "equivalent energy" from:

$$E_s(t) = T_s \sum_{i=1}^{i=n} (p_{(s+n)_i}^2 - \overline{p_n^2}) \tag{4.8}$$

where $p_{(s+n)_i}$ was the i$^{\text{th}}$ element of pressure waveform, n was the last sample point and $\overline{p_n^2}$ was the mean squared noise pressure from noise samples obtained from the level of p^2 immediately before or after the call (McCauley, 2001). Mean-squared pressure within the 5 and 95% region of the total cumulative energy curve (Figure 4.5.3B) was then calculated (Malme *et al*., 1986) thus standardising the averaging time to that at which 90% of the energy from the entire signal (less noise) had passed. The call length was taken as the time for 90% of the signal energy to pass. A power spectral density of each call was produced to observe spectral peak frequencies compared with each calculated energy level (Figure 4.5.3C) and aid confirmation of repetitive calling by individual fish.

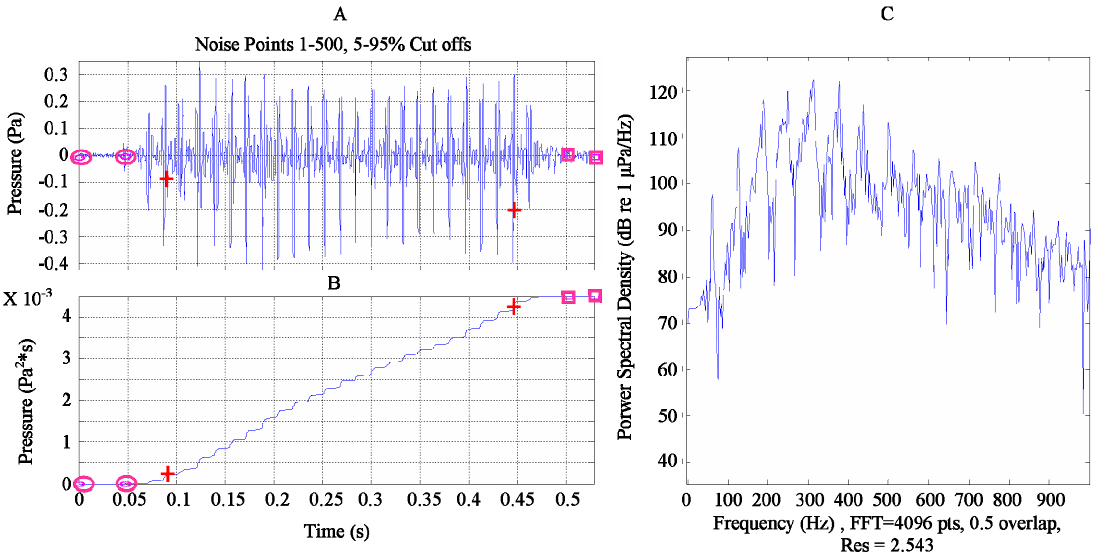

Figure 4.5.3. *Example sound pressure level calculation of an A. japonicus Category 2 long call. A) Waveform of example call with noise removal zone 500 points before (circles) and after (squares) shown. Crosshairs mark the 5 and 95% energy points within the analysed region. B) Cumulative energy of the call showing pressure squared per second with 5 and 95% region markers shown. C) Power spectral density of call. Process developed by R. McCauley, Centre for Marine Science and Technology, Curtin University from techniques described by Malme (1986) and Greene (pers. comm.).*

Trends from the plotted SPLs against $\log_{(10)}$(range) were calculated using least squares linear regression to compare observed transmission losses with that of estimated from spherical (20log(range)) and cylindrical (10log(range)) spreading where r is measured in metres (Urick, 1983). Although surface reflections were observed, in the context of source level calculation spherical spreading was considered as a minimum estimate for transmission losses expected in 20 m depth water at ranges of less than 100 m. The estimate of losses due to geometrical spreading provides a minimum loss on which to base initial calculations (Sprague and Luczkovich, 2004). Losses due to absorption and water movement were considered of negligible impact.

4.5.3. Results

Localisation of calls from the hydrophone array data reported in Section 4.4 produced the locations of 213 calls (65 and 148 Category 1 and 2 calls respectively). Several calls of each category were deemed unreliable for source level analysis due to overlap in adjoining calls, background vessel noise, or insufficient number of sample points for noise removal. The remaining calls, and others subsequently localised after the study in Section 4.4, which contained sufficient sample points either side of the signal for background noise analysis, were analysed for SPLs. Of the total localised calls 53 Category 1 and 112 Category 2 calls at ranges of between approximately 20 and 100 m offered signals of sufficient clarity to analyse SPLs. One fish in particular was tracked and reported in Section 4.4, which eventually produced 65 calls for SPL analysis in this study.

Figure 4.5.4 illustrates the effects of transmission loss with range of Category 1 calls. Source levels and regression determined transmission loss of call categories are shown in Table 4.5.1. Category 1 call waveforms originating from different fish were often disparate in structure. The first pulse in many waveforms was of lower amplitude than the second, while other calls displayed pulses of equal amplitude. The resultant linear regression of SPLs from all the 53 Category 1 calls gave a mean squared pressure source level of 163 (±15.5 95% confidence limits in the dB domain) dB re 1µPa and estimated spreading losses of 25.39 log(r) (Figure 4.5.4, Table 4.5.1). The variation in waveform structure was highlighted by the relatively low correlation with the transmission loss curve when using an equation of best fit of the form RL = AlogR + B, ($R^2 = 0.412$).

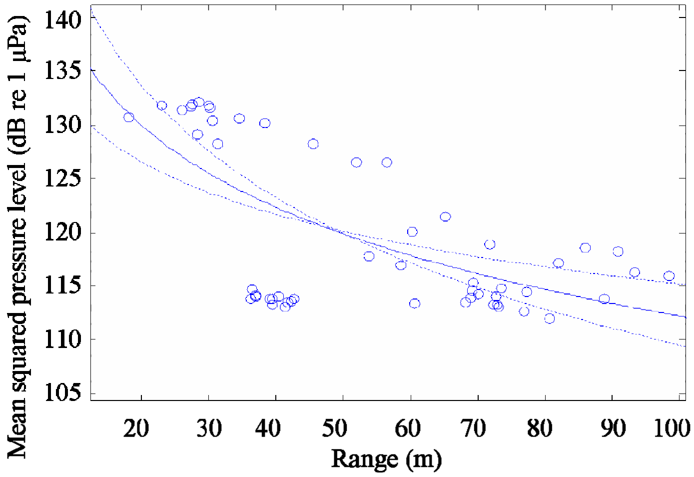

Figure 4.5.4. *Detected sound pressure levels with range for 53 Category 1 calls. Continuous line illustrates linear regression model of transmission losses with 95% confidence limits of source level shown (dotted lines).*

Table 4.5.1. *Extrapolated call sources levels for each category of A. japonicus call from least squares linear regression. Values display source levels (mean squared pressure) and equivalent spreading losses together with 95% confidence limits and the curve correlation with data points.*

Call Category		Orientation	Number Calls	Source Level (dB re 1 µPa) (± 95% confidence limits)	Transmission loss (log(r)) (± 95% confidence limits)	R^2
Category 1	All	N/A	53	163 (147.7, 178.6)	-25.39 (-34.55, -16.24)	0.4214
Category 2	Individual	N/A	65	172 (163.3, 180.2)	-23.94 (-29.69, -17.4)	0.6081
	All	N/A	112	172 (168.4, 176)	-23.74 (-25.89, -21.58)	0.8235
Category 3	All	N/A	28	157 (154, 160.3)	-23.04 (-26.62, -19.47)	0.8756
	One pulse	Towards	7	156 (150.7, 161.9)	-18.67 (-26.4, -10.94)	0.8853
		Away	4	152 (144.2, 158.9)	-19.17 (-27.19, -11.15)	0.9814
	Two pulse	Towards	3	163 (98.25, 226.7)	-27.53 (-101.7, 46.62)	0.957
		Away	10	154 (150.1, 158)	-18.81 (-23.52, -14.1)	0.9271

Category 2 calls exhibited less variation in calculated source level than those of Category 1 (Figure 4.5.5) and 95% confidence limits produced a source level range of only 7.6 dB re 1µPa around the determined mean of 172 dB re 1µPa from all calls (Table 4.5.1). SPLs from all calls exhibited similar ray multi-path interference features to those observed from the tracked individual (Section 4.4), evident as the received signal level oscillating with range. However, interference appeared to be more pronounced towards ranges between 50 and 100 m (i.e. see Figure 4.5.5 black dotted line). Correlation with the transmission loss curve of all Category 2 calls and those of the individual caller alone were higher than that of Category 1 calls (Table 4.5.1).

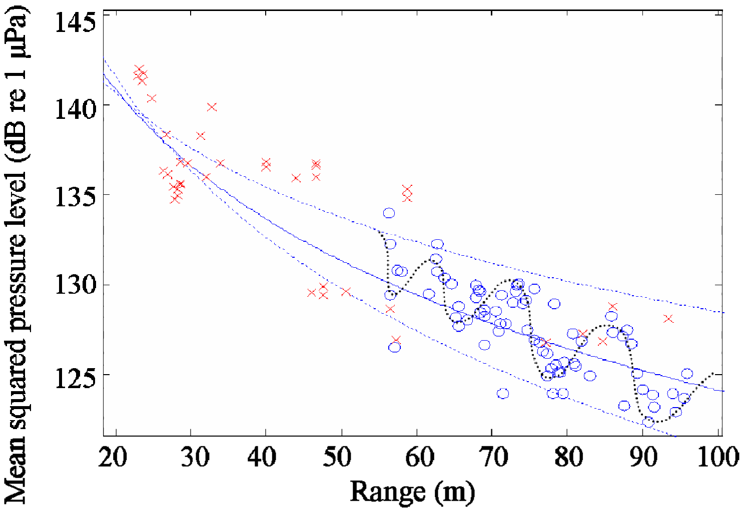

Figure 4.5.5. *Detected sound pressure levels with range for Category 2 calls. Calls of a tracked individual fish (○) and those of all remaining fish (x) are shown. Continuous line marks the linear regression determined transmission losses with 95% confidence limits (dotted lines). Possible interference trend is hsown by black dotted line.*

Ground truth data of Category 3 call ranges posed fewer complications. During localisation recordings an individual fish emitting Category 3 calls approached the bottomed hydrophone. The range at this time was determined by geometry from the first surface reflection received by the hydrophone and the known water depth. Section 4.4 highlighted the difficulty in observing surface reflections within multiple pulse calls.

However, Category 3 calls of *A. japonicus* often comprised only one or two swimbladder pulses. At such close range, high pulse repetition rate, and water depth initial surface reflections were clearly visible after the call was completed. As the call was emitted within a metre of the hydrophone, the fish must have been swimming close to the riverbed and it was assumed that the fish continued at the same depth. The individual provided both single (n = 11) and double (n = 17) pulse calls, at a variety of ranges (though Category 3 calls of a higher number of pulses were not analysed due to issues in confirming range).

As the fish swam past the hydrophone, recordings provided a comparison between orientations of head towards and away from the receiver based on the assumption that the individual was swimming forwards and not drifting with the current at an alternate orientation. It is possible that the fish altered direction during the course of its calling and did not swim directly towards and away from the hydrophone. However, the consistency of call time with range suggests that a direct route was the case, at approximately 0.5 ms^{-1}. Figure 4.5.6 displays the source level with range for a fish swimming towards, and past, the hydrophone. The o and x indicate 1 and 2 pulse calls respectively, emitted by the same fish as it approaches (blue) and departs (red) from the hydrophone. Once the fish was approximately 10 metres past the hydrophone the calls were emitted in such quick succession with very little range variation, that neither toward or away orientation was assumed.

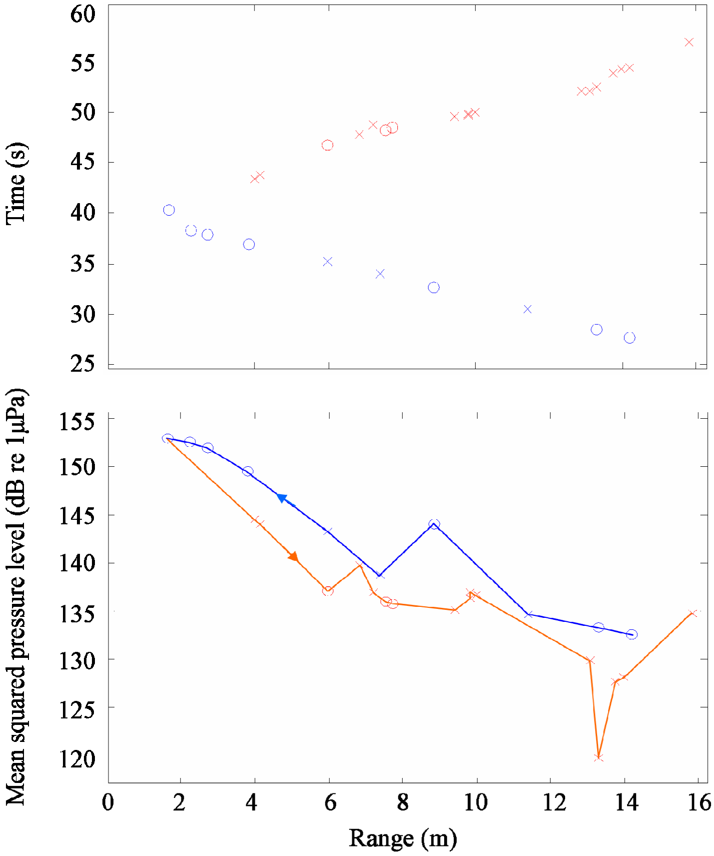

Figure 4.5.6. *Time of fish calls with range highlighting the order of 1 pulse (o) and 2 pulse (x) Category 3 calls, as the fish approached (blue) and then passed (red) the hydrophone (top). Sound pressure levels against range as detected by the bottomed hydrophone (bottom). The order of calls is indicated by arrows. Many calls not suitable for range analysis have been omitted.*

The regression model for all Category 3 calls produced a source level of 157 dB re 1µPa (95% confidence limits of 154 and 160.3), comparatively lower than both Category 1 and 2 calls. Calculated source levels of calls employing two swimbladder pulses in both orientations were greater than those of the single pulse calls (6.2 and 2.4 dB re 1µPa greater towards and away from the hydrophone respectively) and in both types of call the source levels were greater with fish direction orientated towards the hydrophone (greater by 4.7 and 8.5 dB re 1µPa greater for single and double pulse calls respectively) (Table 4.5.1, Figure 4.5.6). Calls from both orientations displayed a high correlation with the determined transmission loss curve (Table 4.5.1). The lower correlation observed when all Category 3 calls were tested together against transmission losses corroborates the received SPL difference due to fish orientation (Table 4.5.1).

Figure 4.5.7 shows the distribution of mean squared pressure source levels back calculated from the recorded SPLs and range, based on spherical spreading losses to the receiver only. It is thought that the tight distribution of the Category 2 calls was due to the fact that more than half the calls were emitted by one fish. By comparison, the Category 1 call source levels were derived from a number of fish. In addition, the variability observed in short calls (between 1 and 4 pulses) has a greater effect than that found in Category 2 long calls (11-32 pulses). The emissions of Category 3 calls, by a fish facing towards and away from the hydrophone, affected the source level distribution.

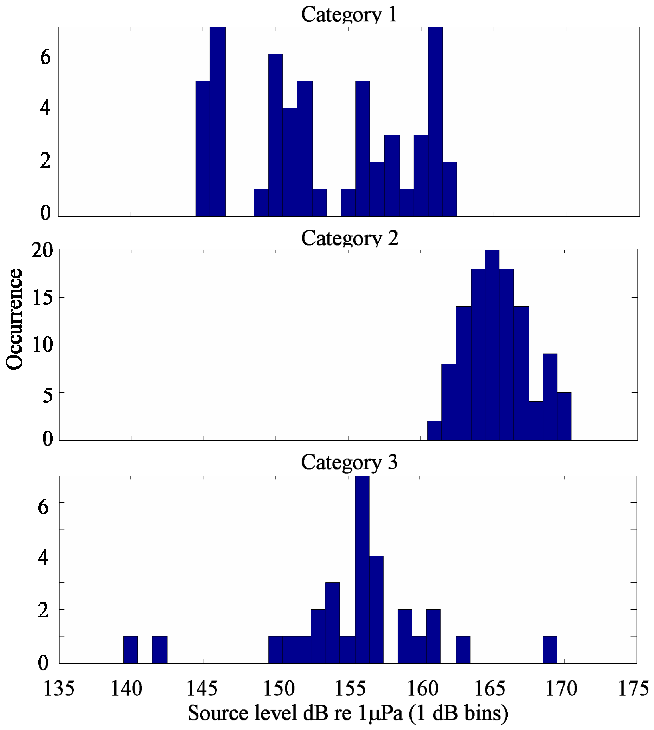

Figure 4.5.7. *Distribution of source levels from recorded sound pressure levels for each A. japonicus call category based on spherical spreading losses only.*

The regression models employed to calculate the call category source levels were repeated to determine the sound energy levels (SELs) and peak-peak pressure source levels for each category together with their respective R^2 values (Table 4.5.2). In each category the varying length of the call is responsible for the differences between mean squared pressure and sound exposure level source levels between the categories. This is because mean squared pressure (dB) = equivalent energy (dB) plus 10log(call length).

Table 4.5.2. *Values of source levels with standard deviation, based on recorded values. Mean squared pressure source levels using 20log(r) losses are shown, followed by regression calculated source levels based on mean squared pressure, S.E.L equivalent energy and peak-to-peak pressures. Calculated losses with confidence limits and correlation coefficients together with mean call length are shown for each call category.*

		Call Category		
		1	**2**	**3**
Source level (mean squared pressure) 20log(r) transmission loss (s.d.)		153 (5.673)	165 (2.233)	156 (3.93)
Mean squared pressure (dB re 1μPa)	Source level (95% c.l.)	163 (147.7, 178.6)	172 (168.4, 176)	157 (154, 160.3)
	Transmission loss (log(r)) (95% c.l.)	-25.39 (-34.55, -16.24)	-23.74 (-25.89, -21.58)	-23.04 (-26.62, -19.47)
	R^2	(0.4214)	(0.8235)	(0.8756)
S.E.L. (dB re 1μPa^2.s)	Source level (95% c.l.)	152 (138.2, 166.1)	165 (156.2, 173.2)	136 (132.1, 139.4)
	Transmission loss (log(r)) (95% c.l.)	-22.85 (-31.11, -14.6)	-21.83 (-27.2, -16.46)	-17.35 (-21.2, -13.51)
	R^2	(0.6386)	(0.6407)	(0.7383)
Peak-peak pressure (dB re 1μPa)	Source level (95% c.l.)	183 (172.6, 194.9)	194 (189, 200.6)	167 (164.8, 169.9)
	Transmission loss (log(r)) (95% c.l.)	-25.15 (-31.71, -18.58)	-27.17 (-30.77, -23.57)	-16.13 (-18.77, -13.49)
	R^2	(0.7716)	(0.8602)	(0.8304)
Mean call length (s) (s.d.)		0.05375 (0.0211)	0.34568 (0.0632)	0.01835 (0.0145)

Background noise levels during the course of these recordings were observed at 108 dB re 1μPa, however, during the course of research in Mosman Bay noise levels which include vessel traffic and/or calling *A. japonicus* have been known to reach 148 dB re 1μPa. The background noise has significant impact on the detection range of a call to both intended recipient and observer (McCauley, 2001, Sprague and Luczkovich, 2004). For simple comparative purposes a maximum detection range of the regression determined mean squared pressure source level was calculated for each call category (and confidence limit values), based only on spreading losses and running the call out to broadband ambient noise (Table 4.5.3). This does not account for call frequency band or fish hearing critical ratios at the call frequencies.

Table 4.5.3. *Maximum detection ranges (r) for all call categories (black) and associated source level 95% confidence limits (lower-blue, upper-red) for two levels of background noise calculated from spherical, cylindrical and regressions determined from the data.*

		Detection Range (m)			
		Regression calculated transmission loss and noise levels (dB re 1µPa)		Spherical transmission loss and noise levels (dB re 1µPa)	
Call Category	Source level (dB re 1µPa)	110	150	110	150
1	147	31	1	39	0.4
	163	123	3	231	2
	178	503	13	1,383	14
2	168	275	6	426	4
	172	396	8	660	7
	176	571	12	1,024	10
3	154	81	1	81	1
	157	112	2	117	1
	160	152	3	167	2

4.5.4. Discussion

A simple resolution to accurately determine fish call source level would be a concurrent, calibrated audio and visual recording, 1 m from the hydrophone at the time of vocalisation, similar to that reported by Sprague and Luczkovich (2004). However, behaviourally unbiased, *in situ*, ground truthed recording of fish calls, in dark or turbid waters, at such short ranges is improbable. Furthermore, disparities in source level, both between fish and from individual callers denote that a range of observed call SPLs is required to model the species source levels. Thus the ability to precisely locate individuals and obtain accurate source ranges of species-specific calls is the most appropriate method for recording source levels. The localisation of fish from arrival-time differences in their calls using a passive acoustic hydrophone array facilitated accurate determination of source range, a prerequisite for the calculation of fish call source level. Observation of recorded calls at various locations developed the relationship for estimated transmission losses with range.

Source levels of 163, 172 and 157 dB re 1μPa (mean squared pressure) with associated confidence limits for *A. japonicus* call Categories 1, 2, and 3 respectively have been established. Transmission losses observed in the linear regression models for Categories 1 and 2 were within acceptable range of spherical spreading to 'practical' spreading loss (Coates, 1990), a theoretical loss considered satisfactory to ranges of around 50 m in water depths of approximately 20 m (Cato, 1998).

Category 1 calls displayed comparatively low correlation with the least squares regression model due to the variability of the call structure compared to that of the other call categories. The difference in amplitude between the first two pulses of a call varied significantly throughout all three call categories. This variation had greater impact on source levels of calls comprising fewer pulses. Therefore determined confidence limits of 147 and 178 dB re 1μPa with a correlation of $R^2 = 0.4214$ for the best fit (25.39log(range)) transmission losses were deemed a reasonable estimate of source level range.

Source levels increased through Categories 3, 1 and 2 as more pulses were included in calls. This was due to an increase in amplitude over the first three pulses of several calls. Section 4.2 highlighted that in many long Category 2 calls the first one, two or three pulses were of lower amplitude than subsequent pulses. To corroborate this, double pulsed Category 3 calls when the fish was orientated towards the hydrophone produced source levels comparable with those of the Category 1 calls (Table 4.5.1). Lagadere and Mariani (2006) observed similar traits in the short calls of *Argyrosomus* *regius* and suggested these were less intense than long grunts, a hypothesis in agreement with findings of this study. After the initial pulses the detected maximum amplitude often reached a plateau thus Category 2 call source levels were more stable than those of Category 1. By inference the high correlation of Category 3 call source level within the single and double pulse trends were due to the single source origin. Whether this trait is characteristic of an individual is unknown.

The *in situ* source level of a *B. chrysoura* was reported at a maximum mean squared pressure of 135 dB re 1µPa (Sprague and Luczkovich, 2004). *B. chrysoura* typically grow to a maximum size of 30 cm (Fishbase, www.fishbase.org/Summary/SpeciesSummary.php?id=1165). Barimo and Fine (1998) reported a maximum *in situ* source level of 130 dB re 1µPa for the oyster toadfish (*Opsanus tau*), a species of maximum recorded length of 43.2 cm (Fishbase, http://www.fishbase.org/Summary/speciesSummary.php?ID=3069&genusname=Opsanus&speciesname=tau). As these species are considerably smaller than *A. japonicus,* and source levels of swimbladder generated calls are related to fish size (Connaughton *et al.*, 2000), this difference was to be expected. McCauley (2001) reported calls of mean source level (mean squared pressure) of 149 dB re 1µPa from sources speculated to be *Protonibea diacanthus*, a smaller Sciaenidae of similar sound production mechanism to *A. japonicus*. This is of comparable level to sounds (148 dB re 1µPa) from similar waters, reported by Cato (1980), which were also speculated as a Sciaenidae, likely *P. diacanthus*. Both reports of speculated *P. diacanthus* sounds comprised calls of similar spectral peak frequencies (250 - 400 Hz) to those of *A. japonicus* (Section 4.2). *P. diacanthus* caught in a recent study around the Northern Territory were of comparable length to the >1 m *A. japonicus* captured in the Swan River (Farmer, 2008, Phelan, 2008). However, sampling conducted in Cape York Peninsula waters in 1999 and 2000 captured predominantly 750-799 and 600-649 mm specimens, respectively. It is not known whether the sounds reported by Cato (1980) and McCauley (2001) originated from similar sized fish.

The *A. japonicus* sounds reported here were of greater intensity than previously reported fish source levels. The authors suggest several possible causes for this:

- Firstly the sizes of *P. diacanthus* callers were unknown and if of considerably smaller size may contribute to the intensity difference.

- Secondly, previous reports of fish source levels have estimated transmission losses at 20log(*r*), in contrast with this study where transmission losses were calculated through best fit regression and found to be greater (Table 4.5.3). The substitution of 20log(*r*), for transmission loss, reduced the *A. japonicus* source level for categories 1, 2 and 3 as 153, 165 and 156 dB re 1µPa, respectively (Table 4.5.2).

- Thirdly, the most comparable sound between those of *A. japonicus* above and those reported of *P. diacanthus* differ in length and structure. McCauley (2001) noted that the *P. diacanthus* calls persisted for longer than a second, in contrast with the 0.35 s *A. japonicus* calls. *P. diacanthus* calls reduced in amplitude in the latter half of the call (McCauley, 2001), similar to that of the Atlantic cod (*Gadhus morhua*), which was suggested to exhibit lactic acid build up in the sonic muscle (Nilsson, 2004). The greater call length and possible lactic acid build up are suggested to account for the lower intensity. However, if the sound energy levels from Table 4.5.2 were to be calculated using a 20log(r) transmission loss the determined source level becomes 162 (± 2.7) dB re 1µPa^2.s, more comparable with the 150 dB re 1µPa^2.s reported by McCauley (2001) for speculated *P. diacanthus*.

This study has provided evidence of *in situ* fish call directivity. Trends of Category 3 calls recorded as the fish passed the hydrophone displayed a 5 to 8.5 dB re 1µPa difference between orientations. Therefore, if the assumption of forward motion is correct, and the individual was not swimming against a current, calls were greater in front of the caller than behind. This finding is in contrast with Barimo and Fine (1998), who hypothesised that directivity in sound production of *O. tau* would be reduced in the direction of the fish ears and observed a 3 to 5 dB re 1µPa decline in source level moving from the rear to the front of the fish. The reason for this difference is unknown. However, when considering biomass estimates from passive acoustic surveys it may be necessary to observe general orientation of fish whilst aggregating, although statistical based methods will account for source directivity patterns. For example, if a species such as *A. japonicus* exhibit lekking behaviour and aggregate to spawn shortly after high tide (Section 4.2) it may be necessary for them to swim against current to maintain a relatively stationary position. This could result in a population orientated head on to an upstream hydrophone, positively biasing the overall SPLs.

Although *A. japonicus* calls contain energy across the frequency bandwidth 50 to 1000 Hz the tone burst dominates the call such that often one or more of the spectral peak frequencies are 10 dB re 1µPa greater than other amplitude modulated frequencies. As a result ray multi-path interference was observed in the source levels (Figure 4.5.5). This was observed to be more prominent at the farther regions of the test ranges; however, this may be due to the cluster of calls emitted by one fish rather than close range measurements where the effect was masked by the variability of different callers.

Call source levels relative to background noise determine the maximum detection range of a call from its intended recipient. Table 4.5.3 has shown some estimates of maximum detection range at various ambient noise levels given different models of losses for each category of call. From a fisheries perspective this may have specific impacts on the spawning success rates of an aggregation. *A. japonicus* in Mosman Bay form aggregations of low density calling by comparison with other reports (Sprague and Luczkovich, 2004), thus individuals can often be detected at comparatively long ranges, even at the height of calling (Section 4.4). During periods of low background noise, such as the above recordings taken at ambient noise levels of approximately 108 dB re 1µPa, regression models predicted a maximum detection range of 151, 490 and 140 m for calls of Categories 1, 2 and 3 respectively. These detection ranges are for simple comparison only as they do not consider the differences between call and ambient noise frequency content, and do not account for the unknown fish hearing, critical thresholds.

Periods of high background noise were frequent at the study site, as vessel traffic in Mosman Bay is prominent. Vessel noise of water skiers directly above calling fish has reportedly masked calls at distances of less than the water depth (Section 4.4) and a vessel SPL greater than 150 dB re 1μPa would mask all calls at distances of near 1 m (assuming spherical spreading). Therefore, for abundance measurements from fish call counting the ambient noise conditions need to be monitored and hydrophone detection ranges altered accordingly.

4.5.5. Conclusions

Recorded SPLs of calls at known ranges have provided determination of *in situ* source levels for *A. japonicus* calls as 163, 172 and 157 dB re 1μPa (mean squared pressure) for Category 1, 2 and 3 calls respectively. Confidence ranges have been suggested based on observations of interference with transmission losses and variability produced by different callers. The source level of an *A. japonicus* call is dependent not only on fish size, temperature and seasonal time (Connaughton *et al*., 2000, McCauley, 2001, Section 4.3), but also acoustic propagation, orientation and call category. When considering call source levels as a method for estimating biomass it is therefore necessary to determine a species source level range at a given temperature and spawning maturity before progressing further.

4.5.6. Future Work

The next step to utilise the source level of *A. japonicus* calls is to begin the process of absolute biomass estimates. Hydrophone array studies have provided an insight into separation distances and distributions of calling *A. japonicus* in Mosman Bay. Proposed future work involves the combination of the determined source level ranges and distributions with call counting techniques to estimate the number of calling *A. japonicus* within the hydrophone detection range. The issue of determining the ratio of calling/non-calling fish remains to be resolved absolutely. In an ideal world this would involve an isolated aggregation of randomly distributed sized males and females of known number. Thus the number of calls could be related to the overall population. In the absence of this possibility, ratios will have to be repeatedly obtained from *aquaria*.

4.5.7. Acknowledgements

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4.6. Estimating numbers of calling fish from their vocalisations using a single hydrophone.

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ABSTRACT:

Long term monitoring of vocalisations by soniferous fishes provides a powerful means of 'observing' fish populations without creating any bias in their natural behaviour. In many species calls are pertinent to functions associated with spawning. If calls do not overlap, call counting techniques can provide estimates of fish numbers using determined consistent calling rates. Alternatively, as sound pressure levels produced by calling fish have been shown to correlate with egg production, and by proxy, the number of spawning fish, they offer a relative measurement of the population. Transferring the recorded sound pressure levels into absolute numbers of calling fish and therefore total biomass requires the identification of the sound transmission properties of the spawning area; the source level of calls; quantification of ambient noise sources; call characteristics of temporal patterns; and an estimate fish spatial distribution. However, caller range is a defining variable in call sound pressure levels. Calls produced by a spawning aggregation of A. japonicus in Mosman Bay, Swan River, Western Australia were recorded by an array of hydrophones throughout the evening of the 5th March, 2008. Low levels of caller density facilitated determination of up to 15 callers within the detection range, using call counting techniques. Once calls overlapped significantly the sound pressure levels, as recorded by a bottomed hydrophone, produced estimates of the number of A. japonicus callers using a combination of call counting techniques and call partial pressures over a given period.

Keywords: *call counting, abundance, sound pressure levels, source level*

4.6.1. Introduction

In some species sound pressure levels (SPLs) of calling fish have been related to egg collection data (Luczkovich *et al.,* 1999b), from which relative spawning numbers could be inferred. However, estuarine spawners, such as *A. japonicus* may aggregate at periods of high tidal streams, such that tidal currents remove eggs and larvae into open water, rendering egg collection techniques ineffective. In contrast, passive acoustic recording of fish sounds offers the opportunity to monitor sound production, and by proxy, spawning levels, despite adverse environmental factors.

Assuming average call rates are maintained by individual fish then the total number of species characteristic calls recorded within an area may correlate to the number of fish calling. In many species of Sciaenidae, it is only the male which possesses functional sonic muscles, with which it produces sounds (Griffiths and Heemstra, 1995, Connaughton *et al.,* 2000). Therefore, if the call rate is known, and the percentage of males within the population that call at any given time can be estimated, then the total number of calling males can be determined. An estimate of the total number of fish in the aggregation can then be made if the sex ratio of the population is known. Such ratios of callers to total population become more complex if both sexes of the species are soniferous, as the ratio between males and females emitting calls must be assessed.

When overall calling reaches a certain rate, calls from different individuals merge. During such a chorus, techniques of call counting become problematical and can underestimate numbers of vocalising fish. An alternative method of estimating calling numbers is the contribution of an individual call to the overall SPLs (McCauley, 2001, Sprague and Luczkovich, 2004, Section 4.5). However, to determine contributions to SPLs one must first determine an average call source level, the acoustic propagation at the time and site of recording, the spatial distribution of fish, background noise levels, and the vertical fish position in the water column, since this greatly impacts call transmission (McCauley, 2001).

During previous studies a range of call source levels for *A. japonicus* was determined, spatial distribution between callers during quiet periods of calling were calculated and the location of callers in the water column identified (Sections 4.4 and 4.5). It is the aim of this section to investigate techniques of estimating the number of *A. japonicus* within the detection range of the hydrophone.

4.6.2. Methods

An array of hydrophones was set out in Mosman Bay on 5th March, 2008 to record spawning related *A. japonicus* vocalisations. Details of the array deployment, data acquisition and subsequent fish localisation can be found in Sections 4.4 and 4.5. This section is primarily concerned with recordings taken by a single bottomed hydrophone in 18.5 m of water (tide dependent) using an HTI-90U hydrophone (High-Tech Industries, MA, USA), attached to a Centre for Marine Science and Technology (CMST) – Defence Science and Technology Organisation (DSTO) designed sea-noise logger (Figure 4.4.1, R_1). Details on passive acoustic data acquisition and processing are given in Section 4.1. To limit vessel noise during this study, data were passed through high- and low-pass filters at 100 and 1000 Hz respectively.

As background noise and temperature affect the ambient SPLs and fish source levels (Connaughton *et al*., 2000, Section 4.4), and both have been shown to vary throughout the spawning season in Mosman Bay (Section 4.3), estimates of spawning numbers have only been made from a single evening's calling on 5th March 2008. Once all environmental variables which affect *A. japonicus* call characteristics have been evaluated it is anticipated that the technique can be applied across an entire spawning season. All values reported in this section are the mean squared SPLs over the period in question.

To maximise recording of the highest number of fish calling within the area, while limiting likelihood of a vessel passing, the evening's recordings were separated into two minute segments. Segments containing excessive vessel noise, causing masking of calls, (Section 4.4) were rejected. In order to assess the variation of ambient biological noise during the course of the evening the remaining two minute segments provided a cross-section of SPLs from before spawning began, until after it finished. The analysed segments encompassed periods of few callers, several callers where individual calls could be distinguished, and periods where calls overlapped sufficiently to significantly increase background noise levels during the two minute period. Analysis was conducted on Category 1 and 2 calls of *A. japonicus* (Section 4.2), due to the regularity of call rates in these categories. Category 3 calls were found to be sporadic, and less defined in terms of their repetition and use (Section 4.2). As such, during the current study, segments displaying significant evidence of Category 3 *A. japonicus* calls were discarded.

During periods where calls could be distinguished from each other and from background noise the number of audible calls in each segment was counted. To provide comparable estimates the waveform and spectrogram for the corresponding segment were analysed to count the number of distinguishable calls and each method compared.

If a typical male emits calls at a consistent rate then the number of overall detected calls can be related to an estimate of the number of fish calling (e.g. at a call interval of 4 s at total number of 60 calls in a minute suggests 4 fish are present calling). Therefore the total number of detected calls were multiplied by the mean estimated call interval for individual *A. japonicus* (Section 4.2) and divided by the duration of section analysed to give the first estimate of calling fish numbers. As call spectral peak frequencies are to an extent individually characteristic, and detected pressure amplitude is related to range (discounting interference), waveforms and spectrograms were analysed to discriminate between individual fish sources to provide a comparison (Section 4.2). Segments where calls overlapped to produce a constant background noise (such as during times of peak chorus calling) were not analysed using call counting techniques.

Received mean squared SPLs and cumulative energies were determined for each two minute segment by the removal of sound energy not attributable to fish calls. In order to remove background, ambient noise a sample section, containing a minimum of 500 detected pressure readings of ambient noise only and no fish calls, was identified within each segment. This sample section provided ambient noise SPLs to be removed from the overall segment SPLs, as per the technique outlined in Section 4.5.2. This ambient noise may have been biological only, or include noise from a distant vessel at approximately constant range (i.e. consistent pressure levels over the two minute segment). However, during chorus periods, where calls merged, it was not always possible to locate a sample period containing a minimum of 500 points without calling present. To compensate for the absence of clear ambient noise with no fish calling present, periods free of calls were identified throughout the evening and those considered to most closely resemble the noise present in the section under scrutiny were assumed as the ambient noise. The mean squared pressure of this noise sample was subtracted from the overall squared pressures to produce cumulative energy attributable to fish calls for each segment, background noise contribution, and the mean squared SPLs without any ambient noise (Section 4.5.2).

Figure 4.6.1 highlights the incremental growth of cumulative energy with time, in particular, the variation in gradient of the energy curve due to near consistent noise such as a vessel at constant range (Figure 4.6.1A), changing noise such as a passing vessel (Figure 4.6.1.B) and *A. japonicus* calls (Figure 4.6.1C, expanded inset). Figure 4.6.1B highlights the complexity of accounting for SPL contribution from passing vessels or vessels which started engines during the two minute segment (Figure 4.6.1C). For example, the first 15 seconds of the recording shown in Figure 4.6.1C, where noise from a distant vessel has been removed, displayed several distant calls. However, once a vessel at closer range started up, such calls were masked on the waveform and barely visible on the cumulative energy curve as they contributed comparatively lower energy, (Figure 4.6.1C, black ovals). The spectral content of the two sections of this recording are shown in Figure 4.6.1Ci, and ii where the vessel contribution around 100 Hz in the first 15 seconds (i) has been accounted for by noise removal, but additional noise from the second vessel in the latter section has not (ii). Vessel noise spectra was often centred between 200 and 300 Hz, at similar frequencies to calls of *A. japonicus* and so could not be filtered out.

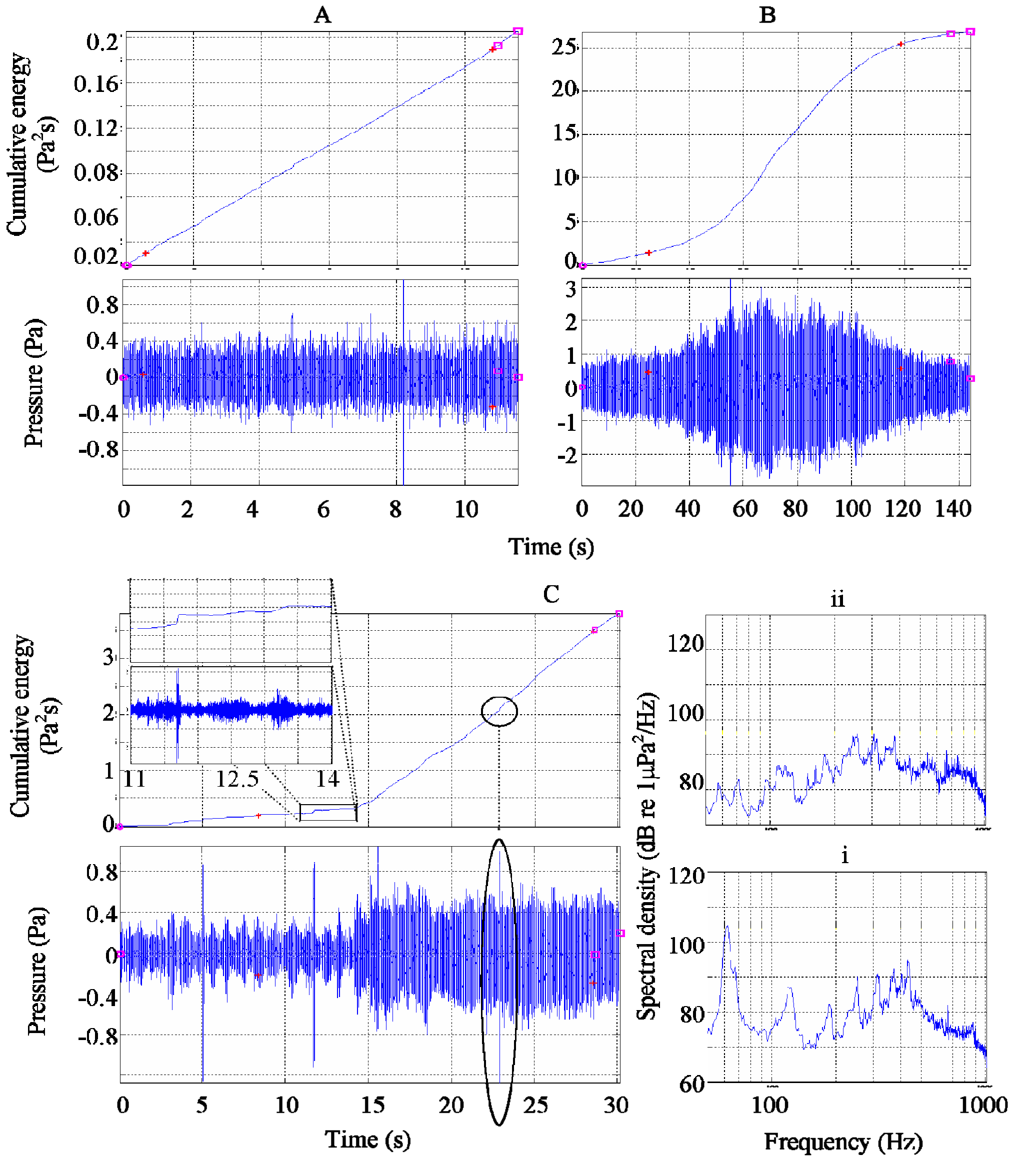

Figure 4.6.1. *Cumulative energy (upper) and waveforms (lower) for noise samples taken at approximately (A) 17:30 (little biological noise and a distant vessel at constant range), (B) 18:00 (vessel passing the hydrophone at a range of approximately 100 m) and (C) 18:30 (distant vessel and distant callers during the first 15 seconds, followed by the engine running of a nearer vessel for the following 15 seconds masking the distant callers). Spectral content between 50 and 1000 Hz are shown for the first (i) and second (ii) 15 seconds of C. Pink dots represent the boundaries of noise samples used in analysis and red crosses mark the 5 and 95% energy boundaries.*

To replicate fish call SPLs a repetitively calling fish was simulated by modelling a call of known source level in Matlab. Details of how the individual call was replicated can be found in Section 4.7.3.1. A duty cycle was created from the determined call repetition rate. This calling period is shown by Figure 4.6.2 where a 16 pulse, 0.29 s (call time t_c), 172 dB re 1μPa source level Category 2 call, was repeated every 3.2 s (duty cycle T_{dc}). These acoustic characteristics are typical of *A. japonicus* Category 2 calls, as determined in Sections 4.2 and 4.4.

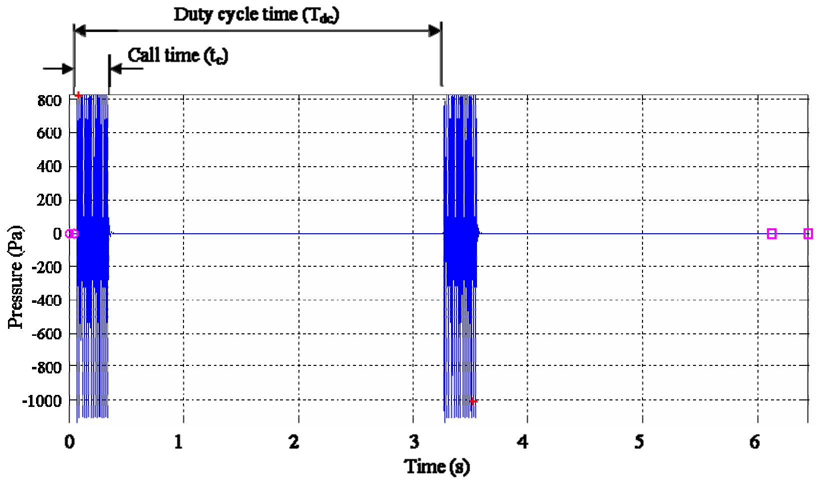

Figure 4.6.2. *Pressure waveform of a simulated A. japonicus call, highlighting the call duration and duty cycle time for a repetitive caller.*

The call was repeated for the duration of the two minute segment and SPLs were calculated for this hypothetical caller at ranges of 25, 50, 100 and 200 m from the hydrophone. Mean squared SPLs at these ranges were determined as 130, 124, 118, and 112 dB re 1 μPa respectively, for the two minute period.

4.6.3. Results

Samples of ambient noise without *A. japonicus* calls were found to differ very little in SPLs throughout the evening with predominant noise variations were due to vessel presence. It was only in the late evening, once nearly all vessel noise ceased, that evidence of shrimp clicks became commonplace and background noise reduced to a minimum of 91 dB re 1µPa, in comparison with nearly 110 dB re 1µPa, early in the evening. Vessel noise was sporadic, sometimes lasting for only a few seconds. The presence of vessel noise was accounted for in chorus level analysis by either elimination of the period including vessel noise from the segment, or rejection of the segment altogether. All calculations were adjusted for the segment time lost due to vessel noise. When calling density was such that all calls merged, the contribution of vessel noise to overall SPLs was complex to determine precisely, thus an estimate of vessel noise was made from aural scrutiny.

During segments where calls could be discriminated from each other, comparing direct counts of audible calls with counts derived from scrutiny of waveform and spectrograms, produced similar estimates of total call numbers, with a mean difference of -1.0 % (s.d. = 0.07 %, max. = 13 %, n = 31). Therefore only the numbers determined from waveform call counting have been shown. Figure 4.6.3 shows 30 s samples from four segments recorded throughout the evening. The cumulative energy from six distant calls (A), seven calling fish; including one at approximately 30 m range (B), many calling fish at varying ranges upwards of 50 m (C), and many shrimps clicks with distant Category 1 calls (D) show the difference in SPLs generated by different combinations of calling fish.

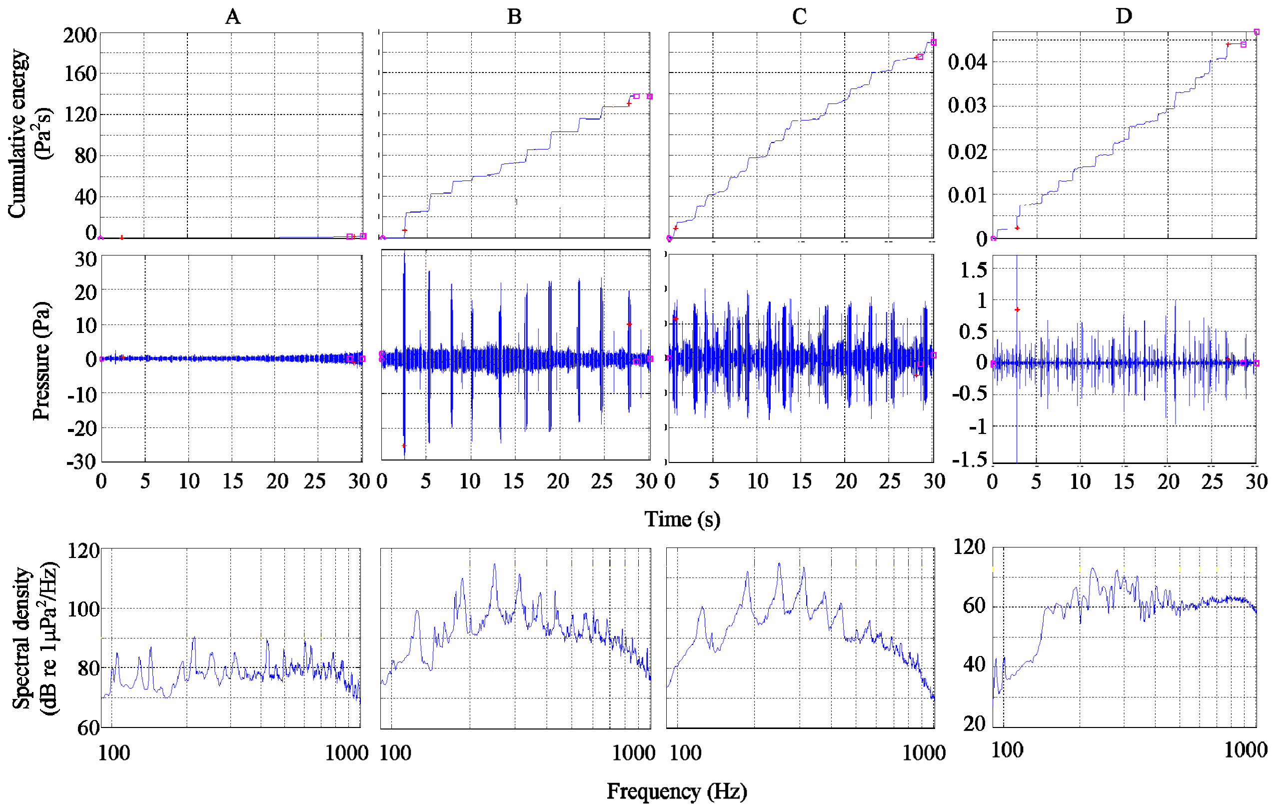

***Figure 4.6.3**. Cumulative energy (top), waveform (middle) and power spectral density (bottom) from thirty second periods of: A) few vocalising fish at 17:00; B) vessel noise interrupting vocalising fish (one fish at 25-35 m range) at 19:00; C) high density of calling fish at > 35 m range where calls overlap and background noise is dominated by distant fish calls at 22:30; D) no vessel noise, many distant Category 1 calls at similar maximum amplitudes to several shrimp clicks.*

Call counting techniques were able to identify up to approximately 300 calls per minute for *A. japonicus* Category 1 calls alone, and 150 for combinations of Category 1 and 2 calls. Discrimination between calls was based on call separation, amplitude and duration. At typical call intervals determined in Sections 4.2 and 4.4, of approximately 2 and 4 seconds respectively for Category 1 and 2 calls, the number of calls equated to approximately 10 individual callers in both cases. The comparison of spectral content and waveform amplitude was able to estimate up to a maximum 15 callers in an individual two minute segment.

Figure 4.6.4 illustrates the discrimination between callers via waveform amplitude and spectrogram characteristics, over a 17 s period, where calls predominantly did not overlap. In this example nine callers were identified which, when compared to the *A. japonicus* source level ranges (Section 4.5), facilitated the estimation of fish ranges as shown in Figure 4.6.5. During the early evening, when Category 2 long calls were prevalent, a maximum of 15 individual fish were determined to be calling during any 10 second interval. At times of chorus level calling, where calls overlap, it was speculated that more than 15 callers were present, though this could not be confirmed.

The mean squared SPLs of 135 (s.d. = 0.7) and 132 (s.d. = 0.4) dB re 1μPa for the black and grey callers in Figure 4.6.5 respectively, signified ranges of approximately 35 m (confidence limits of 25 and 55 m) and 19 m (confidence limits of 4 and 68 m) from the determined source levels and transmission losses in Section 4.5. Fish were not often at such close range to the hydrophone and at the height of spawning the waveform amplitudes of closer calls were predominantly between 10 and 20 Pa (approximately ranges of 100 and 50 m respectively).

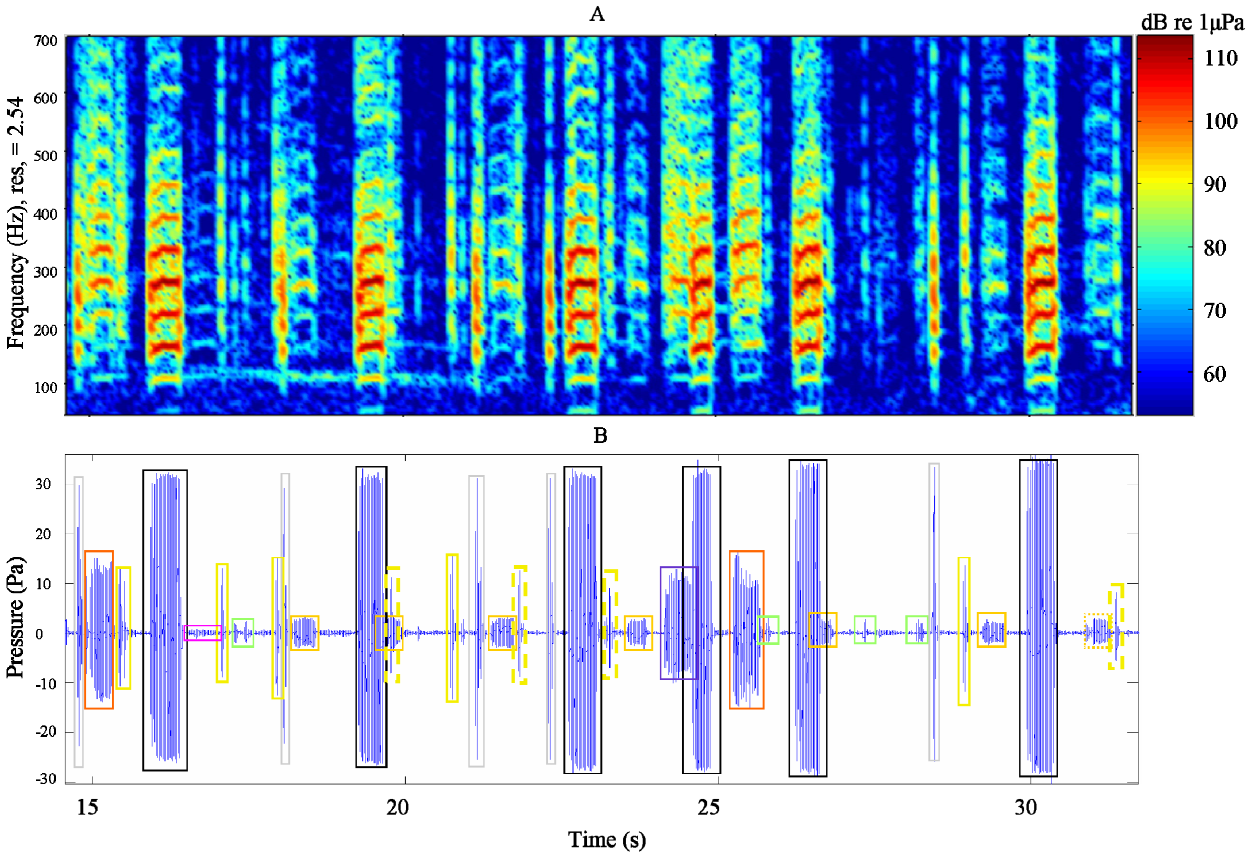

Figure 4.6.4. *Spectrogram (A) and waveform (B) of 30 seconds of calling, as recorded by a bottomed hydrophone in 18.5 m of flat water at 19:02 on 5th March 2007. Coloured continuous lines surrounding Category 1 and 2 calls in waveform highlight individual fish calling repetitively. Dotted lines represent calls from fish speculated to be the same fish as the equivalent coloured lines. Spectrogram frequency resolution was 2.54 Hz.*

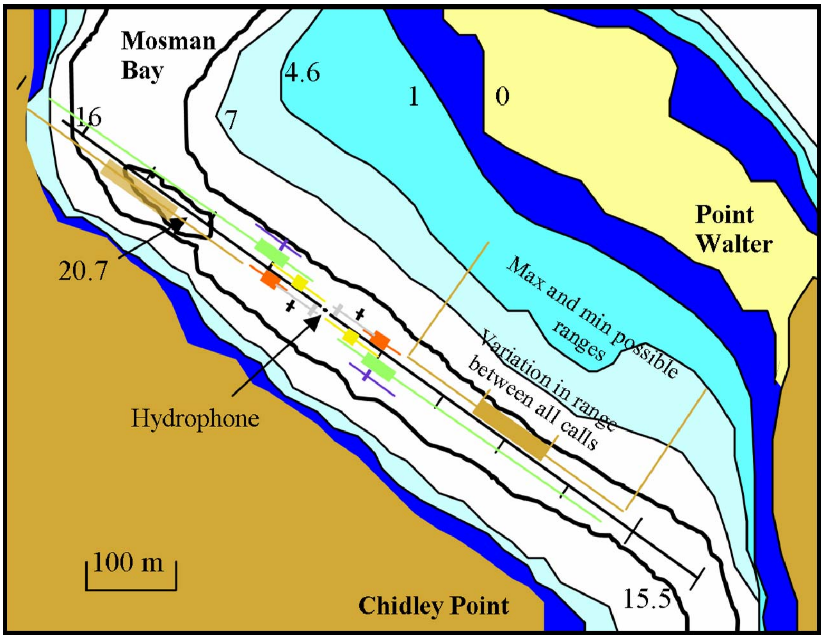

Figure 4.6.5. *Map of fish ranges for seven of the eight fish shown in Figure 4.6.4 (black, grey, yellow, red, brown, green, and purple) determined by the recorded sound pressure levels and regression calculated transmission losses applied to the source level ranges. Fish locations have been assumed as either up or downstream of the hydrophone. Continuous thick lines represent boundaries of maximum and minimum range from all calls using spherical spreading losses. Thin lines represent the maximum and minimum possible ranges of each fish using the 95% confidence limits for source level and transmission loss determined in Section 4.5. Water depths are shown for various contours and land is marked in brown, while the range is shown by the graduated black line. Scale bar ticks are 100 m apart.*

The SPLs, total number of calls and estimated number of individual fish calling (where determined) in each segment are shown in Figure 4.6.6. Between 18:30 and 19:00 the number of calling individuals and total calls observed varies comparatively little (7 to 10 callers emitting 135 to 245 calls). However, the SPLs increased as the calling fish approached the hydrophone (Figure 4.6.6, 19:00 to 20:00). At 19:00 a single fish, calling at approximately 30 m range appeared and SPLs rose to 126.5 dB re 1µPa (localisation information can be found in Section 4.4). While other fish maintained relatively consistent ranges the nearer fish moved from approximately 30 to 60 m range and the SPLs dropped to 118 dB re 1μPa. This decline illustrates the effect that range of a single caller has on the overall SPLs. Such range effects are further highlighted by the green line in Figure 4.6.6, showing the SPLs of the simulated caller in Figure 4.6.2, at 25, 50, 100 and 200 m ranges, each for a two minute segment.

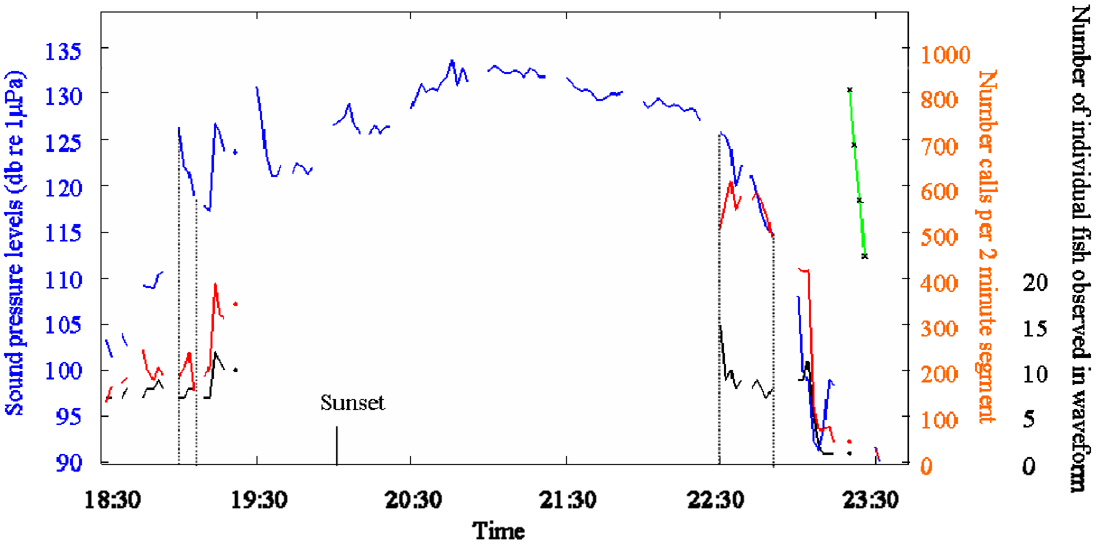

Figure 4.6.6. *Sound pressure levels (blue), total number of recorded calls (red), and number of individual repetitively calling fish observed in the pressure waveforms (black) for each segment between 18:30 and 23:35. Times of interest are highlighted by dotted lines. The green line illustrates the variation in SPLs from a simulated Category 2, A. japonicus call repeated every 3.7 seconds throughout the two minute segment at 25, 50, 100 and 200 m range.*

SPLs remained consistently around 130 dB re 1μPa between 20:40 and 21:30, peaking approximately one hour after sunset. This period of peak calling was dominated by multiple calling fish, rather than a single, close range caller. However, during this period, calls overlapped and could not be counted individually, thus SPLs could not be related to the exact number of fish. At 22:18 Category 1 short calls appeared and by 22:30 dominated the waveforms, with only the occasional Category 2 long call present (Figure 4.6.2D). As vessel noise was virtually non existent by this time, and short call duration is less than 0.1 s (Section 4.2), calls could be counted more efficiently. Between 22:32 and 22:48 the number of callers and calls remained similar, while the SPLs dropped from 125.3 to 114.8 dB re 1μPa, as the fish range increased.

By 23:12 only one caller remained, emitting a short call every 2.15 s (s.d. = 1.3 s) over the two minutes, slowing to 2.93 s (s.d. = 1.6 s) between calls at 23:22. Despite the comparatively large abundance of calls, due to the higher call rates, the Category 1, short calls were estimated to be at ranges in excess of 400 m, generating lower SPLs than early evening Category 2 calls. At this time biological noise of shrimp clicks were also prevalent, contributing to the cumulative energy (Figure 4.6.2D).

4.6.3.1. Estimation of caller numbers during times of merging calls

To theoretically estimate the number of detected callers from their contribution to overall SPLs, each stationary fish is considered to call at a constant rate for the duration of the two minute segment, similar to the simulated caller in Figure 4.6.2. In addition, it has been assumed that all callers are of the same size and therefore emit calls at approximately the same source level. If all non-call noise is removed, the total mean squared pressure over the call duty cycle (T_{dc}) will be equal to the total transmitted mean squared pressure multiplied by the ratio of call time to duty cycle time, given by:

$$\left\langle p^2 \right\rangle_{Total\,dc} = \left\langle p^2 \right\rangle_{Total\ \ transmit} \cdot \frac{t_c}{T_{dc}} \tag{4.9}$$

As the call rate is considered constant throughout the segment the total call time to segment time will approximate to the same ratio as call time to duty cycle. From eq. 4.2 Parseval's theorem the total mean squared pressure is equal to the sum of the partial mean squared pressures (p_i) and therefore the pressure received at the hydrophone from all callers becomes:

$$\left\langle p^2 \right\rangle_{\mathrm{Re}\,ceived} = \sum_{i=1}^{N} \left\langle p_i{}^2 \right\rangle_{Total\ \ i\ \ received} \tag{4.10}$$

where N is the number of transmitters.

If the range is equal or less than the water depth then spherical spreading used as a minimum estimate for transmission losses. Although regression modelling of transmission losses in *A. japonicus* call localisation gave greater than spherical losses, spherical spreading losses have been assumed for the purpose of these calculations:

$$\left\langle p^2 \right\rangle_{Total\ i\ received} = \frac{1}{r^2} . \left\langle p^2 \right\rangle_{Total\ transmit} \tag{4.11}$$

where *r* is the caller range. Substitution into Equation 4.10 gives:

$$\left\langle p^2 \right\rangle_{\mathrm{Re}ceived} = \left\langle p^2 \right\rangle_{Total\,dc} . \sum_{i=1}^{N} \frac{1}{r_i^2} \tag{4.12}$$

Due to transmission losses the number of callers required to create the same SPLs as a single caller at 1 m increases with range by a ratio of 4*N* for every doubling of the range, where *N* is the previous number of callers. For example, spherical spreading transmission losses mean 1 caller at 25 m gives the same time averaged, mean squared SPLs as 4 callers at 50 m, or 16 and 64 callers at 100 and 200 m ranges respectively (assuming calls are not in phase).

Figure 4.6.7 illustrates the received SPLs for segments where the number of calls and callers were determined. Although the relationship between the caller numbers and SPLs was significant ($p<0.0005$), there was also significant overlap between the SPLs received from a small number of callers and SPLs during peak calling. For example, within 95 % confidence limits a time averaged segment SPL of 130 dB re 1μPa could theoretically be explained by anything more than nine callers, as shown in Figure 4.6.7. The SPLs were therefore more dependent on the range of the callers than the number of them.

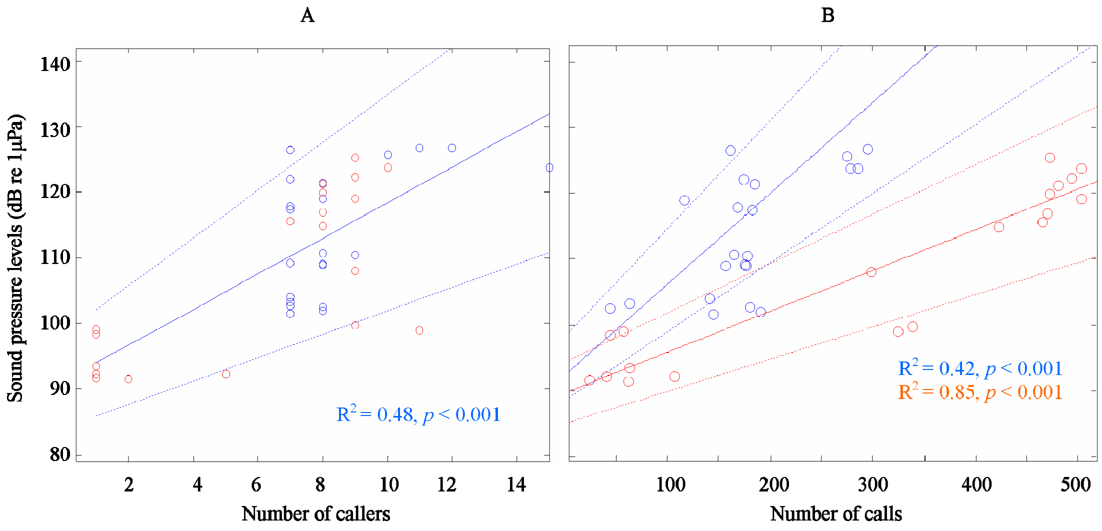

Figure 4.6.7. *Sound pressure levels against the determined number of callers (A) and total number of calls (B) per segment during periods of low density calling prior (blue) and post (red) peak chorus time. The mean trends (continuous line) with 95 % confidence limits (dotted line) are shown.*

The two curves shown in Figure 4.6.7B represent the total number of calls counted within each two minute segment. The difference between the gradients of the pre- (blue) and post-spawning (red) values highlight the difference in contribution of individual calls to overall SPLs due to the differing call lengths of Category 1 calls (generating the greater number of calls post spawning, red) and Category 2 calls (the longer calls which dominated the period immediately prior to peak chorus, blue). Although Category 1 calls were present in large numbers prior to peak calling, this was predominantly during afternoon hours, when many segments were distorted by vessel noise and have therefore not been included in this analysis.

For ease of calculation the following analysis assumes that all received call related SPLs are produced by Category 2, long calls. As a higher number of Category 1 calls (and therefore a higher number of fish) originating at the same source distance would be needed to produce the same SPL the assumption of Category 2 calls only would lead to an underestimate of fish numbers.

The required number of callers at range r to produce the recorded SPL is restricted by the available calling area (the centre of the river channel is a finite area), compared to the spatial separation exhibited between *A. japonicus* callers (localisation in Section 4.4 noted an approximate 25 m separation distance during low density calling). Therefore a range limit may be determined, above which calls are not considered to contribute to the SPLs, because an improbable calling density would be required to create the required SPL.

A minimum number of callers within the range restricted area can then be determined for the recorded SPLs. So for N callers at ranges $< r$:

$$\sum \frac{1}{r_i^2} > \frac{N}{r^2} \tag{4.13}$$

Localisation data (Section 4.4) estimated a minimum separation distances between calling fish, during quiet periods of calling of approximately 25 m. If such separation was consistent throughout the later, high density calling, it may be possible to predict fish distribution throughout the area surrounding the hydrophone. The development of a probability density function of calling *A. japonicus* spatial distribution can provide the most likely population which meets the criteria of recorded SPLs and maximum calling fish density. For example, the 20:42→20:44 segment of recording produced a mean squared pressure of 131 dB re 1μPa (Figure 4.6.6). This SPL could be matched by two example scenarios shown in Figure 4.6.8. The first scenario being one caller at 30 m, three at 60 m and four at 120 m range and the second scenario, 36 callers at 100 m. Such scenarios illustrate the necessity to determine the likely distribution as a number of possibilities would meet the SPL criteria. Due to time constraints the development of probability density functions for the spatial distribution of calling *A. japonicus* has been deemed future work.

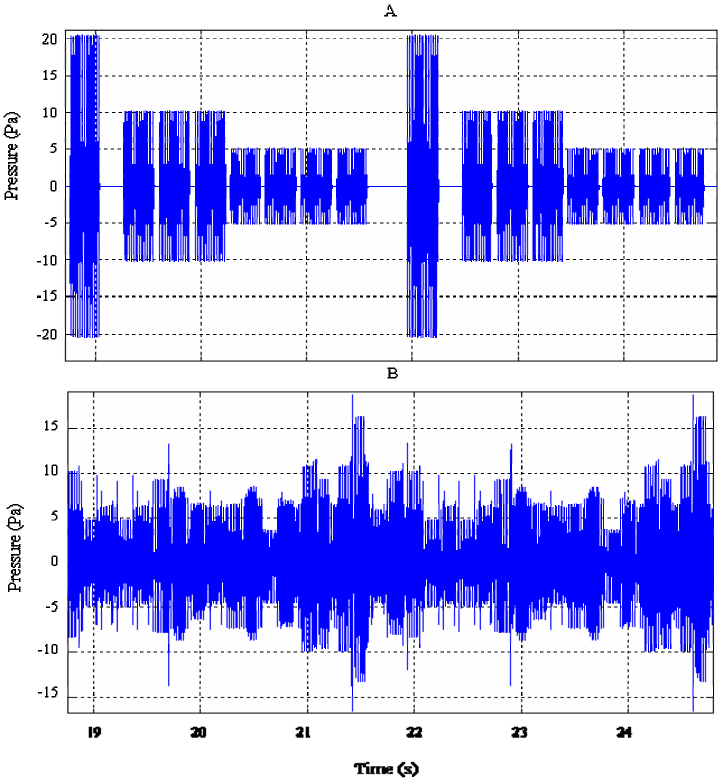

Figure 4.6.8. *Example pressure waveforms for two simulated scenarios of A. japonicus callers creating two minute time average mean squared pressure levels of 131.3 dB re 1µPa. Sound pressure levels created by one caller at 30 m range, three at 60 m and four at 120 m (A), and thirty six callers at 100 m range (B).*

From the known source level of a typical call (Section 4.5) the minimum number of callers at any given range to produce a received SPL can be determined. For example, the minimum number of callers to produce mean squared SPLs of 131 dB re 1µPa over the two minute segment, with no fish closer than 100 m is 36 (a possible resultant waveform of which is shown in Figure 4.6.8B). However, the pressure waveform may clearly demonstrate a caller at considerably closer range than other fish, similar to that of the waveform in Figure 4.6.2B. If the signal-to-noise ratio of a close caller can be considered great enough to assume that interference from the pressure amplitudes produced by background calls has little effect on the pressure amplitude of the close caller then a minimum range can be estimated for the close caller. Alternatively, no single call in Figure 4.6.2C exhibits pressure amplitude greater than 20 Pa. If little destructive interference is assumed for the closer calls, then this would suggest that conservatively, no fish called within 50 m range of the receiver.

Figure 4.6.9 shows figuratively a combined method of call counting and SPL call contributions to estimate a maximum number of fish calling. This technique would involve an iterative process of sequentially removing the contribution from the nearest fish to the total chorus level. One fish in Figure 4.6.9B is calling at significantly lower range than the others.

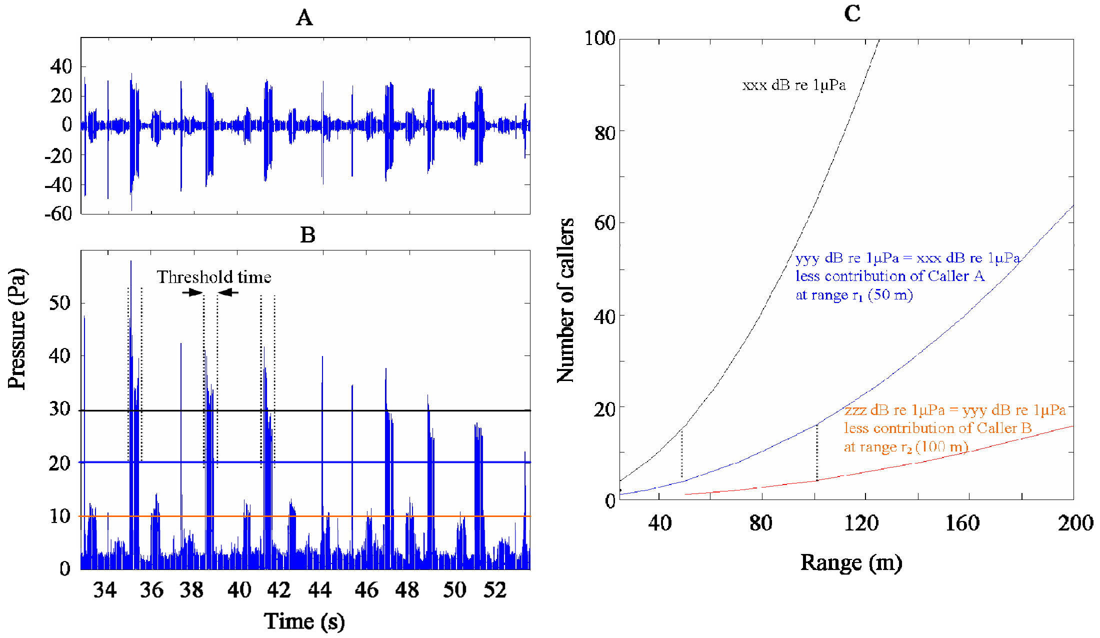

Figure 4.6.9. *Pressure waveform (A) and absolute pressure (B) for 20 seconds of chorus calling in Mosman Bay, as recorded by a bottomed hydrophone in 18.5 m of water. Threshold time marks the length of a call over which automated programs would search for pressure amplitudes greater than the determined pressure threshold (black, blue and red lines in B). Minimum number of callers at minimum range r to produce a given sound pressure level (C). Black, blue and red lines denote the theoretical number of callers at various ranges to produce arbitrarily chosen sound pressure levels and display the removal of call contributions from callers A and B above a threshold level.*

An automated program can detect calls from the near fish by searching for any pressures levels exceeding a set threshold (derived from the known source level and an estimate of transmission losses) for a given time (derived from the typical call lengths), shown by the absolute pressure in Figure 4.6.9B. Caller A, exceeding the 30 Pa black line threshold is at <30 m range, based on the Section 4.5, regression model, lower 95% confidence limits for this recorded SPL. Any signals meeting similar criteria could be attributed to a single fish at range r.

An example case is shown Figure 4.6.9C where the recorded SPL of xxx dB re 1μPa could be explained by approximately 15 fish at 50 m range or 100 fish at 120 m. However, the removal of call contributions from a <50 m range fish leads to a remaining SPL of yyy dB re 1μPa and a subsequently lower number of fish at given ranges greater than 50 m (Figure 4.6.9C). If another fish (Caller B) could be determined at a second threshold the process could be repeated (there was no such fish above the blue threshold in Figure 4.6.9B). The removal of Caller B pressure contributions would further reduce the SPLs to zzz dB re 1μPa. The process would be repeated until the distribution of the remaining amplitudes in the pressure waveform showed statistical improbability that another individual caller could be separate and the remaining pressure waveform was effectively due to background calling from a number of fish at ranges greater than that of the last removed caller.

By combining the number of removed callers, the minimum number of fish at minimum caller range r and the maximum possible number of fish at maximum range caller R, it may therefore be possible to give the boundaries of fish numbers capable of producing the recorded mean squared SPLs. As discrimination between the nearer fish and the background calling improves, the more accurate the abundance range estimate will be.

4.6.4. Discussion

Call counting techniques were more successful during low density calling occurring towards the beginning and end of the calling cycle, accounting for all detected calling fish (Figure 4.6.6). In the hour before sunset Category 2 calls increased in numbers

(Section 4.2), but could still be discriminated from each other. However, at this time, vessel noise consistently masked long range calls of low signal-to-noise ratio. Thus a number of calls could not be counted. During periods of peak calling, call counting techniques underestimated the number of calls, due to merging; however, calls at comparatively close range could be discriminated.

Post peak calling, Category 2 call numbers fell and Category 1 calls could be counted at greater range than early evening Category 1 calls (up to ~500 m). As background noise at this time was low, comprising predominantly shrimp clicks which occupy brief periods on the pressure waveform, call counting was effective. Figure 4.6.3D illustrates the short duration of shrimp clicks and Category 1 calls, in comparison with Category 2 calls, and therefore the ability to discriminate a large number of calls. Category 1 calls in the late evening, however, have been associated with post courtship behaviour in some Sciaenidae (Lagadere and Mariani, 2006, Section 4.2), thus the number of fish which have already courted, emitted similar calls, and left the vicinity is unknown. Therefore, although call counting at this time provides a minimum estimate of caller numbers, it may not be representative of the aggregation numbers at the height of spawning.

Call counting from the cumulative energy curve displayed good discrimination of calls at close range. The energy curve gradient increased significantly, in response to a consistent gradient due to constant vessel noise. However, at increased caller range the contribution to overall cumulative energy of fish calling compared to vessel noise was not significant.

The received SPLs from calling fish have been shown to be range dependent. It has been shown that only a small number of fish could be responsible for the minimum and peak received SPLs at the beginning and height of calling respectively.

Therefore the authors propose that neither call counting techniques nor call contributions to overall SPLs alone should be used to estimate maximum fish numbers during chorus levels of a soniferous aggregation. However, a combination of the two techniques can provide an estimate of numbers between set range limits. An automated program may determine SPLs equivalent to a fish at close range and estimate a minimum range of the closest caller. Further scrutiny of waveforms and spectral content can evaluate whether such SPLs are due to a close, single fish or constructive interference from multiple callers at varying ranges. By using the maximum and minimum boundaries above, together with the probability of fish spatial distribution, it is speculated that future work will determine likely densities of calling fish and estimate numbers using the contribution of partial pressures of individual calls to the overall, time averaged SPLs.

4.6.4.1. Variables for future consideration in abundance calculations

McCauley (2001) emphasized the need to determine fish spatial distribution in abundance estimates. However, in aggregations of fish exhibiting even limited mobility the distribution of fish around the hydrophone over time has been shown to be a complex variable. Due to transmission losses the received SPL from a call varies significantly with range. A single call emitted at close range contributes SPLs equivalent to multiple callers at greater range. *A. japonicus* have been shown to exhibit low levels of mobility while vocalising (Section 4.4 and 4.2), with one localised fish travelling at speeds of approximately 0.25 ms^{-1}, equating to 30 m over a two minute period. During one two minute recording segment a fish moved between ranges of approximately 25 and 50 m. Similar movement by another fish over three segments contributing to overall SPLs varying between 131 to 121 dB re 1μPa. If all fish exhibited such movement while vocalising SPLs would vary considerably, and the complexity of the variation will be dependent on the movement of the fish. For example, random movement by a large number of individuals at differing ranges may have little effect on the mean SPLs over time. However, a uniform alongstream motion, as has been hypothesised for *A. japonicus* (Section 4.4), would have significant implications for SPLs as the fish approach and leave the hydrophone. Therefore the length of segment time chosen to determine SPLs has significant impact on the estimated number of callers.

The sporadic presence of vessel noise in Mosman Bay causes significant issues in the estimation of callers via SPLs. Once *A. japonicus* calls had risen to chorus levels it was not always possible to confirm whether vessel noise was present as it would have been masked by the chorus. As such, it was not always possible to quantify the contribution of vessel noise to SPLs. The estimate of callers using cumulative energy is therefore dependent on the noise samples used. As vessels in Mosman Bay have been shown to generate noise of bandwidth encompassing frequencies of *A. japonicus* calls they are of significant cause for concern when using SPL to estimate numbers. Figure 4.6.2 shows that distant calls contribute very little cumulative Pa and are easily masked by a distant vessel.

McCauley (2001) highlighted several physical variables which require assessment before biomass estimates can be made of chorusing fish, including fish position in the water column as this greatly impacts the call transmission. Section 4.3 localised fish emitting Category 2 calls at, or near, the riverbed. It has been speculated that male *A. japonicus* call from the riverbed to attract females (Section 4.2), behaviour similar to that of other vocal species, such as Atlantic cod (*Gadhus morhua*) (Nilsson, 2004, Fudge and Rose 2008). Therefore SPL variation due to source location in the water column water depth has not been considered as an important factor here with the consistent fish calling depth removing this potential sound transmission variable.

The results above assume that a fish calls consistently throughout the sample segment. Previous studies *in situ* and *in aquaria* of *A. japonicus* have shown that this is not always the case. Callers employ a relatively consistent call repetition rate (Section 4.4), however, periods of cessation occur, lasting between several seconds and tens of minutes (Section 4.2, calls *in aquaria*). A true estimate of the number of calls requires the probability of cessation within the recording time to estimate the ratio of callers to non-callers over any given period in time.

Category 1 and 2 calls emitted by different fish occur during the same period and add to the complexity of estimating caller numbers. At times of peak calling it is not possible to discern the ratio of Category 1 to 2 calls, thus at the moment only an estimation is possible. Variance in the mean squared pressure source levels are affected by the length of the call. Longer calls contribute greater energy to the time averaged overall SPLs (Section 4.5.5). Call lengths often vary not only between callers, but one individual has been shown to emit calls of varying length and structure within a short space of time (Sections 4.2.4.1 and 4.4.3, respectively). The difference in pressure level contribution due to call length becomes more significant when comparing contributions due to Category 1 and 2 calls. Category 2 calls are typically 6.5 times the length of Category 1 calls and were of greater amplitude (Section 4.2.4.1). Both categories have been shown to occur throughout the evening therefore an adjustment is required to account for the different call energy levels.

Final abundance estimation requires determination of calling to non-calling members of the entire aggregation. Juvenile *A. japonicus* do not possess the sonic muscles to vocalise (Griffiths and Heemstra, 1995, Section 4.2) and during previous sampling surveys of Mosman Bay only mature *A. japonicus* were caught (Farmer, 2008). As this study concerns a spawning aggregation, which by definition includes only mature *A. japonicus,* then juveniles were not considered. It has been assumed that as all males are present in the bay to spawn, they all emit Category 2 calls to attract females. However, it is not known if all males continue to call with Category 1 calls afterwards, or if some have already left the vicinity. It is possible that some males may remain silent while spawning, or exhibit sneak-spawning behaviour similar to that of sockeye salmon (*Oncorhynchus nerka*) remaining in seclusion until just before spawning (Foote *et al.*, 1997). Although the sample was small (n = 62), spawning season sampling in a recent study in Mosman Bay produced a male: female ratio of 1.3:1 (Farmer, 2008). Thus call counting techniques were able to determine a maximum of 26 spawning *A. japonicus*, within approximately 100, 000 m^2 (Figure 4.6.5, across stream range restricted by depth) equivalent to approximately 3, 850 m^2 per fish (assuming a random distribution of callers and recipients). Subsequent increases in SPLs show that more fish were present, later in the evening, when call counting techniques were underestimating the number of recorded calls.

4.6.5. Conclusions

Due to the length of *A. japonicus* calls, the interval between calls of an individual fish and the ability to separate two calls occurring at the same time (Section 4.2) call counting techniques have currently determined up to a maximum of 15 calling individuals and therefore 26 fish in total during periods of low density calling. However, once chorus levels have been achieved and calls overlap a correlation between SPLs and caller numbers has not been shown. The possibility of estimating a maximum number of fish between arbitrary ranges has been shown, although further work is required to improve confidence. This would enable fish density over a portion of a spawning site to be estimated, and this extrapolated over the dimensions of the full spawning site. Sporadic vessel noise masks calls at varying ranges, thus the call detection range of the hydrophone requires adjustment for the given background noise.

4.6.6. Future Work

The issue of determining the ratio of calling/non-calling fish remains unresolved. Ueng *et al*. (2007) reported differences between sound production by male and female *A. japonicus*, in captivity. However, if not all males call, it is necessary to determine what proportion does call, when cessation occurs and how long it lasts, and whether the fish remain in the vicinity during periods of cessation. In an ideal world the observation of such behaviour would involve an isolated aggregation of randomly distributed males and females of known number. Thus the number of calls could be related to the overall population. In the absence of this possibility, calling ratios will have to be repeatedly obtained from aquaria. In such controlled conditions it may be possible to confirm vocalisation with underwater video techniques.

4.6.7. Acknowledgements

The authors would like to acknowledge the Fisheries Research and Development Corporation (FRDC) for project funding and the Western Australian Marine Science Institute (WAMSI) for a PhD top-up scholarship. Mal Perry and Frank Thomas supplied endless technical and field support in the acquisition of acoustic data on which this study

was based. Cordelia Moore, Ben Saunders and Wojceich Klonowski provided excellent support in the field on long protracted evenings.

4.7. Protocols for recording and reporting fish sounds.

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ABSTRACT:

Modern hardware developments are allowing routine seasonal monitoring of calling fish. The spawning calls of fish and their associated functions offer a great deal of information about the behaviour and dynamics of the callers and the aggregations they originate from. Sound production by fishes is often species and environmentally specific. Many species of fish use ecological cues to define their calling patterns, thus different environmental conditions can result in calls of differing character. In order to monitor and understand how fish behave and respond to their environment in the long term it is necessary to compare calls on varying temporal and spatial scales. Such comparison requires the knowledge of all pertinent variables available and an understanding of the effects each imposes on the resulting call characteristics. The example of mulloway (Argyrosomus japonicus) calls is used to illustrate the relationship of call and/or behavioural characteristics with source and receiver positions, water depth and temperature, bottom structure, time of day, salinity, tides, and supplementary catch data.

Keywords: *passive acoustics, long term monitoring, acoustic characteristics*

4.7.1. Introduction

It is only recently that hardware developments have allowed long-term monitoring of marine animal calling behaviour. The following discussion highlights some of the variables affecting the analysis of fish sounds together with the reasons why researchers should report all possible information when relaying their findings, and examples of different environmental influences on recorded calling behaviour. Examples to illustrate each variable have been taken from research conducted on *A. japonicus* in Mosman Bay, Swan River, Western Australia.

4.7.2. Methodology

Recordings of *A. japonicus* calls have been conducted at various times and locations in Mosman Bay, Swan River (Section 4.1-6). Omni-directional HTI-90U and -96min hydrophones were attached either to Digital Audio Tape (DAT) recorders (Sony D8 or D100) or Centre for Marine Science and Technology (CMST) – Defence Science and Technology Organisation (DSTO) designed sea-noise loggers. DAT recorded acoustic data were transferred to digital files by means of a DP430-FFT Analyser (Data Physics Corporation) at a sample frequencies ranging between 5 and 15 kHz. The data were processed using Matlab® programs developed by the CMST and passed through high (50 Hz) and low (1000 Hz) pass filters to limit noise effects of hydrophone movement and shrimp clicks. Further detail on data acquisition, processing and analysis techniques have been outlined in Sections 4.1 to 4.6.

4.7.3. Dynamics affecting call characteristics

4.7.3.1. Source and Receivers Positions

Figure 4.7.1 displays resultant surface reflected ray-paths of a call which can vary with hydrophone depth and have been shown to change the recorded waveform of a fish call (Section 4.4, Figure 4.7.1B). The call initiation peak (CIP), defined as the first detected voltage amplitude peak associated with the call (Section 4.4), has been used to determine the start of a call, (shown in Figure 4.7.1B at 1). Hydrophones positioned at different depths, but the same horizontal range, receive the direct path signal of the CIP at different times such as the waveforms received by R_2 and R_3 at 1) and 2) in Figure 4.7.1B (note, in this case R_1 was positioned at a different range and has only been time synchronised to the CIP of R_2 for illustration purposes). Once reflected paths arrive at the hydrophone significant variations in the waveform are observed 3-6). For example, the inverted surface reflection of the CIP exaggerates the first negative amplitude peak in R_3 at 3, and creates a local minimum in the detected pressure amplitude of R_2 at 4. By comparison the bottomed hydrophone (R_1) does not receive the reflected path for a period of time, such as the entire first pulse of the swimbladder at 7).

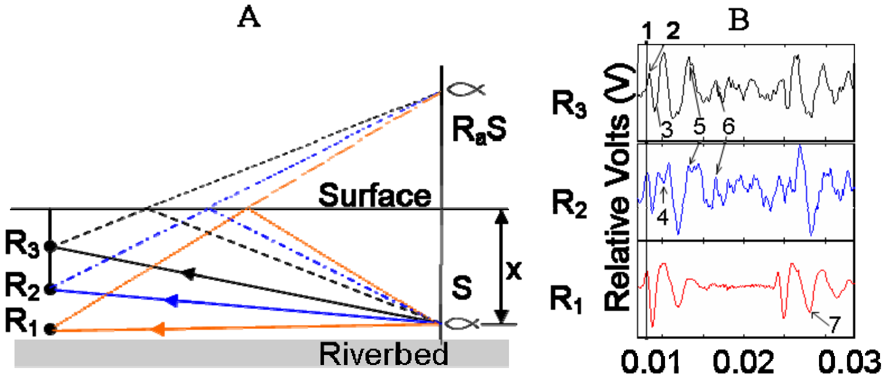

Figure 4.7.1. *Direct and first surface reflected ray paths of a riverbed call in 20 m of water to three hydrophones (R_1, R_2 and R_3) at varying depths (A). Original source S and imaginary source from surface reflection R_aS are also shown. B) Waveforms as recorded by bottomed, 10 and 5 m depths hydrophones (R_1, R_2 and R_3 respectively – R_1 has been time synchronised for illustration purposes only).*

If the difference between the direct path and surface reflection (i.e. compare Figure 4.7.1A, continuous and dotted lines) is sufficient, the arrival of the first, phase inverted, surface reflected signal does not alter the temporal position of the CIP, thus 1) and 2) are purely the direct path. However, with increased range and reduced hydrophone depth the beginning of the inverted surface reflection can arrive at the hydrophone before the direct path arrival reaches its initial amplitude peak. As the inverted surface reflection possesses a negative gradient, the resultant peak of the combined direct and reflected path occurs earlier than that of the direct path alone, thus 2) would occur earlier. The likelihood of this occurring increases with decreasing difference between direct and reflected path distances (i.e. either hydrophone or source is nearer a reflecting surface or range is increased). From the simple comparison between the first pulse of R_1 and those of R_2 and R_3, surface reflections can be seen to affect perceived pulse duration, pulse period and amplitude. Subsequent to the final direct pulse, surface reflections can arrive after the direct arrival finishes, leaving call length to the subjective judgement of the observer. To counter this effect Malme (1986) deemed the 5 and 95 % values of the cumulative intensity time product of the call, to be the start and end points for standardisation of signal lengths. Therefore the depth of the hydrophone has significant implications for the resulting waveform and the information drawn from it. The depth of the recording hydrophone should therefore be reported and where possible, maximised. True emitted waveforms can often be determined from de-convolution of the signal and may provide information on caller range, however, this can be complex and outside the scope of the study (McCauley, 2001).

Lagadere and Mariani (2006) proposed that short *A. regius* calls are of lower amplitude than long calls. A similar phenomenon was reported in the detected voltage amplitudes of Mosman Bay *A. japonicus* (Section 4.2); however, it was observed that in several instances surface reflections arriving after the second pulse had increased the amplitude peaks of the first cycles of subsequent pulses. As a result the initial one, two or three pulses in these long calls were of lower amplitude than the successive ones (as shown in Figure 4.7.2). Such effect are much like those seen in Figure 4.7.1B (R_2 and R_3 second pulse peak) when compared to a signal without reflected paths (R_1). However, not all amplitude variations were attributed to surface reflections, but the variation of sonic muscle tension (detail on sonic muscle tenions effects can be found in Section 4.2), thus both possibilities should be considered. Rome (2005) noted that after a twitch, sonic muscles do not have time to relax completely, thus further twitches may contract the muscles to a greater extent than the first. Therefore later pulses can possess greater tension and produce waveforms of greater amplitude.

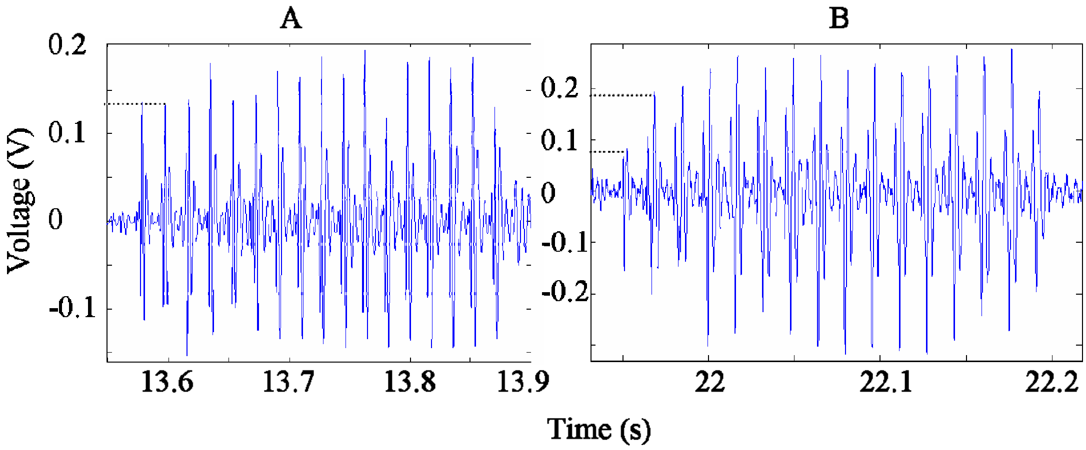

Figure 4.7.2. *Waveforms of two Category 2a long A. japonicus calls. In the first call (A) the initial pulses were of similar amplitude (dotted line), whereas in the second call (B) the first pulse was of significantly lower detected amplitude (dotted lines).*

Calls of *A. japonicus* have been split into several categories and types (Section 4.2). The arrival-time of surface reflections of *A. japonicus,* Category 2, long calls are often difficult to observe as they occur within the waveform structure of subsequent swimbladder pulses (Section 4.4). However, *A. japonicus* short calls comprising few pulses, or fish calls which exhibit widely separated pulses (McCauley, 2001, Connaughton *et al*., 2000), may reveal surface reflections more easily. Although localisation is not possible using a single hydrophone, if accurate time separation and comparative energy levels between direct and surface reflected arrivals can be identified these can provide estimates of range and source depth if the receiver depth is known (Cato, 1998, McCauley, 2001). It is therefore prudent to include reports of surface reflections where possible. In the example shown in Figure 4.7.3, the time between the direct and surface reflected ray paths is such that, for a bottomed receiver in 18.5 m water, the fish was within 1 m range of the hydrophone. In this instance the recording, together with the surface reflection, provided the call source level and the position of the fish near the riverbed (Section 4.2).

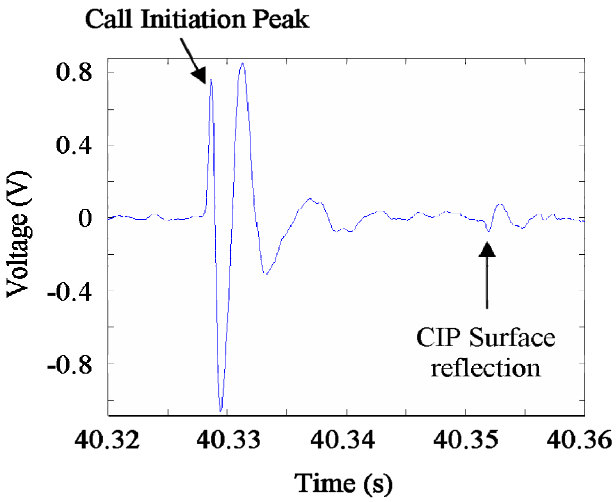

Figure 4.7.3. *Waveform of a single pulse Category 3 A. japonicus call with surface reflection highlighted. Call was recorded with a silt substrate bottomed hydrophone in 18.5 m water in flat conditions at approximately 19:10 on the 5th March, 2008, 2 hours 20 minutes post high tide and 20 minutes pre sunset.*

Call spectral peak frequency provides recipients with information on characteristics of the caller size (Gilmore, 2002). The incorrect recording of spectral peak frequency can therefore distort the inferred characteristics of the calling fish, for example, an incorrectly inferred fish size. Where calls such as those of *A. japonicus* contain a tone burst and sidebands of amplitude modulation which dominate the spectral density, interference at differing ranges and receiver depth can cause the spectral peak frequency to change. To illustrate this point a simple model was generated to produce a click similar in structure and frequency to that of an *A. japonicus* call, with the source waveform and power spectra shown on Figure 4.7.4A and B. The single click was given a spectral peak frequency of 275 Hz (Figure 4.7.4B).

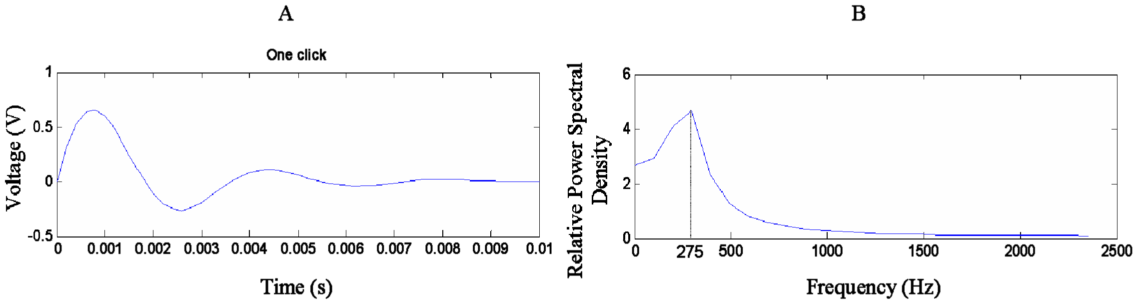

Figure 4.7.4. *Generated model for the single pulse of a swimbladder from a single click similar to that in frequency to* *A. japonicus,* *including waveform (A) and power spectral density (B).*

The model click was repeated 20 times at a repetition frequency of 55 Hz, to imitate a Category 2a, continuous long call (Section 4.2). A possible calling scenario was created using this source, where a vocalising fish was recorded by a mid-water hydrophone at three ranges and received call frequency spectra calculated (as shown in Figure 4.7.5A, B and C) including the direct and surface reflected paths. In each scenario the magnitude and phase shift of the bottom reflection was considered.

In the first scenario (Figure 4.7.4A) the fish and hydrophone were farthest apart so that the differences between direct and surface reflected path distances were small. If the fish was located at the same depth, but closer to the hydrophone, surface reflected path angles changed significantly, increasing the arrival-time difference between direct and reflected paths, but also between the reflected paths. In the third instance it was assumed that the fish vocalised over hard substrate, such that a third multi-path reflected by riverbed and water surfaces had a significant effect.

The resulting frequency spectra in the three scenarios changed the perceived spectral peak frequency, producing results of 275, 210 and 165 Hz respectively, dependent on the relative position of hydrophone and fish, and habitat in which the fish is based. This illustrates that the substrate over which a fish calls can affect the perceived frequency content of the call.

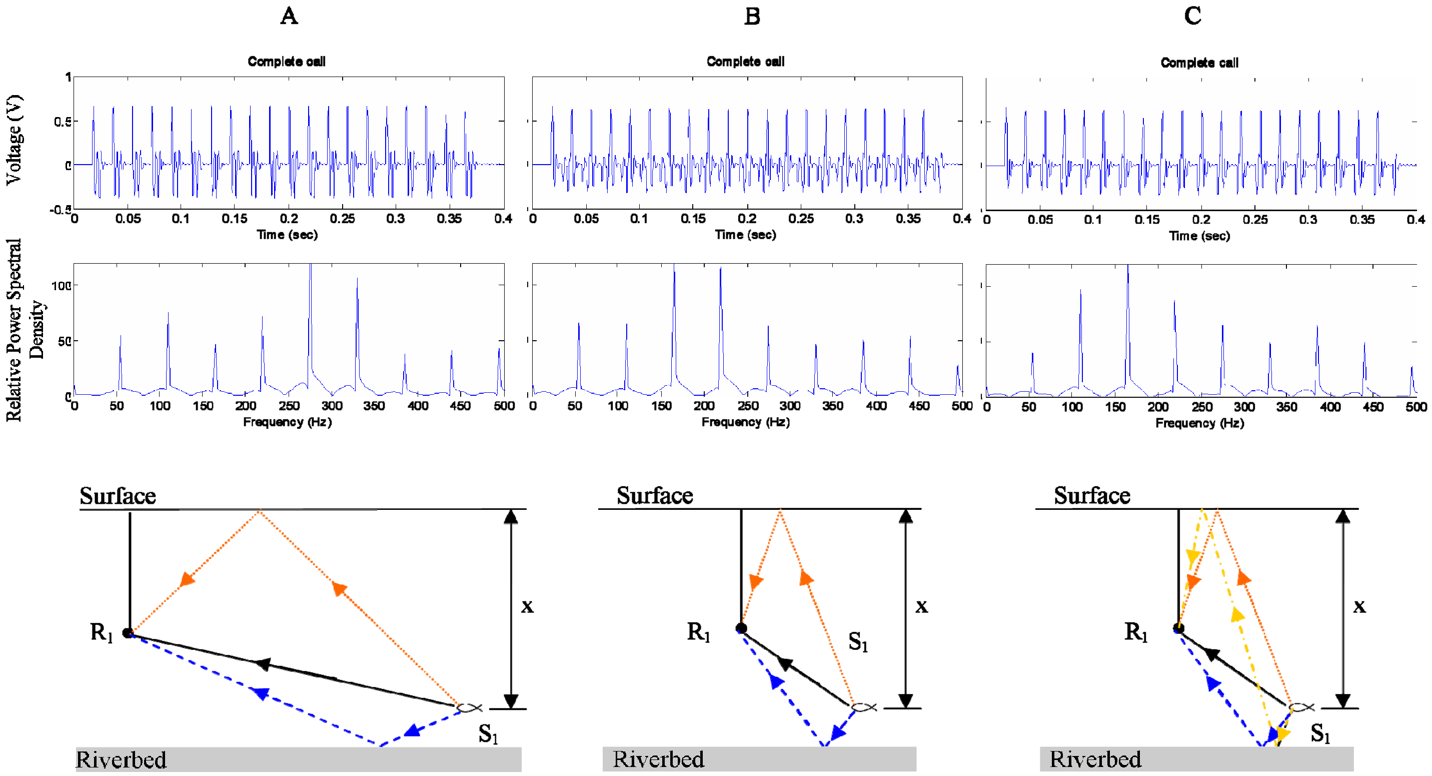

__Figure 4.7.5.__ Three example scenarios are shown (A, B and C) demonstrating the waveform of the call (top), the frequency distribution (middle) and relative fish/hydrophone positions with associated call ray paths (bottom). In each scenario the source S_1 is positioned at depth x at a distance from receiver R_1. In scenario A the fish and receiver are far apart and in B they are positioned closer together such that surface (red) and bottom reflections (blue) are observed in the waveform. In the third scenario the riverbed is of sufficiently hard substrate that a reflection from bottom and surface (orange) affects the waveform and therefore call spectra.

In many reports spectral densities of recorded calls are smoothed for ease of viewing. The smoothed curve is affected by the relative energy levels at the spectral peak frequency and those peaks of the sideband modulation, thus creating a slightly different curve at different ranges, depths and substrates. It is therefore recommended that the spectral peak frequency and the closest frequencies of the sideband modulation are reported and that frequency spectra curves remain unsmoothed in an attempt to identify whether spectral peak frequency has been affected by interference.

4.7.3.2. Range effects on source level

Source level ranges for the three categories of call emitted by *A. japonicus* were reported in Section 4.5. During this study one fish was observed repetitively emitting calls at varying ranges from the receiver. An estimate of propagation loss was made from 65 localised calls, shown in Figure 4.7.6, and a species Category 2 mean squared pressure source level of 172 (95% c.l. = 168.4, 176) dB re 1µPa was determined using least squares regression fitted to measured values. However, assuming the fish consistently emitted calls of comparable intensity, the sound pressure levels displayed distinct signs of constructive and destructive interference at the various ranges, as shown in Figure 4.7.6 (dotted line).

The difference between the two calculated source levels would lead to considerable difference in abundance estimates if applied to the technique of individual call contributions to overall SPLs recorded during fish choruses (Section 4.6). The 95% confidence limits in the regression determined mean squared pressure source level of *A. japonicus* Category 2 calls varied by 11 dB re 1µPa (Section 4.5). This is a significant variation when considering the contribution of an individual caller to overall ambient noise SPLs. McCauley (2001) highlighted the need to understand propagation losses of a call from source to receiver, in order to quantify the contribution of a call to overall SPLs. The trend in transmission losses for this Category 2 calls was determined to be greater than spherical ($-23.94\log r$ for an individual monitored fish and $-23.74\log r$ for all fish tested). Applying spherical spreading to individual recorded levels determined a mean source level of 165 (s.d. = 2.2) dB re 1µPa (compared to the determined 172 dB re 1µPa). Therefore, although the identification of a species call source level is an important step towards the estimation of biomass using passive acoustic techniques it is important to ensure that a sufficient number of individual calls from different callers have been recorded at a variety of ranges to accurately assess the effects of propagation on transmission losses, and to gauge normal source level variability between different fish.

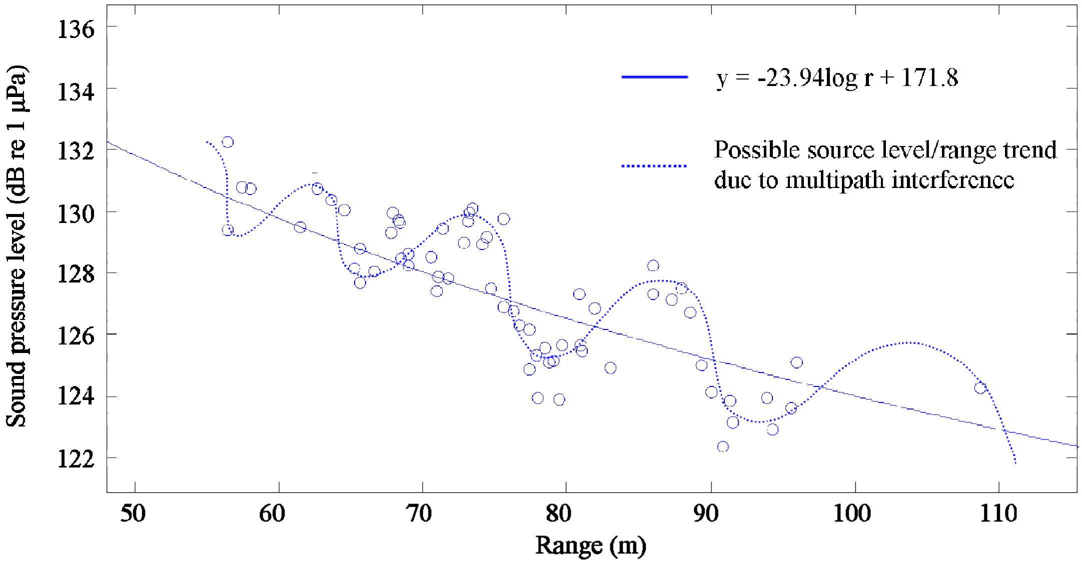

Figure 4.7.6. *Variation with range in sound pressure levels of calls emitted by a repetitively vocalising riverbed positioned fish as determined by a bottomed hydrophone in relatively uniform silt substrate, 18.5 m below flat water surface on the 5th March, 2008, 2 hours 20 minutes post high tide and 20 minutes pre sunset.*

Three variables affecting sound transmission are the riverbed substrate, bathymetry and on occasion, sea state. Mosman Bay comprises a relatively uniform, silt substrate riverbed, offering low acoustic reflectance (Jensen, 1997) and comparatively simple ray multi-path interference. However, other species of fish may vocalise in areas of more complex bathymetry and/or over a harder, more acoustically reflective substrate affecting calls produced in that area. Nassau grouper (*Epinephelus striatus*) for example, spawn and vocalise around coral reefs in the Caribbean, which have hard limestone seabed, though limestone converts significant portions of acoustic energy into shear waves (Moulton, 1958, Hazlett and Winn 1962, Aguilar-Perera and Aguilar-Dávila, 1995, Jensen, 1997). In addition, wind levels at the time of recording affect the state of water surface which in turn bears an impact of the multi-path transmission of the call signal.

4.7.3.3. Background Noise

Mosman Bay is subject to high levels of vessel traffic repetitively passing above calling fish, often limiting the discrimination of fish calls. Figure 4.7.7 highlights such masking, showing constant vessel noise (horizontal lines) overlying fish calls. In the case of Figure 4.7.7 some distant calls are barely visible on the waveform, while closer calls may be more easily detected. Although a vessel passing 20 m overhead may not prevent a fish calling it has a significant impact on the maximum detection range of the call for a recipient fish (Section 4.4). At times of high background noise fish calls may be completely masked even at close range (Section 4.5). In prolonged periods of high ambient noise this masking may have a significant effect on the spawning success rate of the calling fish and therefore has important ramifications for the management of essential fish spawning habitat. Alternatively, fish may alter their behaviour to counter such noise, such as caller position, or timing of calls. It may be possible that fish possess the capability of increasing call source level to compensate for increased background noise, similar to the Lombard vocal response observed in whales (Scheifele *et al.,* 2005, Holt *et al.,* 2009). Understanding the effect of anthropogenic activities, such as vessel noise, is an important factor in maintaining the sustainable ecology of the fishery. Therefore, providing comparative background noise data illustrates the acoustic environment a fish has acclimated to vocalising in.

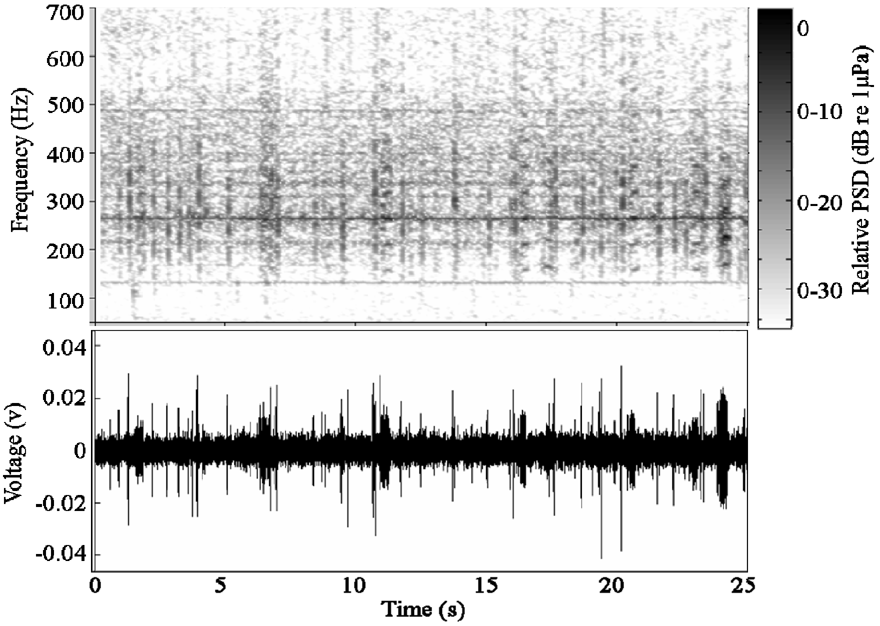

Figure 4.7.7. *Spectrogram and waveform of a recording taken at approximately 21:30, 17th January, 2007 illustrating the effects of contributing vessel noise at frequencies similar to that of the signals produced by fish. Horizontal dark lines in the spectrogram result from passing vessels.*

Background noise, however, is not always vessel generated. During Mosman Bay acoustic survey on the 20th December 2007, 25 knot easterly winds generated waves of sufficient amplitude and regularity to oscillate moorings in close proximity to where *A. japonicus* regularly vocalise and spawn. The resultant effect was that virtually no fish calls were audible in recordings above the noise of the mooring chains (author *pers. obs.*).

At the beginning of a Mosman Bay evening spawning cycle *A. japonicus* individual callers can be detected at distances in excess of 100 m. At times of high call density where vocal signals are dense enough to become a background noise, severe reduction in the detection range of an individual caller has been reported to as little as a few metres (McCauley, 2001, Sprague and Luczkovich, 2004, Section 4.2). Therefore noting the ability to separate calls and the recorded difference in amplitude between a single call and aggregation background noise (considering calls as incoherent signals) provides an inference of the density of calling fish, the range for detection of a call by the intended recipient and therefore an insight into the spawning behaviour of the aggregation.

4.7.3.4. Recording vessel movement

Calls of *A. japonicus* are often emitted by individuals at approximately consistent rates, similar to other species (Mann and Lobel, 1998, 2002, Luczkovich, 1999, Parsons *et al*., 2006a, Section 4.2). Thus a change in relative amplitude of calls from an identified individual can offer information on the relative movement of the fish. Many opportunistic recordings are taken by drifting vessels. It is therefore prudent to note the drift (direction and speed) of the recording vessel to aid in establishing fish movement. If the repetitive calls from an individual fish can be considered of similar amplitude, and the vessed speed and transmission loss determined, then it may be possible to infer whether a fish is stationary or not. For example, on Figure 4.7.8 the voltage amplitude of *A. japonicus* Category 1, short calls increase steadily with time by approximately 1.5 times, from 0.37 to 0.55 V. During this recording the vessel from which it was taken, drifted upstream at approximately 0.25 ms^{-1} (approximately 10 m overall). If the caller was comparatively stationary and ray paths could be assumed to parallel to each other, the fish range would have varied from appromixately 30 to 20 m. In addition, variation in call amplitude may imply varying degrees of movement by the fish. For example, vocalising *A. japonicus* have been shown to exhibit slow along stream movement while attempting to attract a mate (Section 4.4), which would produce a similar waveform to Figure 4.7.8 if detected by a stationary (rather than mobile) vessel.

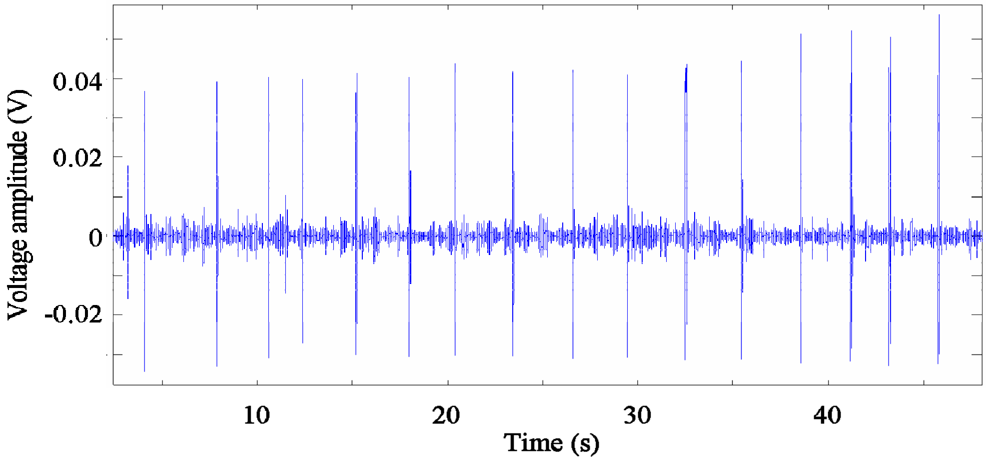

Figure 4.7.8. *Amplitude variation of Category 1, short calls thought to be emitted by a single fish over a 50 second period as a vessel drifted upstream at a rate of 0.25 ms^{-1}. Hydrophone positioned 4 m below the surface in approximately 19 m of flat water above relatively uniform silt substrate at 19:57 on 17th January 2006, 2 hours 30 minutes pre high tide and 30 minutes post sunset. Detected amplitude increases gradually as the calls proceed implying a reduction in range between caller and receiver.*

4.7.3.5. Temperature

Spawning of *A. japonicus*, along the coastline of Western Australia, has been shown to be linked with temperature (Farmer, 2008). In Mosman Bay temperature has been shown to be a driver of the commencement and cessation of the spawning season (Section 4.3). Significant correlation was also observed between maximum sound production trends and those of temperature as the season progressed (Section 4.3). Observation of water temperature at time of recording, relative to seasonal maximums, can help identify the relative expected level of spawning associated with recorded SPLs. Thus the comparison of recordings at the same location with supplementary water temperature data increases knowledge of which environmental factors drive vocalisation, and by proxy spawning.

However, the relationship between temperature and vocalisation levels is not simple. Connaughton *et al*. (2000) reported a 5.9 dB re 1μPa increase in weakfish disturbance calls associated with a 12.5 °C temperature increase ($R^2 = 0.50$, $p = 0.0001$), due to increased muscle contraction rates at the higher temperatures. Such a temperature related source level increase will have significant implications in abundance estimated from SPLs of soniferous fish. Hydrophone recordings in Mosman Bay showed *A. japonicus* sound production over the evening time averaged, 250 Hz centred one third octave, increased from approximately 75 to in excess of 100 dB re 1 $\mu Pa^2/Hz$ between the start and height of the spawning season. During this period temperatures ranged from 18.5 °C to greater than 26 °C (Section 4.3, Figure 4.7.9). When estimating fish numbers it is important to consider what proportion of such SPL increase is due to greater numbers of callers and what is due to the temperature driven increase in mean SPL of an individual caller. Therefore the same source level can not be used in the estimation of absolute biomass until the effects of temperature on the species source level ranges have been quantified and the temperature at the time of survey recorded.

Variations in termperature also affect the spectral peak frequency of a call. Connaughton *et al.* (2000) associated increases in water temperature with increases in the spectral peak frequency of weakfish calls, as the sonic muscles contract more rapidly. Contraction and relaxation rate of sonic muscle fibres is an influential factor in the spectral peak frequency of a swimbladder generated fish call (Connaughton *et al.*, 2000), which is in turn dependent on the rate of calcium Ca^{2+} transfer in the muscle (Rome, 2005). Temperature changes affect the rate of calcium Ca^{2+} transfer within muscle tissue and thus the rate of contraction and relaxation (Rome, 2005). An example of positive correlation between temperature and spectral peak frequency in the current study was illustrated by the increase of *A. japonicus* call spectral peak frequency with temperature increase, as recorded at the beginning and height of spawning, shown by the 2006-7 spawning season in Figure 4.7.9A and Section 4.3. Although it was acknowledged that frequency variation could have been caused by vocalisations of different sized fish during the course of the season, a high level of significance (R^2= 0.516, $p<0.0001$ for the 2006-7 season) was observed between temperature and spectral peak frequency.

Fine-scale call frequency variation was also observed throughout individual evening spawning cycles. Using time averaged frequency distributions for recordings during the Mosman Bay 2007-8 spawning season Figure 4.7.9 illustrates examples where call spectral peak frequencies rose and then fell (B), remained constant (C), fell (D) or rose (E) during the course of an evening spawning cycle. Whether these frequency variations were driven by temperature changes is undetermined.

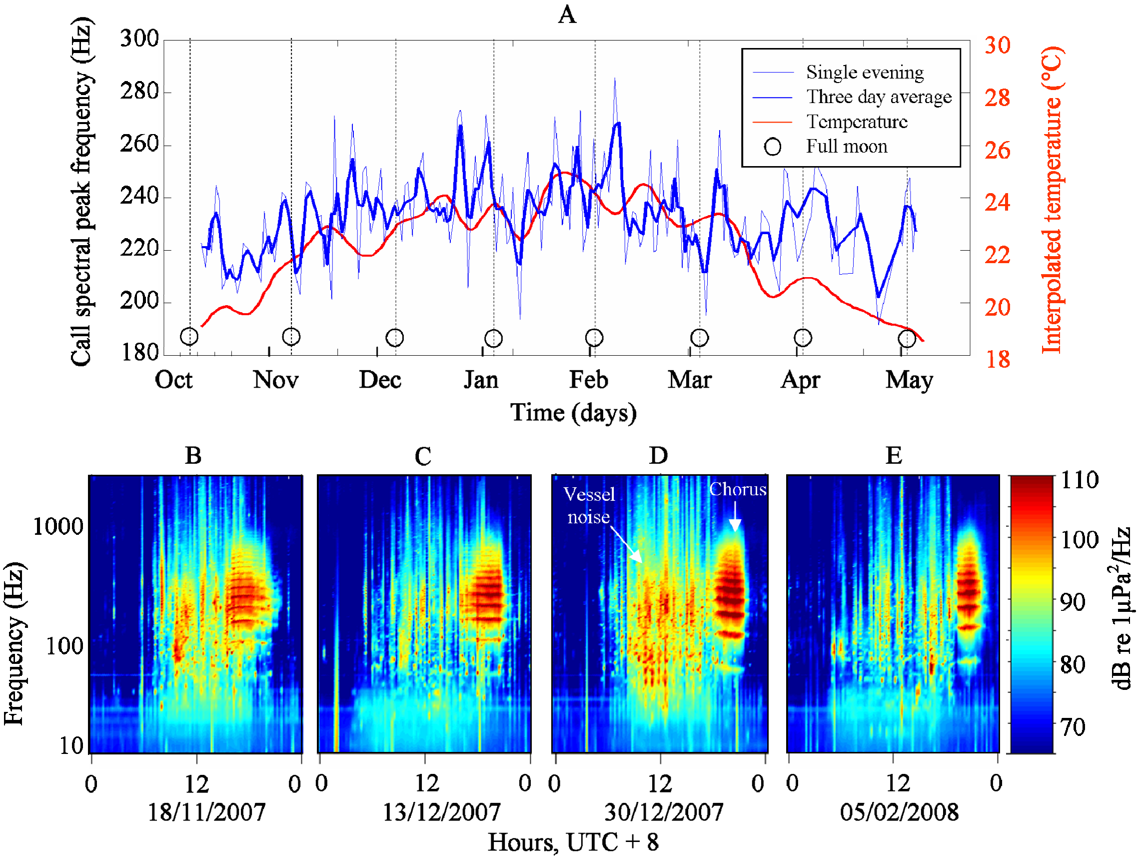

Figure 4.7.9. *Call spectral peak frequency averaged over an evening and using a three day running average (thin and thick blue lines, respectively) and temperature (red) over the Mosman Bay 2006-7 A. japonicus spawning season (A). Day long spectrograms displaying frequency distribution of sound from vessel noise and fish chorus (2007-8 spawning season). Dominant spectral call frequencies during the course of an evening chorus are shown to rise and fall (B), remain constant (C), fall (D) and rise (E).*

4.7.3.6. Light levels and time of day

Ueng *et al.* (2007) noted light related circadian rhythms in *A. japonicus* sound production. The SPLs produced from calls at dusk and dawn led to the suggestion by Ueng *et al.* (2007) that *A. japonicus* is a nocturnal species. However, recordings of *A. japonicus* at Fremantle, TAFE aquaculture facilities where two captive, spawning broodstock *A. japonicus* are habituated in near perpetual twilight showed calls from throughout the day, ceasing before sunset (Section 4.2). Mosman Bay *in situ* recordings, displayed significant sound production several hours prior to sunset, illustrating that nocturnal behaviour alone is not the case (Figure 4.7.10, Section 4.3). Although there was a strong correlation between sound production and time of sunset, with mean seasonal maximum chorus levels approximately 1 hour post local sunset, calling ceased completely prior to midnight, without the dawn chorus. Given the afternoon/evening calling in Perth and nocturnal activity in Taiwan it has now been proposed that *A. japonicus* adapt their behaviour to the environmental conditions around them and that light is only one variable affecting species behaviour (Section 4.3). It is therefore important to report the time of day and location to help determine the environmental cues pertinent to sound production.

In addition, it is important to relate the survey to the relative time of the season, as chorus times can vary as the season progresses (Section 4.3). For example, in Figure 4.7.10 the sound production has been zeroed to the time of sunset. Throughout the spawning season the intensity and time of the calling varies, not only relative to our daily clock, but also to time of sunset which follows seasonal changes given as the declination of the sun from the earth's equator. A full season of recording is suggested to provide the long term relationship between environmental drivers such as light levels, and sound production during spawning.

Daily maximum SPL times in Mosman Bay displayed a close relationship with the time difference between sunset and high tide (Figure 4.7.10, Section 4.3). Farmer (2008) proposed that *A. japonicus* spawn at high tide to ensure eggs are taken out into open water with the ebb tide. It is likely that the timing between call categories which are associated with differing spawning behaviours vary with tide and should therefore be reported.

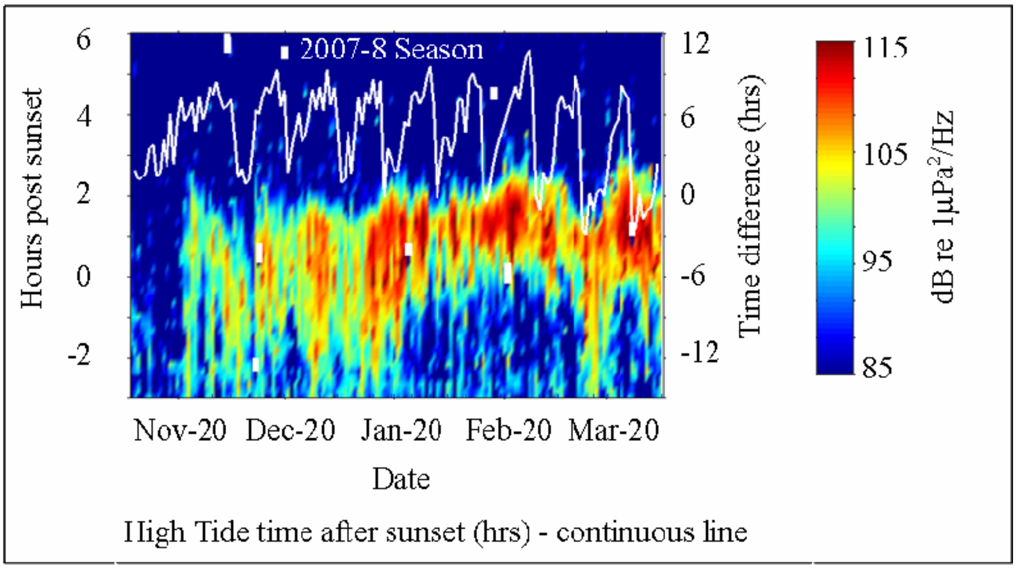

Figure 4.7.10. *Seasonal average time and intensity of daily A. japonicus sound production compared with that of high tide (white line) and the time difference between high tide and sunset between 19th October, 2007 and 23rd March, 2008.*

4.7.3.7. Salinity

Adult *A. japonicus* are marine animals, and in Western Australia enter estuaries once salinity levels are sufficient (Griffiths *et al.,* 2005). Levels of *A. japonicus* sound production in Mosman Bay have been positively correlated with salinity, explaining significant portions of deviance in sound production across a season (Section 4.3). Where significant declines in salinity were observed the average SPLs also decreased. It was unknown whether this signified an overall reduction in caller numbers, lower individual call rates or a migratory movement away from the hydrophone vicinity; however, it highlights environmental conditions correlated with spawning activity which should be reported with the fish calls where possible.

4.7.3.8. Seasonal variations in fish anatomy

Minor decreases in SPL of individual weakfish (*Cynoscion regalis*) calls throughout the spawning season have been reported (Connaughton *et al*., 2000). It was proposed that this reduction was due to the depletion of sonic muscle mass, possibly due to muscle atrophy toward the end of the spawning season (Connaughton *et al*., 1997). Intuitively the muscle depletion may also reduce achievable muscle tension and so affect the call spectral peak frequency, though this requires confirmation.

The constraint of swimbladder movement by free space in the body cavity may also affect call characteristics such as spectral peak frequency and SPLs, thus where possible, traits such as feeding time, and seasonal levels of fat content or gonad size should be recorded. Spawning maturity also impacts on the nature of the emitted calls. *A. japonicu*s is a batch spawner and has been observed to exhibit spawning maxima on two to four day cycles (Farmer, 2008, G. Jenkins, TAFE, Fremantle, *pers. comm*.). Similar spawning related trends were observed in sound production with local maxima every 3.97 days (Section 4.3). It is possible that call rates of certain individuals or call categories provide information on the spawning maturity of the individual caller. However, although it cannot be confirmed whether the males and females vocalising in Mosman Bay are the same individuals each evening, the identification of day-to-day trends in long term sound production is an efficient way of observing spawning cycles, in comparison with more labour intensive methods, such as fertilised egg and larvae sampling.

4.7.3.9. Sound production mechanism

Call characteristics are dependent on the sound producing mechanism employed by the fish. Within extrinsic methods of swimbladder excitation, notable differences in call characteristics can be observed (Connaughton *et al*., 2000, Nilsson, 2004, Parsons *et al*., 2006a, Section 4.2). Muscles which are inefficient produce lactic acid quickly and so generate shorter calls before fatigue (Rome, 2005). Lactic acid build up is also possible in well adapted species where long calls such as Atlantic cod 'hum' decline in pulse repetition frequency at the end of the call (Nilsson, 2004). *A. japonicus* and *A. regius* are biologically similar species with similarly located sonic muscles around the posterior two-thirds and three-quarters of the swimbladder respectively (Griffiths and Heemstra, 1995, Lagadere and Mariani, 2006, Section 4.2). However, *A. regius* calls recorded in the Gironde Estuary, France, lasted a minimum of 1000 ms (Lagadere and Mariani, 2006), over twice as long as that of *A. japonicus* in Mosman Bay (Section 4.2). Whether such difference is due to biological mechanism efficiency or adaptation to environmental conditions is unknown, but may be revealed by more detailed evaluation of muscle properties. Therefore when comparing sound production by different species it is advantageous to compare not only the call characteristics, but also those of the sound producing mechanism to determine whether behaviour is biologically or environmentally driven.

4.7.4. Conclusions

A number of biological, environmental and seasonal variables have been shown to significantly affect call characteristics and by proxy, the behaviour and/or biology of the fish. Thus monitoring of species distribution and fish ecology using the observation of calling behaviour requires supplementary data recording all influential variables as well as ground truth information on the function of the calls. When environmental variables are reported over seasonal time scales it is possible to derive models identifying which descriptors contribute to the deviance in sound production and therefore offer a better understanding of the species ecology. Although it is accepted that under survey conditions not all parameters can be recorded the authors' feel that as many of the parameters detailed above should be observed and reported. These variables are highlighted in Table 4.7.1.

Table 4.7.1. *Physical, biological and environmental variables affecting the recorded characteristics of fish sounds, together with their respective influence and importance on the calls.*

	Variable/measure	Impact	Importance
Transmission	Source and receiver position	Received call characteristics of intensity, duration, spectral peak frequency	Inferred size of the caller, species call characteristics, caller range
	Surface reflections	Provides a range estimate if the water depth is known, and therefore source level data.	Confidence in source levels and resultant abundance estimates.
	Spectral peak frequencies	Ray multi-paths can affect the spectral peak frequency of a call.	Inferred size of callers and the associated preferences of female recipients.
	Estimated range	Source level due to multi-path interference.	Inferred size of the caller and abundance estimates.
	Substrate and bathymetry	Acoustic reflectance becomes increasingly complex with substrates of high acoustic reflectance and varying bathymetry	Significant implications for localising fish as complex bathymetry affects confidence in CIP selection. Identifying substrate helps determine spawning Essential fish Habitat.
	Background noise (biological or anthropogenic)	Levels of call masking	Highlights the density of the aggregation.
			Shows how an individual may be detected, located and chosen.
			Whether anthropogenic activity affects calling or detection of fish sounds, and resultant impact on spawning success.
	Vessel movement	Amplitude of calls with range	Inferred mobility of the caller and therefore behaviour during spawning.
Environmental	Sunset/Lunar phase/Tidal ranges	Time and intensity of sound production and, by proxy, spawning.	Ecological trends in spawning behaviour due to environmental drivers.
	Temperature	Biological spawning requirements	Commencement, cessation of spawning season and diel behaviour
		Sonic muscle contraction rates	Alters the source level of a call and thus affects size estimate of individual and abundance estimates from SPL contribution
			Alters call spectral peak frequency and thus inferred fish lengths
	Salinity	Biological spawning requirements	Commencement, cessation of spawning season and diel behaviour
Biological	Seasonal variations in biology	The ability to produce sound and the effect on characteristics.	Seasonal behavioural patterns both vocal and spawning.
	Biology of sound production mechanism	Acoustic characteristics of calls	Ability to compare species in terms of function and occurrence, together with the relationship to associated behaviour.

4.7.5. Acknowledgements

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4.8. Preliminary findings of passive acoustics applicability to alternate species.

Deployments of mid-water and seafloor hydrophones (Section 4.1, Figure 4.1.2) near *S. hippos* spawning aggregations revealed no evidence of vocalisation. These findings were in line with the expectation that *S. hippos* is not a soniferous species.

Although vocalisation in Glaucosomatidae has not yet been reported *G. hebraicum* possesses bi-lateral intrinsic muscles connecting skull and swimbladder typical of swimbladder related sound production mechanisms (Vu, 2007, M. McCauley, Curtin University, *pers. comm.*). Biochemical assessment of Citrate synthase (CS) and L-lactate dehydrogenase (LDH) activity (enzymes associated with energy supply for muscle contraction) in *G. hebraicum* swimbladder muscles suggested their involvement in sound production (Chiu, 2006). Furthermore, swimbladder vibrations and noise have been heard on two occasions by scientists when tagging *G. hebraicum* (M. Mackie, Department of Fisheries WA, *pers. comm.*). Ambient noise levels due to wind and wave motion in open water are likely to be greater than those in Mosman Bay. Therefore the detection ranges of recording *G. hebraicum* which spawns in shallow, nearshore waters (Mackie *et al.*, 2009) are speculated to be smaller than those of *A. japonicus* in Mosman Bay, due to the likely cal signal to noise ratio. Therefore, when investigating small numbers of open water spawning fish, hydrophones need to be accurately located within a small detection range of the site.

The deployment of sea noise loggers to observe *C. gerrardi* during active acoustic surveys (December 2006 and February 2007) revealed no evidence of vocalisation. However, the schools observed formed outside the spawning season when the fish may not have reason to call. The structure of the swimbladder of *C. gerrardi* suggests that the species is soniferous and it is possible that vocalisations are only produced during spawning. Future targeting of this species at spawning aggregations is anticipated to confirm vocal capability and the efficacy of passive acoustics to monitor them.

Spawning aggregations of *P. auratus* have, as yet, not been targeted for passive acoustic monitoring. Anatomical evidence suggests that the species may be soniferous and future investigations are planned to study *P. auratus* aggregations in the Cockburn Sound.

4.9. Conclusions

Research conducted during the course of this study has taken existing techniques of passive acoustic monitoring, applied them to a relatively unstudied species of Sciaenidae and furthered several techniques in a rapidly emerging discipline.

An ideal study site for assessing passive acoustic techniques to monitor soniferous fish is one which possesses low acoustic complexity with boundaries of simple or non existent reflectance (i.e. low bathymetric complexity, a bottom of low reflectance and a flat water surface), with easy access for study. Ideally the number of callers would build from a low density where individuals can be distinguished easily to high levels where calls occur in choruses and cannot be individually discerned. The study site used during this project within the Swan River meets these criteria and is ideal for further studies of *Argyrosomus japonicus* using passive acoustic techniques.

4.9.1. Equipment feasibility and processing capabilities

The acquisition of shallow, short term passive acoustic data (for example, an evening spawning cycle of an estuary based aggregation) requires little equipment and can be deployed from small, trailerable vessels. Midwater recordings can be acquired from moored or drifting vessels using only a hydrophone, pre-amplifier and tape recorder. Recordings taken from the riverbed, deeper open water or over long term periods may require sealed, autonomous recordings systems such as the CMST-DSTO underwater noise logger (Section 4.1). The dimensions of logger equipment are such that they may be deployed by hand, from small vessels (see Section 4.1 for example deployment configurations).

During long term acoustic surveys the sea-noise loggers can be deployed to record for periods ranging up to a year. Upon retrieval data may be downloaded from the hard drive and after maintenance checks and battery replacement may be re-deployed. Thus the passive acoustic recording of fish vocalisations is a low maintenance and non-labour intensive method of data acquisition. The rapid process of recovery, data retrieval, maintenance and redeployment can be repeated for the life of the equipment.

Although processing and analysis of acoustic data is complex and requires specific programs and specialist training, once species calls have been characterised a level of automation is possible (Sirovic *et al.*, 2009*,* Sections 4.2 and 4.6). Thus processing time and complexity of data from successive seasons may be reduced. Therefore, once the recording equipment has been developed, set up costs met and preliminary processing conducted, the low level of deployment and maintenance means that passive acoustics is a cost effective method of monitoring aggregations over several year periods. Because of the ease of data collection, passive acoustic techniques are one of the few methods for sustaining continuous decadal period observations.

4.9.2. Standard protocols

During the course of this study the results and conclusions of this and previous reports have led to the designation of an initial set of standard protocols for recording and reporting fish sound production (Section 4.7). The objective of such standardisation was to facilitate comparison of calls at various locations and times of season by different research bodies. Several variables which are often unreported have been shown to affect call characteristics. Many variables such as relative time to high tide or water salinity are supplementary to acoustic data, and whilst they may not directly affect acoustic characteristics they may indirectly do so by varying fish behavioural characteristics (Section 4.3). In contrast, physical variables such as hydrophone depth and water temperature have been shown here (Section 4.7) and in previous studies (Connaughton *et al.*, 2000) to affect received call characteristics. There is therefore the necessity to accurately record and report, not only fish sounds and associated behaviour, but all available supplementary variable data.

4.9.3. Call characterisation, ground truthing and behavioural bias

When employing passive acoustic techniques it is first necessary to identify the origin and purpose of the call. This includes the determination of call timing, ground truthing and an assessment of induced behavioural bias.

In the case of, *A. japonicus* aggregations within Mosman Bay several categories and types of call were documented and aligned with possible functions. Calling, as an announcement of a spawning cycle or readiness to spawn, has been reported in other species (McCauley, 2001), and were speculated as dominance related, hierarchical calls by *A. japonicus* (Section 4.2). Such calls may provide a cue for spawning between male and female fish, and may also enable researchers to gauge relationships between spawning and variables such as light levels and high tide (Sections 4.2 and 4.3). Courtship calls and calls of attraction have been shown to elicit reproductive behaviour between individuals (Luczkovich *et al.,* 1999b, 2000), offering significant information on the spawning success and population of an aggregation. *A. japonicus* was observed to exhibit a greater vocal repertoire of courtship calls than was expected (Parsons *et al.*, 2006a, Section 4.2). Similar observations in other species have been reported as an increasing number of in-depth acoustic studies of fish vocalisations are conducted (Nilsson, 2004). As some species use call acoustic characteristics to discriminate between fish for mate selection such as the bi-colour damselfish (*Pomacentrus partitus*) (Myrberg *et al.*, 1993), the extent and meaning of vocal repertoire requires documentation. Opportunistic observation of *in situ* calls of interaction may be rare.

As a small, silent, immobile object, a hydrophone may go unnoticed by the vocalising fish. At times of low ambient noise the hydrophone detection ranges have been shown to extend into at least hundreds of metres while visibility is often restricted to substantially less (Section 4.4). Thus a fish may be recorded from a considerable distance. Passive acoustics therefore offers the ability to observe fish, via their calls, without inducing a behavioural bias, in contrast with techniques such as baited video (attracting fish to the video with unquantified bait plumes), diver tows or active acoustic survey (often inducing diver or vessel related avoidance), which alter natural behaviour.

The ideal confirmation of species-specific calls and their functions may be achieved through *in situ* simultaneous video observation. However, given the visual conditions in which many vocalising species spawn (dark, or turbid waters), the comparison of call acoustic characteristics between field and aquaria recordings may be the most practical method of ground truthing call function. The analysis of calls *in aquaria* provided ground truthing for *A. japonicus* calls. The observation of calls of differing category and their timing compared with that of egg production has added corroborative data to the speculated call categories and associated functions of Mosman Bay *in situ* vocalisations. The limitation of call comparison in such controlled conditions is the restricted vocal behaviour and repertoire exhibited by some species in captivity (Midling, 1993, Midling *et al.,* 2002) and possible effects of captivity on the acoustic characteristics of a call, for example the atrophy of sonic muscles (Section 4.2).

4.9.4. Long-term observation

The long term acoustic observation of spawning related sound production together with supplementary environmental data offers significant information on the ecological variables which influence spawning times and intensities. Species often require specific conditions in which to spawn. For example, it has been reported that *A. japonicus* along the coast of Western Australia spawn in temperatures above 19 °C (Farmer, 2008). The comparison between temperature and sound production can determine the commencement of spawning. As water temperatures increase it is possible to relate increases in sound production to the temperature (Section 4.3), allowing for the associated increase in caller Sound Pressure Levels (SPLs) with temperature (Section 4.7). The observation of sound production with temperature may determine optimum spawning temperatures for a species and whether there is a cut off at which a temperature increase results in no further rise in sound production.

The variation of an individual's call SPLs and spectral peak frequency as a result of temperature effects on sonic muscle contraction rate and tension has recently been documented (Connaughton *et al.*, 2000). Long term recording at the same location provides information with which to assess how call characteristics change throughout the season both concurrently and concomitantly with temperature change (Section 4.4). Due to the effects of fish size on call spectral peak frequency and SPLs an assessment of the distribution of fish sizes throughout the recording period is required for accurate comparison. Once ground truthed, these relationships are expected to stand for successive years of monitoring.

Ueng *et al.* (2007) reported *A. japonicus* to be a nocturnal animal based on recorded sound production of captive individuals, however, Mosman Bay datasets showed soniferous behaviour up to several hours before sunset. Section 4.3 highlighted several variables influencing sound production in Mosman Bay *A. japonicus* in relation to peak calling times and intensities. The variation in time of sound production compared with high tide illustrates that rather than being strictly nocturnal, the species may adapt behavioural patterns to optimise the ecological conditions they spawn in. Such observations may provide information on the optimal spawning conditions for *A. japonicus* such as light levels, temperature, salinity and current, to be used to promote production in aquaculture.

Repeat deployment of autonomous, acoustic loggers, at the same location provides long term data series of high temporal resolution for successive spawning seasons. Aggregations can therefore be monitored over periods of years to observe responses to long term dynamics such as climate change or fishing pressures. The analysis of season long Mosman Bay passive acoustic datasets of *A. japonicus* vocalisations together with supplementary environmental data have shown season-wide responses of fish sound production and by proxy spawning levels to variations in environmental conditions.

4.9.5. Localisation of individual fish and source levels

This study has reported the movement of individual fish within an aggregation area using an array of hydrophones. The technique of arrival-time differences to locate animals is rarely used in such fine-scale studies. Aggregations often occur in locations of acoustic complexity, due to surface acoustic reflectance and bathymetry (such as hard substrate coral reefs). Passive acoustic localisation of fish calls requires development and testing in an acoustically simple environment and evaluation of the spawning site (Section 4.4). The study of *A. japonicus* calls highlighted the effects of caller density on the performance of localisation. As caller density increases the ability to discriminate between calls reduces and, therefore, so does localisation. Thus spawning aggregations of high caller density pose more complex processing issues than those of low density and require assessing.

Once localisation techniques are developed and hydrophones calibrated the array provides information on fish movement and separation between callers. Behavioural information from caller position such as calling from the riverbed or the midwater similar to that of *A. japonicus* may have specific implications for their function (Section 4.4). Observation of repetitive calling by individuals leads to understanding the relationship between calling fish, such as their separation distances, and how they react to external influences such as vessel noise (Section 4.4).

The localisation of an individual results in a known range from the recording hydrophone. Thus source levels can be derived for the types of call emitted (Section 4.5). The passive acoustic localisation of *A. japonicus* allowed determination of source level ranges for all three categories of call emitted by *A. japonicus* during spawning. The verification of source level is rarely reported and is one of the first steps towards absolute biomass estimates from passive acoustic recordings, as well as providing minimum ranges at which an intended recipient might hear a call of attraction.

4.9.6. Biomass estimation

The application of known call source levels, together with call counting techniques facilitated counting and ranging of a maximum of 15 callers within a hydrophone range of approximately 500 m, during low density calling in Mosman Bay. However, abundance estimates based on individual call contributions to overall SPLs requires further development. However, a system to determine broad limits of maximum and minimum callers within given range boundaries has been outlined in Section 4.6.

4.9.7. Acoustically Baited Remote Underwater Videos (ABRUVs)

Baited Remote Underwater Video (BRUV) techniques are often employed in the observation of marine life (Watson *et al.*, 2005, 2007, Watson and Harvey, 2007, Moore, *in prep*.). However, the use of underwater video techniques to survey low density aggregations of fish in dark and/or turbid waters is limited in range to a few metres and observation fish is often only a fortuitous event (Sprague and Luczkovich, 2004, Fudge and Rose, 2008). Autonomous broadcasting of calls of attraction to prospective spawners from a non-threatening object, simultaneously recording visual data, offers the opportunity to observe natural interaction between fish. If individual fish can be drawn to within metres of a simulated call source, visual data can provide ground truth data on vocalisation, size, source levels and behaviour. The variation of the broadcasted call characteristics, such as spectral peak frequency, can provide valuable information on the preferred attributes a recipient desires, inferred from the quantity and nature of the fish attracted by each type of emitted call.

4.9.8. Supplementary noise sources

As a by-product of recording fish calls, concomitant sources of noise are often of interest to researchers studying other local projects. Three examples of such data were noted during this study. Dolphin calls were often noted during recording sessions as resident Swan River dolphins passed through Mosman Bay. Studies of the local dolphin population would gain significant benefit from an acoustic ‘gateway’ to monitor individuals as they pass the hydrophone. Long term datasets have shown varying levels of vessel and land based noise. The recording of current anthropogenic noise levels can create a baseline with which to monitor the effects of future increase use of the river and the possible effects on the local ecology. Snapping shrimp noises were present throughout all datasets and are an important trophic group for the health of the river. Future monitoring of shrimp populations may help provide a picture of the distribution of the group.

4.9.9. Summary

The passive acoustic recording of fish vocalisations offers non-invasive, behaviourally unbiased, spatially identifiable datasets on soniferous species of fish in timeframes ranging from seconds to entire seasons. The field technique is low maintenance, cost effective and non labour intensive, requiring little biological sampling and resulting in no impact on the surveyed habitat. It is possible to monitor fine-scale individual movement, broad-scale migration, population and to an extent temporal size distribution of an aggregation by observing their calls. Methodological conclusions drawn from techniques applied to *A. japonicus* can be extended to most soniferous species given the appropriate environmental acoustic conditions. However, the application of passive acoustic techniques to study a new species requires considerable initial effort to characterise call repertoire and behaviour.

5. General Discussion

5.1. Context

Australia has a rapidly expanding population and increasing number of fishers spread over a vast coastline (McPhee *et al*., 2002). In recent years technological advances have greatly improved fishers' ability to target and catch otherwise inaccessible fish from off-shore fisheries, increasing pressure on fish stocks. As such, the management and monitoring of a fishery requires efficient, cost effective means of acquiring data while using minimal time and effort.

The characteristics of fish spawning aggregations often leave them vulnerable to overfishing (Mackie *et al.*, 2009). For example, the formation of large stationary aggregations at known locations such as those of *Seriola hippos*, the courtship vocalisations of *Argyrosomus japonicus*, or the residency of fish such as *Glaucosoma hebraicum* around acoustically identifiable seabed 'lumps' can each be targeted by fishers. The repetition of these behaviours allows fishers to target the aggregation over successive years, in some cases systematically reducing the population to collapse (Claydon, 2004). At the same time the seasonal migration of numerous individuals from differing locations to a single area of high density spawning aggregations offers researchers an opportunity to observe and monitor large populations of fish, potentially over extended periods and with reduced effort. In some cases the spawning characteristics which consign a species to increased fishing pressures may lend themselves to a particular type of technique to monitor them.

5.2. Characteristics of differing fish aggregations and the features of individual acoustic techniques employed to study them

Species-specific characteristics exhibited by spawning aggregations have implications for the choice of acoustic technique used to study them. These characteristics and the consequential impact on each acoustic technique are discussed below.

5.2.1. Stationary aggregations

Seriola hippos often form relatively stationary, midwater aggregations comprising large individuals of significant acoustic reflectance and large nearest neighbour distances (Section 3). *S. hippos* aggregations provide a good illustration of the characteristics exhibited by a stationary aggregation.

The repetition of single-/split-beam echosounder surveys throughout a spawning season provides intra- and inter-seasonal comparisons of aggregation size and relative fish density, as demonstrated by the monitoring of spatial area, abundance variations and site preferences of *S. hippos* aggregations in this study (Section 3.2.3). However, standard acoustic techniques of echo-integration and target counting to estimate abundance require assumptions of school temporal uniformity and random distribution within the aggregation (Simmonds and MacLennan, 2005, Section 3.2). Such assumptions are limited by natural mobility and vessel avoidance behaviour exhibited by the aggregation during survey.

The acoustic swath of MBS is sufficient to acquire high resolution backscatter from entire midwater aggregations in a single two or three minute transect, such as those found here with *S. hippos* (Section 3.3 and 3.4). Therefore, researchers are provided with more defined aggregation spatial extents and volume, in minimal time, requiring fewer temporal assumptions than echosounding. Where fish reflectance and separation are sufficient, individual targets such as *S. hippos,* can be discerned, providing more detailed structure of the aggregation (Section 3.4). Due to beam pattern and sample volumes such spatial detail is less readily observed with single-beam echosounding techniques. Thus a single MBS transect may encompass an entire aggregation and acquire greater detail than one hour of single-beam survey. Alternatively, the speed at which backscatter is acquired from the MBS offers the ability to survey a greater number of aggregations within a given period, compared to that of single-beam echosounding.

In the past, volumes of water column backscatter data acquired by each MBS 'ping' have been large enough to reduce the available ping rate. The time between each ping may lead to spatial aliasing in the unsampled volumes of water. Recent surveys employing increased ping rates have minimised the effect of missed targets in this unsampled water (Section 3.5). However, surveys employing high MBS ping rate have also highlighted the multiple detections of the same fish. The cause of these detections is movement speculated to be due to along track vessel avoidance behaviour (Section 3.4), similar to that noted at greater ranges by Soria *et al.* (1996). Therefore even aggregations which appear to be stationary may exhibit some level of vessel avoidance, similar to that of *S. hippos* during February 2007 surveys (Section 3.4).

A significant limitation in single-beam and MBS surveys is species identification, exacerbated by high levels of species diversity and heterogeneous aggregations in Western Australian waters. The application of 3-D TS models for species identification and echo-integration in MBS surveys is complex, species-specific and requires considerable development (Horne *et al*., 2000, Simmonds and MacLennan, 2005, Pena, 2007). The variation in S_V values from an *S. hippos* aggregation, across the swath of a Reson 7125 illustrated some of the complexities of 3-D target strength (Section 3.4.3). In order to quantify the effects of this S_V variation ground truthing of mean length and species composition (particularly in multi-species aggregations or areas of high species diversity) is necessary, typically by stereo video techniques, catch and release sampling, or where feasible, trawling (McClatchie *et al.*, 1999, Simmonds and MacLennan, 2005, Mackie *et al.* 2009).

Therefore, while a stationary, midwater aggregation may be monitored by echosounding techniques for general biomass and seasonal variations, this can be compared against the increased coverage MBS systems provide, and the more rapid and accurate abundance estimates with greater detail on aggregation behaviour and structure. However, the use of MBS systems in fisheries acoustics is still in its infancy and limitations in biomass estimates still require quantification.

5.2.2. Mobile aggregations

The nature of vertical echosounding denotes that fish which are not within the beam geometry, directly below the vessel at the time of survey, will not be sampled. Low levels of mobility or vessel avoidance may result in the 'blurring' of the apparent aggregation spatial extents, particularly in single-beam surveys (Figure 3.2.5). The quantification of such blurring requires further investigation. More pronounced movement inhibits the number of transects acquiring usable data. Surveys of *P. auratus,* which move around shallow (<30 m) waters and exhibit wary behaviour to vessels (Mackie *et al.,* 2009), illustrated that such aggregations are not conducive to a vessel positioned directly above the fish. The aggregations of *P. auratus* avoided the vessel altogether (Section 3.5). As such, species which form mobile aggregations or exhibit comparatively high lateral vessel avoidance are not well suited to survey with single-/split-beam echosounders.

As many MBS systems provide athwartships coverage of up to several times the water depth, lateral vessel avoidance in shallow water can often be observed using typical seafloor mapping mounting positions. At greater depths lateral and vertical avoidance are of less concern, though the high operation frequencies of MBS (hundreds of kHz) and consequential range restrictions mean a towed body may be required (Graham *et al.*, 2004, Trevorrow *et al.*, 2005, Parsons *et al.*, 2006b). By comparison, the lower frequencies generally employed in echosounding provide a greater depth range for surveying. However, Soria *et al.* (1996) quantified example distances at which fish may exhibit vessel avoidance behaviour. This distance may often be less than that of the water depth (up to 100 m) and thus fish flee before the research vessel arrives. Therefore in shallow waters (for example, less than 50 m depth) or in studies of wary species, survey planning may consider alternative system mounting positions (Gerlotto *et al.*, 1998). Sideways mounting of the system allows surveying at a greater distance and therefore less induced vessel avoidance (Section 3.5.3). It is anticipated that such surveys provide researchers with similar data to that of typical (nadir orientated vertically) mounting positions, though this was not evaluated here.

5.2.3. Soniferous species

The use of passive acoustics to monitor a fishery is limited by the capacity of target species to produce sound. The species which exhibit vocal behaviour often aggregate in conditions which reduce the efficacy of traditional sampling techniques such as high turbidity or low light levels. In such circumstances innovative techniques are required for accurate monitoring of species abundance and behaviour. In addition, vocal species which exhibit lekking behaviour, such as *A. japonicus* can spawn in comparatively low densities, calling from the riverbed. As a result it is not always possible to observe targets use active acoustic techniques (Section 3.7).

Once confirmation of sonific capabilities has been attained, and a database of species call characteristics generated, it is no longer a necessity to conduct biological sampling of a survey site. However, further specific ground truthing may be required to identify species at sites of multi-species soniferous fish, emitting calls of similar characteristics (Sirovic *et al*., 2009).

Confirmation of vocal ability may require species-specific temporal and spatial targeting, at considerable effort (Section 4.2). For example, vocalisation of low source level or by aggregations comprising small numbers in areas of high ambient noise, such as shallow open water, may possess small detection ranges. For example, *A. japonicus* calls of 172 dB re 1 μPa source level may be audible several hundred metres away during periods of low background noise (Section 4.4, Figure 4.4.4). By contrast, smaller species such as *Chrysoura bairdiella* (source level of 135 dB re 1 μPa) calls may have a detection range of tens of metres away, given typical levels of background noise in a shallow embayment (Sprague and Luczkovich, 2004). Surveys of *C. gerrardi* conducted outside the spawning season highlight the need for specific temporal targeting as no vocalisations were recorded, despite the fact that vocalisation by this species is highly likely (Section 4.8). Similarly, only anecdotal evidence of vocalisation by *G. hebraicum* has thus far been recorded, despite obvious swimbladder sonic muscles. Therefore in targeting low numbers of sedentary species such as *G. hebraicum* it may be necessary to deploy hydrophones at small inhabited 'lumps' for prolonged periods to observe vocal behaviour. Such deployments at strategic locations may observe activity patterns on key indicator species such as *G. hebraicum* where little is known about the ecology and behaviour of a species worth further investigation.

5.2.3.1. In situ

Once a large aggregation of fish has been shown to be soniferous the mapping of its extents can be a task of moving a hydrophone until produced sounds can no longer be heard. The sustained localisation of individual *A. japonicus* to within 3 m (Section 4.4, Table, 4.4.2) was achieved in this study and highlights the level of accuracy to which the extents of spawning aggregations can be mapped.

Vessel and background noise have been shown to mask courtship calls in some species (Sprague and Luczkovich, 2004, Section 4.4) and may affect behaviour in the long term (Picciulin *et al., in prep*). Passive acoustic recordings are able to evaluate the intensity of vessel noise and determine an estimate of the ranges over which this would mask a marine animal call. *A. japonicus* Category 1, 2, and 3 call source levels of 163, 172 and 157 dB re 1μPa respectively would be completed masked (assuming broadband intensities only) in ranges less than 10 m by noise of 150 dB re 1μPa (Section 4.5, Table 4.5.3). The masking of calls requires investigation as this may have specific impacts on spawning success rates of an aggregation. Thus passive acoustic techniques allow the evaluation of anthropogenic noise levels and temporal overlap with fish spawning calls and behaviour.

The spawning aggregations of *A. japonicus* in the Swan River Estuary combine the susceptibility of Sciaenidae species to exploitation with the location's ease of access for a large number of recreational anglers (Griffiths, 1996, Sadovy and Cheung, 2003, Farmer, 2008). As such, these aggregations may require particular protection during the spawning period. Therefore the soniferous aggregations of *A. japonicus* in Mosman Bay provide an excellent case study to highlight the implications of observing spawning vocalisations for fisheries managers.

5.2.3.2. Aquaculture

In aquaculture, broodstock productivity is often measured by levels of captured eggs. The environmental conditions, such as temperature and salinity, determined as drivers of spawning behaviour from their long term correlation with sound production, can be applied to aquaculture conditions. In *A. japonicus* the regularity of calls in captivity was significantly lower than the wild (Section 4.2), while other species in captivity exhibit a reduction in call repertoire (Midling, 2002). The correlation between variations in vocal and spawning behaviour from the wild and captivity is unknown. However, monitoring vocal behaviour *in aquaria* and comparing with *in situ* data may provide information on the relative condition of the captive brood stock.

5.2.4. Pair spawning/aggregations comprising small numbers of fish

The most problematic aggregations to acquire acoustic data from are small groups, or pairs of fish located around bathymetrically complex seafloor, and which are either not soniferous or emit comparatively few calls of low source levels. For example, small groups of *G. hebraicum* were not easy to discern from the seafloor with single-beam echosounder techniques, due to beam width, the acoustic dead zone and the varying structures near the seafloor around which they reside. The ability to discern benthic targets such as *G. hebraicum* and *A. japonicus* with MBS systems has not been assessed, although the high resolution of along beam sampling (cm) imply that acoustic targets could be detected. Although opportunistic observation and quantification of medium to large aggregations via MBS or single-beam acoustics (Section 3.6) is feasible, species identification of fish in such small numbers via active acoustics alone is impractical and requires associated alternative techniques such as video monitoring. Long-term passive acoustic monitoring may offer ecological and behavioural information on such species that are otherwise problematic to observe (Section 5.2.3).

5.3. Implications for fisheries management

In Western Australia several methods are typically employed to monitor fish stock levels. These include the collection of fishery independent information such as catch and effort data and biological sampling which can be compared using catch per unit effort (CPUE) (Haddon, 2001), yield and egg per recruit models (YPRM and EPRM) (Quinn and Deriso, 1999). Other means of collecting fisheries independent data include video techniques (Watson *et al.*, 2005, 2007, Delacy, 2008) and remote sensing (Santos, 2000, Moore, *in prep.*). Typical methods to control the exploitation of fisheries, such as minimum legal length for retention (MLL; Winstanley, 1990) and temporal and spatial closures (Sadovy, 1996, Mackie *et al.*, 2009) can be dependent on the type and accuracy of species data acquired.

Fishery dependent data is likely to remain the primary source of fishery monitoring data as this is inexpensive and provides long historical datasets. However, as fish stocks decline it is important that data acquisition has minimal impact on the surveyed stocks. Although active and passive acoustic techniques require initial ground truth sampling to characterise species acoustic reflectance and vocal behaviour, respectively, once preliminary studies have been conducted acoustic techniques are non-invasive. At a time when ethical restrictions are tightening and concerns over barotrauma related mortality are increasing, active and passive acoustic techniques offer the capability of monitoring aggregations with significant reductions in survey related mortality. This ability to ‘observe’ fish from a distance, with negligible interaction, facilitates the monitoring of natural population and behaviour with limited survey induced bias. Of particular note are areas where fishery dependent data is not readily available, such as aggregations formed in deep water, turbid estuarine waters or within marine parks. In these areas acoustic techniques provide a non-fishery biased data source with minimal impact on species and its environment.

5.3.1. Cost of the monitoring technique relative to the value of the fishery

The methods of fisheries acoustic techniques employed in this study each demonstrated levels of differing cost, technical expertise, time and logistical support. The efficacy of each technique further depends on whether valuable, supplementary (or sole) information is gleaned at manageable cost and effort, compared to the value of the fishery.

Echosounding provides a relatively inexpensive, easily deployed method to collect data for general abundance estimates. This is particularly advantageous for management of large, relatively immobile aggregations and schools where vessel avoidance is limited. Analysis and preliminary studies may be initially time consuming, though like all complex tasks can be streamlined and formed into routine tasks. Over the past decades single-beam acoustics has been a standard tool in monitoring high value fisheries of large aggregation volumes such as North Sea herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*; Misund, 1997), or in deeper waters orange roughy (*Hoplostethus atlanticus;* Kloser *et al.,* 2002, 2005). As technology has advanced, and become more cost effective, single-beam techniques are more readily available to managers with restricted resources. One example is the observations of a small, high value, limited funded fishery of Nassau grouper (*Epinephelus striatus*) where single-beam techniques determined more pragmatic numbers of spawning fish than previous video and diver census (Ehrhardt and Delevaux, 2007). Thus although single-beam monitoring may be coarse, this is relative to the limitations of previously employed monitoring techniques and can be sustained in the field with low relative effort for multi-year sampling programs.

By comparison with single-beam echosounders, MBS systems are expensive to deploy, require specialist training to operate, produce vast amounts of data and are complex to analyse and interpret. The volume and speed of data acquisition, however, is far greater than other fish monitoring techniques. Recent advances in processing to reduce unnecessarily high sample resolution (Section 3.4), while maintaining sufficient data quality (Malzone, 2008), mean that MBS systems provide a more manageable, if costly, fisheries tool. Thus MBS techniques are most effectively applied to fisheries of high value, or which require high resolution data. Such fine scale detail is ideal for observing small schools, the dimensions of which may not be easily discerned with single-beam techniques. For example, Fernandes (2003) observed clusters of small pelagic schools in the Bay of Biscay, covering whole schools in single transects. The reduction of bottom dead zone volumes using MBS systems could improve data on economically important semi-pelagic stocks such as cod and pollock (Trenkel *et al.,* 2008).

Logistics of data acquisition via passive acoustics is relatively uncomplicated, requiring little vessel capacity and few personnel to deploy/retrieve equipment which can record autonomously for periods up to years. Rapid deployment means that passive acoustic surveys can be conducted concurrently with other surveys of differing focus, at minimal additional cost and effort. Analysis may be complex; although like single-beam data, a level of automation may be achieved. Thus while initial data processing requires building new algorithms and so may be time consuming, in the long term these tools only need to be applied. The low cost and effort involved in deployment, maintenance and retrieval of recording equipment means that in the long term such data acquisition is extremely cost effective. Recreational fisheries, such as those of silver perch (*Bairdiella. chrysoura*), red drum (*Sciaenops oscellatus*) and weakfish (*Cynoscion regalis*) in the Pamlico Sound, North Carolina, benefit from the ease of deployment, mapping and cost efficiency of passive acoustics where alternative methods of observation have provided limited data (Luczkovich et al., 1999a, 1999b, Sprague and Luczkovich, 2002, 2004, Lowerre-Barbieri, 2008).

In each acoustic technique significant time and effort are required to characterise species response, whether this be acoustic reflectance or vocal behaviour. In active acoustics it is necessary to understand how the swimbladder reflects the acoustic beam to relate the response to species size, length and, in some cases orientation. When studying species vocalisations investigation to identify the call characteristics and functions is needed before they can be used to monitor behaviour and abundance.

5.3.2. Monitoring biomass

Single-beam echosounding requires assumptions of fish spatial uniformity over time. This uniformity applies to the aggregation as a whole (i.e. it is assumed the aggregation displays negligible overall mobility) and to the distribution of fish within it. Such assumptions arguably restrict biomass estimates to coarse measurements, unless mobility can be quantified. However, in a low value fishery, such as *S. hippos*, a broad view of the aggregation and its structure over time, providing a coarse abundance index may be all that is required. The study of *S. hippos* has shown that rough estimates of abundance (hundreds to tens of thousands, depending on the site and time of season) were of the order of magnitude expected at the aggregation sites and notable site preference was similar to that in a broad study of the species (Mackie, *et al.*, 2009, Rowland, *in prep.*). Accuracy of single-beam techniques to estimate biomass has been well documented by previous studies of schools, though it should be noted that such confidence is relative to sampling errors (systematic and random); also found in alternative abundance techniques (Hampton, 1996, Simmonds and MacLennan, 2005). However, differences between TS models and *in situ S. hippos* TS data demonstrated the stochastic nature of fish reflectance and the impact on biomass estimates. Similar results were observed in another study where disparities in biomass arose from differing TS models of the same species (Kloser *et al.* 2005). When considering the variability of TS it was felt that *in situ* TS data, which requires significant number of single targets, provided the best model to calculate biomass (Ona, 1990, Korneliussen *et al.*, 2007). Where fish are of sufficient size, or nearest neighbour distance, it may be possible to use methods of target counting to estimate fish numbers, similar to that seen with *S. hippos* aggregations and some small schools of *Centroberyx gerarrdi* (Sections 3.5 and 3.7).

Biomass estimates from single-beam surveys must, however, be compared to alternative surveying techniques. Simmonds (2003) compared stock assessment models of results from acoustic, trawl and larval surveys of North Sea herring (*C. harengus*). All three surveys produced useful data and could be combined to improve the assessment where, when considered separately, the acoustic survey proved to be the most reliable (Simmonds and MacLennan, 2005).

In MBS surveys the spatial extents of fish position can be mapped swiftly and accurately. Schools and aggregations are often elongated, possibly by vessel induced avoidance (*Sardinella aurita*, Gerlotto and Paramo, 2003; *S. hippos*, Section 3.2 and 3.4), thus the across track dimension of data acquisition provides more accurate volume estimation for abundance. Counting of individual targets and determination of aggregation volume can accurately identify the number of acoustic detections of fish, such as the thousands of *S. hippos* over tens of thousands of cubic metres (Section 3.3

and 3.4). In more dense aggregations Weber *et al.* (2007) combined single-beam echo-integration and MBS to estimate numbers of Atlantic herring (*C. harengus*).

Combined nearest neighbour distances and MBS recorded aggregation volumes can provide estimates of fish numbers and discriminate between schools of differing sized fish (*S. hippos* and *P. dentex*, Figure 3.5.1). However, species acoustic target density and, by proxy, packing behaviour can vary over the season, illustrated by the 23.8 m^3 (October) and 13.89 m^3 (February) per acoustic target during *S. hippos* surveys (Section 3.7). In addition, differences in packing density have been observed with changing environments, such as packing density in captivity, compared with that in the wild (Misund, 1993). Therefore abundance estimates produced using packing densities and aggregation/school volumes should be made with caution. Variability of species packing behaviour, even in stationary aggregations requires quantification, possibly with the aid of video techniques. However, it is yet to be determined if variations in observed acoustic density packing were due to differences in size, species and/or behaviour. The advantage of MBS surveys to counter such errors in target counting and density packing is the rapid, repetitive coverage of an aggregation, offering the opportunity to acquire a more statistically accurate estimate. Results from the Reson 7125 survey of *S. hippos* (Section 3.4) suggested that the smaller determined aggregation volumes were due to lack of movement and therefore more analogous to abundance

The geometry of MBS swaths allows the observation of lateral and vertical movement by fish, and therefore more accurate biomass estimates, when compared to single-beam techniques. However, the restricted ping rate in some MBS systems creates spatial aliasing in target data, due to unsampled volumes between pings (Section 3.4). Such aliasing and therefore confidence in estimates can be improved by simultaneous single-/split-beam echosounding, though the extent of improvement in confidence limits is unquantified. High sample resolution of along beam MBS data implies that individual, benthic targets could be discriminated from seafloor and fauna. However, due to the stochastic nature of fish reflectance, species identification currently requires alternative techniques to MBS (Trenkel *et al.,* 2008).

Long term, time averaged sound pressure levels (SPLs) can correlate to calling fish numbers (Luczkovich, 1999a, Section 4.6). If averaged over significant time and a random spatial distribution of fish is assumed the knowledge of relative sound production each year provides a proxy to the long-term state of the population. For example, comparative Mosman Bay sound production between spawning seasons displayed times of spawning peaks and trends, such as January 2005-6 (Figure 4.3.3), when sound production decreased throughout the month.

Numbers of calling fish within the detection range of single hydrophones can be estimated through techniques of call counting to a maximum density of callers determined by the call duration, rate and range (15 callers in the case of Mosman Bay *A. japonicus*). Once the caller density suitable for call counting techniques has been exceeded the contributions of individual calls to overall SPLs to determine caller numbers can be applied (Section 4.5 and 4.6) to offer managers information on fish density within the hydrophone detection range. This density can be extrapolated to the dimensions of the aggregation for absolute abundance estimates. However, the relationship between numbers of fish during chorus calling and SPLs is a complex function which is highly dependent on caller range and category of call. Currently, although broad maximum and minimum boundaries can be drawn, estimating numbers of fish at times of high density calling requires further development. Once finalised, it is anticipated that biomass estimation techniques will offer an effective way to monitor the number of fish within the detection area. Once adjusted for environmental variables (such as temperature) which affect call source levels, passive acoustics offer managers inter- and intra-seasonal comparison of callers.

Absolute abundance estimates can be related to the overall biomass through the determination of calling to non-calling males (in species where only the male is soniferous) and the ratio males to females present. In the case of the Mosman Bay aggregation, where gender ratios have been estimated at approximately 1:1 (Farmer, 2008), this equates to detecting a maximum of 26 fish, via call counting techniques and the knowledge that more fish are present during peak calling.

5.3.3. Ecological information

The variations in echosounder acoustic data can highlight spawning period commencement and cessation times, locations and intensities, together with seasonal and daily activity patterns. An example of such monitoring is shown by the preferred Site 2, *S. hippos* aggregation, where abundance estimates rose from a thousand fish over approximately 10, 000 m^2 in October to nearly twenty thousand over 50 000 m^2 in December/January before dropping again to a few hundred fish in March.

The resolution of MBS data on aggregation structure can provide fine scale detail on behavioural responses to anthropogenic activity (such as along track vessel avoidance, or structural variation after fishing, Sections 3.3 and 3.4). Similar levels of detail have been observed in aggregation dynamics (Misund and Galen, 1992), school structures (Gerlotto and Paramo, 2003) and packing density variation (Misund, 1993, Section 3.7). Researchers can also evaluate the extent of effects to short term fish behaviour from activities such as fishing (Brehmer and Gerlotto, 2001, Section 3.4) and vessel presence (Gerlotto and Freon, 1998, Soria *et al.*, 1996) or possible predator presence (Benoit-Bird *et al.,* 2004, Weber *et al.,* 2007).

Hydrophone recordings over extended periods will detect the seasonal commencement and cessation times of spawning. For example, in Mosman Bay *A. japonicus* sound production begin at threshold temperatures of 18.5 °C lasting typically from October until May. During the Mosman Bay 2006-7 spawning season explained deviance in sound production by temperature, salinity and sunset (contributing 40.4, 38.3 and 28.8 % of the overall explained deviance, respectively) illustrated the effect environmental drivers or correlates can have on spawning levels. Marine animals are not expected to follow our Gregorian calendar and may follow lunar cycles, shown by the correlation in 2006-7 between SPLs and lunar cycles in Mosman Bay (R^2 = 0.652), thus passive acoustics can accurately define timing of events and trends associated with sound production. The long term, continuous data series acquired by passive recordings allow analysis over extended periods rather than surmising possible relationships from point data such as deployed one hour video tapes, or snapshots obtained from an active acoustic survey. Once acquired, long term sound production offers a proxy to spawning responses to gradual environmental changes such as temperature, salinity and habitat on diel, lunar, seasonal or decadal scales.

Over several years the monitoring of sound production with environmental data allows the identification of response to long term trends such as annual temperature change. In the short term, fish localisation offers an opportunity to follow individual fish and observe natural mobility, spatial caller separation, call rate, variation in call repertoire and response to alternate noise sources (shown by the 67 calls emitted and approximately 57 m travelled by a single fish during a four minute period, Section 4.4). Anthropogenic noise can be evaluated for impacts on call masking (Section 4.4) and behavioural changes in fish (Picciulin *et al., in prep*). If anthropogenic noise is found to be excessively detrimental to spawning conditions, areas of low vessel use may be set to limit the impact. In extreme cases, limits on general vessel noise, or more specific sounds such as seismic surveys, may be required to reduce the behavioural impact or permanent damage to fish hearing (McCauley *et al.,* 2002, Popper *et al.,* 2005, Mann *et al.,* 2007).

5.3.4. Areas of essential fish habitat (EFH)

The identification of location and spatial extents of spawning aggregations provides target sites and times around which management of a fishery can be focussed. Specifically, this mapping of spawning behaviour can lead to fishery closure times and zones, location of marine park boundaries, and optimising future monitoring surveys.

Single-beam acoustic techniques offer broad scale sampling of EFH and have been used for decades to relate bottom type to fishing and spawning grounds, such as the trawl fisheries of the Bering Sea (Misund, 1997). This technique also offers a relatively inexpensive means for coarse mapping areas for consideration as Marine Park Zones compared to MBS techniques (Colquhoun and Heyward, 2008). If conducted without *a priori* knowledge of species and habitat, single-beam echosounders offer comparatively opportunistic locating of spawning aggregations outside the spawning season. However, once located, coarse spatial extents of a stationary aggregation can be mapped relatively simply. Aggregation mobility increases the complexity of accurate mapping and decreases confidence.

MBS systems are capable of simultaneously acquiring sufficient bathymetric and backscatter data to identify, classify and monitor EFH around spawning aggregations (Section 3.4.3). The classification of a species EFH allows future surveys, conducted outside the spawning season, to identify potential aggregation sites. The baseline MBS mapping of areas such as the Eastern Fairweather Ground by Greene et al. (2004) was designed to monitor habitat in relation to spawning groundfish. Such mapping via MBS systems provide greatly improved precision compared to that of single-beam echosounders and able to monitor more gradual variations in habitat (Parsons *et al.*, 2007, Parnum *et al., in prep.*).

The locating of calling fishes from their vocalisations provides an indication of species Essential Fish Habitat (EFH) (Rountree *et al.*, 2006). Numerous loggers or a vessel mounted hydrophone can be deployed to map calling fish within an area. The spatial scale over which passive acoustic data is acquired is limited by the acoustic transmission, call source level and the signal-to-noise ratio.

5.4. Summary

Echosounder techniques to monitor fish aggregations are inhibited by the sampling volume and the resultant required assumptions of school temporal uniformity and are therefore recommended to fisheries managers for monitoring relatively stationary aggregations. By contrast, MBS systems sample vast volumes providing considerable data on water column and seafloor habitat offering invaluable fine scale snapshots of the fish structure. The combination of echosounders and MBS systems can alleviate issues of along track avoidance. However, the ability of MBS systems to study small numbers of fish, residing close to the bottom has not been evaluated in this study. The identification of surveyed species using active acoustics is complex and in its infancy, requiring ground truthing via biological sampling or video techniques. The recommendation of this research for active acoustic study would be the concurrent deployment of MBS and single-/split-beam echosounder studies to provide data on abundance, behaviour and response to external impacts (such as fishing) of aggregations.

Passive acoustics techniques are relatively inexpensive, acquire large temporal datasets, and are easy to deploy and maintain. Fisheries managers are then equipped with cost effective, low maintenance, behaviourally unbiased data encompassing spatial and temporal extents, reactions to environmental and anthropogenic variations, and ultimately, absolute biomass estimates, with only preliminary detrimental effect to the aggregation. However, recording vocalisations is only suitable for a select number of species and situations.

Although passive and active acoustic techniques offer significant complementary data, and in many cases provide a substantial portion of the available fishery independent, species and ecological data, it is recommended that the above methods are predominantly used in conjunction with other available monitoring techniques such as video and/or biological sampling so that baseline behavioural data can be obtained.

A general summary of recommended acoustic survey techniques, pertinent to individual different species characteristics can be found in Table 5.4.1.

Table 5.4.1. *Species-specific characteristics pertinent to acoustic surveys, and their effect on individual acoustic techniques.*

	Aggregation characteristic			
	Near stationary	**Mobile (vessel induced or natural mobility)**	**Soniferous**	**Sedentary/small numbers**
Single-/split-beam techniques	Coarse inter- and intra-seasonal change in abundance and spatial extents. Requires temporal assumptions of school uniformity and alternate ground truth data at the time of survey. Can detect large individual targets Easy to deploy at short notice.	Not well suited to data acquisition	Dependent on the mobility and density of the aggregation.	Not well suited to acquire fish reflectance data, but can acquire and relate habitat data.
Multi-beam techniques	High definition of spatial extents, structure and individual behaviour. Aggregations can be rapidly mapped in their entirety providing vast quantities of data Considerable information on individual fish and its behaviour. Significantly reduced survey time. Requires alternate ground truth data at the time of survey. Along track spatial aliasing due to reduced ping rate in some systems. Multiple detections of fish and movement caused by vessel avoidance	Alternate configuration (directed sideways) allows 'viewing' a greater distance inducing less avoidance.	Dependent on the mobility and density of the aggregation.	System resolution should detect individual fish at, or near the seafloor.
Passive recording of vocal behaviour	Requires confirmation of vocal behaviour	Requires confirmation of vocal behaviour	Once initial ground truth data is collected further biological sampling is not required. Remote, long range observation, without creating behavioural bias. Fine-scale mapping of spatial extents, and behavioural timing. Continuous, decadal datasets. Correlation with environmental drivers. Range dependent on ambient noise. Sound production cues applied to aquaculture.	Requires confirmation of vocal behaviour Requires high accuracy of targeting. Long term remote observation can capture short sporadic events.

6. Future Work

6.1. Active acoustics

The three-dimensional modelling of species acoustic target strength relationships is an important contribution to the echo-integration analysis of single-beam and MBS fish school data. There are numerous methods of developing theoretical and practical three-dimensional target strength models (Horne *et al.,* 2000, Jech and Horne, 2002, Pena, 2007). RESON 8125 and 7125 MBS surveys have provided acoustic data from multiple transects of the same aggregation of *S. hippos* at various lateral angles of ensonification. Further analysis of acquired *S. hippos* S_v values with respect to target swath position will provide a better understanding of the three-dimensional aspects of acoustic backscattering of *S. hippos* swimbladder.

If supported by the Western Australian Department of Fisheries the on-going monitoring of *S. hippos* using a recently purchased Biosonics 38 and 120 kHz split-beam echosounder will facilitate the monitoring of spawning aggregation response to sport fishing and any discernible climatic changes over the forthcoming spawning seasons.

In Western Australia *C. gerrardi* has recently been acknowledged as a key indicator for the ecological health of the local environment (Mackie *et al*., 2009). Combined split-beam acoustic and video techniques are anticipated to provide invaluable information on the stocks of *C. gerrardi* around the Geographe Bay region.

The continuance of MBS surveys in this research is subject to the availability of fisheries related MBS systems and success of funding applications. Research targets include: the shallow water investigation of *P. auratus* found each year in Cockburn Sound to observe the performance of a sideways mounted system with vessel avoidance behaviour; and small populations of sedentary and temporarily bottom dwelling species such as *G. hebraicum* and *A. japonicus* respectively. The latter will provide ground truth data of separation distances between calling *A. japonicus* observed by passive acoustic hydrophone arrays and assess the ability to discriminate targets from the riverbed.

Comparisons may also be made between the fine scale classification of habitats about which the spawning aggregations reside from MBS seafloor bathymetry and backscatter.

6.2. Passive acoustics

The use of passive acoustic recording of fish vocalisations in fisheries based research is an emerging technique requiring development. Issues have been itemised for future investigation at every level of research during the above study.

Although species call categories have been ground truthed as originating from *A. japonicus* and call functions have been speculated, the visual evidence of exact function and associated physical behaviour is still required. Atlantic cod (*Gadhus morhua*) and haddock (*Melanogrammus aeglefinus*) have shown elaborate courtship behaviour with wide vocal repertoire (Hawkins and Amorim, 2000, Nilsson, 2004). A specific call associated with a direct spawning event, such as a call to instigate a vertical spawning rush would provide a tangible record of spawning success. A number of methods to confirm exact call function have been highlighted. Simultaneous video and audio recording of TAFE spawning *A. japonicus* broodstock will confirm the exact time and physical behaviour involved with courtship. Although inhibited vocal behaviour has already been noted by these subjects (Section 4.2.3) vocalisation may occur at the exact time of spawning.

Section 4.5 idenitified the source level of various categories of *A. japonicus* calls and a simple estimation of the range at which these calls may be detected during varying levels of ambient noise. In murky water determination of the direction and distance of the male emitting sounds may be critical to the female. However, as yet the method by which *A. japonicus* detects sound pressure levels and direction has not been determined (ie sound pressure or particle motion). Close to the sea surface the particle motion will tend to take place normal to the surface and directional information will be lost. The affect this has on detection range and source localisation by the recipient requires *in situ* assessment using a hydrophone sensitive to particle velocity and acceleration has been identified as future work.

It has been suggested that the replaying of pre-recorded *A. japonicus* Category 2 calls of attraction may draw females to the speaker. The deployment of Acoustically Baited Remote Underwater Videos (ABRUVs), which would concurrently record audio and visual (using low light level or infra red cameras) while also emitting pre-recorded calls, may provide visual *in situ* evidence of *A. japonicus* spawning behaviour.

It is anticipated that multiple deployments of ABRUVs emitting calls of varying acoustic characteristics will determine whether acoustic cues are used during mate selection and if so, what the characteristics of those cues are. Variation of call characteristics (allowing for inherent limitations of underwater speakers) such as peak spectral frequency replayed on the ABRUV may invoke differing behaviour (e.g. differing frequencies may attract differing sized fish).

The *in situ* effects of temperature variations on call characteristics require quantification. Current environmental data contributed by the Swan River Council for analysis with Mosman Bay logger datasets are based on a weekly sampling regime. Future long term deployments of CMST loggers to Mosman Bay will include a temperature logger sampling riverbed water temperature at the hydrophone location every hour. The resolution of this sampling will facilitate the calculation of mean call spectral peak frequencies for given temperatures. The temperature data recorded concomitantly with the deployment of a localisation hydrophone array will facilitate the evaluation of calculated call source levels against temperature.

Research at Mosman Bay has provided a significant insight into the spawning behaviour of *A. japonicus* in the Swan River; however, it has not proffered the whole picture. Several spawning sites around the Swan and Canning River systems have been noted by recreational fishermen and biological sampling in previous studies (Farmer, 2008, Gaughan *et al.*, 1990). Strategic deployment of acoustic noise loggers along the Swan and Canning River systems will offer the opportunity to monitor *A. japonicus* biomass, behaviour and response to environmental and anthropogenic variables throughout the river. Such concurrent recordings would facilitate the mapping of the Swan River for *A. japonicus* populations and help determine preferred spawning habitats, relative timings of spawning commencement/cessation, size distributions and any possible migratory patterns along the river.

Subject to funding, passive acoustic surveys of *A. japonicus* will be extended to known coastal water spawning site locations. Mapping the distribution and populations is an important basis for the monitoring of the species throughout Western Australia. In the future funding is to be sought to conduct acoustic surveys in New South Wales and South Australia where *A. japonicus* aggregations are currently monitored employing alternative techniques (Taylor *et al.* 2006, Taylor and Suthers, 2008, Ferguson *et al.*, 2008). Furthermore, the global distribution of *A. japonicus* encompasses developing nations such as India, Pakistan and China (Griffiths and Heemstra, 1995). The development and application of passive acoustic techniques to monitor a commercially important species in such nations will provide significant aid to the security of an economically important food source.

Further studies are necessary to confirm soniferous behaviour in three of the focus species in the above study. Subject to the application and approval of support and funding it is anticipated that research will include:

- long term (spawning season) deployment of sea noise loggers at locations around Geographe Bay known to be inhabited by significant numbers (tens) of *G. hebraicum* during the spawning season.

- larger number of *C. gerrardi* forming in aggregations facilitates a more *ad hoc* approach to obtaining acoustic recordings. It is anticipated that opportunistic targeting of *C. gerrardi* aggregations during WA Fisheries surveys will confirm vocal behaviour.

- determination of primary spawning sites for *P. auratus* in the Cockburn Sound. Early evening recordings taken shortly after high tide will help determine whether vocal behaviour is included as a cue for spawning.

Once the application of fisheries based passive acoustic techniques has been developed it will be possible to apply them to other commercially important soniferous species within State and National waters. Currently known vocal genera can be found in Appendix 6.1 (McCauley, 2001).

There are at least 17 species of Sciaenidae represented in Australian waters, only two of which, *ca* 10%, are found in temperate waters (Appendix 6.2; Farmer, 2008). Tropical Sciaenidae species in Australia, are represented predominantly by *Johnius* and *Nibea* genera (Sasaki, 1992a, 1992b; Yearsley *et al*., 2006) typically <650 mm, and not targeted by recreational or commercial fishers. These species do, however, contribute significant bycatch to prawn trawling operations (Farmer, 2008). In contrast with other tropical Sciaenidae the black Jewfish (*Protonibea dicanthus)* has a maximum total length of ca 1500 mm (Phelan, 2002). *P. dicanthus* contributes significantly to commercial, recreational and subsistence catch in the Northern Territory, Queensland and north-west Australia (Phelan, 2008, Phelan *et al.,* 2008). McCauley (2001) speculated that *P. dicanthus* would produce sounds via a series of knocks and has since been shown to produce pulse related sounds similar to those of *A. japonicus* at times of spawning (Mok, 2006). *P. dicanthus* is therefore an excellent prospective subject for future passive acoustic study and subsequent comparison of acoustic data with tag and release programs already in place in the Northern Territory waters (Phelan 2008). Other Australian species of Sciaenidae are likely soniferous and may require monitoring as a record of species diversity.

It is anticipated that past, present and future fisheries based passive acoustic techniques developed at the CMST will culminate in the inception of a passive acoustic handbook. This work will outline the monitoring of a soniferous species from identification of a spawning site to fine scale monitoring individuals within aggregations and seasonal monitoring of biomass. The content will be based on the study of *A. japonicus* as an example species and include associated techniques required in ground truthing physical behaviour and acoustic characteristics.

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Appendices

Appendix 1.1

List of Publications

Parsons, M.J.G., McCauley, R.D., Mackie, M., Siwabessy, P.J., and Duncan, A.J. Localisation of individual mulloway *(Argyrosomus japonicus)* within a spawning aggregation and their behaviour throughout a diel spawning period, **ICES Journal of Marine Science** vol. 66, pp**.**

Parsons, M.J.G., McCauley, R.D. and Mackie, M., Characterisation of a mulloway *(Argyrosomus japonicus)* spawning aggregation using passive acoustic techniques**. 2nd International Conference and Exhibition on Underwater Acoustic Measurements: Technologies and Results,** *conference proceedings,* Crete, June 20-25th, 2007.

Parsons, M.J.G., Parnum, I.M., McCauley, R.D. and Mackie, M., Multi-beam visualisation of Samson Fish *(Seriola hippos)* aggregations and spawning habitats in Western Australia (invited). **2nd International Conference and Exhibition on Underwater Acoustic Measurements: Technologies and Results,** *conference proceedings,* Crete, June 20-25th, 2007.

Parsons, M.J.G., McCauley, R.D. and Mackie, M. 2006, Spawning sounds of the mulloway (*Argyrosomus japonicus*)**. Australasian Acoustical Society, *conference proceedings, Christchurch, New Zealand, November 19th-22nd.***

Parsons, M.J.G., McCauley, R.D., Siwabessy, P.J. and Mackie, M., (2006), Evaluation of multi- and single-beam acoustic backscatter data collected from a samsonfish *(Seriola hippos)* spawning aggregation in Western Australia. **8th European Conference of Underwater Acoustics,** Portugal, June 12th-15th, 2006.

Parsons, M.J.G., McCauley, R.D. and Mackie, M. 2005, *Preliminary analysis of single-beam acoustic data of fish spawning aggregations along the Western Australian coastline***. Australian Acoustical Society, *conference proceedings, Busselton, WA, November 9-11th.***

Parsons, M.J.G., McCauley, R.D. Mackie, M., and Lewis, P., 2009 Contributor Chapter 2 (Materials and Methods), & Chapter 4 (Acoustic Methods) of FRDC 2004/051 Management and monitoring of fish spawning aggregations within the west coast bio-region of Western Australia **(eds Mackie, M.C., McCauley, R. Gill, H. and Gaughan, D.). Fisheries Research and Development Corporation and Department of Fisheries, WA.**

Parsons, M.J.G., Siwabessy, J.P. and Parnum, I.M., (2007), Seabed habitat mapping using single beam and multibeam sonar systems. **Australian Coral Reef Society Conference,** Fremantle, October 9th-11th.

Parsons, M.J.G., Parnum, I.M., Siwabessy, J.P. McCauley, R.D. and Mackie, M.C. (2007), Applications of acoustics in monitoring fish schools and essential fish habitat. **Australian Coral Reef Society Conference,** Fremantle, October 9th-11th.

Appendix 2.1

Table App.1. *Nomenclature*

α	Absorption coefficient (dB per unit distance)
ϕ	Angle of target to the vertical (°)
λ	Wavelength (m)
θ	Angle of incidence (°)
	Off-axis beam angle (°)
ρ	Acoustic density
ρ_{A}	Fish density (fish.n.mi^{-2})
ρ_{V}	Fish density (fish.n.mi^{-2}.m^{-1})
σ	Backscattering cross-section (m^{2})
τ	Pulse duration (s)
a	Target strength related constant specific to species and frequency
	Area (m^{2})
A	Unit area (m^{2})
A_d	Script denoting densely populated area of aggregation
A_t	Script denoting total aggregation
b	Target strength related constant specific to species and frequency
b	Fish body length (m)
B	Biomass (tonnes/n.mi^{2})
$\mathrm{B_h}$	Horizontal beginning of echogram ‘school’
$\mathrm{B_v}$	Vertical beginning of echogram ‘school’
BS	Backscatter (dB)
c	Sound Speed (ms^{-1})
c.l.	Confidence limits
C	Passive acoustic system calibration coefficient (dB re V/µPa)
CIP	Call Initiation Peak
d	Length (m)
E	Signal energy
$\mathrm{E_h}$	Horizontal end of echogram ‘school’
$\mathrm{E_v}$	Vertical end of echogram ‘school’
f_b	Frequency band (Hz)
FL	Fish fork length (cm)

GAM	Generalised additive model
hyds	Hydrophone sensitivity (dB re V/μPa)
H	Altitude of aggregation (m)
I	Intensity
L	Intensity level across a given frequency band (dB re 1μPa^2/Hz)
L	Fish length in target strength calculation (cm)
L_{50}	Fish length at first maturity (mm)
M_h	Maximum height of aggregation
M_l	Maximum length of aggregation
N	Number of fish
NASC	Nautical Area Scattering Coefficient (m^2/n.mi^2)
p	Pressure (Pa)
r	Range (m)
R	Receiver
R^2	Correlation coefficient between two variables
R	Range (m)
s_A	Nautical Area Scattering Coefficient (NASC) (m^2/(n.mi)^2)
s_V	Volume backscattering coefficient (dB)
s.d.	Standard deviation
SPL	Sound pressure level
SEL	Sound exposure level
t_c	Call duration (s)
T_{dc}	Duty cycle duration (s)
T	Signal length (s)
TS	Target strength
V	Volume (m^3)
W	Fish weight (kg)
z	Depth band (m)

Appendix 2.1

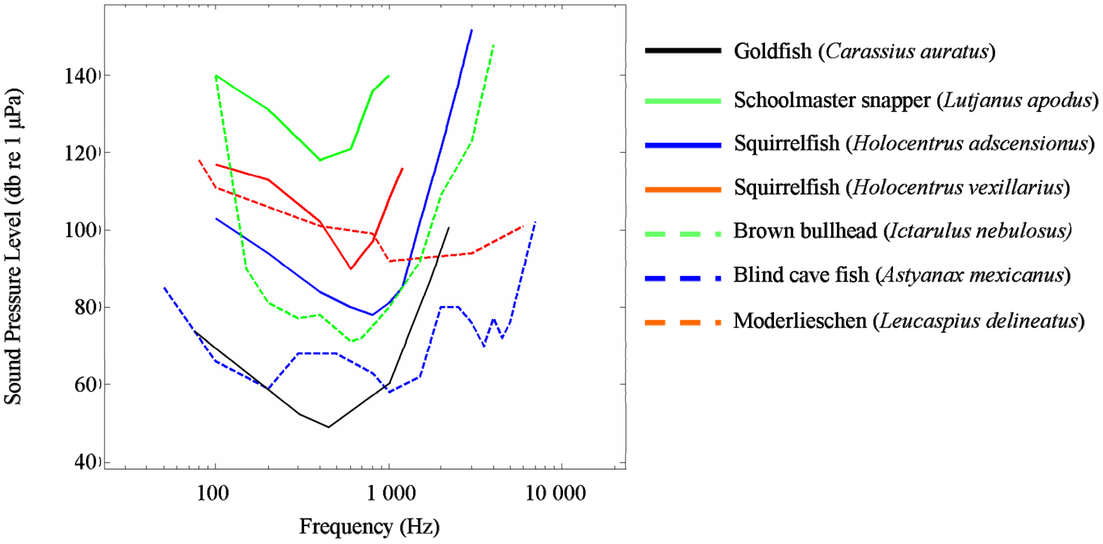

Figure App.1. *Example ranges of fish hearing thresholds (adapted from Popper et al., 1973). Continuous represent examples of ostariophysines and dashed lines represent non-ostariophysines.*

Appendix 3.1

Calibration of the Simrad EQ60 single-beam echosounder was conducted using the suspended reference sphere method (Foote *et al.,* 1987) at the CMST marine research site in Jervoise Bay, Cockburn Sound. A schematic of the calibration mounting is shown in Figure App. 2. A section of the experimental site comprised a 3 m high pier extending into 8 m depth of water. The EQ60 was mounted on the bow of the Curtin 4 m outboard vessel, which was in turn attached between pier supports for stability. The transducer head was located 0.8 m below the surface, directed vertically downwards. Above the water surface three 1 m poles extended horizontally from the transducer head mount at 120° angles between each pole. Along each of these poles ran a 6.2 m fishing line, of adjustable length, hanging over the end and down into the water where all three lines were combined at a depth of 5 m. Attached to the line at the inverted apex was a tungsten carbide target sphere of diameter 3.8 cm (for calibration of the 38 kHz transducer), 4.2 m directly below the transducer head.

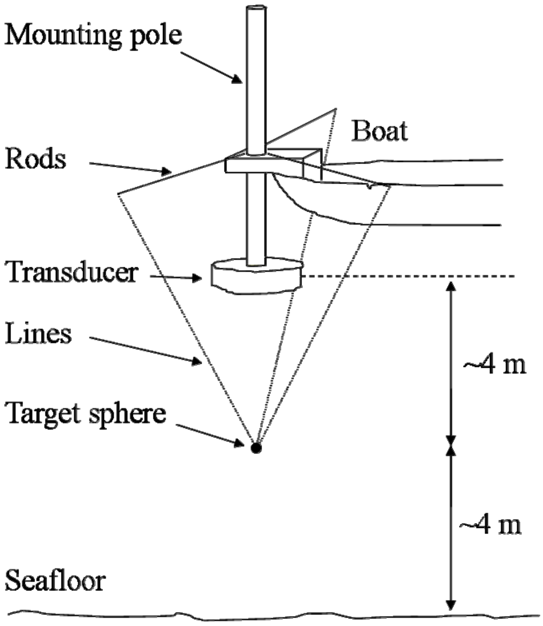

Figure App.2. *Schematic of initial calibration mounting for Simrad EQ60 echosounder*

With the transducer in active mode the fishing lines were adjusted until a maximum backscatter reading was detected to ensure the target sphere was in the centre of the acoustic beam. Each pulse duration was tested for one minute to ensure replication of the maximum target strength value from the lowest power (100 W) to the maximum (1000W) to identify any power dependence and therefore linearity in the power settings. The 38 kHz and 200 kHz frequencies were tested separately and together to determine any interference. Data were then imported into Echoview for processing and the calibration offsets for the system for both 38 and 200 kHz beams were calculated. From the calibration data, S_A corrections were calculated as per the Simrad user manual. However, results were found not to be in line with known backscattering coefficients for the tungsten carbide target sphere, and so re-calibration of the Simrad EQ60 was deemed necessary.

Appendix 4.1

To quantify temporal variations in the logger datasets from the March 5th 2007 hydrophone array survey a continuous recording was conducted at ambient conditions before a replication of experimental thermal conditions was created. The hydrophone was heated via 500 W lamps (Figure App. 3) for 4 hours, before being cooled in water from 20 to 16 °C to replicate all possible temperatures of evening riverbed water temperature (Figure App.3C). During this experiment a 1 kHz sine wave (generated and counted by an Agilent 33220A and 53132A respectively) was applied to the recording system and continuously logged (Figure App.3B). Calculated sample and temporal drift coefficients were then applied to each relevant dataset and correlated with implosion signals to remove temporal drift.

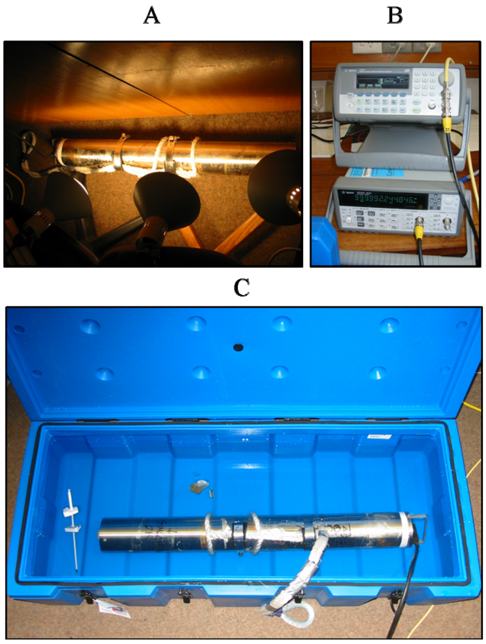

Figure App.3. *Heating of hydrophone equipment via three 500 W halogen lamps (A). Agilent 33220A and 53132A signal generator and counter respectively (B). Water cooling tub with hydrophone housing and thermometer (C).*

Appendix 4.2

A sixth hydrophone (HTI-90U), recording at a depth of 10 m (5 m vertically below hydrophone R_4) was originally intended to create redundancy in three-dimensional localisation calculations. The two hydrophones deployed from the same vessel recorded into separate channels of a D100 Sony DAT recorder. A single pre-amplifier unit was used with each hydrophone detected signal running through a separate channel in the unit, before continuing to the DAT recorded. However, during *in situ* recording, significant noise was noted on the lower hydrophone channel. Post experimental testing of equipment revealed signal crossover in the pre-amplifier unit from the channel of the 5 m deep hydrophone, to the channel of the 10 m hydrophone. This extent of this 'crosstalk' was not quantified as it was considered detrimental to the 10 m hydrophone and thus this data was discarded. At no stage during testing was any significant signal crossover observed to the channel of the 5 m hydrophone.

The *in situ* response of the two unaffected hydrophones, recording at different depths, onto the same DAT recorded, but using separate pre-amplifiers (R_2 and R_3) can be seen in Figure App. 4A. When a 1 kHz signal (generated by an Agilent 33220A) was directed into the hydrophone 2 channel (R_3, red line) no signal was recorded on the hydrophone 1 channel (R_2, blue line), and vice versa. However when the same signal was connected to the channel of the 5 m deep hydrophone R_4 (Figure App.4B, blue line) the signal was still observed on the second channel, due to crosstalk from one channel to the other in the pre-amplifer (Figure App. 4C). When the signal switched channels the crosstalk was not observed from the second channel to the first (Figure App.4C, after 8.5 s). Therefore data from the 10 m deep hydrophone was discarded.

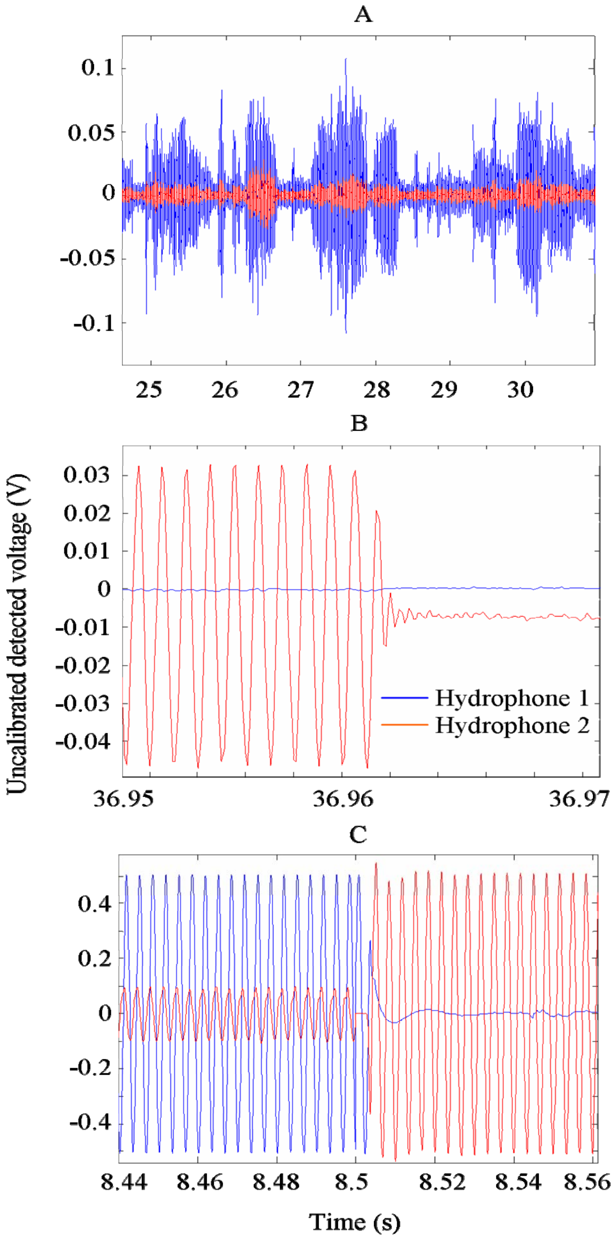

Figure App.4. *Hydrophone detected voltage outputs with time of three uncalibrated sets of responses from the following scenarios: Two in situ hydrophones using separate pre-amplifiers, displaying no cross-talk between recordings (A); and then the same two hydrophones recording ex situ with a 1 kHz signal applied to the hydrophone 2 channel until approximately 39.96 s (B); Two hydrophones using a single pre-amplifier box recording ex situ as a 1 kHz signal is switched from the hydrophone 1 channel to the hydrophone 2 channel at approximately 8.5 s (C).*

Appendix 5.1

Table App.2. *Examples of genera of fish reported or likely to exhibit vocal behaviour (Table reproduced from McCauley, 2001).*

Family	Common name	genera	Notes
Ariidae	Catfishes	*Arius spp*	*A. thalassinus* common in northern Austrlia
Batrachooididae	Frogfishes	*Batrachomoeus, Halophryne*	Reportedly produce 'croaking noises'
Berycidae	Nannygai, red snapper	*Centroberyx, Beryx*	Possess swimbladder muscles and morphology likely to be involved in sound production.
Ephippididae	Batfishes	*Drepane, Platax, Zabidius*	Grunts reported in *Platax*
Glaucosomatidae	Perchlet	*Glaucosoma*	*G. hebraicum* reported as vibrating and emitting sound by fisheries researchers. *G. hebraicum* and *G. burgeri* possess muscles attached to anterior of swimbladder.
Holocentridae	Squirrelfish	*Myripristis, Ostichthys, Sargocentrum, Neoniphon*	Specialised hearing structures and many species reportedly produce sound.
Macrouridae	Whiptails, rattails, grendiers	15 genera (deep water)	Specialised hearing structures and many species reportedly produce sound.
Monacanthidae	Leatherjackets, triggerfish	27+ genera	Swimbladder extends to dorsal surface in many species and may be struck by dorsal spine.
Pomacanthidae	Angelfishes	*Apolemichthys, Chaetodonotplus, Pomacanthus*	Reports of grunting of similar fashion to *Platax*
Pomacentridae	Damselfishes	15 genera	Known to produce 'pops' and aggressive grunts
Pempheridae	Sweeps	*Parapriacanthus, Pempheris*	Reported as possessing swimbladder muscles
Priacanthidae	Big eyes	*Cookeolus, Priacanthus, Pristigenys, Heteropriacanthus*	Specialised hearing adaptations and swimbladder mucles
Sciaenidae	Croakers	*Argyrosomus, Austronibea, Johnius, Protonibea*	Chorus behaviour observed in *A. japonicus* and *P. diacanthus*. Other species reported as sound producers.
Scorpaenidae	Scorpionfish, stonefish	33 genera	Numerous Sebates spp. Reported as producing sound using swimbladder muscles
Serranidae	Cod	*Epinephelus*	*E. striatus* reported as possessing red swimbladder

			drumming muscles
Terapontidae	Grunters	*Pelates, Terapon, Pelsartia*	Muscles attached to the anterior of the swimbladder
Triglidae	Gurnards, sea robins	15 genera	Some species possess muscles attached to the swimbladder
Zeidae	Dories	*Cyttus, Zenposis, Zeus*	Swimbladder muscles reported in *Zeus faber*

Appendix 5.2

Table App.3. *Sciaenidae fishes of Australian waters separated into reported presence in tropical and temperate waters (table reproduced from Farmer, 2008).*

	Scientific name	Common name
Tropical		
	Atrobucca brevis	Orange Jewfish
	Atrobucca nibe	Longmouth Jewfish
	Austronibea oedogenys	Yellowtail Jewfish
	Johnius amblycephalus	Bearded Jewfish
	Johnius australis	Little Jewfish
	Johnius borneensis	River Jewfish
	Johnius laevis	Smooth Jewfish
	Johnius novaeguinae	Paperhead Jewfish
	Larimichthys pamoides	Southern Yellow Jewfish
	Nibea leptolepis	Smallscale Jewfish
	Nibea microgenys	Smallmouth Jewfish
	Nibea soldado	Silver Jewfish
	Nibea squamosa	Scaly Jewfish
	Otolithes ruber	Silver Teraglin
	Protonibea diacanthus	Black Jewfish
Temperate		
	Argyrosomus japonicus	Mulloway
	Atractoscion aequidens	Teraglin

Appendix 6.1

Table App.4. Breakdown of author contribution to individual papers

(this table does not include all the thesis advice provided by the author's supervisor and associate supervisors)

Section paper	Author	Role
Section 3.2 Seasonal spatial and temporal patterns of *S. hippos* aggregations, west of Rottnest Island using single-beam acoustics	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Aiding acquisition of acoustic backscatter data Paper review and comments
	Michael C. Mackie	Aiding acquisition of acoustic backscatter data Paper review and comments
	Paul J. Lewis	Aiding acquisition of acoustic backscatter data
Section 3.3 Evaluation of acoustic backscatter data collected from Samson fish *(Seriola hippos)* spawning aggregations in Western Australia Using a Reson 8125 multi-beam sonar	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Paper review and comments
	Michael C. Mackie	Aiding acquisition of acoustic backscatter data Paper review and comments
	Paulus J. Siwabessy	Development of Matlab programs for the discrimination of individual fish targets.
	Iain M. Parnum	Data acquisition and operating Reson multi-beam system.
	Paul J. Lewis	Aiding field acquisition of acoustic backscatter data
Section 3.4 Multi-beam visualisation of Samson Fish *(Seriola hippos)* aggregations and spawning habitats in Western Australia using a RESON 7125 Seabat multi-beam sonar	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Iain M. Parnum	Data acquisition and operating Reson multi-beam system. Programming and data processing of seafloor backscatter.
	Paulus J. Siwabessy	Matlab processing to discriminate individual targets.
	Michael C. Mackie	Aiding acquisition of acoustic backscatter data Paper review and comments
	Robert D. McCauley	Paper review and comments
	Paul J. Lewis	Aiding acquisition of acoustic backscatter data
Section 3.5 Preliminary findings of active acoustics applicability to alternate species.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the

		following roles.
	Robert D. McCauley	Paper review and comments
	Michael C. Mackie	Aiding acquisition of acoustic backscatter data Paper review and comments
Acquisition and analysis of underwater passive acoustic data has predominantly employed a suite of equipment and Matlab programs designed by the Centre for Marine Science and Technology predominantly contributed to by Robert D. McCauley.		
Section 4.2 Characterisation of mulloway (*Argyrosomus japonicus*) spawning sounds.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds. Paper review and comments
	Michael C. Mackie	Acquisition of passive acoustic data. Paper review and comments
	Paulus J. Siwabessy	Development of Matlab programs for the identification and characterisation of individual fish calls.
Section 4.3 An assessment of temporal variations in mulloway (*Argyrosomus japonicus*) vocalisations in Mosman Bay, Swan River throughout successive spawning seasons from a single hydrophone.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Michael C. Mackie	Paper review and comments
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds. Considerable advice on the analysis of long-term acoustic data. Paper review and comments
	Cordelia H. Moore	Theory and data processing behind ecological modelling.
Section 4.4 Localisation of individual mulloway (*A. japonicus*) within a spawning aggregation and their behaviour throughout a diel spawning period.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Michael C. Mackie	Paper review and comments
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds. Paper review and comments
	Alec J. Duncan	Development of Matlab code to localisation sounds using the arrival-time difference method.
Section 4.5 *In situ* source levels of mulloway (*Argyrosomus japonicus*) calls**.**	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds.

		Paper review and comments
	Michael C. Mackie	Paper review and comments
Section 4.6 Estimating numbers of calling fish from their vocalisations using a single hydrophone.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds. Paper review and comments
	Michael C. Mackie	Paper review and comments
	Alec J. Duncan	Theory on the contribution of incoherent fish calls to overall sound pressure levels.
Section 4.7 Protocols for recording and reporting fish sounds.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Development of the majority of Matlab programs for the analysis of recorded underwater sounds. Paper review and comments
	Michael C. Mackie	Paper review and comments
Section 4.8 Preliminary findings of passive acoustics applicability to alternate species.	Miles J.G. Parsons	Experimental design, data acquisition, processing, analysis, discussion and reporting, assisted/supervised by co-authors through the following roles.
	Robert D. McCauley	Acquisition of passive acoustic data Paper review and comments
	Michael C. Mackie	Acquisition of passive acoustic data Paper review and comments

Appendix 7.1

Letters of request to reproduce previously published data or figures

The following letters have been sent via post and e-mail in order to contact copyright owners of previously published work to request authorisation to use adaptations of their work in the reproduction of figures in this thesis. Each author has been acknowledged in the appropriate figure.

RE: Request for authorisation to reproduce Table 1.1 from your PhD thesis Comparisons of the biological and genetic characteristics of the Mulloway *Argyrosomus japonicus* (Sciaenidae) in different regions of Western Australia

Dear Dr Farmer,

I am currently writing my PhD thesis entitled 'An investigation into active and passive acoustic techniques to study aggregating fish species'. I would like to reproduce Table 1.1 from your thesis to include in my Appendices. The table will be acknowledged in the title as a reproduction of your work and will not be published outside the thesis.

Once completed, the thesis will be made available in hard-copy form in the Curtin Library and in digital form on the Internet via the Australasian Digital Thesis Program. The material will be provided strictly for educational purposes and on a non-commercial basis. Further information on the ADT program can be found at http://adt.caul.edu.au.

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Full acknowledgement of the ownership of the copyright and the source of the material will be provided. I would be willing to use a specific form of acknowledgement that you may require and to communicate and conditions relating to its use.

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I look forward to hearing from you and thank you in advance for your consideration.

Kind regards,

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RE: Request for authorisation to reproduce data from 'Sound detection and processing by fish: a critical review'

Dear Dr Popper,

I am currently writing my PhD thesis entitled 'An investigation into active and passive acoustic techniques to study aggregating fish species'. In the appendices I have given examples of hearing ranges for six fishes. I would like to use some data from your paper 'Sound detection and processing by fish: a critical review', 1973, to produce this figure as part of one appendix.

Once completed, the thesis will be made available in hard-copy form in the Curtin Library and in digital form on the Internet via the Australasian Digital Thesis Program. The material will be provided strictly for educational purposes and on a non-commercial basis. Further information on the ADT program can be found at http://adt.caul.edu.au.

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I look forward to hearing from you and thank you in advance for your consideration.

Kind regards,

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RE: Request for authorisation to reproduce 'Experiences with multi-beam sonar in shallow tropical waters' *Fisheries Research*, vol. 35, no. 1-2, pp. 143-7.

Dear Dr Gerlotto,

I am currently writing my doctoral thesis entitled 'An investigation into active and passive acoustic techniques to study aggregating fish species', which I am undertaking at Curtin University of Technology in Perth, Western Australia. In the literature review I have given examples of mounting positions for multi-beam sonar. I would like to adapt and reproduce one of the figures from your paper 'Experiences with multi-beam sonar in shallow tropical waters', 1998, as an example of such configurations.

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RE: Request for authorisation to reproduce a figure from '*A Practical Manual for Aquatic Biomass Estimation*,. FAO, Rome. 1983. 240 pp.

Dear Dr Johannesson,

I am currently writing my doctoral thesis entitled 'An investigation into active and passive acoustic techniques to study aggregating fish species', which I am undertaking at Curtin University of Technology in Perth, Western Australia. In the literature review I have given examples of a transducer beam pattern an it interaction with fish swimbladder acoustic reflectance. I would like to adapt and reproduce one of the figures from your FAO handbook 'A Practical Manual for Aquatic Biomass Estimation', as an example os such interaction.

Once completed, the thesis will be made available in hard-copy form in the Curtin Library and in digital form on the Internet via the Australasian Digital Thesis Program. The material will be provided strictly for educational purposes and on a non-commercial basis. Further information on the ADT program can be found at http://adt.caul.edu.au.

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I look forward to hearing from you and thank you in advance for your consideration.

Kind regards,

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