Muresk Institute

The response of marine finfish and invertebrates to seismic survey noise

Jane Leah Fewtrell

This thesis is presented for the degree of Doctor of Philosophy of Curtin University of Technology

October 2003

Abstract

The oil and gas industry is of major economic importance to Australia. Offshore seismic surveys are an essential component of exploration for fossil fuel reserves. Offshore seismic surveys involve the use of arrays of air-guns that produce repetitive high energy, low frequency sound. There is increasing concern about the effect that the noise generated by a seismic survey has on the surrounding marine life.

Various species of captive marine fish and one species of squid were exposed to the noise from a single Bolt PAR 600 B air-gun with a 20 cui firing chamber and a source level at 1 m of 203.6 dB re 1 μPa mean squared pressure. Ten trials were conducted in Jervoise Bay and two were carried out off the coast of Exmouth. A different noise regime was used in each trial, however most involved the use of approach-depart scenarios to simulate an actual seismic survey and a 10 second duty cycle. Noise levels received by the animals ranged between 128 – 192 dB re 1 μPa mean squared pressure.

Behavioural observations of the fish and squid were made before, during and after air-gun noise exposure. The physiological stress response of the fish was monitored by measuring plasma cortisol and glucose levels before and after noise exposure. The sensory epithelium was removed from the ears of the fish prior to, immediately after and up to 86 days after air-gun noise exposure and examined using a scanning electron microscope.

No statistically significant physiological stress response in fish was detected as a result of the air-gun noise exposure regimes used.

Significant damage to the ciliary bundles of the sensory epithelium of the sacculus was observed in pink snapper (*Pagrus auratus*) that had been exposed to air-gun noise between 144 - 191 dB re 1 μPa for 1.71 hours. No regeneration of the hair bundles was observed 58 days after exposure to air-gun noise. However, evidence of regeneration was observed between 58 and 86 days after noise exposure.

Behavioural observations suggested that as air-gun noise levels increase, fish respond by swimming faster, in tighter groups and towards the bottom of the water column. Significant increases in alarm responses were observed in fish and squid to air-gun noise exceeding 158 - 163 dB re 1μPa. An increasing proportion of alarm responses were also observed as the noise level increased. A decrease in the frequency of alarm responses for repeated exposures was observed in squid and some fish.

The implications of these findings are discussed with comparisons of noise levels measured from an actual 2678 cui seismic survey air-gun array.

I dedicate this thesis to my family;
Eileen, Malcolm, Chris, Elsie & Fred Fewtrell
and to
Matthew Marshall.

STATEMENT OF SOURCES

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from published or unpublished sources has been acknowledged in the text and a list of references is given.

Jane Fewtrell **October 2003**

STATEMENT OF ACCESS TO THE THESIS

I, the undersigned, the author of this thesis, understand that Curtin University of Technology, Perth, Western Australia, will make this thesis available for use within the university library and allow access to users in other approved libraries. All users consulting this thesis must sign the following statement:

"In consulting this thesis I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author, and to make proper written acknowledgment for any assistance which I have obtained from it."

Beyond this, I do not wish to place any restrictions on the access to this thesis.

Jane Fewtrell **October 2003**

Acknowledgments

If I mentioned everyone that has helped me along the way, this section would be longer than the actual thesis. Therefore, what follows is a tribute to the people who I couldn't have done this without.

To my supervisor, Dr Robert McCauley whatever I say will not be enough to show my appreciation for your support and imparted wealth of knowledge, from the very beginning to the very end of this thesis. It has been such a long process, thankyou for your patience.

Thankyou to Professor Louis Evans and Dr Yuri Tsvetnenko for your assistance in the write up of this thesis and encouragement along the way. Thanks to all the staff at the Aquatic Science Research Unit for their support and putting up with a very distracted technician, especially in the final stages. In particular, thankyou to Dr Glen Whisson for his support, assistance and understanding (and fantastic coffees!).

My appreciation to the Australian Petroleum Production and Exploration Association (APPEA) for initiating this work and sponsoring the project. Thankyou to the Australian Research Council for providing a scholarship to financially support me. Also to the US Minerals Management Service for providing financial support to further the fish ear work.

For statistical assistance, thankyou to Jane Sneesby and Associate Professor Joan Osbourne.

To Professor Arthur Popper – the fish ear guru! Your knowledge, advice and enthusiasm were invaluable.

To Sandra Payne and Dr Lionel Martin – thanks for understanding.

Special thanks to all the people who helped with the success of the Jervoise Bay and Exmouth trials, especially Peter Mazzucato, Ross McLaren-Nicole and Julie

Murdoch. You all made long days seem a lot shorter.

I appreciate the expert advice and technical support that was provided by the Curtin University and UWA electron microscope technicians, James Brown, Elaine Miller and Steve Parry.

Thanks to Shirley Slacksmith from the WA museum for sharing her knowledge on mollusc anatomy – and showing me my first real statocyst!

For proof reading the final draft – thanks to Alan Marshall whose insomnia was no doubt 'cured' whilst trudging through these pages. Your time and expertise is appreciated.

A huge thankyou to Matthew Marshall, who not only looked after me during the toughest times, but also was by my side when ever I needed help (which was alot!).

Finally, thankyou to my family; your belief in me and unconditional support ensured that I never gave up (dad, you can finally use your new computer!).

Table of Contents

List of Tables

List of Figures

List of Plates

Publications arising from this research

McCauley, R.D., Fewtrell, J., Popper, A.N. (2003) High intensity anthropogenic sound damages fish ears. Journal of the Acoustic Society of America. 113 (1), pp 638-642.

McCauley, R.D., Fewtrell, J., Duncan, A.J., Adhitya, A. (2002) Behavioural, physiological and pathological responses of fishes to air-gun noise. Bioaccoustics, the International Journal of Animals Sound and its Recording. 12, pp 318-321.

McCauley, R.D., Fewtrell, J., Duncan, A.J., Jenner, C., Jenner, M., Penrose, J.D., Prince, T.I.T., Adhitya, A., Murdoch, McCabe, K. (2000) Marine seismic surveys – a study of environmental implications. APPEA Journal 2000, pp 692 – 708.

McCauley, R.D., Fewtrell, J., Duncan, A.J., Jenner, C., Jenner, M., Penrose, J.D., Prince, T.I.T., Adhitya, A., Murdoch, McCabe, K. (2000) Marine seismic surveys: analysis and propagation of air-gun signals; and effects of air-gun exposure on humpback whales, sea turtles, fishes and squid. Centre for Marine Science and Technology, Curtin University of Technology, R99-15, Perth, 185 pp.

Chapter 1

Introduction

1.0 INTRODUCTION

1.1 The oil and gas industry in Australia

The oil and gas industry is of major economic importance to Australia, with production presently valued at AUD $16 billion per annum and seventy percent of the country's liquid fuel requirements being produced domestically (APPEA 2002). Seismic surveys are an essential component in the exploration for fossil fuel reserves, and are used world wide, for both onshore and offshore exploration (McCauley 1994). In Australia, ninety percent of oil and gas production is offshore (APPEA 2002).

1.1.1 Seismic surveys

Offshore seismic surveys involve the use of a noise source, usually an array of air-guns towed slowly behind a ship. At regular intervals each of these air-guns produce a high intensity, low frequency noise that is directed towards the seabed. The reflected sound is received by a series of hydrophones, processed and converted to graphical images. These images are used to determine the probability of the presence of fossil fuel reserves in the area (Deffenbaugh 2002). Although the acoustic energy is directed towards the seabed, considerable energy is propagated horizontally, travelling for many kilometers from the source (Greene 1985; Caldwell and Dragosnet 2000).

In 1998, offshore seismic survey data acquisition in Australia was at record levels, with more than 960 000 line km of data being recorded, mainly from the Bonaparte, Browse and Carnarvon Basins (Petrie et al. 2001). There has been reduction in offshore seismic survey activity between 1999 and 2002 (Table 1.1) (Petrie et al. 2001; Petrie et al. 2002). However, continual exploration activity exposes extensive areas to intense sound, raising concern about short- and long-term effects on the surrounding environment (McCauley 1994; Ketchington 2000; Deffenbaugh 2002).

Table 1.1: Offshore seismic survey activity in Australia for 1999 – 2002 (Petrie et al. 2001; Geoscience Australia 2002; Petrie et al. 2002). Data for 2 dimensional (2 D) and 3 D dimensional surveys are presented separately.

Year	2 D data (line km)	3 D data (km^2)
1999	83 277	42 956
2000	129 858	22 605
2001	6 278	18 529
2002*	3 334	4 854

* 4^{th} quarter data not included for 2002

At present Australian seismic survey activity is governed by State and Commonwealth legislation. Legislation varies between States but is modelled on the Commonwealth legislation (DME 2000). The Commonwealth legislation and regulations that are relevant to seismic surveys are as follows:

- *Petroleum (Submerged Lands) Act, (1967)*
  - use of approved energy sources (non explosive sources);
  - requires notification and provision of certain information prior to seismic surveys;
- *Great Barrier Reef Marine Park Act, (1975)*
  - specifically precludes any mineral exploration or recovery within the defined Great Barrier Reef region;
- *Historic Shipwrecks Act, (1976)*
  - provides for the protection of historic wrecks and relics;
- *Environmental Protection and Biodiversity Conservation Act, (1999)(EPBC)*
  This Act replaces the *Environment Protection (Impact of Proposals) Act, (1974)*; *National Parks and Wildlife Conservation Act, (1975)*; *Whale Protection Act, (1980)*; and the *Endangered Species Protection Act, (1992)*. Relevant sections of the Act state that:
  - where actions are likely to have 'significant impacts on a matter of national environmental significance' approval from the Commonwealth

Environmental Minister must be sought. The options for the minister are: assessment on preliminary documentation; public environmental report (PER); environmental impact statement (EIS); public inquiry; or an accredited process that is, accreditation on a case by case basis;

- matters of national environmental significance include: nationally threatened species and ecological communities; migratory species; and Commonwealth marine areas;
- protected areas including conservation zones and the Australian whale sanctuary must be provided for; and
- protection and recovery of species or communities listed as vulnerable or endangered must be accommodated.

In addition, the activities of the Australian petroleum industry are overseen by the Australian Petroleum Production and Exploration Association (APPEA). The potential threat of seismic surveys to the marine environment is recognised by the oil and gas industry in Australia and APPEA have published a code of environmental practices which is voluntarily adhered to (APPEA 1996). At present management agencies put restrictions on seismic survey activity only when marine mammals are affected.

APPEA's code of environmental practice was originally published in 1978 and is periodically updated to accommodate new information and legislation (APPEA 1996). The code of practice outlines recommendations for companies to follow to ensure that exploration activities are conducted in an environmentally friendly fashion. The aim of these guidelines is "to explore the hydrocarbon resources for the benefit of the community in an environmentally responsible manner, minimising impacts on the natural and cultural environment, and other marine / resource users" (APPEA 1996). The Western Australian Department of Minerals and Energy has produced guidelines that are specific for seismic exploration in Western Australia. A main focus of these guidelines is the western rock lobster fishery (Seow et al. 1993).

The noise produced by offshore seismic surveys has the potential to significantly affect individuals and communities in a number of ways. These include: a lethal effect; sublethal pathological damage; changes in behaviour; and interference with acoustic communication (McCauley 1994; Rusby 1995). Due to inter- and intraspecific influences between aquatic species, seismic survey noise has the potential to indirectly affect entire marine communities (McCauley 1994).

1.2 Resources at risk

Under the United Nations Convention on the Law of the Sea, Australia has the rights and responsibilities for over 16 million km^2 of ocean and therefore, a vast array of diverse marine life (Vernon 1995). As seismic survey activity continues, the areas covered will further encroach onto areas with conflicting uses, especially the fishing industry (commercial, recreational and traditional sectors) and tourism.

Australian commercial fisheries have a gross value of AUD $1.74 billion per annum and are therefore important to the primary industry base supporting the Australian economy (Caton 2002). Potential flow on effects of seismic survey noise on commercial fish stocks could have ramifications for the commercial fishing sector. Further, the recreational fishing industry, worth an estimated AUD $3 billion per annum, could be affected by seismic survey noise (FRDC 1999-2000).

Tourism is a growing industry in Australia with international visitors coming to view and interact with Australia's marine biota (McCauley 1994). In 1996, at a conservative estimate, 3.8 million tourists visited Australia and 17% partook in marine recreational activities (12% diving, 3% fishing, 2% whale watching) (FRDC 1999-2000).

1.3 Thesis rationale

Seismic surveys have for some time been under scrutiny from environmentalists and professional fishermen for the possible effects that they may have on marine animals and the indirect affects that may have on higher level predators and the surrounding environment.

The information available to the Australian oil and gas industry to design an environmental regime for offshore exploration is based on interpretation of data that is not necessarily relevant to the type of noise produced during seismic surveys and the species exposed to them. When considering the ecological and economical importance of fish and invertebrates, and that in Australia there are over 3400 species of marine fish and many more species of invertebrates, it is surprising that so little data exists on the effect of seismic surveys on fish and, in particular invertebrates (Myrberg 1990).

To provide effective environmental management of seismic survey activity, the industry must be provided with information on the potential effects on relevant marine life.

This project was undertaken as part of an APPEA sponsored program, conducted by the Centre for Marine Science and Technology (CMST), Curtin University of Technology. The full project was titled: "Investigation of the Environmental Effects of Offshore Seismic Survey Activities" and encompassed a number of concurrent studies. This thesis presents the findings from experiments conducted to observe the behavioural, physiological and pathological response of fish and invertebrates to seismic survey noise.

At the onset of the project it was decided that direct observation of unrestrained fish and invertebrate responses to operating seismic vessels would leave many questions relating to possible physiological or pathological effects unanswered. Thus, the project concentrated on exposing animals contained in sea cages to controlled air-gun noise regimes so as to investigate physiological implications and pathological effects in addition to observing behavioural responses.

1.4 General aim of research

To investigate the effect of seismic survey noise on Australian species of marine fish and invertebrates.

1.4.1 General and specific objectives

i) To investigate the physiological response of marine teleost fish to a known noise regime, similar to seismic survey noise.

Specific objective:

- To determine the effect of air-gun noise on the stress response of two species of marine teleost fish, silver bream (*Rhabdosargus sarba*) and pink snapper (*Pagrus auratus*).

ii) To investigate the impact of a known noise regime, similar to seismic survey noise, on the hearing apparatus of marine teleost fish.

Specific objectives:

- To determine the effect of air-gun noise on the sensory epithelium of the saccule of pink snapper (*Pagrus auratus*).
- To determine if regeneration occurs to the damaged areas of the sensory epithelium of the saccule of pink snapper (*Pagrus auratus*) after exposure to air-gun noise.

iii) To observe the behavioural response of marine fish and invertebrates to a known noise regime, similar to seismic survey noise.

Specific objectives:

- To determine if air-gun noise induces alterations in vertical and horizontal positioning, swimming patterns and alarm responses of select marine fish shoals and squid (*Sepioteuthis australis*).
- To determine correlation between behavioural alterations in select marine fish shoals and squid (*Sepioteuthis australis*), and air-gun noise level.

Chapter 2

Literature Review

2.0 LITERATURE REVIEW

2.1 Introduction

Seismic survey techniques were originally developed by the oil and gas industry and have been used in the exploration for fossil fuel reserves since the 1930's (Deffenbaugh 2002). In Australia they have been used for over 40 years and yet there is still a dearth of information that exists on the effects of seismic surveys on the marine environment.

This literature review focuses on the documented and possible effects of seismic survey noise on fish and invertebrates. Where relevant, studies on effects from noise sources other than air-gun noise are also cited. The final section outlines the environmental implications of offshore seismic surveys based on available literature.

2.2 Seismic surveys

Offshore seismic surveys involve the use of high intensity, low frequency sound waves being directed towards the seabed. The sound source is usually an array of air-guns towed at 6 – 10 m depth, behind a purpose built ship. Sleeve exploders and gas guns, water guns and sparkers are occasionally used instead of air-guns (McCauley 1994). The sound waves travel through the seabed and are then reflected off boundaries between strata layers of varying physical and chemical composition (Falk and Lawrence 1973). The reflected signals are recorded by rows of hydrophones that are towed behind the ship in a 'streamer'. The recorded signals are then processed to provide graphical information on the composition and structure below the seabed. This 'map' can be used to determine the probability of fossil fuel reserves being found in a certain area (Falk and Lawrence 1973).

Seismic survey vessels travel at approximately 4-6 knots along predetermined survey lines (Fig. 2.1). The air-guns are fired every 6-20 seconds depending on required signal spacing and vessel speed. Seismic surveys can be arranged to give either a 2 or 3 dimensional result. Generally, 3D surveys cover a much smaller area in much more detail than 2D surveys, sometimes surveying an area for periods of weeks or months, resulting in higher resolution of the surveyed area (McCauley 1994; Ketchington

2000). The track lines used for 3D seismic surveys are usually closer together than the track lines of a 2D survey (1-10 km apart), sometimes being as close together as 50m (Dalen and Knutsen 1987). Due to the higher cost of using a 3D survey they are usually used to define potential and existing hydrocarbon fields rather than for speculative exploration (McCauley 1994). As a result of the high concentration of seismic shooting in a smaller area, 3D surveys would be expected to have a greater impact on animals in permanent residence in an area (McCauley 1994).

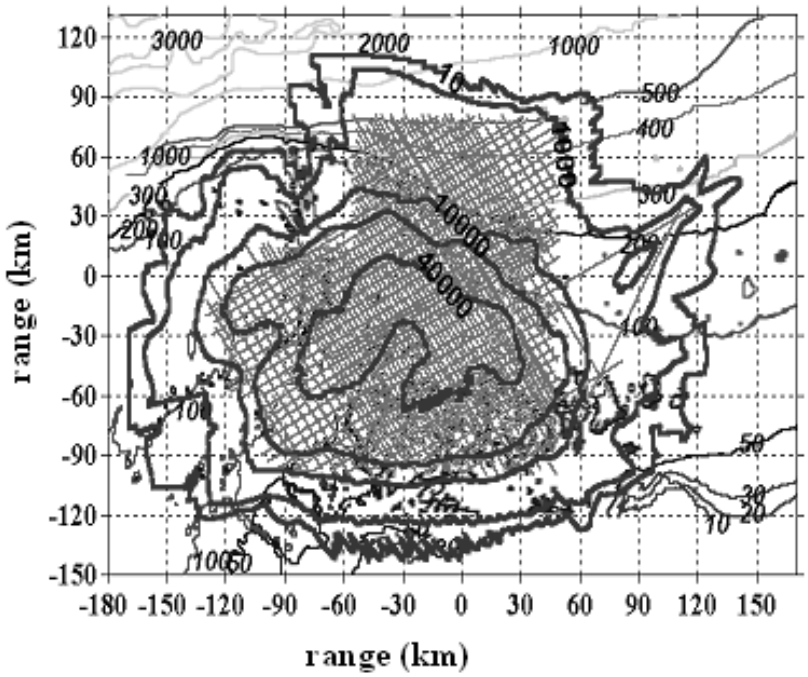

Figure 2.1: Grid of survey lines from a 2D seismic survey that ran for a period of 121 days. Straight grey lines represent track lines followed by the seismic vessel. Numbers on contour lines represent number of shots exceeding 155 dB re 1 μPa^2.s at specified distance from the centre (x=0, y=0) of the survey area (see Chapter 4 for description of units).

The spectral content of the sound signals used in a seismic survey depends on the depth of penetration needed and the composition of the underlying geological structures (Falk and Lawrence 1973). A compromise between resolution and penetration is usually required as resolution is inversely related to wavelength while the sound attenuation in geological structures is directly proportional to wavelength (McCauley 1994). Most of the sound energy produced during a seismic survey is in the range of 10-300 Hz. The sound level produced depends on the air-gun array

design, capacity, operational air pressure and array depth but generally is between 230-255 dB re 1 μPa* at 1 m below the source. It should be noted that air-gun arrays are directional in their sound input. For any specified array the source level in the horizontal plane is typically tens of decibels lower than the source level directly below the array (McCauley et al. 2000). For long range sound transmission the horizontal array source level is the most important. Each array signal (or shot in industry terms) is very short, at most, tens of milliseconds in duration near the source (Wardle et al. 2001).

Seismic survey activity is concentrated in areas that have the highest potential for holding fossil fuel reserves (McCauley 1994). In Australia, calculations made by the Bureau of Mineral Resources predict that the offshore basins of the north west of Western Australia have the most potential for discovery of oil reserves, in particular the Boneparte and the Carnarvon Basin (Robertson 1988). According to their calculations, the Carnarvon Basin also has the greatest potential for undiscovered gas reserves (Robertson 1988). Figure 2.2 shows a map indicating the major hydrocarbon basins in Australia.

* 230-255 decibel relative to 1 micro Pascal

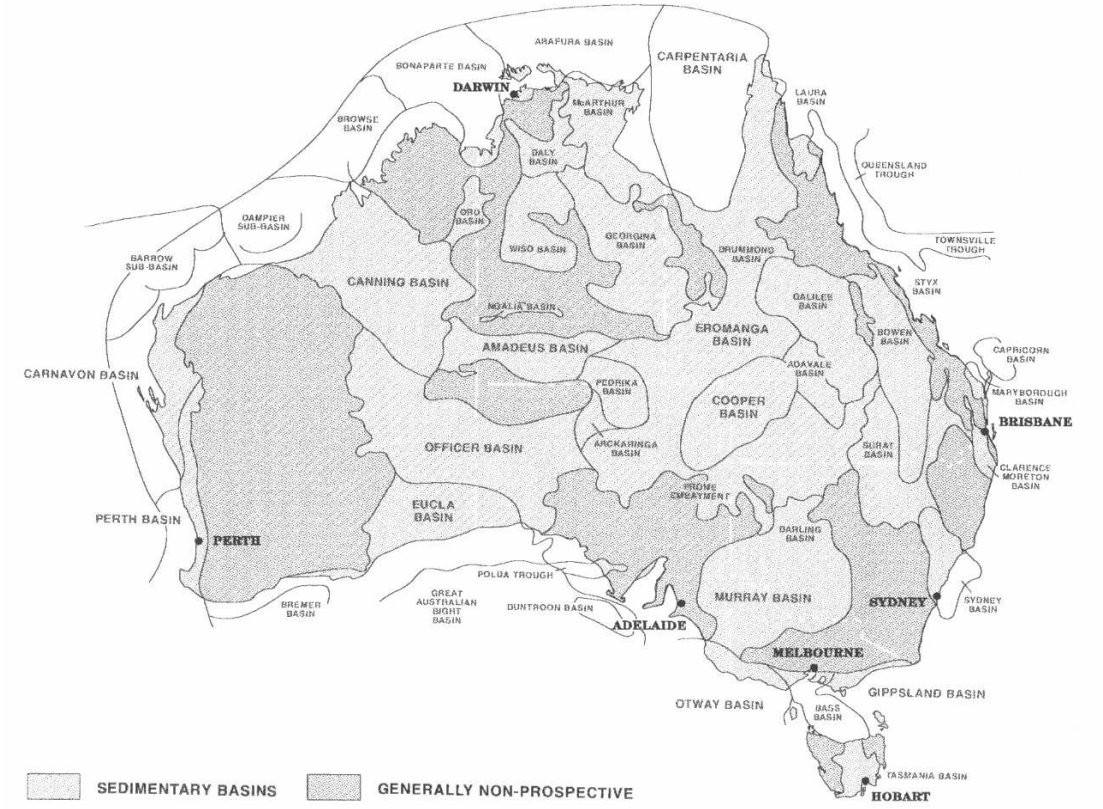

Figure 2.2: The main hydrocarbon producing basins of Australia (McCauley 1994)

2.2.1 Air-guns

Since 1969, sources of acoustic energy used in offshore seismic surveys have included compressed air, gas explosion devices and electrical discharges (McCauley 1994). These sources replaced explosives, which had been previously used in seismic surveys. Air-guns are by far the most commonly used acoustic energy source for offshore seismic surveys, especially in Australia as they produce a highly repeatable acoustic signal that is considered safer to the environment, seismic vessels and workers than explosives (McCauley 1994; Dragosnet 2000; Wardle et al. 2001).

Figure 2.3 is a diagrammatic representation of a generalised air-gun. The operating procedure of an air-gun is briefly outlined below. High-pressure gas is continually supplied to the air-gun through the gas inlet into the operating chamber. The gas is then fed into the firing chamber via the shuttle orifice. In the charged state the solenoid valve blocks off the air passage which vents to the back face of the piston in the operating chamber (Fig. 2.3). The area of the shuttle piston in the operating

chamber is slightly larger than the area of the shuttle piston of the firing chamber, which results in a small net force in the direction of the firing chamber. This maintains the seals of each chamber. When the air-gun is triggered by a suitable electric signal, the solenoid valve is opened admitting air to the lower side of the operating chamber piston. The net forces acting on the piston force the shuttle into the operating chamber thereby releasing the air from the firing chamber through the exhaust ports. The amount of air released will be dependent on the volume of the firing chamber, which can range in volume, up to 10 L. Once the air has been released the solenoid valve closes and high pressure gas enters the operating chamber and forces the shuttle piston downwards thereby sealing the chambers and leaving the air-gun ready for the next shot. Most air-guns can complete the charge / discharge cycle in under one second (McCauley et al. 2000).

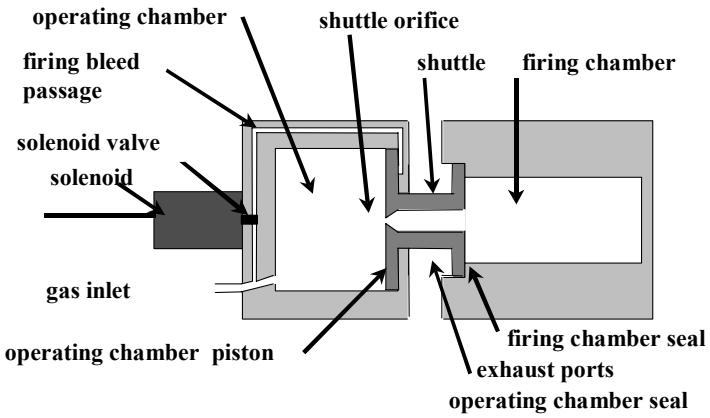

Figure 2.3: Air-gun design. The air-gun is shown in a charged state (McCauley et al. 2000).

The acoustic signal from the air-gun is created by a sudden release of compressed gas into the water through the exhaust ports. The sudden rush of gas and the resulting, rapidly expanding bubble, produces a short signal with a wide frequency band of energy (primary noise pulse)(McCauley et al. 2000). As the bubble of gas rises in the water column it oscillates producing a signal of low frequency and decreasing amplitude. The signal level produced by an air-gun depends on the

function of the air-gun design, capacity of compressed gas, operational pressure and detonation depth (McCauley 1994; Caldwell and Dragosnet 2000).

The primary characteristics of a seismic signal which geophysicists require, are the ability of the signal to penetrate deep into the earth and for the reflected signal to be distinctive, or be easily recognisable, amongst background noise. Air-gun sources produce an impulsive signal with a clearly defined edge, which allows travel times to be relatively easily determined. There are other signal types that could be used, for example lower level, longer coded signals as used in land based seismic surveys. Such signals are also produced by marine vibrators (Smith and Jenkerson 1998).

During a seismic survey air-guns are usually towed in an array. This increases the amount of acoustic energy available, focuses the energy and aids in suppressing bubble pulses, so that better penetration of the sea floor occurs and the bubble pulse signal is minimised (Dragosnet 2000).

A diagrammatic representation of an air-gun signal indicating the main characteristics is shown in Figure 2.4. Complications arise when describing an air-gun signal, as there are many ways in which the signal could be measured. Features of the signal that may be biologically significant are peak pressure, signal energy and duration. The frequency spectrum of the signal is also biologically important as the audible frequency ranges for marine animals varies greatly (Popper and Fay 1993). A biologically important characteristic of the noise produced by a seismic survey is that marine life will be exposed to an impulsive (short and intense) signal rather than continual noise (McCauley 1994; Gausland 2000).

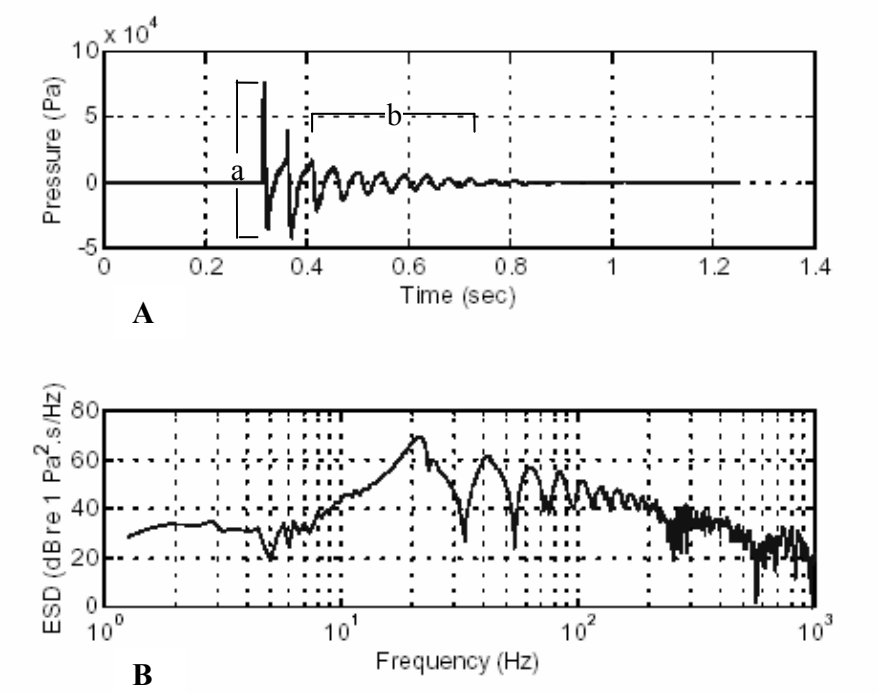

Figure 2.4: A) A representative air-gun signal; a) Primary pulse, b) Bubble pulse. B) a typical energy density spectrum (Duncan and McCauley 2000). See Chapter 4 for description of units.

Previous studies describing air-gun signals have used peak values (positive or negative peak values, mean peak value or peak-peak value), mean squared pressure or a measure of the signals energy (Greene 1985; Santulli et al. 1999; Wardle et al. 2001). Peak values are particularly relevant when applied to the mechanical transduction process that occurs in sound processing in vertebrates and some invertebrates. However, peak measurements give limited information on the sound that is actually perceived by the animal as no consideration is given to the temporal element of sound processing. Measures that include signal length will give additional information on how the signal is perceived by the animal but signal start and end points must be accurately defined. Signal length can be affected by factors such as, the variable levels of background noise, propagation phenomena or different analytical device protocols resulting in biased results (McCauley et al. 2000).

Definition of units used to measure air-gun signals is important, as the same signal can give measures with up to 30 dB difference using different units (McCauley et al. 2000). Unless otherwise stated, units used to describe air-gun signals in this thesis

are mean squared pressure (dB re 1 μPa), referred to as root mean square (rms) levels by several researchers (see McCauley et al. 2000 for mathematical definition).

2.3 Underwater acoustics

Sound travels in longitudinal waves and in any medium will consist of pressure fluctuations and particle motion (Rogers and Cox 1988; Gausland 1998). The ratio between pressure and particle motion will remain constant for a standing or stationary wave or from a planar source as would occur in the ocean for a receiver at a sufficient range from the source (Popper and Coombs 1980). Near to a sound source spherical waves are produced. With spherical waves the ratio between pressure and particle motion changes at varying distances from the sound source. As the sound source is approached the ratio of pressure to particle motion decreases. Therefore, pressure and particle motion are out of phase with each other. The phase difference between pressure and particle motion will depend upon the distance from the sound source. As the distance from the source increases the difference in phase between pressure and particle displacement decreases until the spreading wave is essentially planar at a receiver. The distance at which pressure and displacement become almost equivalent and beyond is termed the 'far field', anything closer is known as the 'near field' (Urick 1983b; Rogers and Cox 1988). The distance of the far field from the sound source is dependant on the frequency of the sound or the source dimensions, with sounds of lower frequency having further distance to travel before reaching the far field (Gausland 1998).

As the extent of the near field is inversely related to the compressibility of the medium, and the fact that most underwater noises are of low frequency, the near field of underwater sound is much more extensive than for airborne sound.

The speed, and wavelength, of sound is approximately 4.5 times greater in water than in air, that is approximately 1500 ms^{-1} in water compared to 330 ms^{-1} in air (Rogers and Cox 1988).

Reflection and scattering are apparent in underwater sound. The water-air interface of

the surface provides an excellent reflector for sound. This gives rise to the Lloyd's mirror effect where the reflected, phase inverted signal from the sea surface causes considerable destructive interference with the directly radiated signal, as a receiver moves closer to the sea surface (Urick 1983b; Rogers and Cox 1988). In contrast, the ocean bed is a variable reflector of sound. The reflection of the sound from the ocean bed will depend on its composition and the angle of incidence of the sound (Rogers and Cox 1988; Gausland 1998). The water column has many good sound scatterers, for example, animals, swim bladders, man-made objects, and gas bubbles (McCauley 1994).

Refraction of sound occurs when the speed of sound changes, causing the sound to bend. In water, sound speed gradients occur due to differences in temperature, salinity and pressure (Rogers and Cox 1988). These gradients cause the sound to bend in the direction of the slower sound speed inducing channelling of sound into regions of excellent (convergence zones) and poor propagation (shadow zones) (Urick 1983b; Rogers and Cox 1988).

As a result of the excellent propagation of sound in water, background (ambient) noise is usually high since natural sources can sum over larger areas (Rogers and Cox 1988). Variations of up to 30 dB in natural ambient noise are normal events (Cato and McCauley 2002).

2.4 Effects of noise on marine fish and invertebrates

Sources of ocean noise are numerous but can be broadly categorised into biological and non-biological sources. Biological sources include; fish, invertebrates and marine mammals while examples of non-biological sources are wind, shipping, rain and earthquakes (Myrberg 1978; McCauley 1994). Ambient levels of sea noise are highly variable but generally, in the frequency range of 10-1000 Hz, they average between 80-120 dB re 1 μPa (Tavolga 1971; Rusby 1995).

Research into the effect that air-gun noise used during seismic surveys has on marine life is very limited, with the majority of the work being conducted on marine

mammals. This is surprising, as it is well documented that chronic and acute sounds of high intensity can have detrimental effects on the behaviour and physiology of humans and other terrestrial animals (Fletcher and Busnel 1978; Kryter 1985). Evidence suggests that teleost fish are most sensitive to sound within the range produced by seismic surveys (McCauley 1994; Engas and Lokkeborg 2002). Previous studies have indicated that the effects that sound has on marine animals are extremely variable depending on species and the characteristics of the sound (McCauley 1994; Deffenbaugh 2002).

Besides signal intensity, two characteristics of particular importance when determining the effect of air-gun noise on marine fish and invertebrates are the pulse duration and the waveform shape of the signal. Air-gun signals typically have a short pulse length and a rapid rise time (McCauley 1994). Studies on fish have shown that: i) signals with a rapid rise time have a more noticeable impact on behaviour (Blaxter et al. 1981b; McCauley 1994) and ii) signals with a short pulse length (i.e. < 0.2 seconds) are not perceived as intense as sounds of longer duration (Hawkins 1981; Popper and Fay 1993).

There are three main ways in which seismic survey noise may affect marine fish and invertebrates; physiologically (functional changes in organs and processes), pathologically (tissue damage) and behaviourally. The majority of previous studies into the effect of seismic survey noise on fish and invertebrates have concentrated on observing overall behavioural and abundance changes in populations in their natural environment (Greene 1985; Dalen and Knutsen 1987; Pearson et al. 1987; Skalski et al. 1992; Engas et al. 1993; Lokkeborg and Soldal 1993; Wardle et al. 2001). Adult and larval fish held in cages have been used to identify pathological effects such as hemorrhaging, ruptured and damaged organs (for example, acoustic detectors) and death induced by air-gun noise and underwater explosives (Falk and Lawrence 1973; Greene 1985; Dalen and Knutsen 1987; Holliday et al. 1987; Pearson et al. 1992; Santulli et al. 1999). Other studies suggest that the noise from air-guns and underwater explosives may elicit a physiological stress response in exposed fish and some invertebrates (Sverdrup et al. 1994; Santulli et al. 1999).

2.4.1 Physiological effects

A major physiological effect that seismic survey noise may have on fish and invertebrates would result from the stimulation of the stress response. Observations of a detectable stress response in fish when exposed to acute or chronic sounds have been reported in the literature (Santulli et al. 1999; Bart et al. 2001).

In the natural environment a stress response usually benefits `immediate' survival. However, in an environment that suddenly changes as a result of anthropogenic activities, where the stressors may be severe or prolonged and chances of escape or avoidance limited, the consequences of the `immediate' survival stress response could have detrimental effects on the animal's health (Pickering 1992).

2.4.1.1 The stress response

Stress is defined as the response reaction by an animal to a stimulus that may somehow alter the animal's homeostatic state (Barton and Iwama 1991). The stress response is a mechanism that has evolved under natural selection pressures to enable an animal to cope with a potentially hostile environment (Pickering 1989a). A key element in the response is a switch from an anabolic state, one in which energy is being taken up and stored, to a catabolic state, one in which the energy reserves are broken down (Pickering 1989b). The mobilised energy is then utilised to avoid or overcome the immediate threat.

It is important to note that stress may have a cumulative effect on the animal. Therefore, a sequence of otherwise sublethal stressors can be harmful if the time period between these disturbances is not long enough for the animal to recover (Carmichael 1984; Sigismondi and Weber 1988; Pickering 1992; Power 1997).

For convenience, the responses to stress in fish and invertebrates have been classified into primary, secondary and tertiary, depending on the level of organisation of the response (Mazeaud et al. 1977; Barton et al. 1986; Barton and Iwama 1991). As the physiological response of invertebrates to seismic survey noise was not studied in this project this section concentrates on the physiology of the stress response in fish.

2.4.1.1.1 Primary responses

The primary stress responses are mediated through the neuro-endocrine system. Stress stimuli are detected by the nervous system which stimulates the endocrine system (Ellis 1981). The resulting alterations that occur (primary effects) are:

i) release of adrenocorticotropic hormone (ACTH) from the adenohypophysis and,

ii) release of `stress hormones' (catecholamines and corticosteroids) from the interrenal area (George 1977; Mazeaud et al. 1977; Wedemeyer and McLeay 1981; Schreck 1990a).

The two major primary stress responses in fish are displayed in Figure 2.5 and are described below.

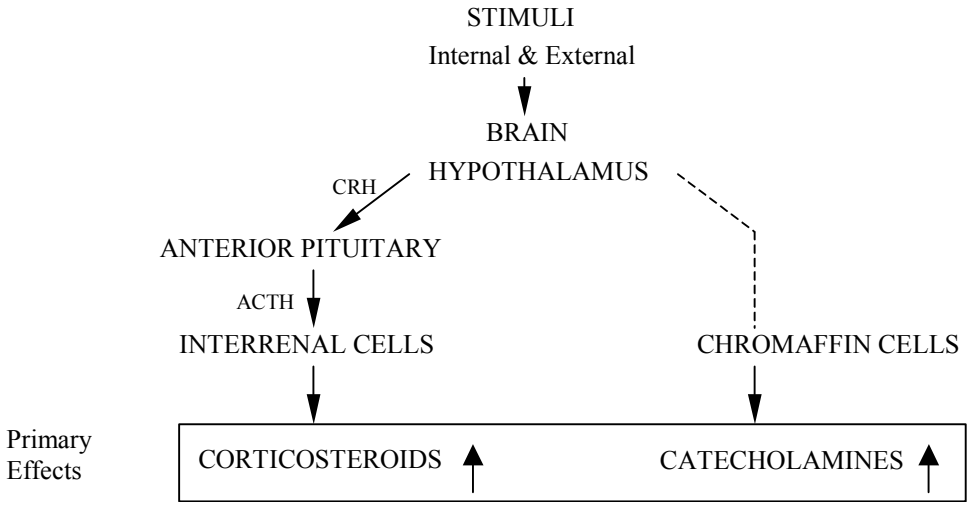

Figure 2.5: The general biochemical pathway for the primary response to stress in fish (Mazeaud et al. 1977).

It must be emphasised that many other components of the fish endocrine system are almost certainly influenced by environmental stress, either independently or via these two pathways (Pickering 1992).

The process on the left side on Figure 2.5 is referred to as the *hypothalymic-pituitary-interrenal axis* (HPI) (Donaldson 1981). This mechanism results in the primary

corticosteroid stress response in fish and, as the name suggests, involves the hypothalamus, the pituitary gland and the interrenal region of the fish.

The stress input is perceived by the hypothalamus which responds by stimulating the anterior pituitary (adenohypophysis) by secreting a neuropeptide, cortico releasing hormone (CRH) (Peter and Fryer 1981; Wendelaar Bonga 1993). CRH is transported by axons from the hypothalamus to the pituitary gland (Fryer and Maler 1980; van der Boon et al. 1991). As there are still many uncertainties about the role of the hypothalamus in the stress response of fish, the pituitary gland is the highest level of the axis which can be used for the evaluation of the primary response to stress.

The adenohypophysis is responsible for the synthesis and secretion of adrenocorticotropic hormone (ACTH) (Donaldson 1981; Wendelaar Bonga 1993). An increase in CRH from the hypothalamus stimulates the synthesis and secretion of ACTH from the adenohypophysis (van der Boon et al. 1991). ACTH originates from the precursor hormone, proopiomelanocortin (POMC) (Wendelaar Bonga 1993). POMC is biosynthesised in the ACTH cells (corticotropes) of the *pars distalis*. ACTH is created by cleavage of POMC (Donaldson 1981; Wendelaar Bonga 1993).

An increase in ACTH stimulates the interrenal region to synthesise and secrete corticosteroids. Corticosteroids are not stored, but synthesised as they are required (Sandler and Idler 1972; Pickering 1992). In the majority of cases the teleost interrenal region is embedded in the anterior portion of the kidney, although variation from this can occur (Nandi 1962; Yoakim and Grizzle 1980; Wedemeyer et al. 1990; Wendelaar Bonga 1993). The release of cortisol from the interrenal tissue displays large variations in relation to stress, daily rhythm, sexual maturity and season (Rance et al. 1982; Pickering and Pottinger 1983; van der Boon et al. 1991). Cortisol secretion is controlled by a feedback system, that is, the circulating level of cortisol has a direct effect on the pituitary gland (inhibitory at high levels) and the sensitive hypothalamic nuclei (Donaldson 1981; van der Boon et al. 1991). Cortisol, as with other steroid hormones, acts specifically via the DNA of it's target cells (van der

Boon et al. 1991). The major target organs for corticosteroid action appear to be the liver and gills (van der Boon et al. 1991).

The major corticosteroids that have been identified in teleostean blood plasma are cortisol and cortisone (Idler and Truscott 1972). Cortisol is released from the interrenal cells and is generally found in higher concentrations than cortisone. Cortisone is produced by 11*B* -hydroxysteroid dehydrogenation of cortisol (Donaldson and Fagerlund 1972). There is evidence of other corticosteroids present in teleosts, however it is generally accepted that cortisol is the appropriate corticosteroid to monitor to quantify a stress response (Donaldson 1981).

Following activation of the HPI axis, levels of circulating cortisol may be elevated for days (Thomas and Robertson 1991). The magnitude of the elevation of cortisol and the length of the response will depend on the level and duration of the applied stressor. Cortisol levels may return to basal levels even though the stressor is still being applied. This indicates adaptation by the fish to the new conditions and involves not only a reduction in circulating cortisol levels but also a reduction in the number of cortisol receptors in target tissues (Pickering 1992). However, care must be taken in reaching this conclusion. A reduced level of circulating cortisol may be the result of stress that is too severe or too prolonged which could result in degeneration of the interrenal and corticotropic tissue and the fish is entering the exhaustion stage where it is unable to synthesise corticosteroids (Wedemeyer and McLeay 1981; Pickering 1992).

The HPI axis has been utilised to evaluate stress in teleosts in response to; culture methods, disease outbreaks, disease treatments and water pollutants (including noise) (Donaldson 1981; Barton and Iwama 1991; Santulli et al. 1999). It may be particularly useful in testing the effects of two or more sublethal stresses applied simultaneously as the response reflects the integrated effect of the several components (of the axis).

The right side of Figure 2.5 represents the *adrenergic response* to stress in teleosts which is instigated by the sympathico-chromaffin system (Mazeaud et al. 1977; Mazeaud and Mazeaud 1981; Ungell et al. 1984) which together with the HPI axis, form the major primary neuro-hormonal disturbances in response to stress in fish.

In mammals catecholamines are secreted by the chromaffin cells within the adrenal medulla, however fish lack this organised structure (Mazeaud and Mazeaud 1981). Instead the chromaffin cells can be found in several organs which may differ between species (Nandi 1962; Holzbauer and Sharman 1972; Mazeaud and Mazeaud 1981; Reid et al. 1998). In teleosts the chromaffin cells are contained primarily within the anterior or head of the kidney (postnephron), often in association with the walls of the posterior cardinal veins and intermingled with interrenal cells (Yoakim and Grizzle 1980; Mazeaud and Mazeaud 1981; Nilsson and Holmgren 1993).

Catecholamines (neurotransmitters of the autonomic nervous system) are synthesised in both non-neural chromaffin cells and adrenergic neurons by identical processes (Randall and Perry 1992). The three catecholamines; dopamine, noradrenaline and adrenaline, are synthesised within the chromaffin cells via the Blaschko pathway (Blanschko 1939; Randall and Perry 1992; Reid et al. 1998). Note that the concentration of dopamine does not rise significantly in response to stress (Randall and Perry 1992).

The elevation in catecholamines may last for hours and may exhibit different patterns according to the species and nature of the stress (Mazeaud and Mazeaud 1981; Randall and Perry 1992).

While it is known that the neural stimulation of chromaffin tissue and consequent release of acetylcholine contributes to the elevation of plasma catecholamines, the intermediate steps are unknown (Randall and Perry 1992; Nilsson and Holmgren 1993). It is likely that a high level of carbon dioxide and a low blood pH (or a closely related variable) are important stimuli for catecholamine release (Aota et al. 1990; Randall and Perry 1992; Wendelaar Bonga 1993). Under normal conditions changes

in blood-borne factors may control catecholamine secretion, whereas cholinergic innervation may mediate the rapid responses of the chromaffin cells to stressors (Wendelaar Bonga 1993; Reid et al. 1998).

As with corticosteroids, circulating catecholamines may return to basal levels despite the continuation of the factor/s that caused the release originally (Chester Jones et al. 1969; Randall and Perry 1992).

The action of catecholamines is more rapid than that of corticosteroids as catecholamines are stored in a readily available form, and do not have to go through the cascade of hormonal events that is involved in the synthesis and release of corticosteroids (CRH → ACTH → cortisol) (Pickering 1992). Dramatically elevated levels of catecholamines can be detected in some species of teleosts within two minutes of the onset of the stressor (Mazeaud and Mazeaud 1981).

These two primary neuroendocrine responses stimulate primary effects that result in a number of biochemical, physiological and immunological changes that have been described as secondary effects.

2.4.1.1.2 Secondary responses

The increase in circulating neuro-hormones, corticosteroids and catecholamines, that is, the primary effects of stress, induce many disturbances of metabolism and osmotic balance in fish. These changes are referred to as secondary effects and occur as the fish tries to return to homeostasis (Wedemeyer and McLeay 1981). Both the HPI and the sympathetic-chromaffin system operate simultaneously and therefore, in the past confusion has arisen over which primary effect stimulates what secondary effect (Mazeaud et al. 1977). Figure 2.6 indicates relationship between primary and secondary effects.

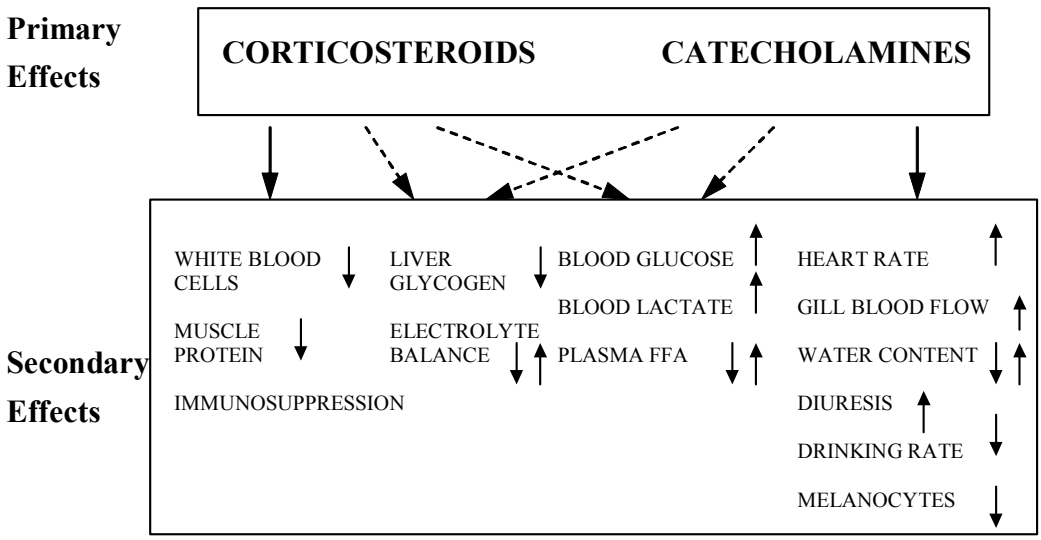

Figure 2.6: Relationship between primary and secondary effects (Mazeaud et al. 1977).

Generally, the alterations that occur in fish as a result of primary effects, that is secondary effects, are:

i) hematological changes, including leucopenia (Ellis 1981) and thrombocytopenia (reduced blood clotting time) (Casillas and Smith 1977; Smit and Schoonbee 1988);
ii) structural and metabolic changes, for example depletion of muscle and liver glycogen, hyperglycaemia, hyperlacticemia and oxygen debt (Wedemeyer and McLeay 1981) and skin colour changes (Fujii 1993);
iii) osmotic disturbances, resulting in blood chemistry changes such as hypochloremia (Eddy 1981).

The control of the ion-regulatory processes in both fresh and saltwater teleosts is dependent on corticosteroids (Henderson and Kime 1987; Evans 1993). Cortisol stimulates the proliferation of chloride cells in the intestinal epithelium and the gills. It also promotes Na^+/K^+-ATPase activity in these chloride cells, the driving force for monovalent ion transport in both fresh and saltwater teleosts (Wendelaar Bonga 1993). Cortisol secretion is stimulated transiently during migration of euryhaline fish (Laurent and Perry 1990). During stressful stimuli, when the level of cortisol increases, one function of cortisol is to control the hydromineral disturbances that

occur in response to stress exposure (Eddy 1981; Wendelaar Bonga 1993). Some osmotic disturbances that occur in response as a result of changes in levels of cortisol and adrenalin to stress are; hemoconcentration / hemodilution, electrolyte shift and increased diuresis (Donaldson 1981; Mayer-Gostan et al. 1987; Wendelaar Bonga 1993).

When a stressor is severe and persistent, an ionic or water overload can occur within minutes of the applied stressor (Bone et al. 1995b). The overload may be so severe that the fish may not recover (Mazeaud and Mazeaud 1981). This is an example of a maladaptive effect of the stress response.

Changing levels of circulating catecholamines and corticosteroids have numerous physiological effects both direct and indirect, all of which lead to either increases in, or maintenance of, energy turnover and oxygen supply under adverse conditions (Mazeaud and Mazeaud 1981; Epple et al. 1989; Aota et al. 1990).

An increase in cortisol, catecholamines (adrenaline and noradrenalin) and the release of glucogon from the pancreatic islets of Langerhans result in an increase in blood glucose levels (hyperglycaemia) and plasma lactate levels (Wedemeyer and McLeay 1981; Yokote 1982; Randall and Perry 1992; Foo and Lam 1993).

Cortisol aids the fish when it is exposed to long term stress by inducing hyperglycemia. This is accomplished by stimulating gluconeogenesis in the liver, that is the production of carbohydrates from a non-carbohydrate source (Idler and Truscott 1972).

When glucose levels are low, or when an increase in circulating glucose is required, the chromaffin cells release adrenalin and noradrenalin which act on the liver resulting in an increase in the rate of the conversion of glycogen (a starch like glucose polymer stored in the liver) to glucose (glycogenolysis) (Vijayan and Leatherland 1989; Pickering 1992; Randall and Perry 1992).

The Islets of Langerhans are small structures which occur in diffuse foci in the pancreatic tissue, in which they form the endocrine portion (Epple 1969; Brinn 1973). The structure and position of the pancreas and islet tissue will vary between groups of fish (Bone et al. 1995b). The islet tissue consists of three different types of cells, that is, A, B and D (Epple 1969). The level of blood glucose is controlled by the secretion of glucagon, from the A type cells and insulin, from the B type cells (Yokote 1982). Increased levels of glucagon augments the rate of glycogenolysis in the liver (Brinn 1973; Yokote 1982). The role of these hormones in the stress response of fish is unclear.

Metabolic disturbances can last for days, long after the neuro-hormone has been cleared from the plasma (Mazeaud and Mazeaud 1981).

Each step of the afferent immune system (involved in the uptake and processing of antigens) and the efferent immune system (responsible for the generation of specific products and activated cells that help protect the fish), from antigen recognition to uptake of the surveillance cells through transportation and processing to the final production of immune effectors, is susceptible to alteration or inhibition by environmental stressors (Ellis 1977; Anderson 1990; Weytes et al. 1999).

Increased pituitary and interrenal activity results in moderate to severe leucopenia (Donaldson 1981; Wedemeyer and McLeay 1981; Sopinska 1983). The white blood cells are involved in the efferent part of the immune system. The mature B lymphocytes release antibodies into the circulation while the T lymphocytes control and modulate antibody production and are involved in immunological memory (Anderson 1990). The administration of corticosteroids to fish causes a decline in circulating lymphocytes; presumably due to similar lymphocytolic properties of corticosteroids to those demonstrated in mammals (Wedemeyer and McLeay 1981; Ellsaesser and Clem 1986). This action may serve to immediately increase the available antibody titre and provide a ready source of protein for glucogenesis; however the deleterious side effects of this secondary stress response in terms of

depression of the immunological system and loss of resistance to infectious diseases are also significant (Ellis 1981; Ellsaesser and Clem 1986; Fries 1986).

Stress may also effect the immune system through modulation of the macrophage activity (Ellis 1981; Pulsford et al. 1994). These cells have a wide range of functions including phagocytosis, presentation of antigens, release of anti-microbial and anti-tumour agents and production of cytokines (Pulsford et al. 1994). Modulation of the activity of these cells should produce marked effects on immunocompetence and potential susceptibility to disease.

2.4.1.1.3 Tertiary effects

The secondary effects described above result in tertiary effects that manifest as whole organism responses (Wedemeyer and McLeay 1981; Adams 1990). These effects include:

i) impaired growth (McCormick et al. 1998);
ii) interference with the reproductive processes (Carragher et al. 1989; Pickering 1992; Schreck et al. 2001);
iii) increase in incidence of disease, infectious and non-infectious (Snieszko 1974; Anderson 1990);
iv) behavioural changes (Schreck et al. 1997) and
v) death.

Cortisol and, to a lesser extent, catecholamines have an effect on the normal regulation of growth and reproduction in fish as they do higher vertebrates (Billard et al. 1981; Pickering 1992). Therefore, as these components of the endocrine system interact, stress will invariably have an effect on both growth and reproduction. Cortisol is thought to effect growth and reproduction by suppressing the secretion of the various releasing factors or hormones (Bonga 1997).

Tertiary effects can be utilised to monitor stress in individual fish or at a population level (Wedemeyer and McLeay 1981). The extent to which the tertiary effects of stress affect fish will depend on the level of primary and secondary responses to the

stressor. The level of the primary and secondary responses to stress and the precise nature of the stress response will vary from family to family and even between species (Mazeaud et al. 1977; Barton and Iwama 1991; Bonga 1997).

2.4.1.2 The effects of noise on the stress response of fish and invertebrates

Although noise exposure has been reported to induce a stress response in fish, evidence for this using actual seismic survey noise is limited. Falk and Lawrence (1973) exposed fish to different explosive and non explosive noise sources that were commonly used for seismic exploration thirty years ago. Air-gun noise was reported to induce a stress response in captive fish (*Coregonus* spp), however the method of detection was not discussed. Knudsen et al. (1992) reported an increase in heart rate in caged fish exposed to air-gun noise whilst constrained in a cage. Sverdrup et al. (1994) exposed Atlantic salmon to underwater detonations (rise time of 40 μs, frequency 500-5000 Hz and pressure amplitude of 2 MPa). Differing patterns of delayed elevated levels in the concentration of circulating cortisol and adrenaline were observed, however the authors concluded that this was probably due to the damage that the detonations caused to the vascular endothelium (Sverdrup et al. 1994). Sverdrup et al. (1994) also observed no significant changes in the levels of plasma chloride. More recently, Santulli et al. (1999) recorded evidence of an increase in levels of primary stress hormones (cortisol) and secondary stress responses (changes in glucose, lactate, AMP, ADP and ATP levels) as a result of exposure of caged European Bass (*Dicentrarchus labrax*) to actual seismic survey noise.

The physiological effects of noise on marine invertebrates are not well documented. Lagardere (1982) reported an increase in growth and reproduction rate in the brown shrimp (*Crangon crangon*) held in sound proofed tanks (noise levels in tank reduced by 35 dB in the 25-400 Hz range and 20 dB in the 400-1000 Hz range). Reduced growth and reproductive rates are known tertiary effects of the stress response (Wedemeyer and McLeay 1981).

2.4.2 Pathological effects

The majority of previous studies have indicated that air-gun noise is not normally

lethal for adult fish and various species of invertebrates (McCauley 1994; Rusby 1995). Deaths have been reported after exposure to shots (3 L gun at distance of 0.5 m) from swim bladder collapse and broken blood vessels in the liver and gonads (Rusby 1995). Using an air-gun of 0.3 L at a distance of 0.5 m resulted in dilated blood vessels in the liver and gonads from which the fish quickly recovered (Rusby 1995). Falk and Lawrence (1973) reported swim bladder damage at a received sound level of 226-234 dB re 1 μPa. Small swim bladder hemorrhages have also been noted in cod (*Gadus morhua*) 6m away from a 220-240 dB re 1 μPa air-gun signal (Rusby 1995). Matishov (1990) observed death in cod and blindness in plaice (*Pleuronectes platessa*) following exposure to a single air-gun signal at a distance of 2-4 m from the gun. Sverdrup et al. (1994) exposed Atlantic salmon (*Salmo salar*) to 10 underwater detonations of approximately 2 MPa (peak pressure) over 70 minutes. No mortality was observed up to 7 days following exposure. However, structural damage to the vascular endothelium of the ventral aorta and the coeliaco mesentric artery was observed. Repair of the observed damage occurred within 7 days post exposure.

Experiments using plankton and larval fish have shown that mortality occurs only at close range (0.5-5 m) to the noise source (222-231 dB re 1 μPa) (Dalen and Knutsen 1987; Holliday et al. 1987; Rusby 1995). While many larvae situated within 2m of the air-gun suffered blood clots, unconsciousness and damage to the swim bladder, kidney and retina, most animals made a full recovery (Dalen and Knutsen 1987). Experiments conducted on the zoeal stage of the Dungeness crab larvae found no significant effect to survival, time to moult or behaviour from air-gun exposures up to 1 m away from the source, with a pressure of up to 231 dB re 1 μPa (Rusby 1995).

The immobility of fish and invertebrate eggs means that they have no control over the intensity of noise that they are exposed to which leaves them susceptible to damage. Dalen and Knutsen (1987) investigated the effect of a small air-gun (640 cm^3 chamber, sound pressure 222 dB re 1 μPa at 1 m) on cod (*Gadus morhua*) eggs, 2, 5 and 10 days after fertilisation. Exposure to the noise at 1 m and 10 m from the air-gun had no significant effect on the hatching success of the eggs or the resulting larvae (measured by feeding success) (Dalen and Knutsen 1987). Holliday et al. (1987) recorded a

significant decrease in the survival of anchovy (*Engraulis mordax*) eggs after exposure to signals from an air-gun array of an energy flux level of 0.60 bar^2 / second. However, the general conclusion of this investigation was that only multiple exposures to full seismic air-gun arrays would have a noticeable impact on anchovy eggs and larvae.

Damage to the auditory system of aquatic animals following exposure to intense noise has been reported in the literature and is a likely consequence of exposure to air-gun noise. Although sound travelling underwater obeys the same laws as sound travelling in air, underwater sound has some unique characteristics. It is likely that these characteristics were a major evolutionary influence on the hearing structures and the various acoustically induced behaviours that aquatic animals possess today (Schellart and Popper 1992). The variation in auditory systems and peripheral mechanisms between species of fish contributes to the unpredictability of the effect that sound at a certain frequency and intensity will have on a particular species (Popper and Fay 1993; McCauley 1994).

2.4.2.1 Hearing in teleost fish

The hearing capabilities of fish vary considerably between species according to their habits and the structural mechanisms they possess to enhance their sensitivity to sound. Generally, from the species that have been studied, most fish can detect sounds within a frequency range of 100-1000 Hz and many are known to detect signals below 100 Hz (Sand and Karlsen 1986; Popper and Fay 1993). Fish can be distinguished in terms of their hearing capability into three groups; Group I possess Weberian ossicles (see below); Group II have some specialised structure (other than Weberian ossicles) that enhances the pressure to displacement transduction and; Group III which possess no specialised structures to enhance hearing capabilities (Schellart and Popper 1992). Groups I and II are known as hearing specialists. Hearing specialists are known to be sensitive to sounds as low as 1-10 Hz while others can detect sounds above 3 kHz and into tens kHz in some cases (Mann et al. 1997; Popper 2000; Akamatsu et al. 2003). The minimum threshold varies widely within these frequencies, with hearing specialists being sensitive to tones of mean

squared pressure as low as 50 dB re 1 μPa and non-specialists (hearing generalists) as high as 110 dB re 1 μPa at their optimum hearing frequency (Fay 1985).

2.4.2.1.1 The acoustico-lateralis system

The mechanoreceptors of the acoustico-lateralis system are responsible for detecting water motions induced by particle motion and pressure fields (Popper and Fay 1993; Bone et al. 1995a). Sound, gravity and linear and angular acceleration of the fish's body can all be detected by different parts of the acoustico-lateralis system (Bone et al. 1995a).

i) Structure

The two main components of the acoustico-lateralis system in teleost fish are the inner ear and the lateral line. The two inner ears are situated on either side of the brain (Fig. 2.7) and consist of an upper and lower part (Popper and Fay 1993; Bone et al. 1995a). The upper part comprises the semi circular canals while the lower part includes the otolith organs (end organs), that is the sacculus, lagena, utriculus and, in some teleost fish, the macula neglecta (Popper and Fay 1993). At the base of each of the three semi circular canals is a swelling (ampulla) that contains sensory crista (Tavolga 1971). Each of the sac-like otolith organs are fluid filled and contain a single calcareous structure, the otolith (Tavolga 1971). Attached to the inner wall of each chamber is the sensory epithelium (macula) which is covered in sensory hair cells surrounded by supporting cells. Tight junctions and desmosomes are situated between cells just below the apical cell membrane (Popper and Hoxter 1981). A gelatinous membrane (otolithic membrane) connects the macula and the otolith and retains both structures in similar positions relative to each other. The lateral line refers to the free, and lines of, sensory cells situated on the head and body of the fish (Flock 1971).

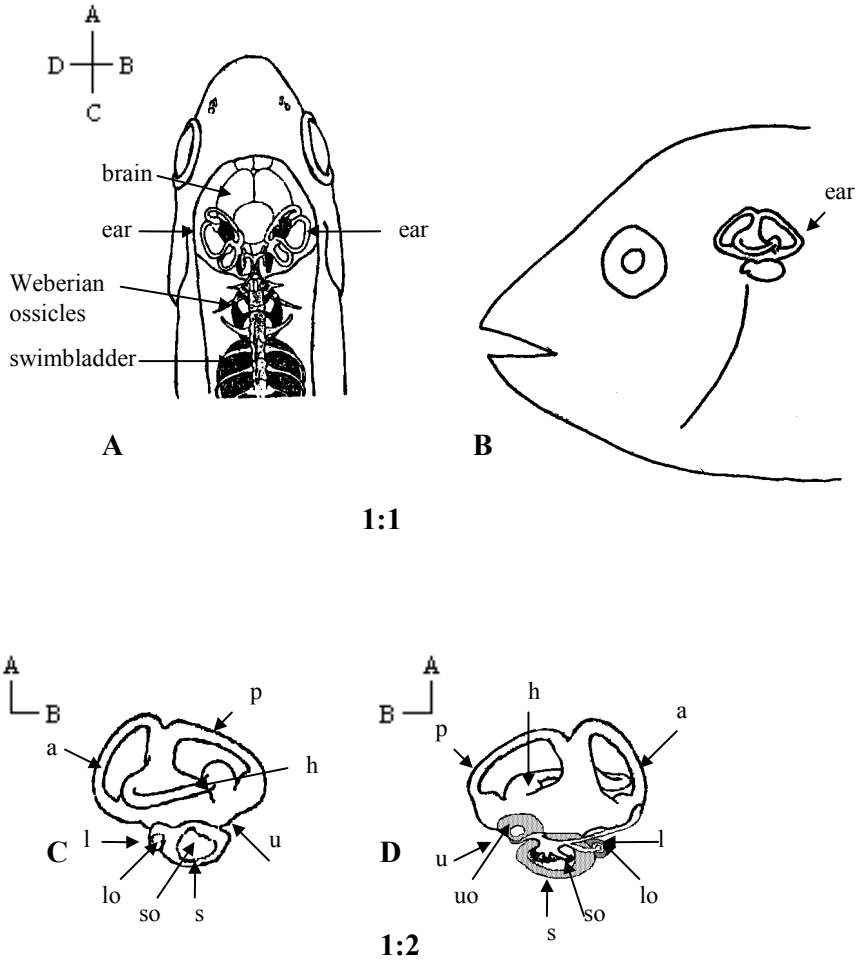

Figure 2.7: A) Dorsal view of teleost fish with inner ear exposed. B) Lateral view of teleost fish indicating position of inner ear. C) Lateral view of inner ear of teleost fish. D) Medial view of the inner ear of a teleost ear. s = saccule; so = saccular otolith; sm = saccular macula; l = lagena; lo = lagenar otolith; lm = lagenar macula; u = utriculus; uo = utricular otholith; um = untricular macula; a = anterior semi-circular canal; p = posterior semi-circular canal; h = horizontal semi-circular canal (Bone et al. 1995a).

The sensory cells of the acoustico-lateralis system are hair cells (Popper and Fay 1993). The hair cells consist of a ciliary bundle and a single kinocilium (9+2 filament) that project into a gelatinous cupula or, in the case of the end organs, the otolithic membrane (Fig. 2.8) (Lowenstein 1971; Popper and Fay 1993). Each bundle comprises approximately 100 cilia (or stereocilia), each consisting of hundreds of cross linked actin filaments enveloped in a plasma membrane (Zhao et al. 1996). Researchers have reported the presence of extracellular filaments, tip links, that

stretch from the tip of each cilia to the side of the neighbouring cilia (Pickles et al. 1984; Zhao et al. 1996; Husbands et al. 1999).

The hair cells are synapsed to two nerve fibres, one that carries information received from the hair cells to the brain (afferent) and one that carries signals from the brain to the hair cell (efferent) which can effectively turn it 'off' (Bone et al. 1995a).

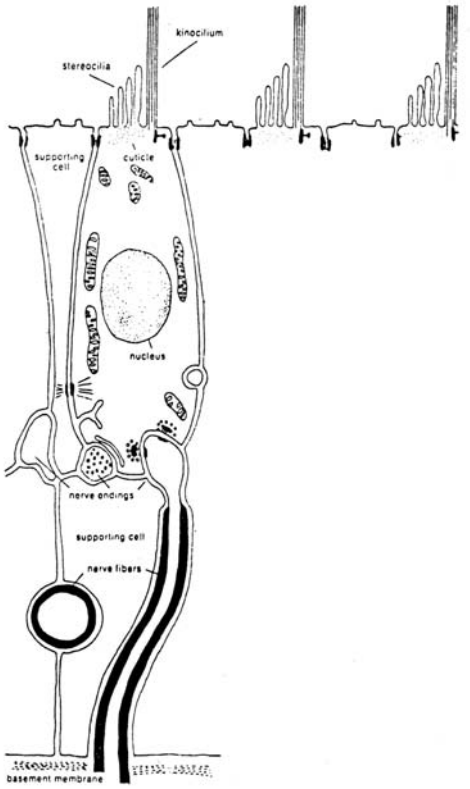

Figure 2.8: Representative diagram of a sensory hair cell from a teleost fish (Popper and Coombs 1980).

Groups of these cells are found as lateral line neuromasts (either free, in pits, grooves or canals), in the ampula of the semi circular canals as crista and on the macula of the end organs. Four types of ciliary bundles, that differ in number and length of the cilium and the length of the kinocilium, have been observed in the end organs of some teleosts (Popper 1981). The hair cells of the macula are arranged in groups of similarly polarised hair bundles (Popper and Fay 1993). The groups of similarly orientated hair cells of the saccular macula in teleost fish are generally arranged in one of five patterns; standard, dual, alternating, opposing and vertical (Fig. 2.9)

(Schellart and Popper 1992; Popper and Fay 1993). From the species studied, it appears that each group of similarly orientated hair cells is innervated by a different section of the saccular branch of the VIIIth nerve (Saidel and Popper 1983; Popper et al. 1988).

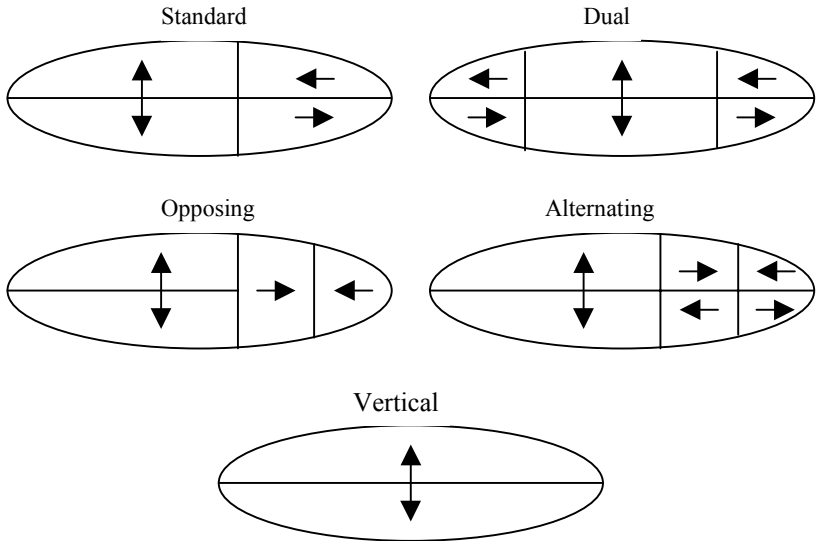

Figure 2.9: Diagram of saccular sensory epithelium showing the five main hair cell orientation patterns of teleost fish (Popper and Fay 1993).

Many fish possess accessory hearing structures that can enhance hearing capabilities (Bone et al. 1995a; Yan 1998; Yan and Curtsinger 2000). These structures involve mechanically coupling pressure fluctuations produced by a gas bubble responding to an impinging sound, to an otolith. Some use the swim bladder and other gas filled structures coupled directly to the otolith, while fish in the superorder Ostariophysi actually have a series of bones, the Weberian ossicles, that connect the swim bladder to the inner ear (Fig. 2.7) (Popper and Fay 1993).

ii) Function

The lateral line does not normally respond to sound pressure, only to relative movement between the fish and the surrounding water, although in some instances pressure-displacement transducers can occur (e.g. clupeoids) (Denton et al. 1979; Bone et al. 1995a). Compared with the lateral line the inner ear of the fish is more sensitive to sound waves and can respond to a wider range of frequencies (Bone et al.

1995a). The cristae of the semicircular canals are involved in the detection of angular acceleration rather than hearing. It is thought that the primary acoustic receptors in the majority of fish are two of the otolithic organs, the saccule and the lagena while the utricle acts as a gravistatic receptor (Bone et al. 1995a). However, at least in one group of fish, the clupeids, the utricle is the major acoustic receptor (Blaxter et al. 1981b). Recent evidence suggests that each otolithic organ may in fact be multifunctional (Popper and Fay 1993).

Sound can reach the ear via two pathways, directly or indirectly. The ear is stimulated directly by the detection of particle motion from an acoustic disturbance (Popper and Fay 1999). Particle motion is more predominant than pressure in the near field. As the body of a fish and water are of similar density both will move similarly when in a sound field. However, as the otolith is three times denser than water, it will move at a smaller amplitude and in a different phase to the body of the fish (Bone et al. 1995a). As the attachment between the otolith and macula is only a gelatinous membrane, that is not rigid, they will move relative to each other (Popper and Coombs 1980). The resulting movement of the otolith against the macula will cause the hair cells to bend (Bone et al. 1995a).

Sound can reach the inner ear indirectly by vibration of the swim bladder or other gas filled structures that the fish may possess, for example optic bulla (Blaxter et al. 1981a). Sound pressure will cause the gas filled structures to vibrate which will transform the sound energy into particle displacement, which causes the otolith to move. In some instances direct mechanical coupling may occur between gas bladder and otolith (McCauley 2001). The pressure component of the sound wave is more predominant in the far field. This indirect signal is used by fish that possess coupling structures between the inner ear and the swim bladder to enhance sensitivity, frequency thresholds and potentially aid in localisation ability (Rogers and Cox 1988; Bone et al. 1995a).

The ciliary bundles have directional properties. Displacement of the otolithic membrane (or cupula, in the case of the ciliary bundles of the lateral line and the

ampullary organs) causes the cilia to bend. The displacement of the cilia results in the opening or closing of transduction channels which maintain the electrical potential across the membrane. It has been suggested that tip links function as gating springs for the transduction channels (Pickles et al. 1984; Hudspeth 1985; Zhao et al. 1996).

If the ciliary bundle is bent towards the kinocilium the transduction channels open. With the subsequent influx of ions (principally K^+, but also Ca^{2+}) the hair cell becomes depolarised which induces excitation (Hudspeth 1989). Hyperpolarisation will occur if the cilia are bent away from the kinocilium, closing the transduction channels and inducing an inhibitory effect. Displacement of the cilia at right angles to the kinocilium / stereocilia axis will have no result (Bone et al. 1995a). The sensitivity of the response at every angle in-between follows a cosine law (Flock 1971; Bone et al. 1995a).

The deflection of the hair cells to an impinging sound and therefore the information gained about the sound will depend on the type of signal and the mechanical properties of the otolith – macula system. These factors include; otolith mass, damping and stiffness, the shape and modelling of the otolith, the degree of coupling between the otolith and the macula and the structure of the sensory tissue. The neuro-biological processes of fish involved in the translation of these acoustic signals, for example level discrimination, frequency discrimination, sound source location, are not fully understood (Fay 1985; Fay 1992; Popper and Fay 1993; McKibben 1999).

It is assumed that the directional cues for locating the sound source come from a combination of processing information about the particle motion and, for hearing specialists, pressure component of the sound (Schuijf 1976). As the distance between the ears of fish is relatively small and the speed of sound under water is relatively fast, it is unlikely that fish are able to use the inter-aural difference in time of arrival to determine the direction of the sound (Rogers and Cox 1988; Popper and Fay 1993). In theory, the differing orientations of the hair cells and macula of the otolith organs could provide the central nervous system with enough information to

determine the axes of the sound propagation (Popper and Fay 1993; Lu 1998; Lu and Popper 1998). Current theories suggest that fish may resolve the phase difference between the particle motion and pressure component of the sound to resolve the 180^{o} ambiguity in direction that would exist from analysing particle motion alone (Schuijf and Buwalda 1980; Fay 1988).

Experimental evidence suggests that the different lengths of ciliary bundles found on the macula may be responsible for detecting differing frequencies with the longer bundles being associated with the detection of low frequency sounds while the shorter bundles appear to be sensitive to higher frequency sounds (Popper and Fay 1993). Another possible mechanism for frequency discrimination could be temporal analysis of the signal which involves the generation of a particular spike rate or sequence of spike intervals associated with a particular frequency (Coombs and Popper 1982; Rogers et al. 1988). Thirdly, frequency regionalisation may exist on the saccular macula due to otolith – macula mechanical properties, with one particular region being sensitive to one or a range of particular frequency (Rogers et al. 1988). A combination of these mechanisms for frequency discrimination is also a possibility.

2.4.2.2 'Hearing' in invertebrates

The hearing ability of marine invertebrates, especially in cephalopods, is a controversial topic (Hubbard 1960; Moynihan 1985; Hanlon and Budelmann 1987; Packard et al. 1990; Popper et al. 2001). In a broad sense most invertebrates can 'hear', that is, most respond in some way to water movement. There are three possible methods by which invertebrates sense particle movement; superficial receptor systems, internal (in most cases) statocyst receptor systems and chordotonal organs (crustaceans) (Budelmann 1992a; Budelmann 1992b). There is no evidence to suggest that invertebrates can sense the pressure component of sound as no species have been identified possessing gas filled cavities associated with sensory structures (Budelmann 1992b).

2.4.2.2.1 Superficial receptor systems

Superficial receptor systems have been identified in most invertebrates. Each receptor system has a single or numerous hair-like projections with a flexible base. When exposed to water motion these hairs bend which sends a signal to the sensory cells. The neurobiology of these systems is poorly understood. Evidence suggests that most invertebrates are sensitive to frequencies from 1-100 Hz with some species being particularly sensitive to vibrations of one frequency (Budelmann 1992b). Decapod crustaceans in particular are sensitive to substrate conducted vibrations of up to 200 Hz (Budelmann 1992a).

Cephalopods have a relatively well developed superficial receptor system which has been likened to the lateral line of bony fish with lines of ciliated sensory cells that run parallel to each other in a longitudinal direction over the head and arms and detect local water vibrations (Hanlon 1990; Packard et al. 1990; Budelmann 1992b). Evidence suggests that these sensory cells are sensitive to vibrations of 0.5 - 400 Hz with very high sensitivity to water displacement (Budelmann 1992b). Ciliated sensory cells are also scattered over the cephalopod body (Packard et al. 1990; Budelmann 1992b).

2.4.2.2.2 Statocysts

Statocysts are present in some form in most invertebrates. They vary in number and location between species and range from the very simple structures found in protozoa to the complex organs found in the higher cephalopods (octopus and squid) which are analogous with the vertebrate vestibular system (Budelmann 1988; Budelmann 1992b).

The basic components common to all statocysts are: i) a mass which is generally denser than the surrounding fluid with a position that depends on the forces applied to it and ii) sensory elements that are mechanically effected by the position of the mass (Budelmann 1977). The primary function of the statocyst is to act as an equilibrium receptor, that is to detect linear (gravity) and, in cephalopods and decapod crustaceans, angular acceleration (Budelmann 1988). However, data exists

from research conducted on cephalopods and crustaceans that components of both the linear and angular acceleration receptor systems of the statocyst are sensitive to vibration and therefore should not be ruled out as being involved in underwater hearing (Budelmann 1988; Budelmann 1992a; Budelmann 1992b).

Very basically, the most common form of statocyst is a fluid filled cavity containing one mass (statolith) or several masses (statoconia). The weight of the mass stimulates the underlying sensory components of the statocyst. The sensory components can be cell organelles, hair cells or cuticular sensory hairs (Budelmann 1992b).

The simplest forms of gravity receptor system can be found in single cell organisms such as ciliates, which utilise cell organelles of high density as statoliths (Budelmann 1988). Other simple forms, which contain statoliths but no known receptor cells, can be found in some lower invertebrates. The most common gravity receptor system found in invertebrates utilise sensory hair cells which are usually in direct connection to an axon (primary sensory organs). Each hair cell carries varying numbers of true kinocilium (unlike fish, hair cells with single kinocilium are rare in invertebrates) which can be polarised, non-polarised or a mixture of both. In a polarised hair cell the direction of the stimulus can be detected as all kinocilia are polarised in the same direction (Budelmann 1988). These polarised hair cells are common in cephalopods.

Cephalopods possess the most sophisticated equilibrium receptor organs found in invertebrates (Budelmann 1977; Budelmann 1992b). The level of complexity of the statocysts is thought to be directly related to the locomotory requirements of the animal (Stephens and Young 1978; Budelmann 1992b). Cephalopods have two bilateral statocysts that are positioned within the cranial cartilage (Fig. 2.10) (Budelmann 1988). Variations in the structure of the statocysts are found between genera but they can be broadly categorised into two forms, that is, octopod (for example the octopus) and decapod (for example the squid) (Budelmann 1988; Budelmann 1992b).

In octopods the statocysts are sphere shaped sacs. They contain a single gravity receptor system that consists of a vertically orientated sensory epithelium (macula) to which a calcareous statolith is attached (Dilly 1976; Budelmann 1988). The sensory hair cells that cover the macula are polarised in a radial pattern towards the macula periphery and are secondary sensory cells that receive innervation from synaptic contact with two types of first order afferent neurons (Budelmann 1988).

Angular acceleration receptors are present in higher cephalopods and occur together in the statocysts with the gravity receptor system. The angular acceleration receptor system is comprised of ridges of sensory hairs (crista) that are arranged on three orthogonal planes and are hence called anterior crista section (CTA), the crista longitudinalis (CL) and the crista verticalis (CV). The crista ridge of the octopod statocyst is divided into nine sections of oppositely polarised hair cells. Each segment is attached to one cupula that protrudes into the statocyst cavity and is sensitive to the fluid movement inside the statocyst. In octopods the size and form of the cupula differ which is thought to be related to the two differing types of locomotory movements, that is slow crawling and fast swimming, that these animals display.

The structure of squid statocyst is more complex than the octopod type due to cartilaginous protrusions (anticrista) from the statocyst walls (Fig. 2.10). The number, position and form of the anticristae varies between genera and evidence suggests that they are related to the speed at which the particular species moves (Stephens and Young 1982; Budelmann 1992b). In fast moving decapods the anticristae can reduce the internal volume of the statocyst chamber considerably and form canals and therefore, restricting the movement of the fluid within the cavity. This presumably leads to reduced angular acceleration sensitivity (Budelmann 1992b).

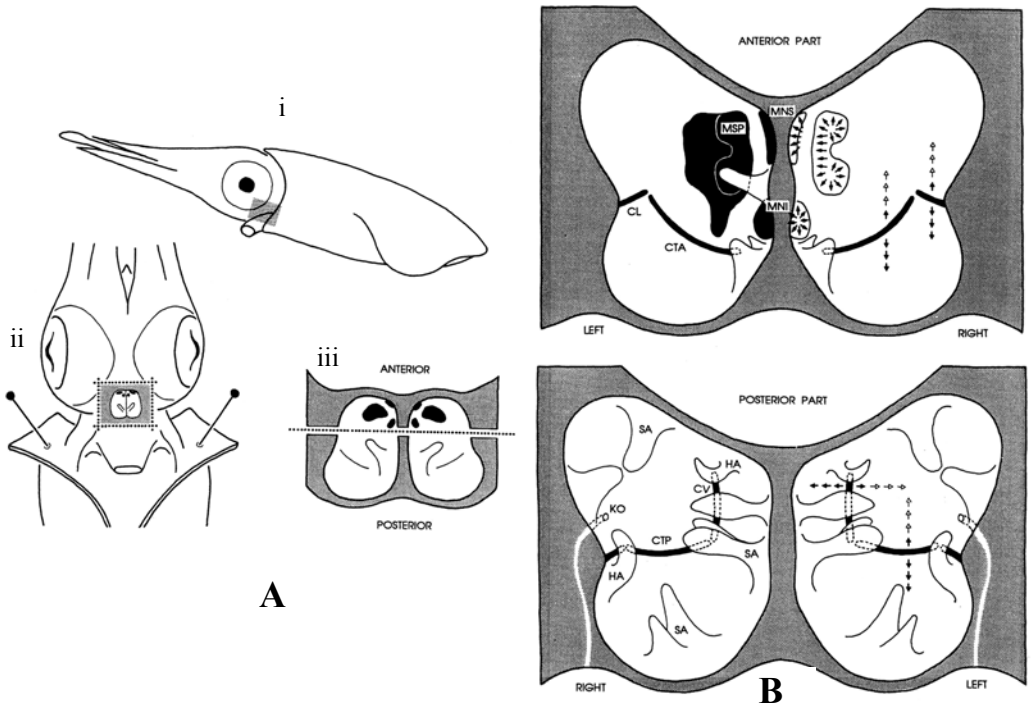

Figure 2.10: The squid statocyst. (A) Position of statocysts in squid. i) Lateral view, ii) Ventral view, iii) Ventral view of statocysts – transverse cut on dotted line and opened (B). (Budelmann 1990).

The gravity receptor system of decapods comprises the macula statica princeps (MSP) the macula neglecta superior (MNS) and the macula neglecta inferior (MNI). A single calcareous statolith is attached to the MSP while statoconial layers are in contact with the MNS and MNI. The hair cells of the maculas are polarised; the MSP and MNI in a radial pattern and the MNS in a fanlike pattern (Budelmann 1988). The information gained from the gravity receptor system does not seem to be dependent on the strength of the stimulus, but rather the excitation pattern of the macula hair cells. The cellular organisation of the gravity receptor system of decapods is basically similar to octopods with all three maculae being innervated by one macular nerve.

In decapods the angular receptor system (crista) is arranged orthogonally but is divided into only four segments of oppositely polarised hair cells. The neural and synaptic organisation of the crista hair cells is complex. There are three types of sensory hair cells that comprise the decapod crista; primary and small and large

secondary cells. There are two types of crista nerves that enter the statocyst separately through the anterior statocyst wall, the crista minor (small medial nerve) and the crista major (a larger lateral nerve). The crista minor synapses afferently with the CTA while the crista major synapses afferently with the CL and CV. Small branches of the crista major have also been associated with efferent synapses to the CTA.

2.4.2.3 Effects of noise on the auditory system of fish and marine invertebrates

Enger (1981) and Cox et al. (1987) exposed codfish and goldfish respectively, to intense sounds that resulted in damage to the sensory epithelium of the inner ear. Hastings et al. (1986) exposed *Astronotus ocellatus* to sounds of frequency 60 and 300 Hz and intensities of 100, 140 and 180 dB re 1 μPa with damage to the inner ear resulting in the fish exposed to 180 dB re 1 μPa at 300 Hz. Damage was restricted to the striola of the lagena and utricle and results suggested that damage was not immediate. However, it is difficult to relate these results to the effect that seismic survey noise has on the auditory system as experiments were conducted with continuous sounds whereas the sounds used in seismic surveys are short and repeated (McCauley 1994). There are reports of inner ear damage to the cod (*Gadus morhua*) after exposure to sound levels of 180 dB re 1μPa and a decrease in nerve activity as a result of exposure to air-gun noise (Rusby 1995).

It has been suggested that the damage to hair cells caused by intense acoustic stimulation is a result of over stimulation of the otolith (Hastings et al. 1996). Regeneration of hair cells after intense acoustic stimulation has been well documented in birds (Corwin and Cotanche 1988; Chen 1996). In fish, evidence of regeneration of hair cells is limited to the striolar regions of the utricle and lagena after damage induced by exposure to gentamicin (Lombarte et al. 1993). Mitosis of the supporting cells of the sensory epithelium is thought to be the major contributor to hair cell regeneration however evidence suggests that supporting cell conversion may also lead to hair cell regeneration (Raphael 1992; Adler and Raphael 1996; Corwin and Oberholtzert 1997)

Experimental evidence suggests that, like other vertebrates, exposure to intense acoustic stimulation can cause a temporary decrease in sensitivity of the fish ear to sounds of certain frequency and amplitude, known as a temporary threshold shift (TTS) (Popper and Clarke 1976). Scholik and Yan (2001) and (2002) exposed two species of fish (*Primephales promelas* and *Leponus macrochirus*) to continuous white noise for varying time periods with a maximum duration of 24 hours. A noise level of 142 dB re 1 μPa with a bandwidth of 0.3 - 4 kHz was used. The results of their studies suggested that the degree of TTS exhibited is dependant on the duration and frequency of the noise and the hearing capabilities of the exposed species. The cellular processes associated with TTS are unknown, however Zhao et al. (1996) observed regeneration of broken tip links in birds and proposed that the time course of repair suggests that broken tip links may be at least one of the underlying processes that results in TTS. The significance of this shift is that affected animals could ignore or misinterpret important acoustic environmental cues (McCauley 1994).

There are no reports in the literature on the effect of intense noise on the acoustic receptors of marine invertebrates. However, vibrational and directional sensitivity of the hair cells of the cephalopod statocyst have been reported (Williamson 1988; Williamson 1989; Packard et al. 1990; Budelmann and Williamson 1994). The similarities between the statocyst of cephalopods and the vestibular system of fish suggests that, if intense acoustic stimulation can damage sensory cells of the fish ear, then this may also be the case for the sensory cells of the cephalopod statocyst.

2.4.3 Behavioural effects

Many of the behavioural changes that may result from exposure to seismic survey noise are likely to be due to any pathological damage and/or the numerous stress induced physiological and biochemical changes that may occur (Schreck 1990). As mentioned above, behavioural measures of stress have been proven as sensitive indicators of the stress response (Schreck 1990a; Schreck et al. 1997). The behavioural alterations induced by stress and pathological damage are likely to have a significant effect on survival of the animal (Schreck et al. 1997). Essential activities

such as food foraging, predator evasion, reproduction, habitat selection and intra and extra species interactions will be affected (Shuter 1990; Winberg and Nilsson 1993; Fox et al. 1997; Schreck et al. 1997; McCormick 1998).

There is very little information about the effect of seismic survey noise on the behaviour of invertebrates. Wardle et al. (2001) exposed an established small reef system to 195-218 dB re 1μPa from a 2.5 L air-gun and observed the resident invertebrates (crabs, starfish and sea urchins) 14 days before, during and after exposure. No significant changes in behaviour were observed and, although escape from the reef was possible, there were no signs of the invertebrates migrating (Wardle et al. 2001). Other experimental evidence suggests that shellfish and crustaceans are relatively immune to air-gun noise (Rusby 1995)

The majority of previous studies have reported behavioural and abundance changes in finfish induced by seismic survey noise (Chapman and Hawkins 1969; Dalen and Knutsen 1987). Engas et al. (1993) conducted perhaps one of the most comprehensive of these studies. The distribution of cod and haddock on the North Cape Bank of the Barents Sea was investigated 7 days prior, 5 days during and 5 days following seismic air-gun activity. Distribution was measured by acoustic mapping, trawling and long line fishing. Acoustic mapping indicated a 45% reduction on cod and haddock numbers in the investigated area (74 x 74 km) with the largest reduction in the actual seismic survey shooting area. These results were supported by the results for trawling (implying that the fish did not simply migrate to the ocean bottom) and long lining. Overall the distribution of cod and haddock was affected with a reduction in catch rate being observed to at least 33 km from the survey area (their sampling limit). A decrease in the average size of the fish caught was also noted. Catch rates and acoustic mapping of the area up to 5 days after the shooting indicated that the area had not recovered. The authors discounted exploitation of the area under investigation or vessel avoidance alone as reasons for the results obtained.

Skalski et al. (1992) reported a 50% decline in catch per unit effort of natural aggregations of rockfish (*Sebates* spp.) after exposure to an estimated 186-191 dB re 1 μPa from a 1639 cm^3 air-gun. Fish were observed to move lower in the water column during air-gun exposure but no dispersal of the fish was recorded. Lokkeborg and Soldal (1993) also reported a reduction of 55-80% in catch rates (trawling and long lining) of *Gadus morhua* after exposure to seismic survey. Avoidance was hypothesised as the reason for the decrease. Other authors have reported *Gadus morhua* being forced to the ocean bottom during seismic survey activity (fright response) (Dalen and Raknes 1985; Dalen and Knutsen 1987).

Pearson et al. (1992) exposed captive rockfish (*Sebates* spp.) to noise from a 1639 cm^3 air-gun. Fish were exposed to noise levels of 160, 180 and 200-205 dB re 1 μPa (mean peak level). The approximate distances from an actual seismic survey air-gun array that these noise levels correspond to are 2.1-12 km, 630-2000 m, and 100-316 m respectively. At 160 dB re 1 μPa (mean peak level) subtle changes on behaviour of the fish were noted, at 180 dB re 1 μPa (mean peak level) tight milling was observed and at 200-205 dB re 1 μPa a startle response (fleeing) was observed.

More recently, Wardle et al. (2001) exposed an inshore reef to noise from three 2.5 L, 2000 psi air-guns. In these trials the air-gun was stationary and fired at a maximum rate of once per minute. The reef and its inhabitant's were observed for one week prior, during and four days after air-gun noise exposure. At 109 m away the fish gave brief C-starts (startle responses) at each air-gun signal but were otherwise unaffected. It appeared that startle responses were not directionally orientated until the fish could actually see the bubble resulting from the released air (peak pressure of 218 dB re1μPa). Other authors have also reported startle responses to air-gun noise (Pearson et al. 1992; Santulli et al. 1999). No emigration of fish from the reef was observed (Wardle et al. 2001).

2.4.3.1 Startle response

The startle response in fish is an involuntary 'reflex' behaviour induced by an adverse stimuli (for example, visual or acoustic), and is characterised by a unilateral

muscular contraction that bends the fish into a 'C' shape or in some cases a 'S' shape (Eaton and Hackett 1984; Godin 1997). This brief stage is typically followed by a propulsive phase where the tail bends in the opposite direction, turning the body of the fish, which is then accelerated forward. Stage three usually involves a period of sustained swimming (Eaton and Hackett 1984; Godin 1997).

2.4.3.1.1 Physiology of the startle response

In most teleost fish the startle response is initiated by the Mauthner cells (M-cells), however, it is important to note that many neurons are involved in the startle response and therefore the presence of M-cells is not essential to initiate the response (Zottoli et al. 1995; Eaton et al. 1997). Nonetheless, it is thought that the involvement of the M-cells in the response is directly related to the reaction time (Domenici and Blake 1997). The neurobiological commands behind slow 'C' starts and 'S' starts are unknown (Domenici and Blake 1997).

The M-cells are a pair of brainstem neurons found in teleost fish and some amphibians (Diamond 1971). Their size, shape and number and disposition of dendrites vary between species, especially between otophysan and non-otophysan fish (Zottoli et al. 1995). Generally, the soma of the M-cells are situated in the brain, under the cerebellum, on either side of the midline (Fig. 2.11). The soma separates into two distinct branches, the lateral and ventral dendrites. The lateral dendrite extends laterally towards the VIIIth cranial nerve while the ventral dendrite curves downward and travels slightly anteriorly (Diamond 1971). The M-cell's axon leaves the soma and extends towards the midline of the medulla. At this point the axons from both M-cells cross and then turn caudally and follow the spinal cord, on the opposite side of their original cell soma, and gradually taper out until they disappear (Diamond 1971). Each axon forms many synapses with motoneurons that innervate muscles along the trunk and tail on the opposite side to the body of the M-cell soma (Diamond 1971; Eaton et al. 1995).

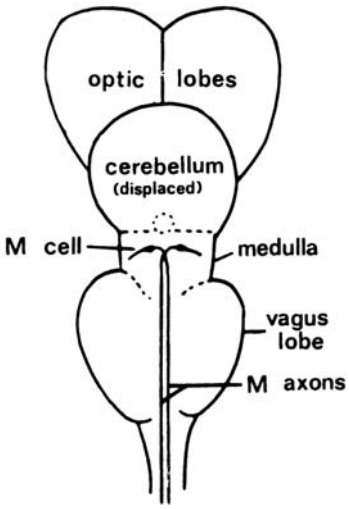

Figure 2.11: Structure and position of the Mauthner cells in teleost fish (Diamond 1971).

There are three main regions for sensory input into the M-cells; the lateral dendrite, the ventral dendrite and the axon cap (Diamond 1971). It is important to note that areas innervated by the posterior lateral line nerve and the spinal cord also provide sensory input to regions of the M-cells. However, as electrical stimulation of these neurons does not usually cause the M-cells to fire, it is probable that they are important in modulating the excitability of the system (Zottoli et al. 1995).

The lateral dendrite receives input from the ear of the fish through the posterior and anterior branches of the VIIIth nerve which terminate in distinct club endings on the lateral dendrite (Zottoli et al. 1995). Studies have shown the connection between the saccule and M-cells, particularly in goldfish. However, evidence suggests that these club endings originate from more than one area of the ear (Popper and Edds-Walton 1995; Zottoli et al. 1995).

The ventral dendrite receives visual input from the eye via the optic tectum. Visual input alone is sufficient to bring the M-cells the threshold (Zottoli et al. 1987)

The axon cap surrounds the initial axonal section of the M-cells and is the site of electronic inhibition of the Mauthner neurons (Eaton et al. 1995). The axon cap

receives polysynaptic input from at least two different types of interneurons, namely the passive hyperpolarizing (PHP) cells and spiral fibers (Zottoli et al. 1995).

There are two known types of PHP cells, the commissural and collateral (Faber and Korn 1978; Eaton et al. 1995). The collateral PHP neurons form feed-forward and feed-back inhibition networks with the M-cells which ensure that only one M-cell fires a single action potential (Eaton et al. 1995; Zottoli et al. 1995). It is important to note that the PHP cells also have an axonal branch into the Mauthner soma and lateral dendrite which are sites of chemical inhibition of the Mauthner cell (Faber and Korn 1978; Eaton et al. 1995). Both types of PHP cells are very sensitive to low level acoustic stimulation (and other sensory input) and will inhibit the M-cell from firing until the strength of the stimulus reaches a threshold that induces saturation of the PHP cells (Eaton et al. 1997). The M-cells increase in excitability, eventually exceeding the PHP cells inhibition which leads to firing of the M-cell. The PHP cells are also thought to be important in determining that the correct M-cell fires to ensure the fish turns away from the stimulus (Eaton et al. 1997). They are also thought to be important in regulating inputs from the sensory afferents (Eaton et al. 1995; Zottoli et al. 1995).

The spiral fibers originate in the hindbrain and travel through the fasciculus longitudinalis medialis and into the axon cap (Nakajima and Kohno 1978; Zottoli et al. 1995). Here the spiral fibers axons wrap around the initial segment of the M-cell's axon where they ultimately synapse with each other, the M-cell axon and the axon hillock (Zottoli et al. 1995). Due to the proximity of the tectobulbar tract to the origin of the spiral fibers, it is assumed that spiral fibers receive and transmit visual input to the Mauthner system (Zottoli et al. 1995).

Eaton et al. (1995) proposed the logical operator XNOR model (EXCLUSIVE-NOR), which demonstrates a possible method for sound localisation of the Mauthner system using the phase model described above.

With acoustic stimuli, the pressure component seems to be important in triggering the fast startle response, at least in ostariophysan fish (Blaxter and Hoss 1981; Eaton and Emberley 1991; Eaton et al. 1997). However, recent evidence suggests that high levels of particle acceleration may be important in the directional activation of the Mauthner system (Casagrand et al. 1999).

Fatigue of the Mauthner system has been observed (Diamond 1971; Kawaski et al. 1996; Oda et al. 1996; Matsui et al. 1997). Evidence also suggests that the latency time of the startle response is effected by temperature and hypoxia (Batty et al. 1993; Domenici and Blake 1997; Temple and Johnston 1997).

2.4.3.1.2 Function of the startle response

The startle response of teleost fish has evolved as an effective mechanism used for evading predators (Godin 1997). A predator approaching a fish will cause particle motion and pressure, which will be detected by the M-cells as, explained above. The M-cell on the same side to the stimulus will fire which induces musculature contractions on the side of the fish away from the stimulus. These contractions bend the fish away from the predator, and will place the fish on a trajectory away (hopefully) from the danger. Observations suggest that the latency time of the startle response in hearing specialists is lower than that of hearing generalists (Zottoli et al. 1995).

Recent evidence has suggested that the trajectory escape path is to some degree under the control of the fish (Godin 1997). It is accepted that the trajectory angle of the escape path is directly related to the direction of the stimulus and, according to some evidence, body size (Domenici and Blake 1993). However, factors such as obstructions, social environment and prior experience with a particular predator can also affect the escape trajectory angle (Godin 1997).

M-cells may also be actively involved in other processes, including hatching, tail flips and other fast response behaviours (Eaton and Bombardieri 1978; Zottoli et al. 1995; Meyers et al. 1998).

2.4.3.2 Interference with acoustic communication

Many fish and some invertebrates are known to produce sounds in various ways. For example, many species of trevally make a grunting noise by grinding gill plates together, while species of gadoids use their swim bladder to amplify muscular drumming (Tavolga 1971; Hendrickson 1977). Some species of decapod crustaceans make loud chirping noises by rubbing their legs against their body (Demski et al. 1973; Bone et al. 1995a). These noises are commonly used in social interactions between individuals of the same species and, less commonly, between different species (Bone et al. 1995a). Even species that are not known to produce noise are thought to use acoustic signals from other species to determine their surroundings (Popper and Fay 1993). Interference with these signals could alter the behaviour of both the animals generating the signal and the intended (and unintended) receivers.

For example, twenty four families of fish are known to transmit acoustic signals when captured or disturbed (distress signals) (Myrberg 1981). The potential function of these signals could be; a warning signal to other prey, a call for help (e.g. mobbing in damsel fish), to attract other predators that may disturb the attacker or to deter the predator long enough to elicit an escape response (Godin 1997; Smith 1997). Acoustic signals are also common cues involved in the courtship behaviour of many fish (for example, batracloid toadfish, *Argyrosomus hololepidotus*) (Hawkins 1986). While the direct effect on these species may be minimal, the potential to affect the future of the species is a possibility.

Interference of these acoustic signals by seismic survey noise is a real possibility as the majority of these signals are of low frequency (5-1000 Hz) and impulsive, similar to an air-gun signal. This similarity could lead to ‘masking’ of the communicative signal (Myrberg 1980; Coombs and Fay 1989).

2.5 Environmental implications of seismic surveys

The impact that a seismic survey has on the fish and invertebrates in an area would depend on many factors. The characteristics of a particular survey, the species that are present in the area to be surveyed and the particular life cycle stage of these

species at the time of exposure are especially pertinent (Deffenbaugh 2002). The implications of any pathological damage caused by seismic survey noise will depend on the extent and position of the damage.

Due to the low attenuation of sound travelling in water, the effects of the noise from a seismic survey could be far reaching. Although most of the sound produced from an air-gun array is directed downwards a considerable amount of energy is transmitted laterally (Greene 1985). Highest levels of noise are found near to the source, however in conditions of good sound propagation the noise from a seismic survey may exceed background noise for hundreds of kilometres from the source (Greene and Richardson 1988; McCauley 1994). While seismic signals are short (tens of ms) near to the air-gun, at much longer ranges, multiple paths cause the signal to stretch over several seconds, where they sound like distant thunder (McCauley 2003). Since they are repeated at 4 – 15 second intervals these signals can cause elevated background noise levels, potentially over large spatial scales.

Most adult fish have the swimming capacity to avoid, at least, the highest intensity signals from a seismic survey. However, larvae, eggs and sessile animals may be unable to escape and are therefore, at a potentially high risk from air-gun signals. Fish in the reproductive stage of their lifecycle tend to be more vulnerable to stress and cortisol is known to have a detrimental effect on reproduction (Billard et al. 1981; Carragher et al. 1989). Many species of fish form aggregations during spawning (e.g. *Hoplostethus atlanticus*, *Pagrus auratus*) (Turner and Newton 1992; Fisheries 2000). If seismic surveys are able to induce a stress response in these animals and / or alter their behaviour then a survey passing through such a breeding ground could have a detrimental effect on fish populations.

Although most of the literature reports that air-gun noise from seismic surveys is not lethal for fish and invertebrates, little information exists on the effects that the reported sublethal changes have on natural populations. For example, damage reported in the literature to blood vessels and sensory organs (e.g. stunning, shift in hearing threshold) may not be lethal under experimental conditions but in the natural

environment these could reduce the affected animals fitness and therefore its chance of survival. No information exists on the long-term effects of seismic surveys on fish and invertebrate populations.

It is not only the animals directly affected by the seismic survey that is, animals that can 'hear' the noise, that will be effected (McCauley 1994). A change in abundance and/or behaviours of the directly affected animals has the potential to affect the whole ecosystem of which it is a part. For example, the predator prey relationships that exist could be compromised if prey is affected by the noise. Even a change in behaviour during a seismic survey as minor as reported by Pearson et al. (1992) that is, tighter swimming schools and changes in swimming depths (Chapman and Hawkins 1969) could have consequences further down the food chain.

2.5.1 Zones of effect

Several authors have used 'zones of effects' to describe the distance from a noise source that a particular response to the noise will occur (McCauley 1994). The zone of effect is defined as the area radiating from a point noise source that will induce a particular response in the experimental marine animal. An important consideration is that for a moving noise source, as is the case with a seismic survey, the total area over which a particular response will be induced would be higher than that for a stationary noise source.

The responses that the zones of effect are classified by are; audibility, masking, behavioural, avoidance, pathological and lethal (McCauley 1994; Erbe and Farmer 2000). A brief definition of each zone and factors that affect the size of the zone are outlined below (for more detail refer to Malme et al.(1989), McCauley (1994) and Richardson et al. (1995)).

2.5.2 Zone of audibility

The zone of audibility is the distance from the noise source that the sound can be perceived by the animal (McCauley 1994). The variation in 'hearing' capabilities of marine animals leads to significant variation in the size of this zone, depending on the species of animals present. The characteristics of the air-gun array, the local

propagation environment and the ambient noise will also play a major role in determining the zone of audibility. It is also important to be aware that there may be seasonal variations in the marine environment, for example salinity and temperature gradients, which will affect the horizontal transmission of sound under water (Urick 1983c). The level of ambient noise, will also affect the distance at which the noise from a seismic survey is perceived (Myrberg 1980; McCauley 1994). Ambient noise, both biological and non biological can mask the noise from a seismic survey, particularly at distances from the array. Ambient noise, predominantly biological noise, is also variable subject to time of day and seasonality (McCauley 1992).

2.5.3 Zone of masking

The zone of masking is defined as the distance from a noise source that results in partial or complete masking of communication and/or acoustic signals of the surrounding marine animals. Factors that affect the distance from a noise source at which masking of sound occurs are similar to the factors that determine the zone of audibility.

The masking of acoustic signals from seismic survey noise would be limited due to the short and intermittent signal emitted. There is evidence to suggest that fish are able to change the characteristics (frequency, intensity and cycle) of their acoustic communicative signals so as to eradicate the similarities of interfering noise (McCauley 1992). Also, animals capable of distinguishing the directional characteristics of an acoustic signal could limit the effect of masking from seismic survey noise. A temporary increase in the hearing threshold of animals close to the array could possibly cause masking of some signals (McCauley 1994).

2.5.4 Zone of behavioural response

The zone of behavioural response is defined as the distance at which a noise source is able to induce behavioural changes in a ‘significant proportion of the surrounding population’ (McCauley 1994). For a noise to induce a behavioural effect it must be audible to the animal therefore, the factors that control the zone of behavioural response are similar to the factors that influence the audible zone. Also, as observed behavioural response to acoustic disturbance is generally directly proportional to the

noise intensity, the propagation environment is of particular importance (Pearson et al. 1992; McCauley 1994). Factors such as stage of lifecycle, sex, habitat (Wardle et al. 2001) and previous exposure to noise, that is, habituation (Chapman and Hawkins 1969; Pearson et al. 1992) can affect an animals behavioural response to noise .

This zones boundary is wide ranging and the behavioural changes observed can be quite subtle. The transition between the zone of behavioural response and the zone of avoidance can be extremely abrupt with animals suddenly fleeing from the noise or gradual, with animals slowly moving away from the sound (McCauley 1994).

2.5.5 Zone of avoidance

The zone of avoidance is defined as the range at which most of the animals from a population show avoidance behaviour from a noise source (McCauley 1994). This zone is influenced by the propagation environment and the characteristics of the air-gun array. However, ambient noise does not limit this zone as noise levels needed to produce this reaction are usually well above ambient noise.

On an individual level, factors that could affect the radii at which avoidance occurs include; stage of lifecycle, specific area of exploration (e.g. spawning ground, protecting young) and territorial tendencies.

2.5.6 Zone of pathological effects

The zone of pathological effects is the range surrounding a noise source at which the air-gun noise will cause pathological damage to the animal (McCauley 1994). As with the zone of avoidance, ambient noise levels have little effect on the area of the zone of pathological effects.

In a real situation animals that are able to avoid the noise would move away from the air-gun array before this zone was reached. Sedentary animals that are unable to avoid the high levels of noise are at the most risk of pathological damage from seismic survey noise.

2.5.7 Zone of lethal effects

The zone of lethal effects is defined as the distance that an animal has to be away from a noise source to cause death. It is generally accepted that air-gun signals are not lethal to adult fish and invertebrates (Falk and Lawrence 1973; Rusby 1995). There are anecdotal reports of air-gun signals having a lethal effect on plankton and fish larvae at close range (i.e. < 5 m) (Kostyuchenko 1971; Holliday et al. 1987; McCauley 1994).

Chapter 3

General materials and methods

3.0 GENERAL MATERIALS AND METHODS

3.1 Experimental overview

A summary of the twelve experiments conducted on fish and squid is given in Table 3.1. Ten of these trials were run inside the Jervoise Bay breakwater. Trials 8 and 9 were carried out in Exmouth Gulf. Note that trials 6 and 7 were carried out on turtles and are therefore not included here.

3.2 Experimental site

Jervoise Bay lies on the coast, at the northern end of Cockburn Sound, south of Fremantle, Western Australia. The Jervoise Bay experimental site was situated inside the breakwater in an industrial shipbuilding complex (Fig. 3.1). The site was a uniform 9 m depth, with a fine muddy bottom. Water temperature during trials ranged from 16.5 – 22.9^{o} C (Appendix 1, Table 2)

Two experiments were conducted in Exmouth, which is approximately 1300 km north of Perth, Western Australia. The Exmouth experimental site was situated in Exmouth Gulf, approximately 300 m offshore from Town Beach. The depth of the site varied (7-10 m) due to the large tidal fluctuations. The sediment of the Exmouth site was fine sand and water temperature during trials ranged between 23.9 – 24.5^{o} C (Appendix 1, Table 2).

3.3 Experimental set-up and exposure regime

3.3.1 Jervoise Bay trials

The experimental set-up at the Jervoise Bay site consisted of one large cage (used for behavioural observations) and five smaller cages (used for physiological measurements). The large cage was located 30 m off a breakwater wall, and was fixed by four chain-rope-chain moorings, one from each corner (Plate 3.1). Railway wheels of 280 kg were used as in-water mooring points. The five smaller cages were suspended from a rope that was fastened to, and extended northwards from, the large cage. The rope and the cages were kept afloat with the use of several buoys.

Table 3.1: Fish and invertebrate experiments carried out, with details of date, species, exposure regimes, sampling undertaken and air-gun regime animals were exposed to on each trial. See Appendix 1 for scientific name, water quality data and time of day for each trial. Fixed exposures (fix) involved a 10 dB signal range with the air-gun moved from 10-30 m off the sea-cage. Approach - depart exposures (a-d) typically began at a distance of 350 – 450 m from the cage (800 m in trial 14) with a 5 - 15 m closest approach achieving a 35-45 dB signal range at the sea cage (70 dB in trial 14). Note that the exposure length for each continual set of air-gun shots is given in hh:mm:ss. The number of approach - departures in each period of air-gun exposure is also given. Note that trials 6-7 were conducted on turtles and are not reported here.

TRIAL	DATE	LOCATION	SPECIES	EXPOSURE TYPE	RESPONSE STUDIED
1	17/02/97	Jervoise Bay	silver bream	fix, exp=1:00:05	no response studied [1]
2	04/03/97	Jervoise Bay	silver bream, striped trumpeter	fix, exp=0:59:59	physiology, behaviour
3	09/04/97	Jervoise Bay	s.bream, pink snapper, striped trumpeter	fix, exp=1:00:33	physiology, behaviour
4	29/05/97	Jervoise Bay	s.bream, mullet, herring	fix, exp=1:00:08	physiology, behaviour
5	04/07/97	Jervoise Bay	s.bream, mullet, squid, cuttlefish	fix, 2 sep by 1:26:25, exp=0:58:56 & 1:01:36	physiology, morphology, behaviour
8	22/10/97	Exmouth	cod, trumpeter, butterfish, wrasse [2]	3 x a-d, exp=1:01:55	behaviour
9	24/10/97	Exmouth	cod, wrasse[2], trumpeter, butterfish, blue spotted emperor, stripy sea perch	2 x a-d, exp=0:34:04	morphology, behaviour
10	17/04/98	Jervoise Bay	squid	3 & 2 x a-d sep by 1:11:17, exp=0:46:47 & 0:22:04	behaviour
11	21/04/98	Jervoise Bay	squid	3 & 3 x a-d sep by 1:12:04, exp=0:46:37 & 0:39:12	morphology, behaviour
12	15/06/98	Jervoise Bay	trevally, jewfish, break sea cod, wrasse[3]	2 & 2 x a-d sep by 1:24:16, exp=0:55:57 & 0:41:57	behaviour
13	19/09/98	Jervoise Bay	pink snapper	2 x a-d sep by 1:12:12, exp=1:05:05 & 0:36:21	physiology, morphology, behaviour
14	16/11/98	Jervoise Bay	pink snapper	4 x a-d sep by 0:15:41 & 1:12:57 & 0:03:51, exp=0:23:12 & 0:28:50 0:26:30 & 0:09:20	morphology, behaviour

Superscripts are:-[1] Trial 1 was a pilot run to test equipment and air-gun configuration, [2] silver streaked wrasse, [3] western king wrasse

Plate 3.1: Jervoise Bay experimental site. The pontoon and large cage can be seen in this picture. The smaller cages used to house fish for physiological measurements were suspended from a rope tied to the top right corner of the large cage.

In trials 1-5 (Table 3.1) the air-gun was operated off a 6 x 2 m pontoon which was fixed on its moorings but ranged between 10-30 m in distance from the sea cages. The gun pressure was dropped or raised accordingly, with the intention of achieving the widest possible range of gun pressure received at the cage (10 dB range; Fig. 4.6, Chapter 4). During these experiments all cage monitoring equipment was cabled back to the air-gun pontoon.

To enable a greater signal range and a more realistic approach-depart scenario than could be achieved using the air-gun suspended from the pontoon anchored 10-30 m off the sea-cage, the air-gun pontoon was towed towards and away from the sea cage using a 4.3 m dinghy fastened to the pontoon's port quarter. This approach was adopted for trials 8-14 (Table 3.1). A start range of 350-450 m and a closest approach of 5-15 m gave a signal range of 35-45 dB at the sea cage (Fig. 4.7, Chapter 4). In trial 14 a start distance of 800 m was used in one approach, which resulted in a 70 dB air-gun signal intensity range. For the approach-depart experimental regime the cage monitoring equipment was all cabled back to the breakwater, with the air-gun

pontoon and monitoring site in radio contact. For approach-depart paths and experimental site lay out refer to Plate and Figure 3.1.

In trials 10-14 the approach-departure exposures were separated by periods with the air-gun switched off (Table 3.1).

3.3.2 Exmouth trials

The experimental site at Exmouth consisted of one large cage (Plate 3.2). For the Exmouth trials the approach-departure scenario was utilised. The air-gun was towed towards the cage using an 8.5 m boat (*Flying Fish*). The equipment required to run the air-gun was carried on board the boat.

During these experiments all cage monitoring equipment cabled back to an anchored 12 m catamaran (*Whale Song*).

Plate 3.2: Experimental site at Exmouth. Pictured is the cage used for behavioural observations and dinghy used to service the cage. The buoys inside the cage were used to support the cameras.

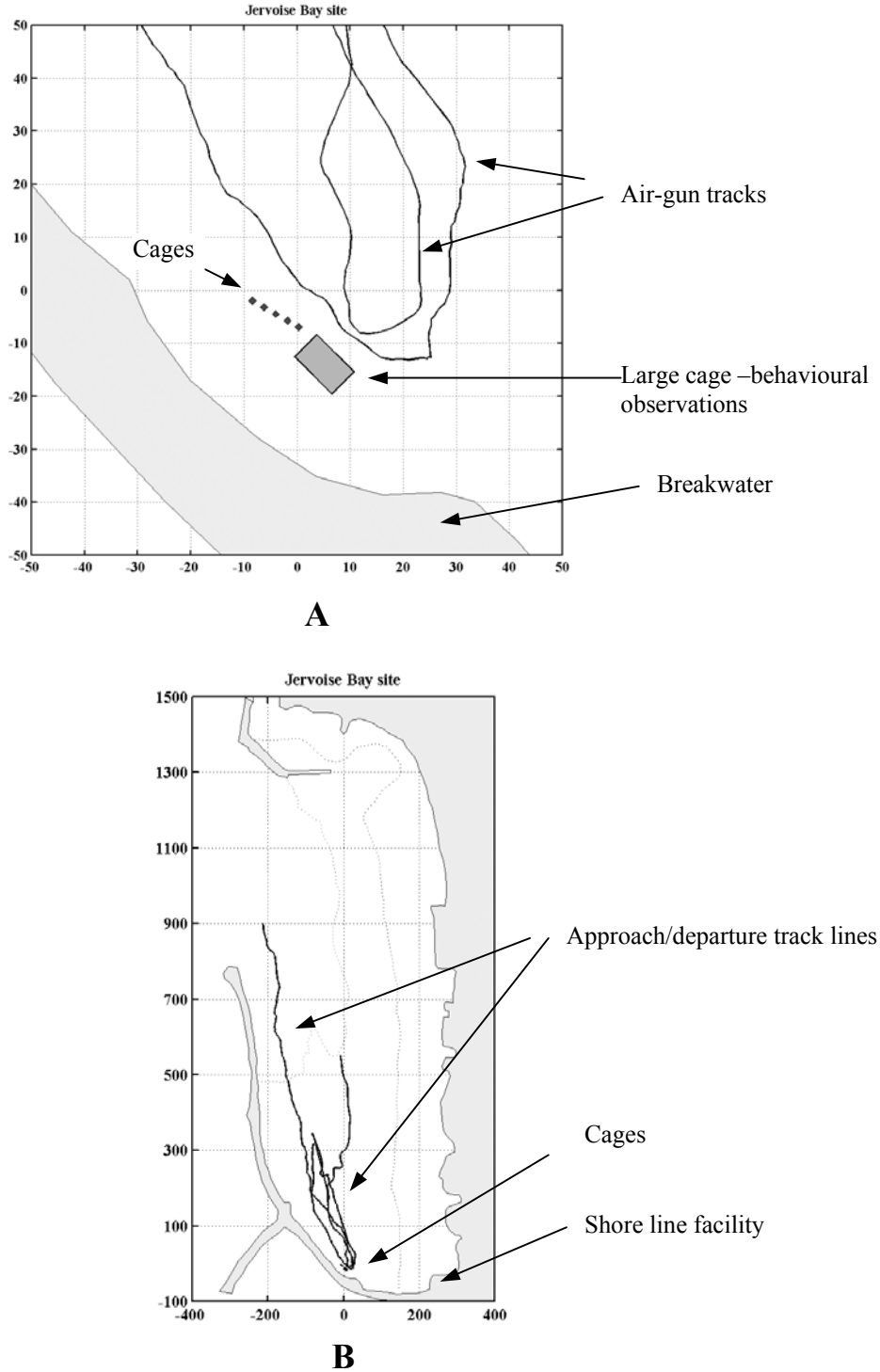

Figure 3.1: Jervoise Bay facility. A) Layout of cages during experiments. The position of the large cage and the five smaller cages used for the physiological measurements can be seen in relation to two approach tracks taken during trials. B) Entire Jervoise Bay facility. Two experimental track lines are shown.

3.4 Experimental animals

Animals were captured using a variety of techniques, involving: baited hooks, squid jigs, trawling, beach seining, gill nets or purchased from commercial aquaculture farms. All wild caught animals were captured locally to where the respective trials were to be conducted. The silver bream and pink snapper used in trials were bought from an aquaculture enterprise. Animal transportation involved an aeration system using a SCUBA feed from compressed air bottles, large plastic bins or tubs and a water pumping system.

All experiments involved accumulating animals over time and acclimatising them to the cage, the presence of divers in the cage and dinghy work around the cage. For Jervoise Bay trials this involved a two to four week period before trials. During the Exmouth trials (8 and 9; Table 3.1) the field work time schedule did not allow this and animals were in the cage for only a few days prior to trials. For all trials animals were fed pilchards or bait fish daily or every second day and the cage was cleaned and checked by divers at least every four days. During the Jervoise Bay trials it was observed that animals learnt to correlate the dinghy arrival with being fed, so that they would come to the surface when the dinghy arrived. This learned behaviour was particularly strong in the squid.

Experimental species, statistics and history are summarised in Table 3.2.

Table 3.2: Numbers, size (mean standard length ± standard error, where available), acclimation history and source of animals held in sea cage for the 12 behavioural trials (fish held in smaller cages for physiological and pathological measures not included). See Appendix 1 for scientific names.

Trial	Species	No of Fish	Standard length (mm)	Acclimation Period in Large Cage (days)	Source
1	silver bream	13	120-150	3	aquaculture
2	silver bream	12	120-150	7-17	aquaculture
	striped trumpeter [1]	≈50	50-55	wild fish	wild
3	silver bream	20	152 ± 4	13	aquaculture
	striped trumpeter [2]	≈ 50	50-55	wild fish	wild
	pink snapper	9	149 ± 8	13	aquaculture
4	silver bream	30	167 ± 10	20	aquaculture
	mullet	24	212 ± 33	7-13	purse seine
	herring	5	147-187	7-13	purse seine
5	silver bream	9	159 ± 6	5	aquaculture
	mullet	10	241 ± 23	5-10	purse seine
	herring	23	186 ± 10	5-10	purse seine
	squid	12		7-18	jigging
	cuttlefish	2		16	jigging
8	black tipped cod	3		3	hook
	Chinaman rockcod	13		3	hook
	western butterfish	20-40		3	bottom trawl
	silver streaked wrasse	15-20		3	bottom trawl
9	long finned rockcod	1		2-3	hook
	Chinaman rockcod	10	200 ± 10	2-3	hook
	blue spotted emperor	3		2-3	hook
	stripey seaperch	10	187 ± 10	2-3	hook
	western butterfish	20-40		6	trial 8
	silver streaked wrasse	15-20		6	trial 8
10	squid	19	185 ± 14	7-10	jigging
11	squid	19	185 ± 14	11-14	trial 10
12	trevally	15		14	hook
	dhufish	1		14	hook
	cod	3	200-350[3]	14	hook
	goatfish	2		14	hook
	wrasse	3		14	hook
13	Pink snapper	50	230 ± 24	24	aquaculture
14	Pink snapper	32[4]	250 ± 8	70	trial 13

Superscripts are:- 1) Striped trumpeters could enter and leave the cage of own accord as 40mm mesh size cage used during acclimation and trial 2) Striped trumpeters could enter and leave cage during acclimation but were trapped using 16 mm mesh liner before trial; 3) All fish escaped from the sea cage during recovery of the liner, therefore all sizes are estimated; 4) Pink snapper from previous trial used, believed fish were missing due to predation.

3.5 Seacages

3.5.1 Construction - Jervoise Bay cages

The Jervoise Bay sea cage was originally constructed as 15 m long, 6 m wide by 4 m deep, composed of eight individually sealed 150 mm diameter PVC pipes, with a flexible join on the long axis to cope with wave motion. Until November 1997 the cage was permanently lined with 40 mm mesh heavy ply trawl net. After the first trial it was realised that a liner in the cage was needed so that fish could be recovered easily after experiments. A liner of 16 mm light net was made and deployed prior to trials, lashed to the heavier net, and recovered with fish after each trial. Due to the lack of material available at the time the liner was made as 10 m long, which transpired to be a suitable length for coverage by the two underwater video systems.

In November 1998 the cage was redesigned, and cut down to 10 m (long) x 6 m (wide) x 3 m (deep) and a light steel frame added to define the underwater section of the net. A new liner of 16 mm mesh was constructed which fitted neatly into the cage. This was deployed before each trial and recovered upon completion.

To reduce predation in the cage by birds and poachers, a cover made of 40 mm trawl mesh was fitted to the cage during the acclimation period and a sign warning of chemical contamination from fish in the cage was attached. Despite these precautions, fish were regularly lost and fishing tackle was routinely recovered from the cage top, sides and mooring lines.

The cages used for physiological measurements were constructed from steel frames and covered in 16 mm mesh. All small cage dimensions were 1 m^3.

3.5.2 Construction – Exmouth cage

The net used in the Exmouth Gulf trials was constructed in a similar fashion to the Jervoise Bay sea-cage. Its dimensions were 6 m x 6 m x 3 m depth, and a 16 mm mesh liner was used. Owing to strong currents during these experiments (up to 1.5 knots) a steel frame for the underwater section of the net was constructed in

Exmouth. The liner was attached to the steel frame. This deployment used a single point mooring off one corner of the cage.

3.5.3 Maintenance

The original large cage at the Jervoise Bay site was periodically cleaned by divers with fouling being a major problem. With the second cage design the net was recovered after each trial reducing, but not eliminating, the fouling problem. In the late summer months fouling was particularly heavy at the site, and a deployment of six weeks in early 1998 (trials 10 and 11) resulted in a weight of tube worms which almost sank the 4.3 m dinghy used to recover the net.

As the Exmouth cage was only deployed for 2 weeks no maintenance was required.

3.6 Sound generation

All exposures involved the operation of a Bolt 600B air-gun with a 20 cubic inch chamber (0.33 L). The air-gun was deployed as shown in Figure 3.2.

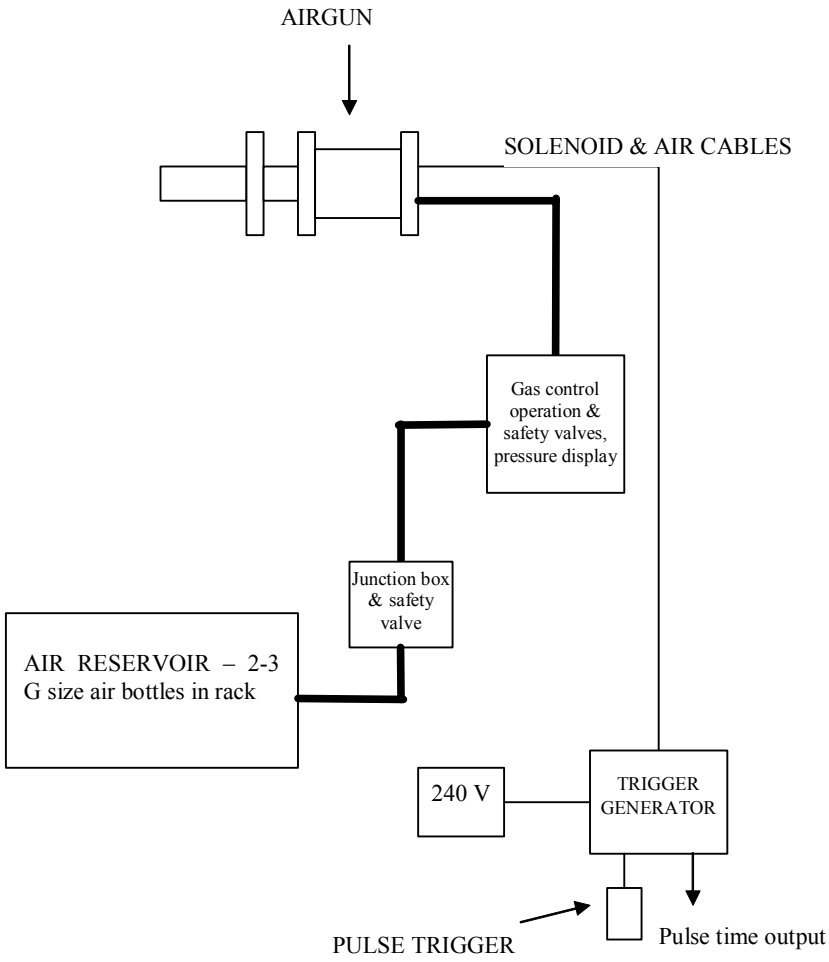

Figure 3.2: General deployment configuration of Bolt 600B air-gun used in trials. Air-gun was towed at a depth of 5 m for Jervoise Bay trials and 3.5 m for the Exmouth trials.

The air-gun was run off three G sized bottles of industrial nitrogen for all Jervoise Bay trials with the air-gun set to release a signal every 10 seconds. The three bottles maintained an operating pressure of 10 MPa for approximately 320 – 350 signals, whereafter the pressure slowly began to drop (Fig. 3.3).

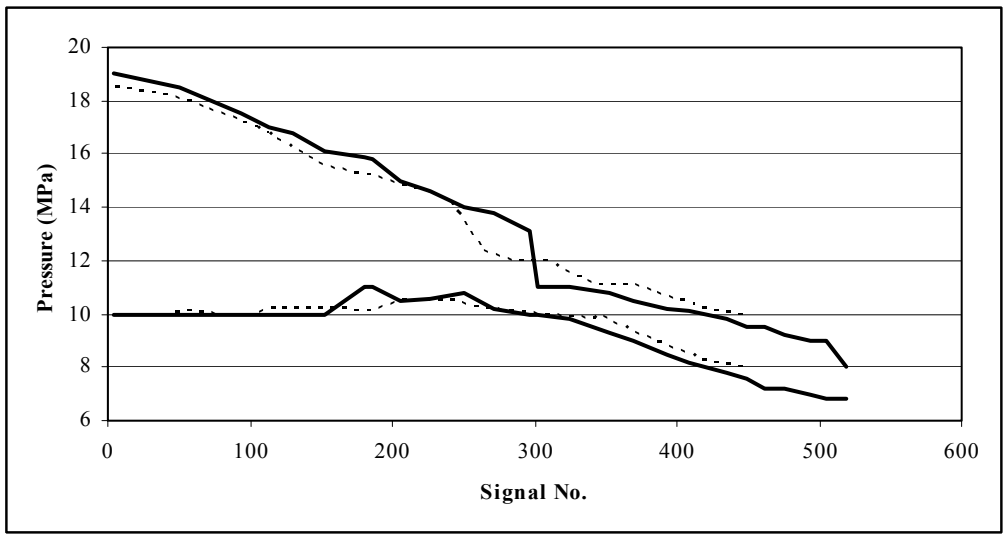

Figure 3.3: Drop in air-gun pressure for Bolt 600 B air-gun, operating with 20cui chamber, run off three G size industrial nitrogen bottles, and maintained preferentially at 10 MPa gun pressure. The upper curves represent the bottle pressure, lower curve the gun pressure (maintained at approximately 10 MPa until shot 320-350). The different line thicknesses represent different trials.

Generally, after 500 signals the gun pressure had dropped to around 7 MPa, with each 1 MPa drop in gun pressure equating to approximately a 1 dB drop in the peak-peak signal level.

For the Exmouth Gulf trials compressed air was supplied to the air-gun by a three stage, 0.19 m^3/min Bauer compressor with two G size air bottles used as a reservoir. The air-gun was set to release a signal every 10 s. Using a 10 MPa gun pressure, the compressor could maintain this rate indefinitely.

3.7 Permits

Experiments were conducted under permits: Fisheries Department of Western Australia, Scientific Authority 29 (animal collection, 1996-1998); Department of Conservation and Land Management permits SF001918 (1996-1997) and SF002294 (1997-1998); and Curtin University Animal Experimentation Ethics Committee permits N-11/96 (1996-1997) and R27-98 (1997-1998).

Chapter 4

Experimental air-gun noise exposure regimes

4.0 EXPERIMENTAL AIR-GUN NOISE EXPOSURE REGIMES

4.1 Introduction

In any study of the impact of noise on marine life an explanation of the methods used to measure the noise levels and of the notation used to express the experimental noise levels is essential, otherwise in subsequent use of the data the results may be misinterpreted.

4.1.1 Measurement of underwater sound

Sound levels can be defined in many ways including the intensity of the sound wave, the frequency and the length of the sound exposure. Acoustic intensity is defined as the average rate of flow of energy through a unit area normal to the direction of the wave propagation and should not be considered a measure of the 'loudness' of the sound (Gausland 1998). Pressure can also be used to define sound levels as sound waves are pressure fluctuations in the propagation medium. Pressure (P) and intensity (I) of a sound wave are related through Equation 4.1.

$$I = \frac{P^2}{\rho_0 c} \qquad (4.1)$$

Where ρ_0 is the specific density of the propagation medium and c is the speed of sound in that medium.

Sound waves from the signals of seismic surveys decrease in amplitude as the distance from the source increases. An approximation to loss is the spherical law, such that the energy of the signal decreases with the inverse of the distance squared. As the sound from seismic survey signals is of low frequency, attenuation is lower when compared with high frequency sound. The main factors contributing to attenuation of noise in the ocean are; geometric spreading, transmission/reflection, absorption (mainly into the seabed in the case of seismic survey noise) and scattering (Gausland 1998). Once sound has been reflected or transmitted the sound wave

characteristics will usually vary from the source signal. At distance from a seismic survey the signal is longer in duration than at the source due to the resultant signal being the summation of many multipath arrivals.

Industry measures air-gun signals as peak to peak pressure. However, there are several other methods of presenting the level of an air-gun signal. Figure 4.1 represents some common characteristics used to measure an air-gun signal. The definitions of these levels are discussed in detail in McCauley et al. (2000).

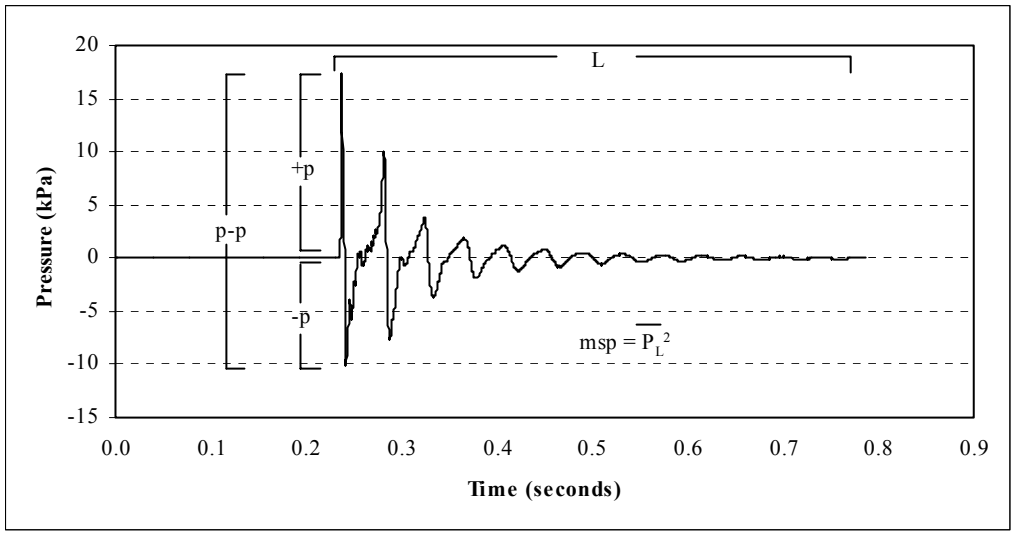

Figure 4.1: A representative air-gun signal and some characteristics that are commonly used in the literature to describe the signal. p-p = peak to peak; +p = peak maximum; -p = peak minimum; L = signal length; msp = mean squared pressure.

The logarithmic decibel (dB) scale is normally used to measure sound. The intensity levels (IL) is defined as in Equation 4.2.

$$IL = 10\log \frac{I_1}{I_0} \qquad (4.2)$$

Where I_1 is the measured intensity level and I_0 is the reference intensity level.

The sound intensity is proportional to the pressure squared so therefore the decibel expression for the sound pressure levels (SPL) is shown in Equation 4.3.

$$SPL = 10\log \frac{P_1^2}{P_0^2} = 20\log \frac{P_1}{P_0} \qquad (4.3)$$

Where P_1 = the measured pressure level and P_0 = the reference pressure level.

The decibel scale is a relative measure and therefore must be expressed with a reference level to be meaningful. In water the pressure reference level is 1 μPa (Urick 1983a).

Signal measurements are usually analysed in the spectral domain with a narrow frequency band, for example, 1/3 octave bands. It is common in the study of impulsive noise (characterised by a transient signal) to use broad band analysis to measure the signal. Broad band units are calculated as the average of the squares of the pressure values that contribute to the signal or the root mean square (rms). As intensity is proportional to pressure squared, when converted to intensity (dB re 1 μPa) the rms value is squared, hence is technically rms^2, or mean squared pressure.

It is sometimes useful to express very short impulsive noise as a measure of the total acoustic energy for the pulse. Richardson et al. (1995) suggested that this was perhaps the most meaningful method of measuring pulsed sounds. Mean squared pressure measures are dependant on the assumed start and end time of the signal and the frequency bandwidth used in the analysis therefore care must be taken to present these parameters in results.

4.1.2 Measuring an air-gun signal

There has been some conjecture about how an air-gun signal should be measured and at present no standard exists. In the literature most researchers have measured the signal in peak to peak pressure or mean squared pressure (e.g. Skalski et al. 1992; Engas et al. 1993; Wardle et al. 2001).

The actual noise levels and characteristics of an air-gun signal are dependant on the gas volume of the operating chamber, the pressure of the gas being supplied to the

air-gun, and the depth at which the air-gun is being towed. At a distance from the source the received sound will be a function of many factors including; background noise, distance from the source and characteristics of the surrounding environment.

The variation in air-gun signal characteristics and methods used to define them makes accurate definition of the signals used in these trials essential if relevant comparisons are to be accomplished.

The aim of the work conducted in this chapter was to characterise the acoustic signal to which the experimental animals were exposed from the Bolt PAR model 600B air-gun. This characterisation would enable comparison with signals from actual seismic surveys and therefore the impacts induced by the noise used in the following trials could be extrapolated to a real situation.

4.2 Materials & methods

Sound levels experienced by fish in the large sea cage were monitored using a GEC-Marconi SH101-X hydrophone situated inside the cage or just outside the centre of the cage's long axis. The hydrophone was located at 3 m depth in the cage centre (trials 2-5), cage apex (trials 8-9), or at the centre of the cages east side (trials 12–14). The vertical difference in sound pressure was also measured in trials 13 and 14 with a shallow hydrophone at 50 cm below the water surface and a deep hydrophone at the bottom of the large cage.

Signals were sent to an impedance matching pre-amplifier with zero gain and recorded on a Sony TCD D8 digital tape deck, also set with zero gain. The tape decks also logged real time, thus allowing precise correlation of air-gun signal to behavioural observations. White noise of known level was recorded to tape with the appropriate equipment settings before each trial. The white noise level was then used to calibrate equipment during analysis.

Two sets of ambient sea-noise measurements were recorded in Jervoise Bay. These recorded 60 s samples at 15 minute intervals for three day periods. Jervoise Bay is a

commercial ship building facility, thus there was some vessel traffic which may have caused elevated sea-noise levels within the Bay. These sets of sequential samples were used to describe the 'typical' and maximum noise exposures experienced by fish held in the experimental facility during acclimatisation periods.

For a full list of recording equipment specifications and details on equipment combinations see Appendix 2.

As mentioned in Chapter 2 there are several methods in which to measure an air-gun signal. For the purpose of this project the following technique was used to analyse the air-gun signals:

i) Capturing the air-gun signals at an appropriate sampling interval (always 4096 samples), usually 96 μs sample rate (10.412 kHz);
ii) Saving this block;
iii) Converting the voltage waveform to pressure units using the recording and analysis calibration values;
iv) Processing the captured sample block for the 1/3 octave levels (Appendix 3).

Then, as outlined in McCauley et al. (2001), the vector given by Equation 4.4 was calculated.

$$Csum = ti.\sum_{To}^{Te} (P_s^2 - P_n^2) \qquad (4.4)$$

Where:

- *Csum* = the vector of the cumulative squared pressure, termed equivalent energy, with the units dB re 1 $\mu Pa^2.s$;
- P_s^2 = the vector of the signal pressure squared (Pa^2);
- P_n^2 = the mean background noise squared pressure level (no air-gun signal). Noise levels were obtained by either taking the mean P^2 value for defined points within the captured sample with no air-gun signal (usually points 1 - 500) or by measuring mean P^2 values between air-gun signals;

- *ti* = the sampling time increment (1/sample rate in Hz);
- *To* = captured block start time;
- *Te* = captured block finish time;

This gave a curve (csum versus time) which began at zero with no air-gun signal input, steadily increased with time as the air-gun signal passed, then flattened out when the background noise level was reached. The maximum value of the curve was then proportional to the total energy in the signal. This maximum value was used to define 5% and 95% cumulative energy values along the curve, which were set as the signal start and end points respectively. These points defined the portion of the signal through which 90% of the signal energy passed, which described all of the dB energy of the signal.

From this analysis a number of signal parameters were obtained including:

i) Peak values (i.e. peak maximum pressure, peak minimum pressure, peak-peak pressure) (dB re1μPa);
ii) equivalent energy (dB re1μPa^2.s);
iii) signal length (seconds);
iv) mean squared pressure of air-gun pulse (dB re1μPa);
v) 90% energy passed (dB re1μPa^2.s);
vi) energy flux maximum (maximum rate change energy) (dB re1μPa^2);
vii) rise times (seconds).

To provide a comparison of a commercial air-gun array with the air-gun used in the trials described in this report an 'air-gun signal matching' program was developed (see McCauley et al. 2001 for full description). Generally, the signals from the air-gun used in this report were compared with signals recorded from a 2678 cui array recorded at two depths (20 m and 40 m). The signals were matched using a weighting system comprising a matrix of the equivalent energy, mean squared pressure, peak to peak level and signal length. The matrix was weighted towards equivalent energy.

4.3 Results

The 600 B Bolt air-gun used in the trials has a source level of 203.6 dB re 1μPa mean squared pressure (222.6 dB re 1μPa peak to peak) at 1 m. The frequency spectra of the signal had highest energy over 20 – 100 Hz, however there was a significant amount of energy over 100 – 1000 Hz. An example of a single air-gun signal as received at Jervoise Bay from the 600B Bolt air-gun at 115 m, and a comparative signal produced by a 2678 cui air-gun array at 1.5 km, is shown in Figure 4.2. The two signals are similar in waveform and frequency content.

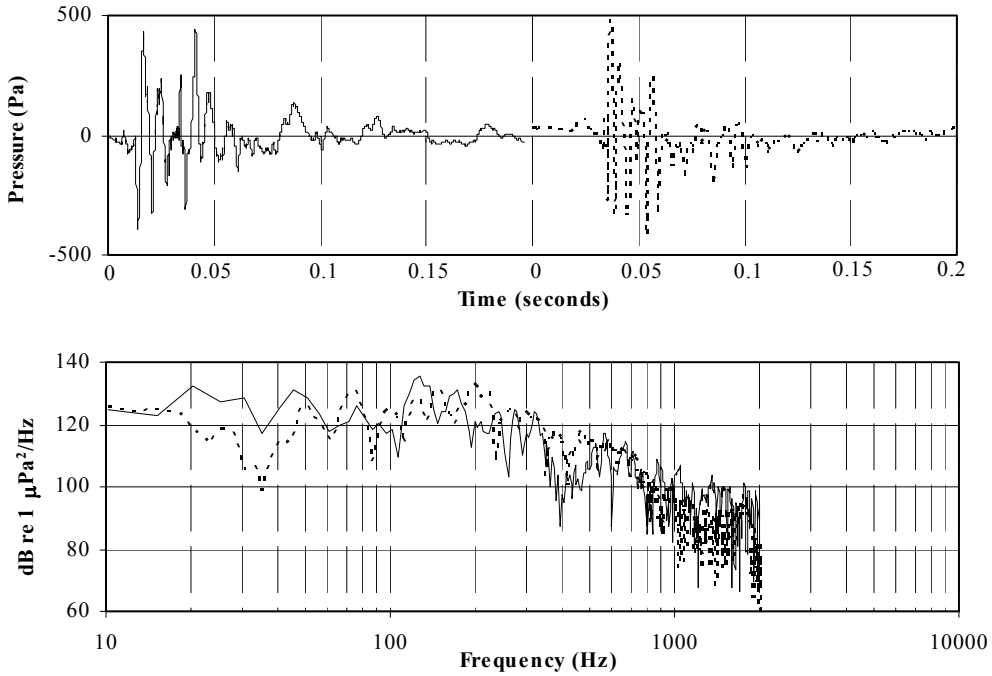

Figure 4.2: Comparative waveforms (top) and frequency spectra (bottom) of air-gun signal: as received 1.5 km from a 2678 cui air-gun array at 68° off the array bow from a hydrophone at 25 m depth (solid lines); and as recorded at the Jervoise Bay sea cage with the Bolt 600 B air-gun at 115 m range (dotted lines). The signals were matched primarily on their total energy.

The comparison between received signals from the Bolt 600B air-gun used in the trials outlined in this report and signal levels received at 20m and 40m depth for an actual seismic survey off Exmouth using a 2678 cui array are shown in Figure 4.3 (based on data of McCauley et al. 2000). From Figure 4.3 it can be seen that at 200 m

from the air-gun at Jervoise Bay the received signal is equivalent to the signal received 1.5-2 km from the 2678 cui array.

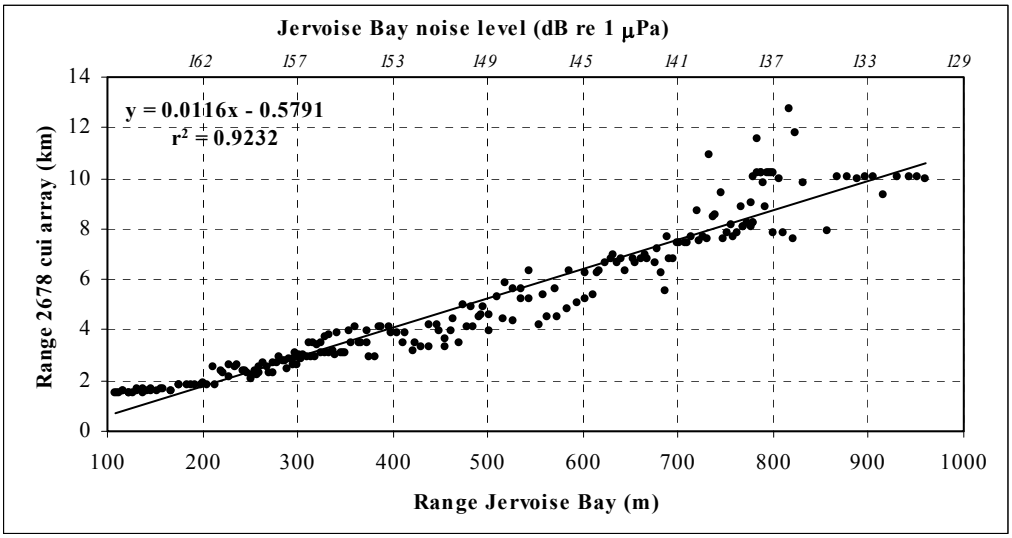

Figure 4.3: Comparative ranges for the single Bolt 600B air-gun as used in the Jervoise Bay trials with a 2678 cui array as measured at 40 m depth off Exmouth. The noise level (dB re 1μPa mean squared pressure) of the Jervoise Bay air-gun at each range step is shown in italics at the top the figure (data from McCauley et al. (2000)).

Two different types of air-gun approach scenarios were used in the trials (Chapter 3). For trials 1-5 the pontoon was fixed to the sea cage with a rope and was pulled closer over the exposure time. For trials 8-14 the approach-depart scenario was used (Chapter 3). An example of the received noise levels resulting from each technique is shown in Figure 4.4. The main difference to note is that the animals were exposed to a wider range of noise intensities using the approach-departure method.

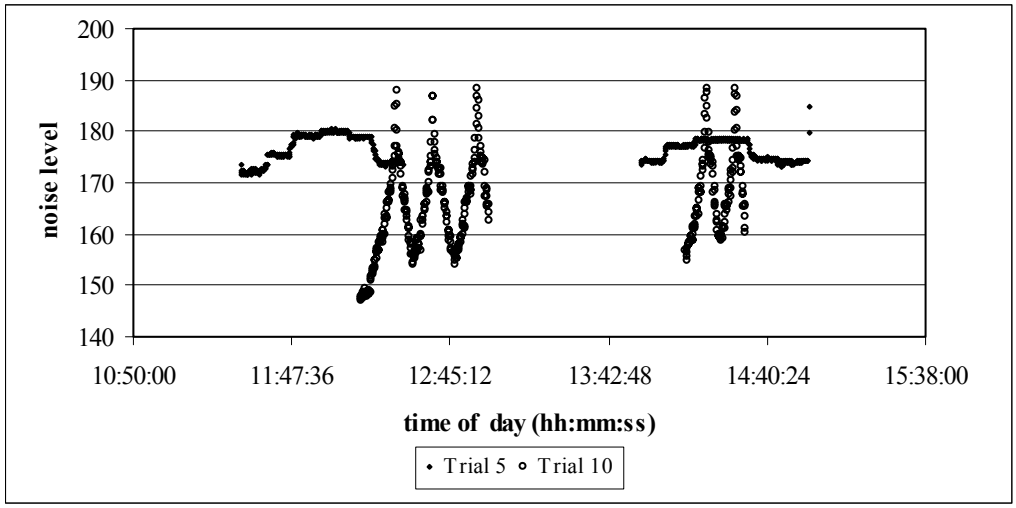

Figure 4.4: Comparison of received noise level (dB re 1 μPa mean squared pressure) for each technique (fixed and approach/depart) used in the Jervoise Bay trials. Trial 5 is an example of the fixed technique with the air-gun pontoon range from 10-30 m off the sea cage. Trial 10 is an example of the approach/depart technique where the pontoon was towed from 350 m to 5 m from the sea cage.

The vertical differences in recorded noise levels are shown in Table 4.1. The sound intensity was higher at the cage bottom than at the water surface.

Table 4.1: Difference statistics for deep hydrophone minus shallow hydrophone equivalent energy for matched shots (decibel statistics calculated by; 1. the conversion of decibels to linear intensities, 2. calculation of statistics, and then 3. the values converted back to decibels).

Source	Horizontal range (km)	Hydrophone Depth (m)		Intensity difference (dB) (deep – shallow)		Mean difference (dB)	95% dB range	No. of shots
		Shallow	Deep	Min.	Max.			
Exmouth	0.5-6.8	3	10	-3.1	8.2	3.77	3.6-3.9	324
Jervoise Bay	0.003-0.7	0.3	9.5	-11.7	23.5	14.3	13.8-14.8	328
Jervoise Bay	0.002-1.2	3	9.5	-25.2	10.9	2.34	2.0-2.6	482

Details of the air-gun noise levels (minimum, maximum, start and finish levels) as measured at the cage, for each trial are outlined in Table 4.2.

Table 4.2: Details of air-gun exposure regimes during trials. Noise levels are given in dB re 1 μPa mean squared pressure. Time given is time of day in hh:mm:ss.

Trial	Run	Start level		Minimum level		Maximum level		End level	
		dB	Time	dB	Time	dB	Time	dB	Time
1	1	183	11:10:44	177	11:14:14	185	11:12:44	179	12:10:49
2	1	171	10:24:18	170	10:30:58	176	11:06:18	174	11:24:17
3	1	170	12:15:35	167	13:05:58	181	12:47:57	168	13:16:08
4	1	175	11:52:52	170	12:33:41	180	12:24:31	173	12:53:00
5	1	174	11:29:03	171	11:29:23	180	12:02:11	174	12:27:59
	2	174	13:54:24	173	14:45:21	185	14:56:00	185	14:56:00
8	1	130	16:31:27	129	16:32:21	182	17:29:42	158	17:33:22
9	1	149	15:43:54	139	15:45:02	178	16:08:24	166	16:17:58
10	1	147	12:12:36	147	12:12:50	188	12:55:23	162	12:59:33
	2	156	14:10:50	155	14:11:29	188	14:18:28	160	14:32:54
11	1	156	11:21:32	156	11:21:50	190	11:47:10	158	12:08:09
	2	157	13:20:13	155	13:35:15	192	13:27:25	156	13:59:25
12	1	154	10:24:02	149	10:52:56	184	10:34:26	156	11:19:59
	2	138	12:44:15	136	12:45:05	182	12:57:19	152	13:26:12
13	1	144	12:45:14	144	12:46:06	191	13:49:59	178	13:50:19
	2	157	15:02:31	149	15:17:53	183	15:26:23	176	15:38:52
14	1	134	11:35:25	134	11:37:46	179	11:55:57	164	11:58:37
	2	134	12:14:18	129	12:14:58	187	12:40:47	169	12:43:08
	3	132	13:56:05	128	14:02:46	183	14:21:25	171	14:22:35
	4	151	14:26:26	151	14:26:26	180	14:34:46	172	14:35:46

Typical and maximum background noise levels that the fish were exposed to during their acclimation period are shown in Figure 4.5. The spikes on Figure 4.5 show the passage of nearby vessels. No vessels passed through the experimental site during trials.

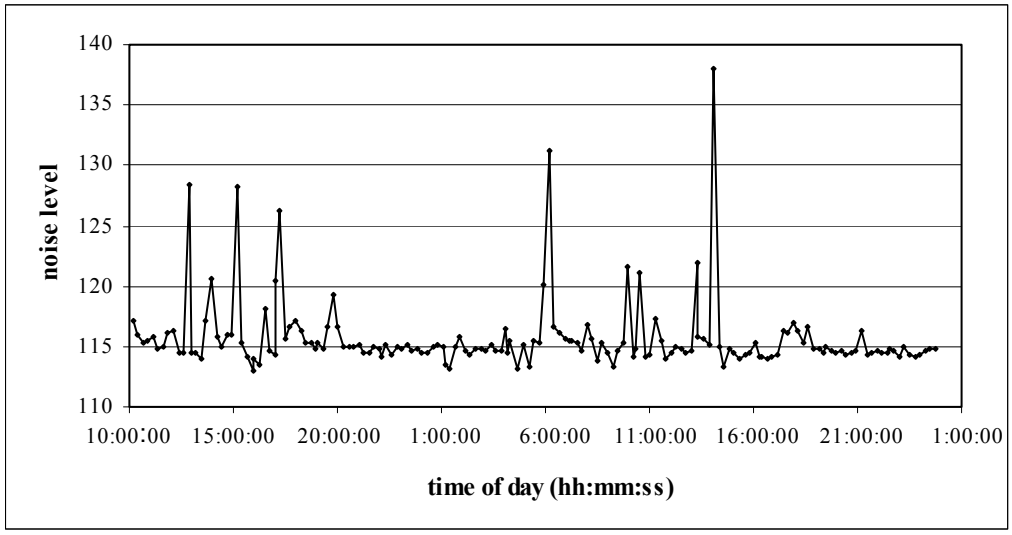

Figure 4.5: Background noise levels recorded in Jervoise Bay during acclimation periods. Noise levels are given as dB re 1µPa mean squared pressure.

The signal intensity for each trial as measured in the large cage at a depth of 3 m is shown in Figure 4.6 and 4.7. The range of noise levels achieved by using the anchored pontoon / air-gun (trials 2 – 5) is shown in Figure 4.6. The range of noise levels achieved by towing the pontoon / air-gun towards and then away from the cage with the dinghy (trials 8 -14) is shown in Figure 4.7. The range of noise levels increased with the approach departure scenario (Fig 4.7). The entire air-gun regime for each trial is displayed, therefore gaps in air-gun signal data points indicate when the air-gun was switched off but the trial was in progress.

The intensity distribution of air-gun signals for each trial, represented as histograms, is shown in Figure 4.8 and 4.9. These figures illustrate that subsequent to trial 5 whereafter the approach / departure method was utilised the fish were exposed to a wider range of noise levels. The fish in trial 14 were exposed to the widest distribution of noise levels.

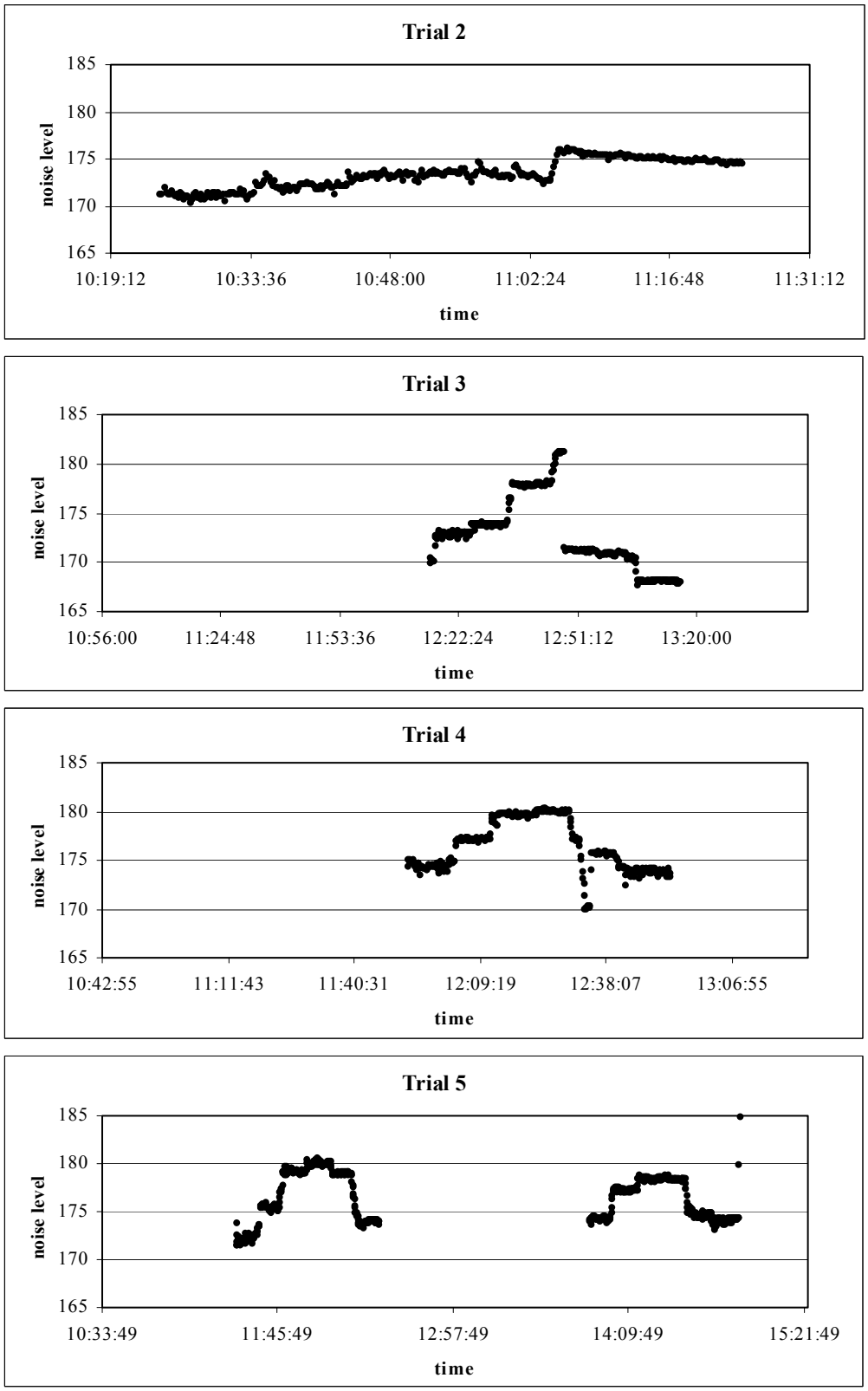

Figure 4.6: Signal intensity received at the large cage for each trial using the anchored pontoon technique (trials 2 – 5). Noise level units are dB re 1 μPa mean squared pressure. Time is time of day (hh:mm:ss).

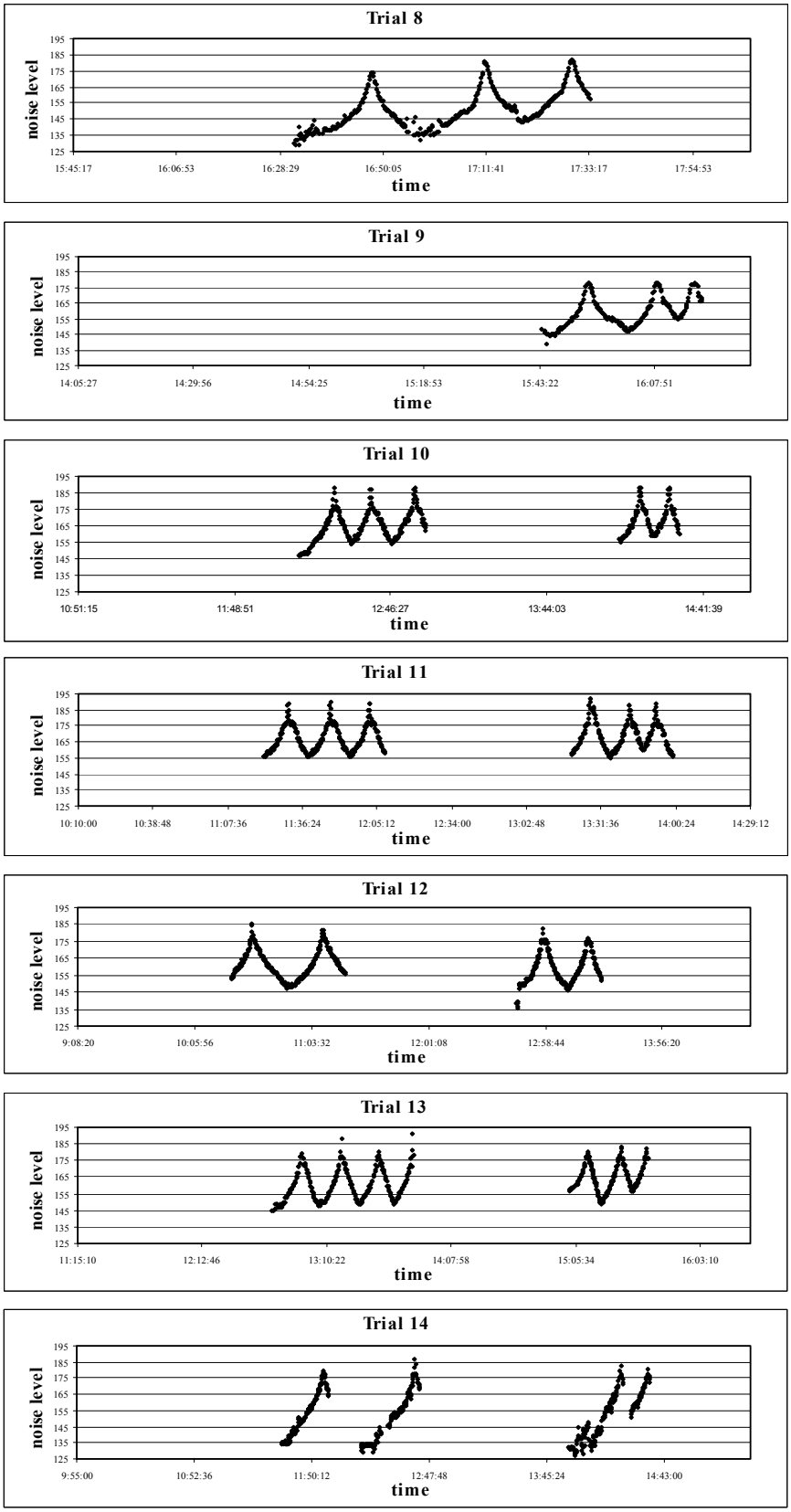

Figure 4.7: Signal intensity received at the large cage for each trial using the towed air-gun (trials 8 – 14). Noise level units are dB re 1 μPa mean squared pressure. Time is time of day (hh:mm:ss).

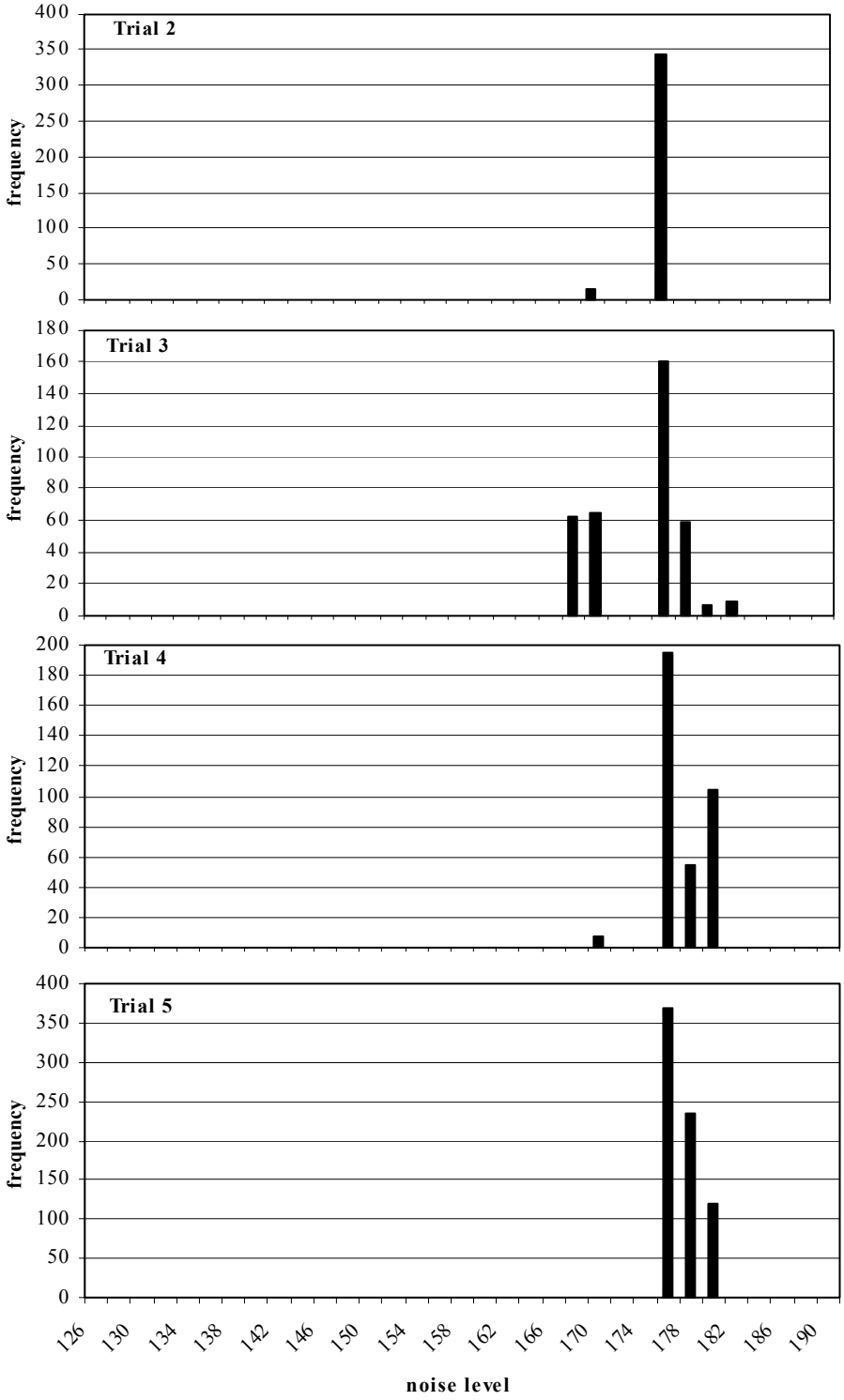

Figure 4.8: Distribution of all air-gun shots (2 dB bins) received at the large cage for each trial using the anchored pontoon technique (trials 2 – 5). Noise level units are dB re 1μPa mean squared pressure.

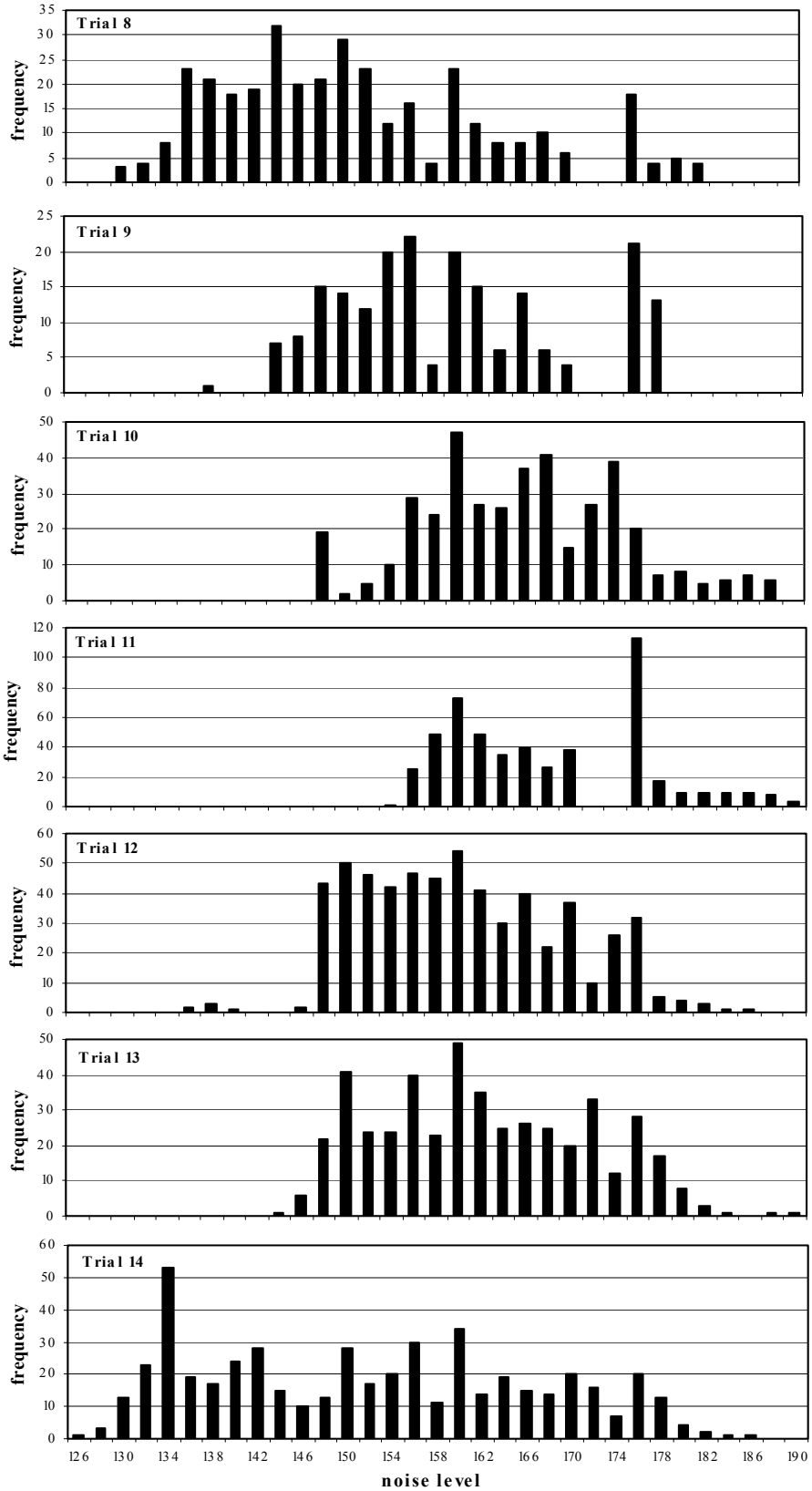

Figure 4.9: Distribution of all air-gun shots (2 dB bins) received at the large cage for each trial using the towed air-gun (trials 8 – 14). Noise level units are dB re 1µPa mean squared pressure.

4.4 Discussion

The fish and squid involved in the trials reported in this thesis were exposed to noise levels between 128 – 191 and 147 - 192 dB re 1 μPa mean squared pressure respectively. These levels were achieved in trials 8 - 14, where the air-gun was towed towards and away from the animals. In trials 2 – 5 the animals were exposed to noise levels ranging from 167 – 185 dB re 1 μPa.

Throughout this thesis received signals from the air-gun have been presented as mean squared pressure. For comparison with other research into the effects of seismic survey noise on marine fish and invertebrates, empirically derived corrections have been calculated (McCauley et al. 2000).

For the results of the trials conducted in this study to be most applicable, it was important to use an exposure regime that simulated the type of noise to which an animal in the vicinity of a real seismic survey would be exposed. The air-gun noise exposure regime in this study was designed to emulate an approaching and departing seismic vessel. The signal rate may also be important when applying results to a real situation. Therefore, as in practice, signal rates typically vary between 5 – 15 seconds, a rate of 1 signal per 10 seconds was used in this study. Other studies (Pearson et al. 1992; Wardle et al. 2001) have used stationary noise sources and unrealistic signal rates which could affect the response of the exposed animals. In trials 5 and 10 – 14 the experimental animals were exposed to two or more (trial 14) periods of air-gun noise, separated by periods with no air-gun noise exposure. This regime was used as it was thought that habituation and/or damage to acoustic receptors may occur and alter the response of the fish or squid in subsequent exposures.

The lower received signal intensities measured at the surface (Table 4.1) are consistent with findings by Greene and Richardson (1988) and can be attributed to Lloyd's 'mirror effect' as described by Urick (1983b). Briefly, sound waves are reflected from the air-water interface as mirror images of the incident sound wave. As these sound waves are exactly the same, but with opposite polarity, they cancel

each other out at the surface resulting in rapid decay of the signal. This phenomenon is especially apparent when the surface wave period/amplitude is low compared to the sound wavelength.

To accurately predict the characteristics of received air-gun signals at a distance from a source requires detailed information and becomes particularly complex with signals generated in shallow water. Information on the receiver and source depth, the water and seabed properties and the water depth along the propagation path are essential (McCauley et al. 2000). The precise nature of the received signal is more accurately obtained from actual measurements taken at the point of receival, as in this report.

It should be pointed out that a 'standard' air-gun array does not exist. Each air-gun array is designed to suit a specific purpose and exploration area, while the signal received at kilometers from the array is largely a function of the local sound propagation environment. Therefore, the signal from an air-gun array will also vary between seismic surveys. Unlike the single air-gun used in the exposure trials in this report, air-gun arrays are designed to suppress the bubble pulse in the vertical plane. However, suppression in the horizontal plane, which is critical for horizontal sound transmission, may not necessarily occur. Hence, the presence of the bubble pulse in the experiments described here may not be different to actual seismic survey sources for receivers exposed to the array horizontal elevations.

The air-gun signals used in these trials and the signals from an actual seismic survey air-gun array were deemed to be comparable. However, when extrapolating the observed impacts from exposure to the noise from the Bolt PAR model 600 B air-gun to a real situation consideration must be given to the differences listed above and the environment in which exploration is to occur.

Chapter 5

Physiological response of fish to air-gun noise

5.0 PHYSIOLOGICAL RESPONSE OF FISH TO AIR-GUN NOISE

5.1 Introduction

The deleterious effects of intense, chronic noise on humans and other terrestrial animals is well documented (Myrberg 1978; Richardson et al. 1995). The majority of studies have concentrated on the effects of noise on behaviour and the auditory system. However, research has also shown that noise affects other aspects of animal physiology, and can induce a stress response. The fish endocrine system displays many similarities to that of terrestrial vertebrates (Bonga 1997) and previous research has indicated that noise exposure does induce a stress response in fish (Sverdrup et al. 1994; Santulli et al. 1999).

The physiological response of fish to environmental stressors has been widely studied (Pickering 1981; Barton and Iwama 1991). Exposure to a stressor results in a cascade of events that can be organised into primary, secondary and tertiary effects depending on the level of organisation of the response (Mazeaud et al. 1977; Barton et al. 1986). Detection of the stressor triggers the primary response, which involves the stimulation of the hypothalamic-pituitary-interrenal axis and the sympathico-chromaffin system resulting in increased levels of circulating cortico-steroids and catcholamines (Mazeaud et al. 1977; Mazeaud and Mazeaud 1981; Reid et al. 1998). The increased level of these hormones induce numerous secondary effects that manifest as changes in a range of metabolic, hematological, hydromineral and structural changes (Barton and Iwama 1991). If the stressor is severe or prolonged and therefore, homeostasis is not achieved, these secondary responses will induce tertiary effects, which involve whole animal and population level responses (Wedemeyer and McLeay 1981; Shuter 1990). On an individual level these include decreased growth, reduced reproductive success, reduced immunocompetence and death (van Weerd 1998; Weytes et al. 1999). At the population level tertiary effects include reduced intrinsic growth rate, recruitment, compensatory reserve and altered species abundance and diversity (Wedemeyer and McLeay 1981; Shuter 1990).

Quantitative assessment of the stress response can be achieved by measuring the various physiological changes that occur (Wedemeyer and McLeay 1981; Barton and Iwama 1991). Circulating levels of the primary stress hormone cortisol are commonly used to detect a stress response in fish (Barton and Iwama 1991). An elevated level of circulating glucose is considered a secondary response to stress (Wedemeyer and McLeay 1981).

The majority of studies on the effects of offshore seismic surveys on marine animals have found that the noise produced during a seismic survey has a significant effect on the behaviour of exposed individuals or populations (Dalen and Knutsen 1987; Pearson et al. 1992; Skalski et al. 1992; Engas et al. 1993). Few have investigated the effect of air-gun noise on fish physiology.

The aim of this section of the study was to determine if air-gun noise could induce a detectable stress response in marine fish using circulating levels of cortisol and glucose as indicators.

5.2 Materials and methods

Blood samples were taken from selected fish species (control and exposed) for monitoring of their stress response to nearby air-gun operations in trials 2 – 5, and 13 (Table 3.1). These fish were housed in 1 m^3 cages kept close to the large sea cage (Fig 3.1). Stocking densities in each cage for each trial were 10 fish per m^3. The fish housed in these cages were collected, acclimated and fed as for the fish in the large sea cage described in Chapter 3.

Each cage represented a sampling time that usually varied with each trial to give the highest probability of detecting changes to cortisol levels. Typically five fish were sampled from each cage, with the blood from each fish being analysed separately and the results averaged for that sampling time. Sampling of fish from each cage was completed as quickly as possible (maximum of 5 minutes) as lifting the cage and netting the fish could have proved to be an additional stressor. A maximum of five fish could be sampled within these time constraints. The order in which the fish were

sampled was recorded and statistically analysed to evaluate the effect, if any, of sampling order on cortisol level.

For all trials blood samples were taken from control fish before air-gun noise exposure. The sampling times for the fish exposed to air-gun varied in each trial (Table 5.1). For trial 5 and 13 the time of day that the fish were sampled was also recorded. In trial 13 two cages, the controls and the fish sampled at 30 minutes after air-gun noise commenced, were sampled again at 6.3 hours and 4 hours after the first sampling respectively. It was assumed that the procedures involved with sampling the fish would induce a stress response and therefore, these cages were sampled twice to indicate the accuracy of the testing procedures. Fish for the second sampling were chosen at random from each cage therefore, as there were 10 fish in each cage, it is not known if the fish subjected to this sampling had previously had blood removed but all fish in these cages had been subjected to previous cage hauling and confrontation with dip nets.

Table 5.1: Blood sampling regime for control (time = 0) fish and fish exposed to air-gun noise. Sample times for exposed fish for trials 2, 3, 4 and 13 were taken from the final air-gun signal of each trial. Sample times for exposed fish for trial 5 were taken from maximum air-gun level.

Trial	Species	Parameter measured	Sample times
2	silver bream	glucose	0, 1.3 hours
3	silver bream,	glucose, cortisol	0, 1 - 1.5 hours; 3, 6, 9, 12 days
	pink snapper	glucose, cortisol	1 - 1.5 hours; 6, 12 days
4	silver bream	cortisol	0[1], 0.5, 24, 72 hours
5	silver bream	cortisol	0[2], 0.5, 1, 2 hours; 2, 4, 6, 8, 10 days
13	pink snapper	glucose, cortisol	0[3], 0.5[4], 1.45, 2.4, 4.5, 22.2, 24.5 hours

[1] 2 cages of unexposed fish (i.e. 10 fish) were sampled to obtain control values; [2] control cage, but different fish (as indicated by clipped caudal fin), sampled again at 10 days following air-gun noise exposure; [3] Fish in this cage were sampled again 4 hours after the initial sampling; [4] Fish in this cage were sampled again 6.3 hours after the initial sampling.

To collect blood samples, the small cages were hauled to the surface and fish were captured from the cages using dip-nets, anaesthetised using 2-phenoxyethanol, and 1 ml of blood was collected from the ventral aorta using a 1 ml syringe. With the exception of trial 2 (see section 5.3.1) samples were immediately centrifuged, separated and the plasma was then stored frozen until analysis.

Due to the nature of the experimental site it was impossible to keep a group of control (unexposed) fish once the air-gun had started. Therefore, after the initial sampling, only blood from exposed fish was analysed for effect of air-gun noise exposure, except in trial 5 where control fish were sampled 10 days after exposure.

Plasma samples were analysed for variation in cortisol levels, using radioimmunoassay kits (CORTICTK-125, P2687) supplied by Sorin Biomedica. Circulating glucose levels were also determined from plasma samples using kits supplied by Sigma Diagnostics (Glucose, procedure No. 510).

5.2.1 Data analysis

Cortisol levels for fish in trials 3, 4, 5 and 13 were calculated using methods described by the kit manufacturers.

All the data was subjected a one-way analysis of variance (ANOVA) using the statistical analysis program SPSS (release 10.0.5 for Windows) to determine if exposure to the air-gun noise resulted in any significant difference in plasma cortisol levels. A one-way ANOVA was also applied to the data to test for significant differences in cortisol levels induced by sampling order.

Prior to ANOVA, normality and homogeneity of variance of the data was assessed using Kolmogorov-Smirnov test for goodness of fit and Levene test respectively. If the assumption for homoscedastic data was not met, then an appropriate data transformation function was applied (Zar 1974). If transformation was not successful (as in trial 4 and 5) a nonparametric test (Kruskal-Wallis) using SPSS was applied to the data

Differences between means were considered significant if $p < 0.05$. Significant differences between means were detected using the Scheffè multiple comparison test.

Plasma glucose levels for fish in trials 2, 3, 13 were measured using methods outlined by the kit manufacturers. Plasma glucose levels of the silver bream in trial 2 were analysed for significant differences using a two-tailed t-test. Glucose levels for trials 3 and 13 were statistically analysed as above for cortisol levels.

Decibel statistics of air-gun levels for each trial were calculated by converting decibel values to a linear scale, calculating the mean and then converting the values back to the decibel scale.

5.3 Results

5.3.1 Trial 2

The silver bream in trial 2 were exposed to 00:59:59 hours of air-gun noise with noise levels that ranged between 170-176 dB re 1 μPa with a mean of 174 dB re 1 μPa.

At the time of conducting trial 2 the facilities for separating the plasma from the blood samples did not exist at the experimental site. Therefore, in trial 2 only, blood was permitted to clot and serum was used in the glucose analysis. As control fish were sampled before air-gun operation, these blood samples were not separated until six hours after sampling. As stated in the manufacturer's instructions approximately 5% of glucose content is lost for every hour that the serum remains with the blood clot (Meites and Bohman 1963). Therefore, the glucose levels measured six hours after sampling were corrected using this assumption (Figure 5.1, control 2).

Circulating glucose levels were significantly higher ($p < 0.05$) two hours after air-gun exposure than prior to exposure for both the measured and calculated control values (Figure 5.1). A larger variation in glucose levels between individuals in the group of fish exposed to air-gun noise was observed than in control fish (Figure 5.1).

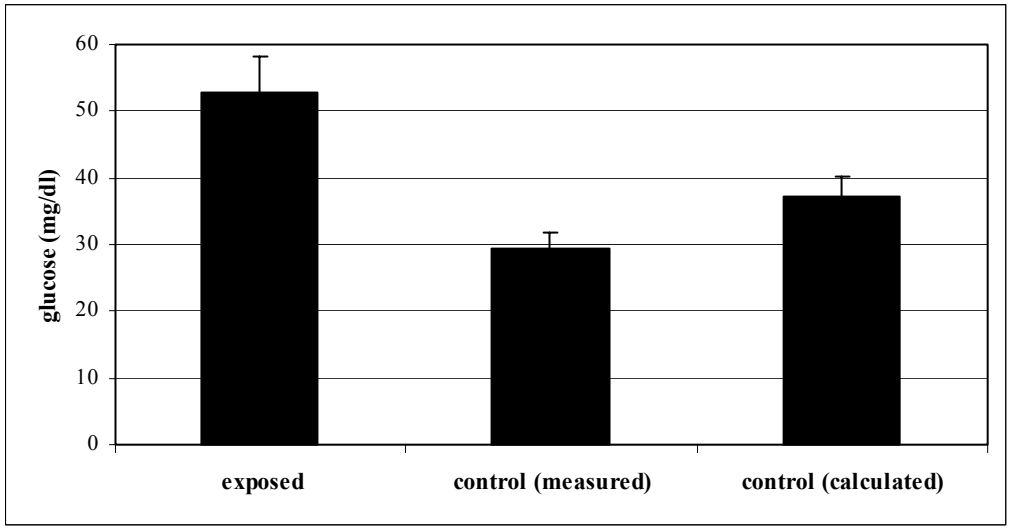

Figure 5.1: Serum glucose levels (mg / dl) of silver bream prior and 2 hours after 00:59:59 hours air-gun noise exposure (mean of five fish ± standard error). Control (measured) is the measured glucose value. Control (calculated) is the calculated value allowing for 6 hours degradation of the sample.

5.3.2 Trial 3

In trial 3 fish were exposed to air-gun noise for 1:00:33 hours with noise levels that ranged between 167-181 dB re 1 μPa. The mean noise level exposure was 174 dB re 1 μPa.

Measured cortisol and glucose levels from trial 3 for silver bream and pink snapper are shown in Table 5.2 and 5.3. Blood samples were taken from control silver bream before exposure, all species 1-1.5 hours after exposure, silver bream thereafter at 3 day intervals for 12 days, and two further samples at six and 12 days for pink snapper.

Table 5.2: Plasma cortisol levels (ng / ml) for silver bream and pink snapper exposed to 1:00:33 hours air-gun noise (mean of five fish ± standard error). Fish were sampled before and 1hour, 3 days, 6 days, 9 days and 12 days after air-gun noise exposure.

Species	Control	Exposed				
		1 hour	3 day	6 day	9 day	12 day
Silver bream	98.65 ± 0.86	91.8 ± 4.43	98.75 ± 1.0	99.48 ± 0.82	98.51 ± 0.73	96.61 ± 2.30
Pink snapper	-	3.27 ± 2.20	-	11.61 ± 6.90	-	3.05 ± 1.60

Table 5.3: Plasma glucose levels (mg / dl) for silver bream and pink snapper exposed to 1:00:33 hours air-gun noise (mean of five fish ± standard error). Fish were sampled before and 1hour, 3 days, 6 days, 9 days and 12 days after air-gun noise exposure.

Species	Control	Exposed				
		1 hour	3 day	6 day	9 day	12 day
Silver bream	58.77 ± 18.19	52.59 ± 11.05	71.08 ± 17.34	90.24 ± 11.41	62.94 ± 1.30	64.55 ± 4.95
Pink snapper	-	79.29 ± 4.20	-	88.9 ± 13.90	-	52.24 ± 4.48

No significant ($p > 0.05$) differences in plasma cortisol or glucose levels were observed in trial 3. However, the control values obtained for the silver bream for both cortisol and glucose were relatively high when compared to the results of trials 2, 4, and 5.

5.3.3 Trial 4

The fish in trial 4 were exposed to 1:00:08 hours of air-gun noise with noise levels ranging between 170 - 180 dB re 1 μPa with a mean noise level of 177 dB re 1 μPa.

No significant ($p > 0.05$) changes in the levels of circulating cortisol were observed at the tested sampling times (Table 5.4).

Table 5.4: Plasma cortisol levels (ng / ml) of silver bream exposed to 1:00:08 hours of air-gun noise (mean of five fish ± standard error). Sampling of exposed fish began 30 minutes after the final air-gun signal.

Treatment	Cortisol (ng/ml)
*Control 1	3.40 ± 2.4
*Control 2	0 ± 0
30 min	0 ± 0
24 hr	5.51 ± 3.5
72 hr	0 ± 0

*Control 1 and 2 were housed in different cages. Control 1 fish were sampled first, followed by control 2.

5.3.4 Trial 5

The silver bream in trial 5 were exposed to two periods of air-gun noise separated by 1:26:25 hours. The duration of the first period of air-gun exposure was 00:58:56 hours and exposed fish to noise levels between 171-180 dB re 1 μPa with an average noise level of 177 dB re 1 μPa. The duration of the second period of air-gun exposure was 1:01:36 hours. Noise levels of the second exposure ranged from 173-185 dB re 1 μPa with a mean noise level of 176 dB re 1 μPa.

Except for the group of fish sampled at the maximum noise level for the first period of air-gun noise (181 dB re 1 μPa at 1.1 hours after the first received air-gun signal), circulating cortisol levels remained constant for all sampling times until six days after exposure to air-gun noise (Table 5.5). The elevated plasma cortisol level at the maximum noise level was entirely due to the plasma cortisol level of the third fish sampled, out of the group of five, which had plasma cortisol levels of 762.5 ng/ml. The other four fish in the group had undetectable cortisol levels. Significantly ($p < 0.05$) elevated cortisol levels were observed ten days after air-gun noise exposure. These elevated levels were also observed in control fish which were sampled on the same day (14 - July, Table 5.5).

Table 5.5: Plasma cortisol levels (ng / ml) of silver bream exposed to 00:58:56 and 1:01:36 hours of air-gun noise, separated by 1:26:25 hours (mean of five fish ± standard error). The first exposed sample was taken when noise levels at the cage were at maximum during the first run.

Treatment	Date (day/month)	Time of day (hh:mm:ss)	Cortisol (ng/ml)
Control 1	4/7	8:00:00	2.19 ± 1.8
[1]Control 2	14/7	12:00:00	262.62 ± 35.2
Max. dB	4/7	13:50:00	152.5 ± 152.5
[2]Max. dB	4/7	13:50:00	0 ± 0
30 min	4/7	14:23:00	0 ± 0
1 hr	4/7	15:00:00	0 ± 0
2 hr	4/7	15:44:00	0 ± 0
2 day	6/7	15:00:00	0 ± 0
4 day	8/7	12:00:00	0 ± 0
6 day	10/7	8:00:00	4.82 ± 3.2
8 day	12/7	13:00:00	4.62 ± 2.5
10 day	14/7	12:00:00	287.74 ± 15.9

[1] Same cage as control 1 but different fish sampled for blood, as indicated by clipped caudal fin. Cage sampled 10 days after first sampling;
[2] Result with 'outlier' (one fish with plasma cortisol levels of 762.5 ng / ml) disregarded

There was no statistical evidence to suggest that the sampling order had a significant effect on cortisol levels ($p > 0.05$).

5.3.5 Trial 13

The noise regime for trial 13 consisted of two groups of air-gun noise exposure separated by 1:12:12 hours of no air-gun noise. The first period of air-gun noise lasted for 1:05:05 hours with noise levels between 144 – 191 dB re 1 μPa and a mean noise level of 172 dB re 1 μPa. The duration of the second period of air-gun noise

exposure was 0:36:21 with noise levels ranging between 149 – 183 dB re 1 μPa and a mean noise level of 172 dB re 1 μPa.

Except for the fish that were sampled twice no significant difference ($p > 0.05$) was detected in circulating cortisol levels at sampling points prior and after air-gun noise exposure (Fig. 5.2). Two cages of fish were sampled twice. In both cases on the second sampling mean circulating cortisol levels were elevated with a large variation observed between individuals (Fig. 5.2). The second sampling of the cage originally sampled at 30 minutes resulted in a significantly higher ($p < 0.05$) plasma cortisol level. Sampling order did not significantly ($p > 0.05$) effect plasma cortisol levels.

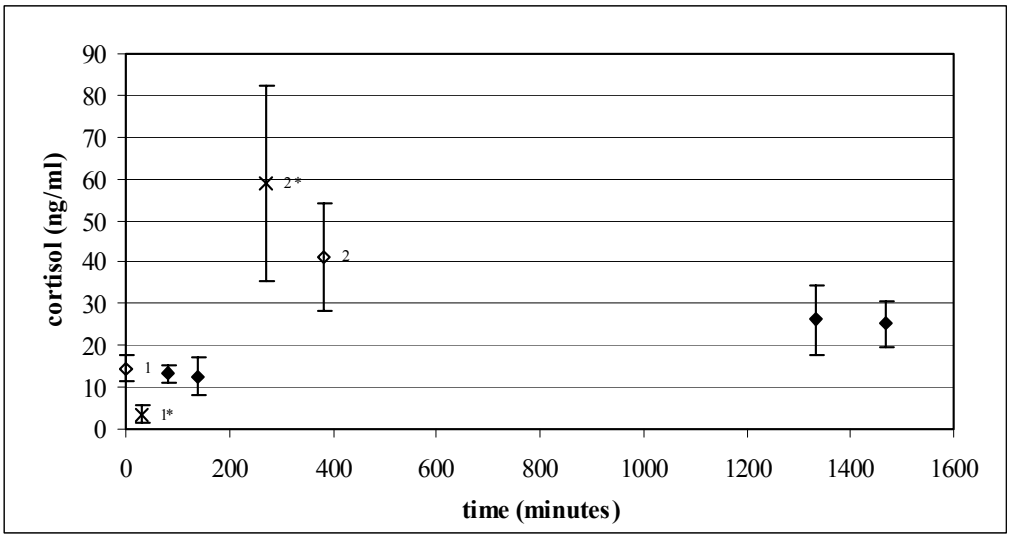

Figure 5.2: Plasma cortisol levels (ng / l) of fish exposed to 1:05:05 and 0:36:21 hours of air-gun noise, separated by 1:12:12 hours (mean of five fish ± standard error). Sampling began 30 minutes after the commencement of exposure to air-gun noise. * Indicates mean values that are significantly different from each other ($p < 0.05$). Hollow symbols and crosses represent groups sampled twice. [1] is first sampling; [2] is second sampling.

Although there was a trend for plasma glucose levels to gradually increase until the 87 minute sampling point and then decrease at the 144 minute sampling point, the changes were not significantly different ($p > 0.05$) (Fig. 5.3).

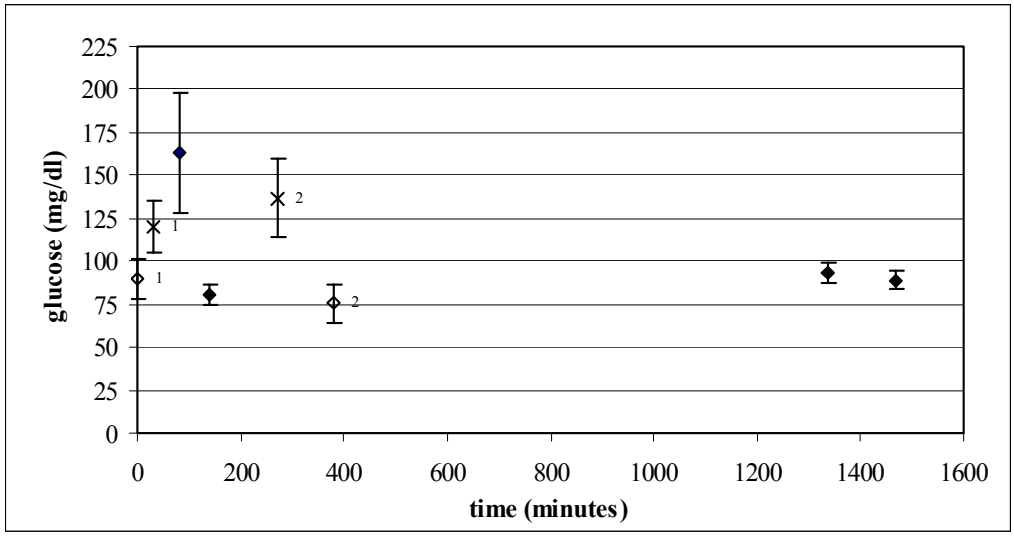

Figure 5.3: Plasma glucose levels (mg / dl) of fish exposed to 1:05:05 and 0:36:21 hours of air-gun noise, separated by 1:12:12 hours (mean of five fish ± standard error). Sampling began 30 minutes after the commencement of exposure to air-gun noise. Hollow symbols and crosses represent groups sampled twice. [1] is first sampling; [2] is second sampling.

5.4 Discussion

The results obtained suggest that exposure to the noise regimes applied to the fish in this investigation did not induce a detectable physiological stress response. Cortisol levels did not vary significantly and, although serum glucose levels of the fish exposed to air-gun noise in one trial did increase, more evidence is required to conclude that air-gun noise induced this response.

Previous studies have indicated that impulsive underwater noise does induce a physiological stress response in fish. Noise sources have included sub sea detonations of explosives (Sverdrup et al. 1994) and air-gun signals (Santulli et al. 1999). Experiments exposing tank held Atlantic salmon (*Salmo salar*) to underwater detonations of approximately 2 MPa in pressure amplitude resulted in delayed increases of plasma cortisol (Sverdrup et al. 1994). The explosions caused damage to the vascular endothelium of the ventral aorta and the coeliac mesenteric artery, which was presumed to cause the delay in observed cortisol levels. However, considering that the control ('pre stress') values were taken only 2 hours following capture and tank transfer it is possible that the initial decrease observed in cortisol values after

noise exposure was due to a declining stress response following the transfer procedures which elevated stress levels (Strange et al. 1977; Swift 1983). Therefore, it is probable that the salmon elicited a normal biochemical stress reaction in response to the acoustic stressor.

Santulli et al. (1999) exposed caged European sea bass (*Dicentrarchus labrax*) to the noise from an array consisting of 16 air-guns, each having a volume of 2500 cui. The cages of fish were situated at varying distances (180 m, 2400 m, 3700 m, and 6500 m) from the seismic survey activity. The actual noise received at each of the cages, and therefore received by the fish, was not documented. Elevated stress levels, indicated by cortisol, glucose, adenylate and lactate, were evident in fish up to 2000 m in distance from the experimental survey transept.

It is generally accepted that measuring circulating levels of cortisol is one of the most reliable methods for detecting a stress response in fish (Barton and Iwama 1991). Therefore, if a stress response was apparent in this study then, according to previous research, elevated cortisol levels should have been detected within an hour of the onset of the stressor (Thomas and Robertson 1991; Waring et al. 1992).

In trials 3 and 4 of this study no significant changes in plasma cortisol levels were observed. In trial 5 the increase in plasma cortisol levels in one of the fish sampled at the maximum noise level during the first period of noise exposure should probably be considered an outlier and therefore ignored. However, it could be indicative of the variation in the sensitivity of the stress response between individual fish and therefore, suggests that a larger sample size should be considered for future research (Pottinger and Carrick 1999). The increase in cortisol levels in trial 5 on day 10 (Table 5.5) was likely to be a result of a pod of wild dolphins observed perusing the caged fish on the sample day immediately prior to sampling.

The fish in trials 3 and 4 were not sampled for blood until 30 minutes after the last air-gun shot. Consequently, the fish were sampled a minimum of 1.5 hours after the first air-gun signal. Even in trial 5, where the first sampling of exposed fish was

during the first period of air-gun noise exposure, fish had already been exposed to 1.1 hours of air-gun noise. In trial 13 blood was sampled within 30 minutes of the first air-gun shot and still no elevation in cortisol levels induced by air-gun exposure were observed. Numerous authors have reported that elevated cortisol levels return to basal values within an hour following a minor acute stressor or one to which the fish have become adapted (Schreck 1981). It has also been reported that a peak in cortisol values can occur within 20 minutes of an applied stressor (Barton and Iwama 1991; Einarsdottir and Nilssen 1996). Santulli et al. (1999) reported that the cortisol and glucose values of European sea bass exposed to air-gun noise returned to basal (control) values within 72 hours after exposure, suggesting that fish recover quickly from acoustic stress. No samples were taken between the period immediately after the noise exposure and the 72 hour sampling point, so it is possible that cortisol and glucose levels did return to basal values prior to 30 minutes after air-gun exposure. Therefore, it is feasible to assume that even if a stress response was present in the current study it could have gone undetected.

The only significant increase in cortisol levels was observed in trial 13 when two groups of fish were sampled twice in the same day, with the second sampling recording a 2-fold increase in mean cortisol levels in one group and a 3-fold increase in the other group. Handling, capture and blood sampling are considered major stressors (Schwalme and MacKay 1991; Braley and Anderson 1992; Waring et al. 1992; Stone and Forteath 1994). Thus, even though the second sampling was separated from the first by a period of 6.3 hours in the first case and 4 hours in the second, the increase in cortisol levels recorded in the second sampling were presumably induced by the first sampling of each cage. The large variation observed in both cortisol and glucose levels (Fig. 5.2 and 5.3) at the second sampling of each cage is likely to be indicative of the different levels of stressor that the fish had been exposed to previously. All of the fish in the second sampling had been exposed to their cage being lifted and held close to the surface, and to being chased with a dip net, while it is likely that some fish had been subjected to the additional stressor of blood sampling. Alternatively, the large variation in plasma cortisol and glucose levels observed between individual fish could be a result of the differing tolerance

levels of individuals to stressors (Mazeaud et al. 1977; Fevolden et al. 1991). These results from the second sampling, and the elevated cortisol levels from day 10 of trial 5, indicate that the lack of a detectable stress response induced by air-gun noise exposure was not due to a physiological defect in the experimental fish or flawed methodology.

It was interesting to note that, although no significant increase in cortisol levels were detected, in trial 3 the control levels of cortisol were relatively high when compared with the cortisol values obtained from the other trials. Other researchers have reported resting (basal) values of circulating cortisol levels similar to the results obtained in this study for trials 4, 5 and 13 (Pankhurst and Sharples 1992; Bollard et al. 1993; Sumpter 1997; Grutter and Pankhurst 2000; Flodmark et al. 2002). The cortisol results for trial 3 suggest that the fish in this trial were subjected to a stressor other than air-gun noise. Although this may have affected the result, if air-gun noise did act as an additional stressor then cortisol levels would still be expected to be higher than control values, as multiple stressors are known to have a cumulative effect on cortisol levels (Barton et al. 1986; Pickering 1992; Power 1997).

The results of glucose analysis were variable but mostly support the information gained by the cortisol results. It is the increase in primary stress hormones, catecholamines and cortisol, acting on the liver of the fish that trigger gluconeogenesis (synthesis of a carbohydrate from a non-carbohydrate source) and glycogenlysis (conversion of glycogen to glucose) that results in an increase in the amount of glucose released into circulation (Randall and Perry 1992). Therefore, it would be expected to observe an increase in plasma glucose levels if a stress response was induced by the air-gun noise. However, in trials 3 and 13 no significant difference was detected in glucose levels.

In trial 2 there was a significant increase in the serum glucose level in fish that were exposed to the air-gun noise. However, the implications of this observation are uncertain. In a properly controlled experimental environment, plasma glucose levels can be a powerful indicator of a stress response. However, in the situation where sea

cages are being used in the open ocean, as in this case, other factors, for example uncontrolled food source, can affect glucose levels. Without cortisol levels or another parameter to reinforce the results obtained, it is not certain if the increase in glucose levels was induced by air-gun noise. In stress response studies more than one stress indicator should be utilised for stronger results (Adams 1990; Mommsen et al. 1999).

It is important to note that the fish used in this study to measure the stress response induced by air-gun noise exposure were hatchery reared. Experimental evidence suggests that physiological responses of fish to a stressor will vary, depending on whether the fish was hatchery reared or wild caught (Wydoski et al. 1976; Woodward and Strange 1987; McDonald et al. 1998). Woodward and Strange (1987) found that hatchery reared fish have a higher threshold to stressful situations than wild fish. This is likely to be due to the fact that the stress response is generally not beneficial in an aquaculture environment. In the natural environment it has evolved to benefit the fish especially in coping with life threatening situations, generally absent in an aquaculture environment, such as seasonal fluctuations in environmental conditions and predation (Pickering 1981; Woodward and Strange 1987). This must be taken into consideration when applying the results to wild populations.

Fish are a very diverse group of animals with vast variations in anatomy and behaviour, which will have an effect on the physiological response to a potential stressor (Barton and Iwama 1991). The majority of the physiological results for this study were taken from silver bream, a fish that in the behavioural studies (Chapter 7) was found to have a relatively mild response to air-gun noise, in particular no alarm responses were observed (Fig. 7.4). It is tempting to speculate that a fish which exhibits a more severe behavioural response to air-gun noise would also show evidence of a stress response. Factors such as age, sex, stage of lifecycle and even social status have been found to influence the intensity and duration of the stress response in fish (Wydoski et al. 1976; Schreck 1981; Fox et al. 1997; McCormick 1998).

The results from this investigation suggest that exposure to air-gun noise does not induce a physiological stress response in fish species. However, this study was conducted on only two species of hatchery reared fish from the same family (Sparidae). Further studies on other fish species from different families are required to evaluate fully the physiological response of fishes to air-gun noise.

Chapter 6

Pathological effects of air-gun noise to hearing systems

6.0 PATHOLOGICAL EFFECTS OF AIR-GUN NOISE ON HEARING SYSTEMS

6.1 Introduction

Several features of the nature of air-gun noise have biological significance relevant to potential acoustic receptor damage. Characteristics such as the pulsed nature of the noise, the relatively short signal duration, the rise time of the signal, the signal energy and the peak pressure displacements contribute to the effect of the noise on the acoustic receptors of marine animals (Blaxter et al. 1981b; Hawkins 1981; Pearson et al. 1992).

Edgar (1981) and Cox et al. (1987) exposed codfish and goldfish respectively, to intense sounds that resulted in damage to the sensory epithelium of the inner ear. Hastings et al. (1996) also observed damage to regions of the inner ear of fish exposed to high intensity, low frequency, and continuous noise. High level sounds have also been shown to cause temporary masking of sound and behavioural changes in some fish (Ha 1985; Pearson et al. 1992). However, it is difficult to relate these results to the effect that seismic survey noise has on the auditory system as experiments were conducted with continuous sounds whereas the sounds used in seismic surveys are short and repeated (McCauley 1994; Gausland 2000).

The variation in auditory systems and peripheral mechanisms between species of fish also contributes to the unpredictability of the effect that sound at a certain frequency and intensity will have on a particular species (McCauley 1994; Hastings et al. 1996). Generally, the fish ear includes three end organs, the saccule, lagena and utricle. Each contains a dense calcareous otolith. In a sound field the differential motion between the otolith and the sensory epithelium (macula) deflects the hair cells that cover the macula resulting in an electrical impulse which induces neurotransmitter release that stimulates the neurons innervating the sensory cells. The signal is then interpreted by the brain as a sound. The saccule is the primary end organ involved in hearing in most fish, although the lagena and utricle may also contribute to hearing sensitivity (Popper and Fay 1999).

The majority of previous studies have indicated that air-gun noise is not lethal for adult fish and various species of invertebrates (McCauley 1994; Rusby 1995). However, evidence exists to show that intense sound can damage fish auditory systems, hence such damage may be a likely consequence of exposure to air-gun noise (Gisiner 1998).

The aim of the research outlined in this chapter was to determine if exposure to air-gun noise could cause damage to the sensory epithelium of the saccule of fish. Observations were carried out to quantify any damage and regeneration processes present.

6.2 Materials and methods

The saccular macula of fish ears were collected for trials 5, 9, 13 and 14. For the air-gun noise exposure regime received at the cage for these trials refer to Chapter 4. Information about air-gun exposure regime not shown in Table 4.2, particularly relevant to ear damage that is, total number of air-gun signals received by the fish and number of shots exceeding 171 dB re 1 μPa is shown in Table 6.1.

Table 6.1: Number of air-gun signals received by the animals in trials 9, 13 and 14. The number of signals exceeding 171 dB re 1μPa mean squared pressure in each trial is also shown.

Trial	No. signals	No. signals > 171 dB re 1 μPa
9	202	34
13	465	104
14	505	64

The tissue from the silver bream in trial 5 was used to refine techniques. In trial 9 (Exmouth) the saccular maculae were dissected from stripey sea perch (*Lutjanus carponatus*) and cod (*Epinephilus rivaltus* and *E. fasiciatus*) before and after exposure to air-gun noise. However, tissue from this trial was not used to quantify damage. Squid statocysts were collected from trial 11 and used to observe morphology. Trials 13 and 14 were designed to observe any damage that air-gun

noise could cause to the saccular sensory epithelium and if regeneration of the epithelium occurred. Pink snapper (*Pagrus auratus*) were used in both trials.

In trial 13 fish were removed from the large sea cage prior to (control) and 18 hours after air-gun noise exposure (exposed). After the conclusion of trial 13 the remaining fish were left in the cage. These fish were used in trial 14. In trial 14 control fish were removed and sacrificed before the start of air-gun noise and then immediately, 11days and 28 days after air-gun exposure.

Fish were sacrificed with an overdose (≈ 250 ppm) of anaesthetic (2-phenoxyethanol) and decapitated. The ears were then exposed and fixed in chilled 4% gluteraldehyde solution buffered in filtered seawater. After fixation the samples were washed in the buffer solution. Excess tissue was dissected away from the sacculi and the membranous sacculi sac was opened. The remaining dissection was carried out under a dissecting microscope.

Fine forceps were used to gently pry the sac away from the otolith. Using this method the actual macula came away from the otolith intact and, as the macula was visible, it was possible to avoid touching the macula with the forceps. In most cases the entire sac was removed. Excess sacculi sac was then dissected away. However, as the membranous structure provided a point to hold the sample without damaging the macula it was not removed completely.

The maculae were then progressively dehydrated through a series of graded acetone solutions (50, 70, 90, 95 and 100%), CO_2-critically point dried and mounted on a stub and sputter coated in gold (2 minutes). The samples were then observed through a PHILIP'S XL 30 scanning electron microscope (SEM).

Statocysts were removed from squid, opened and examined under a dissecting microscope.

6.2.1 Quantification of damage

Damage to the sensory epithelium of the fish sampled from trial 9 was not quantified due to preparation artefacts resulting in an insufficient sample size. However, intact saccular maculae from trial 9 were examined under the SEM. The method used to quantify damage in the fish exposed to air-gun noise in trial 13 involved collating data from the entire macula and is outlined in section 6.2.1.1. The pink snapper in trial 14 were to be used to quantify damage and identify any regeneration of the sensory epithelium following air-gun noise exposure. However, a mesh-like filamentous structure (see section 6.3.1.2) observed covering the majority of the maculae from fish in trial 14 obscured large regions of hair bundles and therefore damage could not be accurately determined using the methods utilised for trial 13. Therefore, a second method was used to quantify the damage in trial 14. This method was also applied to the maculae from trial 13 so that the results from the two trials could be compared. This method is outlined in section 6.2.1.2.

In both methods only damage that resulted in totally ablated hair bundles and holes in the epithelium was quantified. This type of damage was easily distinguished from other types of damage such as preparation damage (Fig. 6.1).

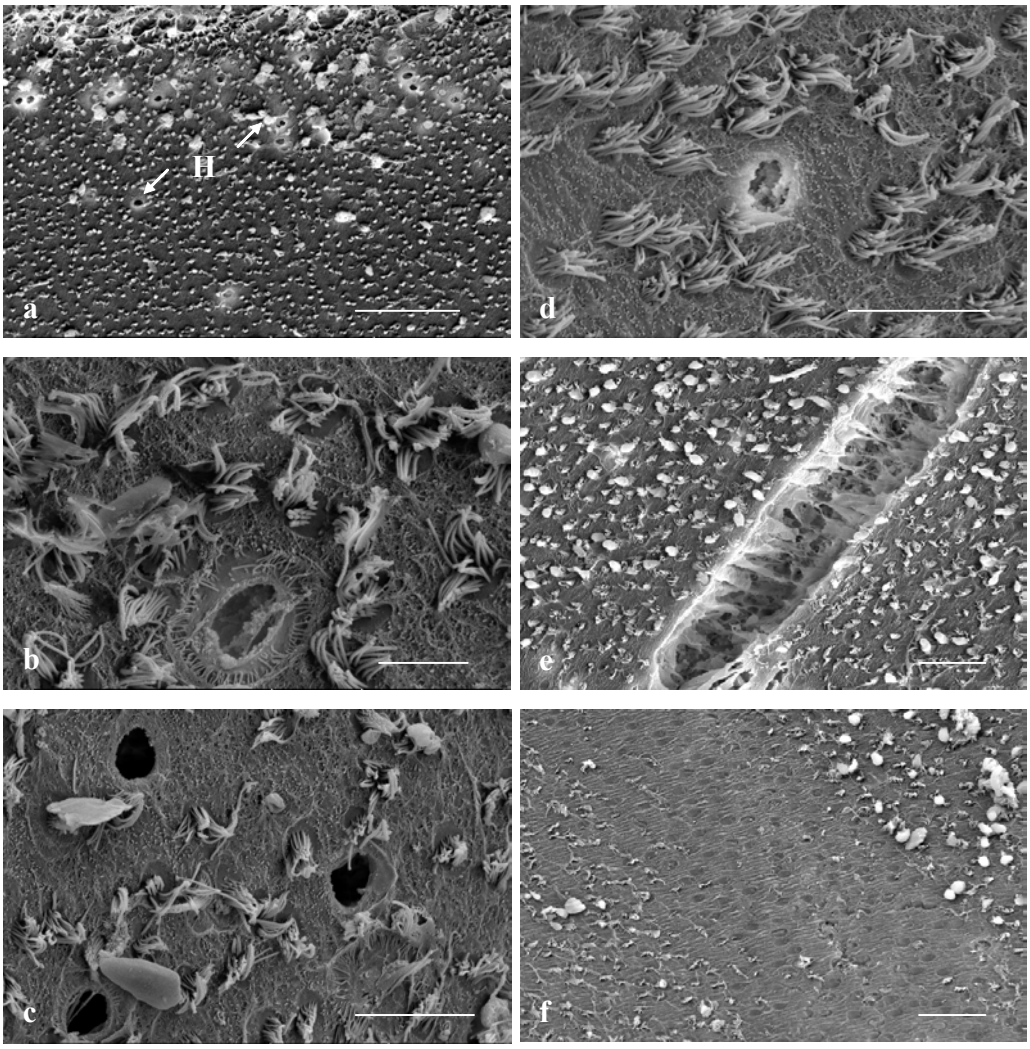

Figure 6.1: Types of damage observed on the saccular maculae. a) Ablated hair cells (H). b - d) Ablated hair cells surrounded by squashed/disorientated hair bundles e) Preparation damage – cracked epithelium. f) Preparation damage – forceps imprint. Scale bars: a, 50 μm; b, 5 μm; c, 10 μm; d, 10 μm; e, 20 μm; f, 20 μm.

6.2.1.1 Trial 13 – entire macula

Maculae from fish exposed and not exposed (control) to the air-gun noise were observed and damage to the sensory hair bundles present on the surface was recorded. The right and left saccular macula from each fish was examined at 80 x magnification. A split screen was then used to observe sections of the macula at 470 x magnification. The 470 x magnification view defined a section. These were designated a letter (a-z) according to their location on the macula (Fig. 6.2). Each section covered an area of approximately 23 500 μm^2. The orientation of each macula in the field of view was kept constant so that the section being observed

would correspond to the identical section of each macula from all fish. The sections of the macula and the label designated to each section can be seen in Figure 6.2.

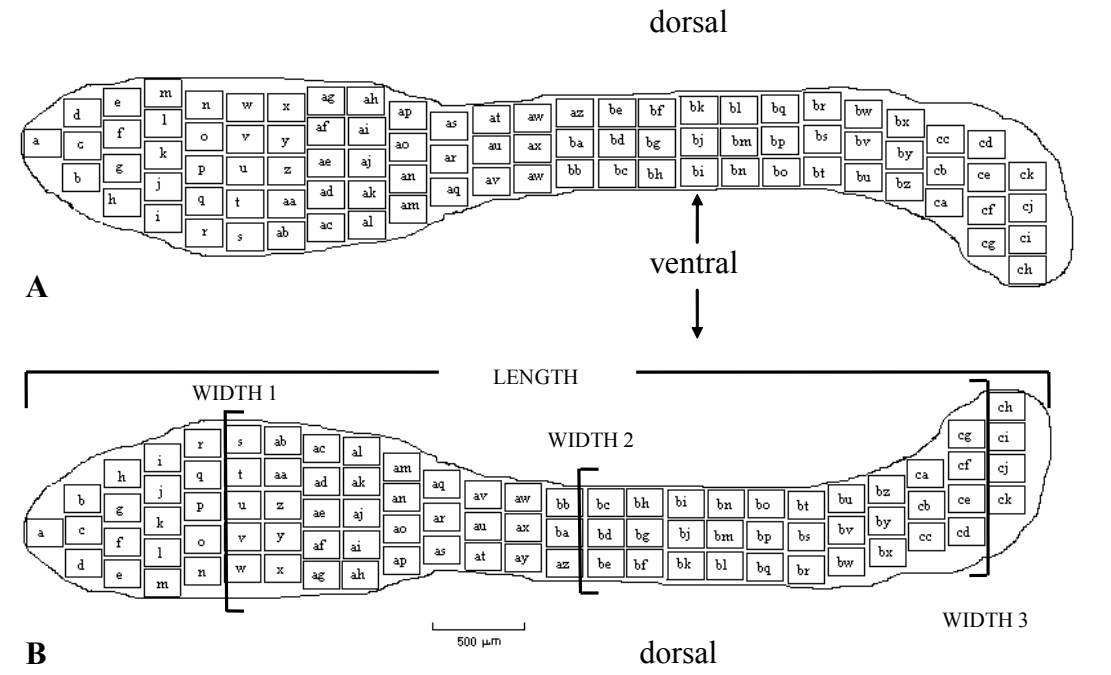

Figure 6.2: Representative diagram of the saccular macula from a pink snapper and the sections that were observed at 470 x magnification for hair cell damage. A) Left macula. B) Right macula. Lines indicate where length and width dimensions were measured (Table 6.3).

The maculae of three control fish were used to determine the total number of hair bundles in each of the above sections and the entire macula.

6.2.1.2 Trial 13 and 14 – transect method

The fish from trial 13 and four groups of fish from trial 14 were divided into 6 groups (group I – VI) (Table 6.2). Group I and II consisted of the control and exposed fish respectively from trial 13. The 'control' fish from trial 14 that is, the fish that were remnant of trial 13 but not yet exposed to air-gun noise in trial 14, were designated group III. A total of 58 days separated the sampling of group II and III. Group IV were the fish sampled immediately after air-gun noise exposure in trial 14. Group V and VI fish were sampled 11 and 28 days respectively after air-gun

noise exposure in trial 14. Another group of fish were sampled 17 days after group VI however, these samples were misplaced before analysis.

Table 6.2: Sampling regime and group designation for the saccular macula of pink snapper in trials 13 and 14. Exposure 1 is air-gun noise exposure in trial 13. Exposure 2 is air-gun noise exposure in trial 14.

Trial	Group	Sample regime	
		Exposure 1	**Exposure 2**
13	I	control	N/A
13	II	18 hours	N/A
14	III	58 days	control
14	IV	58 day + 1 hour	≈ 1hour
14	V	69 days	11 days
14	VI	86 days	28 days

Observation areas were chosen along three vertical transects at the caudal, middle and rostral ends of the macula (Fig. 6.3). If an area was obscured by the filamentous structure then the transect was moved slightly to one side until most of the transect was unobscured. Adjacent digital images of the macula were taken along three vertical transects from Group I - VI fish. The images were taken at 800x magnification (532 x 712 pixels). Each image was then overlaid with 25 x 25 μm gridlines and the number of missing hair cells in each of the 24 x 625 μm^2 grid sections was quantified (Fig. 6.4). Again the only damage quantified was the completely ablated cells that is, pits in the macula.

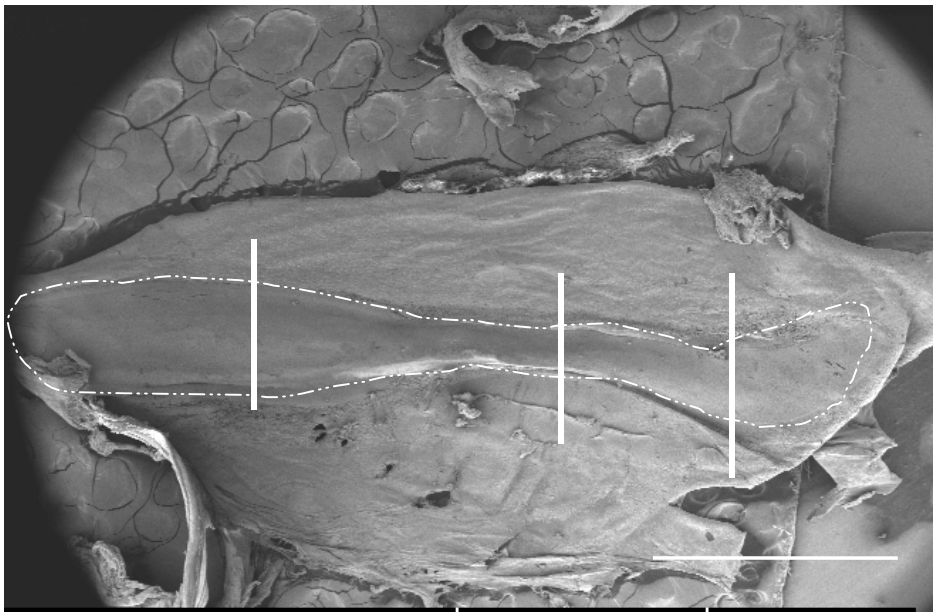

Figure 6.3: Pink snapper right macula (outlined) with the 3 transects used to analyse damage and regeneration. Scale bar: 2 mm.

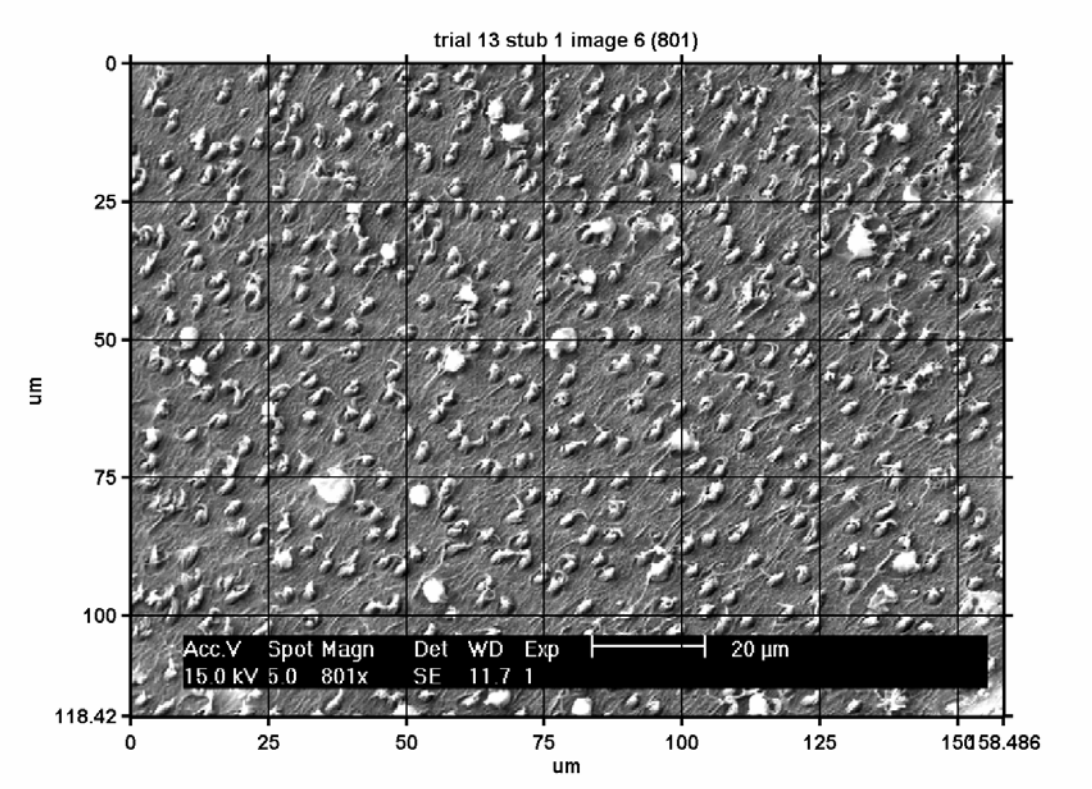

Figure 6.4: A section of a pink snapper saccular macula showing the grid overlay used for quantifying hair bundle damage using the transect method. The total damage in the 24 x 625 μm^2 grid was calculated.

In some cases, areas of the images included artefacts (for example, unidentified tissue or cells) or were affected by charging or contained tears caused by the preparation procedures. Any of the grid squares that contained areas of obscured epithelium were removed from the analysis and were subtracted from the total area searched per image.

6.2.2 Data analysis

All data was analysed using the statistical analysis software SPSS (release 10.0.5 for Windows). Prior to analysis, normality and homogeneity of variance of the data were assessed using Kolmogorov-Smirnov test for goodness of fit and Levene test respectively. If the assumption for homoscedastic data was not met, then an appropriate data transformation function was applied. Differences were considered significant if $p < 0.05$.

The Spearman correlation coefficient was applied to the data on the size of the maculae to determine if fish size was significantly correlated with macula size.

6.2.2.1 Trial 13 – entire macula

The percentage of hair bundles missing in each of the sections was calculated. All percentage data was subjected to an arcsine transformation ($X' = \arcsin \sqrt{X}$) prior to analysis (Zar 1974). An independent samples t-test was applied to the data to test for any statistically significant differences between control fish and fish exposed to air-gun noise.

To test for any significant differences in damage between the different regions of the macula it was divided into 3 sections rostral (a-av), mid (aw-bt) and caudal (bu-ck). A one-way analysis of variance (ANOVA) was then applied to the data to determine any significant differences. Scheffès multiple comparison test was applied to the data to detect significant differences.

6.2.2.2 Trial 13 and 14 – transect method

The total area searched for each image was recorded and the number of missing hair bundles per area was calculated. A one-way ANOVA was then applied to the data to

test for any statistical significance between groups I - VI. A one-way ANOVA was also applied to the data to compare the extent of the damage to the epithelium between the rostral, middle and caudal transects. Scheffès multiple comparison test was applied to the data to detect significant differences.

6.3 Results

The most detailed work on the effect of air-gun noise on the fish auditory system was conducted on the pink snapper in trial 13 and 14. Figure 6.5 displays the auditory system of the pink snapper. The ears of pink snapper were encased in a bony structure with the semicircular canals weaving through channels in the bone. This made removal of an intact ear for morphological investigation impossible. However, reconstruction after removal was achieved with careful dissection and information on basic fish ear structure.

Each of the endorgans was enclosed in a separate chamber. The saccule otolith was substantially larger than the lagena and utricle otoliths. No obvious specialised structures were found in this species that could enhance hearing capabilities.

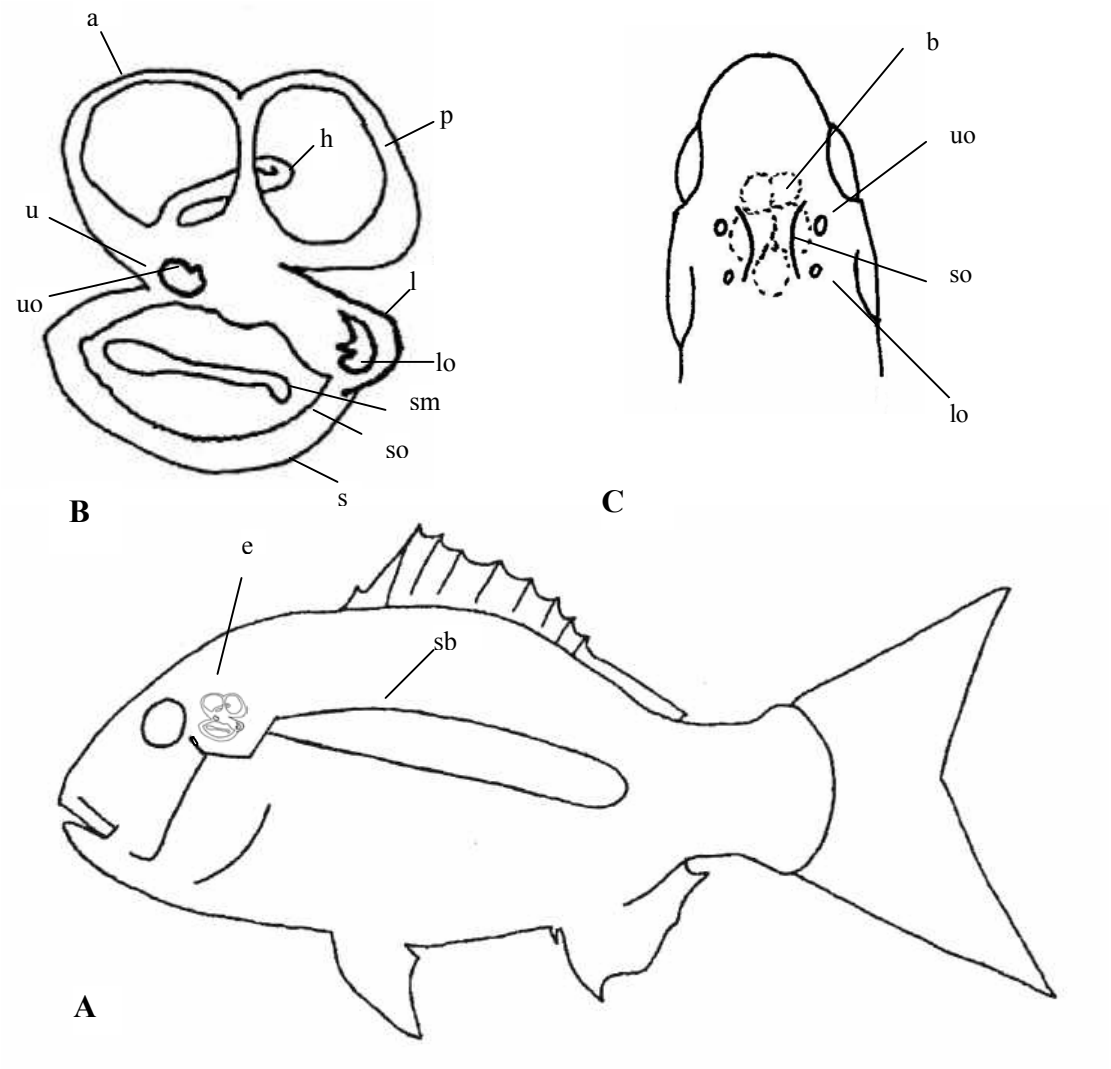

Figure 6.5: A) Position and structure of the pink snapper (*Pagrus auratus*) ear. B) Medial view of the right inner ear of pink snapper. C) Dorsal view of pink snapper with position of otoliths shown (brain removed but position shown by dashed lines). e = ear; sb = swim bladder; b = brain; s = saccule; so = saccular otolith; sm = saccular macula; l = lagena; lo = lagenar otolith; u = utriculus; uo = utricular otholith; a = anterior semi-circular canal; p = posterior semi-circular canal; h = horizontal semi-circular canal

Some characteristics of the saccular macula from the pink snapper are shown in Figure 6.6. Three different types of ciliary bundles were identified and named type F1, F2 and F3, according the description in Popper (1981). Type F1 ciliary bundles were sharply graded and had a kinocilium that was no longer than twice the length of the longest stereocilia (Fig. 6.6 b). Type F2 ciliary bundles have a kinocilium that is

several times longer than the short stereocilia (Fig. 6.6c). Type F3 ciliary bundles were similar to F1 but were longer (Fig. 6.6e and f).

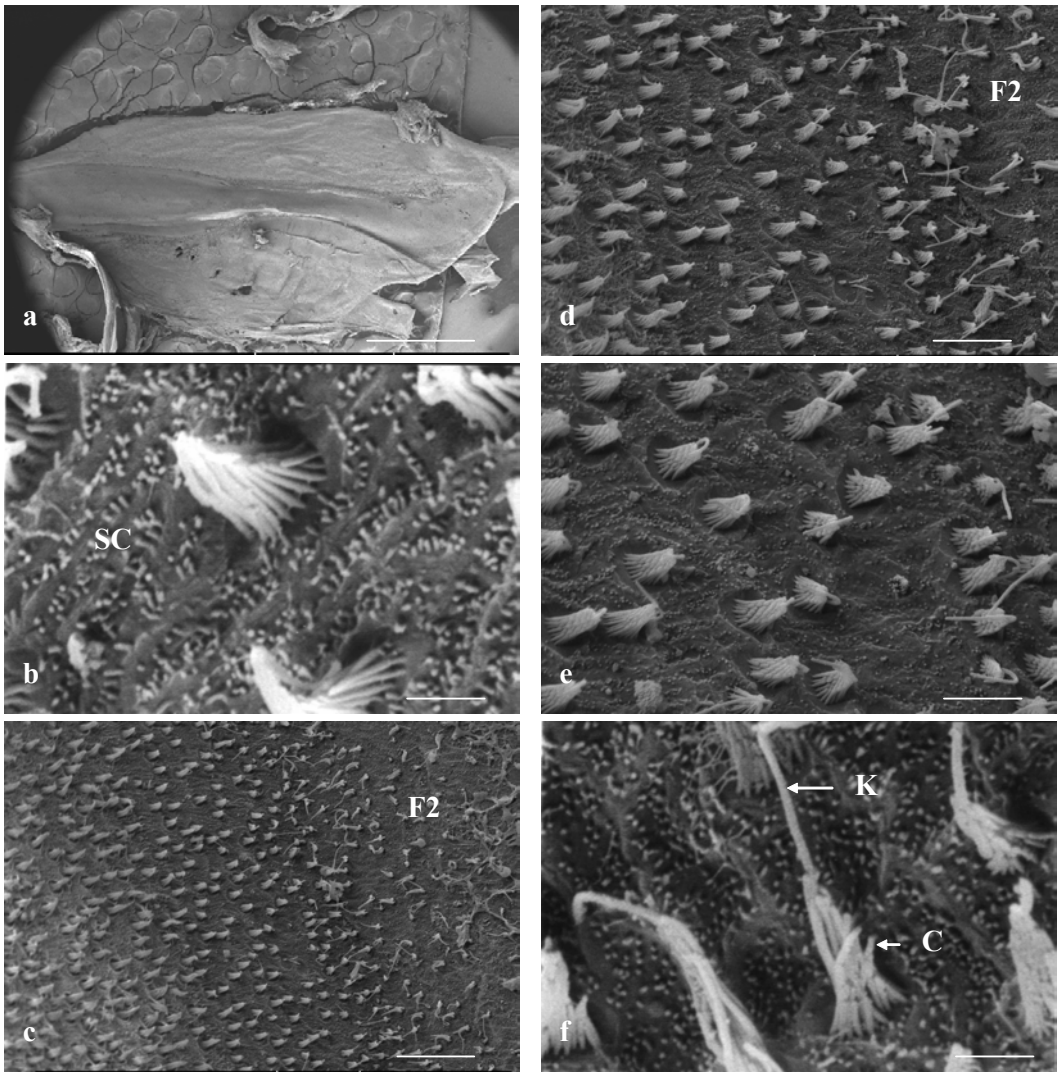

Figure 6.6: Scanning electron microscope images of control pink snapper saccular maculae. (a) Entire right macula. b) Type F1 ciliary bundles. c and d) Edge of macula showing type F2 ciliary bundles in top right corners (F2). e and f) Type F3 ciliary bundles. Supporting cells (SC) can be seen covering the macula surrounding the hair cell bundles. Examples of kinocilium (K) and stereocilia (C) are labelled. Scale bars: a, 1 mm; b, 2 μm; c, 20 μm; d, 10 μm; e, 5 μm; f, 2 μm.

From the density counts of saccular maculae hair cells of three fish from trial 13 that were not exposed to air-gun noise the total number of hair cells on the entire macula was 54613.67 ± 278. Each section of the grid used to quantify damage in trial 13 was found to have a mean hair cell density of 613.64 ± 5.45. Figure 6.7 indicates the hair

cell orientation pattern of the pink snapper saccular macula followed the standard pattern, with four distinct regions of hair cells polarised in a particular direction. The direction of hair cell polarisation was ascertained by observing on which side of the hair bundle the kinocilium was positioned. It was also found that type F1 ciliary bundles were predominant on the pink snapper saccular macula, while the longer type F2 and F3 ciliary bundles were present on the perimeter of the macula (Fig. 6.7).

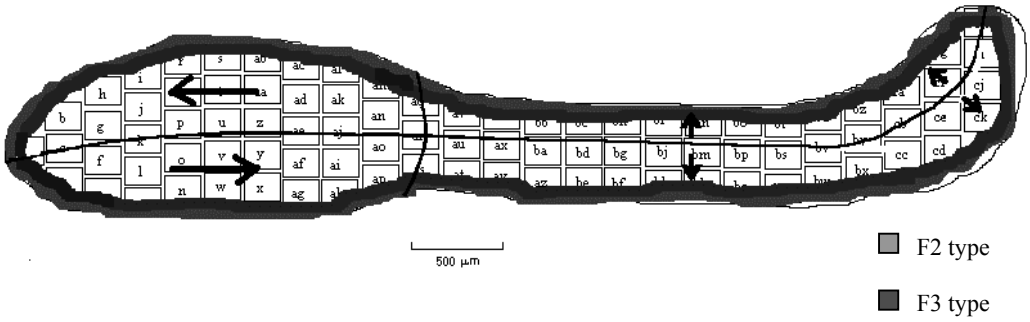

Figure 6.7: Diagram indicating the distribution of the different hair cell types and their orientation on the pink snapper saccular maculae.

Slight variations in shape and size of the macula between individual fish and between left and right maculae from the same fish were apparent (Table 6.3). This resulted in slight alterations in the grid positioning for some maculae. The fish used in trial 13 displayed no significant ($p > 0.05$) correlation between fish length and/or weight and macula width or length, although the small range in the standard length of the fish used (230 ± 24 mm) probably limited the ability to detect such relationships. Variation in maculae dimensions may have been due to placement of macula on stub.

Table 6.3: Measurements of the saccular macula of pink snapper from individual fish from trial 13.

FISH No.	FISH LENGTH (standard length mm)	FISH WEIGHT (gm)	SACCULAR MACULA DIMENSIONS (mm)							
			LENGTH		WIDTH 1		WIDTH 2		WIDTH 3	
			Left	Right	Left	Right	Left	Right	Left	Right
1	257	303.15	6.44	-	1.12	0.96	0.4	0.4	0.48	-
2	245	291.75	6.72	6.8	1.2	0.96	0.48	0.4	0.84	0.88
3	209	250.32	-	5.68	0.96	-	0.48	0.5	0.88	0.88
4	231	274.62	6.50	6.28	0.96	0.84	0.32	0.44	0.83	0.84
5	223	251.59	-	-	-	-	-	-	-	-
6	255	304.71	-	6.8	-	-	-	-	-	0.88
7	205	248.80	-	-	0.96	-	0.4	-	0.88	-
8	218	260.34	6.0	6.5	0.96	0.85	0.4	0.4	0.72	0.88
9	235	280.50	6.4	6.16	0.98	0.8	0.52	0.4	0.8	0.8
10	235	282.26	6.0	6	0.95	0.96	0.56	0.44	0.8	0.92

The saccular otolith of the pink snapper can be seen in Figure 6.8. At low magnification the sulcus, into which the otolithic membrane and macula fit, is shown (S). The SEM images of higher magnification show the rough crystalline surface topography of the sulcus. The images also indicate that pink snapper have saccular otoliths of polymorphic composition (Gauldie 1993).

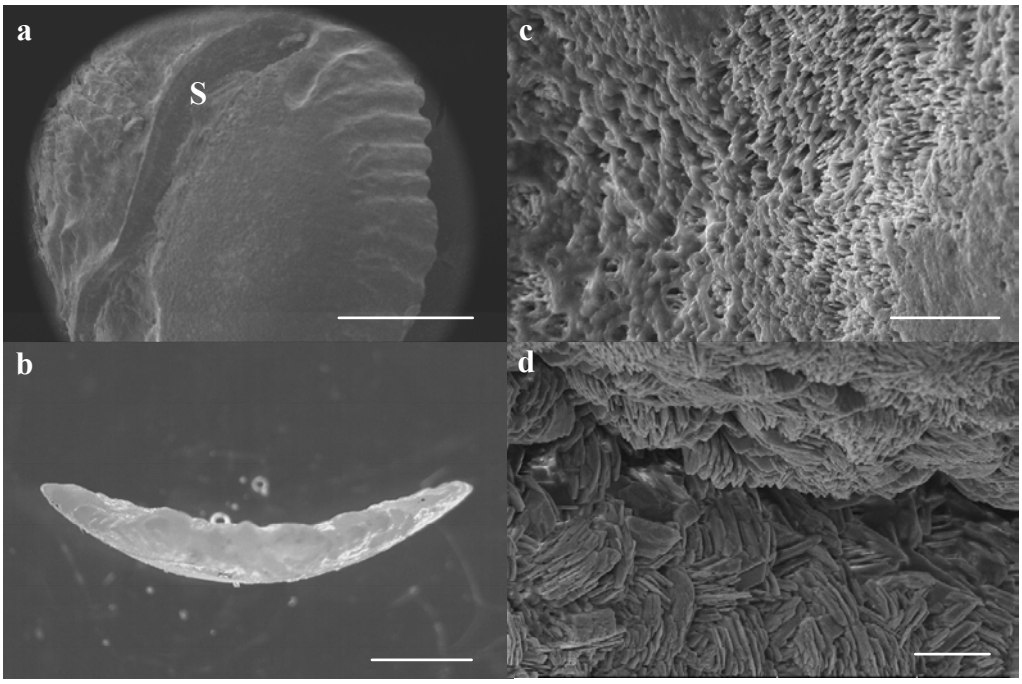

Figure 6.8: Scanning electron microscope images of the right saccular otolith from pink snapper. a) Entire otolith showing sulcus (S). b) Dorsal view of otolith. c and d) Sulcus topography. Scale bars: a, 2 mm; b, 2mm; c, 20 μm; d, 25 μm.

6.3.1 Damage quantification

As mentioned previously, the type of damage recorded was only of completely ablated hair bundles. Hair bundles were often observed to appear 'squashed', especially in areas surrounding completely ablated hair bundles (Fig. 6.1). However, these observations were not quantified.

6.3.1.1 Entire macula

The numbers of completely ablated cells found by counting ablated cells in 23 500 μm^2 grids are represented in Figure 6.9. The regions of damage are concentrated on the middle region of the macula. The areas with the highest levels of damage, that is, bz, bq, cd, bo and be, have a high concentration of type F2 hair cells.

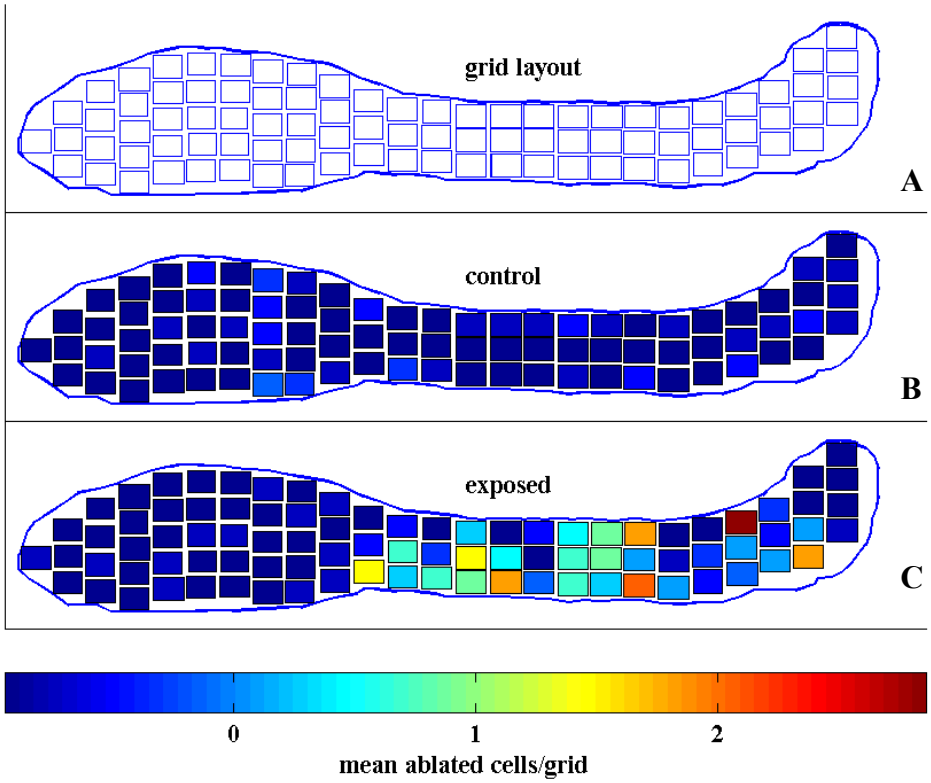

Figure 6.9: A) Grid of macula regions scanned for the ablated hair cells. B) Grid showing colour coded mean number of ablated hair cells per grid for control pink snapper. C) Grid showing colour coded mean number of ablated hair cells per grid for exposed pink snapper. All specimens were from trial 13, control and exposed ablated cell counts shown used mean of five macula each. Colour bar at bottom for middle and bottom plots.

The percentage damage to the maculae of fish from trial 13 is shown in Table 6.4.

Table 6.4: Mean (± standard error) damage to each region of the saccular maculae of fish in trial 13. Damage to the entire macula is also indicated.

Treatment	Region						Entire macula	
	1 (a – av)		2 (aw – bt)		3 (bu – ck)			
	holes / region	% damage (x100)	holes / region	% damage (x100)	holes / region	% damage (x100)	holes/macula	% damage (x100)
Control	5.8 ± 2.8	1.97 ± 0.95	2.2 ± 0.73	1.49 ± 0.5	2.0 ± 0.84	1.92 ± 0.8	10.0 ± 2.51	12.9 ± 1.99
Exposed	8.4 ± 2.6	2.85 ± 0.87	30.0 ± 5.2	20.64 ± 3.6	12.6 ± 1.1	12.08 ± 1.1	51.4 ± 7.57	30.8 ± 2.51

There was significantly ($p < 0.05$) more damage to the saccular maculae in the fish exposed to air-gun noise than the unexposed fish. Significantly ($p < 0.05$) greater damage was found in region 2 and 3 when compared to region 1.

6.3.1.2 Transect method

The presence of a filamentous structure was observed on the majority of saccular maculae sampled after trial 14 (Fig. 6.10). In areas of some samples the filamentous structure was so dense it completely covered the hair bundles (Fig. 6.10g). The filaments had a tendency to attach to hair bundle tips in damaged sections. In some cases the hole left by the ablated hair bundles were covered with a cluster of the filaments, forming matting that covered the hole (Fig. 6.10b). The diameter of the filaments ranged between 0.09 – 0.18 μm.

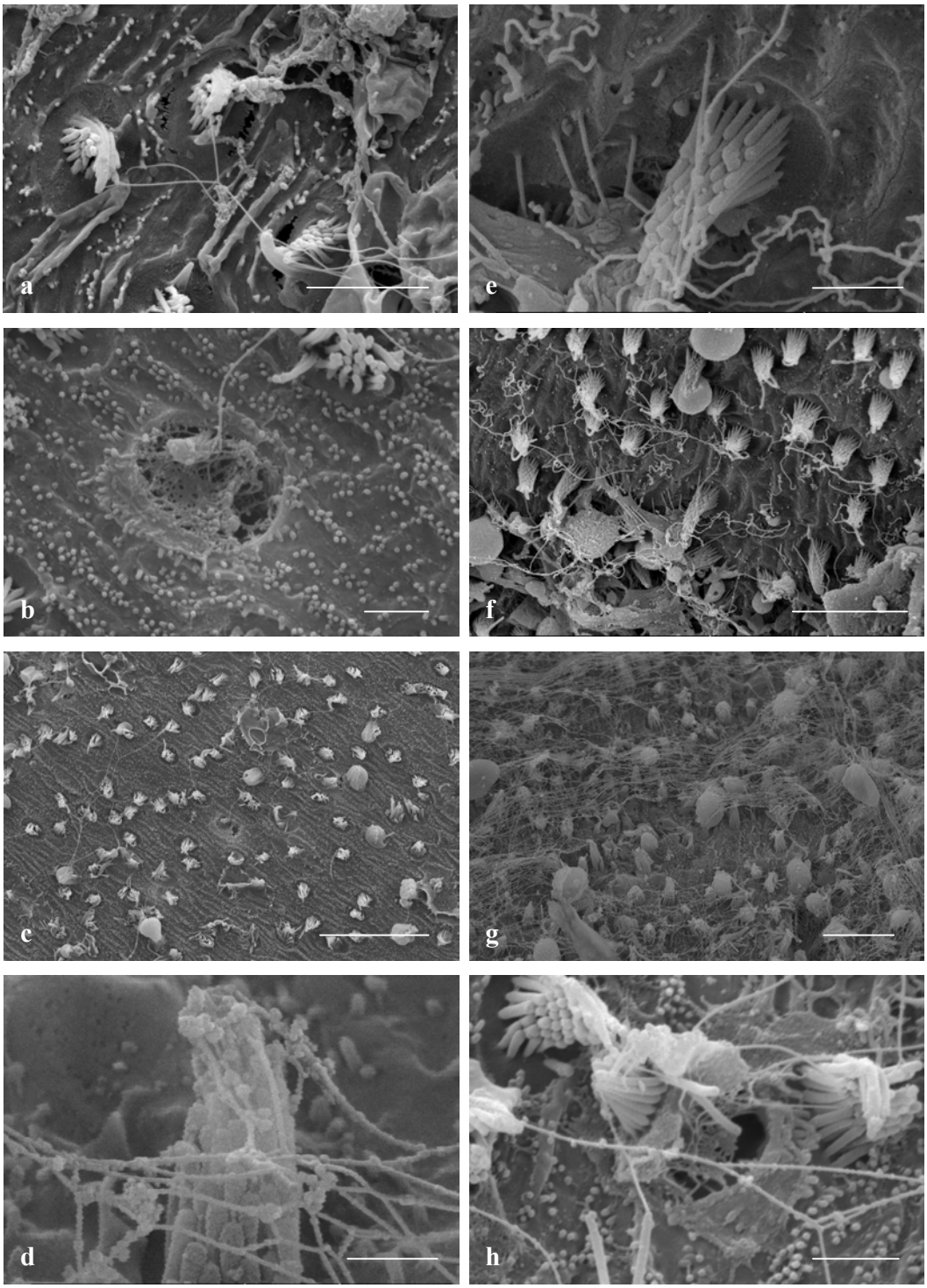

Figure 6.10: Filamentous structure covering the saccular maculae of pink snapper exposed to air-gun noise. Scale bars: a, 5 μm; b, 2 μm; c, 20 μm; d, 1 μm; e, 2 μm; f, 10 μm; g, 10 μm; h, 2 μm.

'Blebbing' was also observed on most of the damaged tissue (Fig. 6.11). This appeared to be a result of the hair cell being expelled from the epithelium.

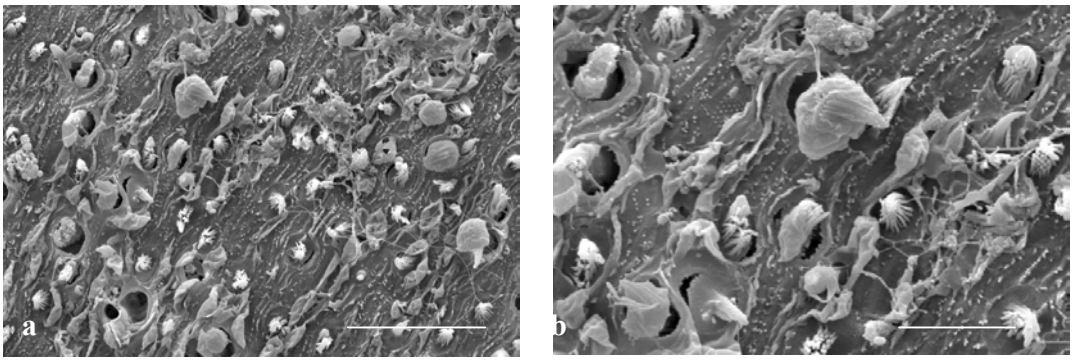

Figure 6.11: Saccular macula samples from pink snapper showing examples of blebbing. Scale bars: a, 20 μm; b, 10 μm.

In order to determine if regeneration of the hair bundles had occurred in the 58 days after trial 13 the 'control' group of trial 14 (group III) was examined and damage was compared to the control (group I) and exposed groups of trial 13 (group II). Damage to group IV – VI maculae was also quantified. The number of holes found in the epithelium per 10 000 μm^2 of the six groups of fish is shown in Figure 6.12.

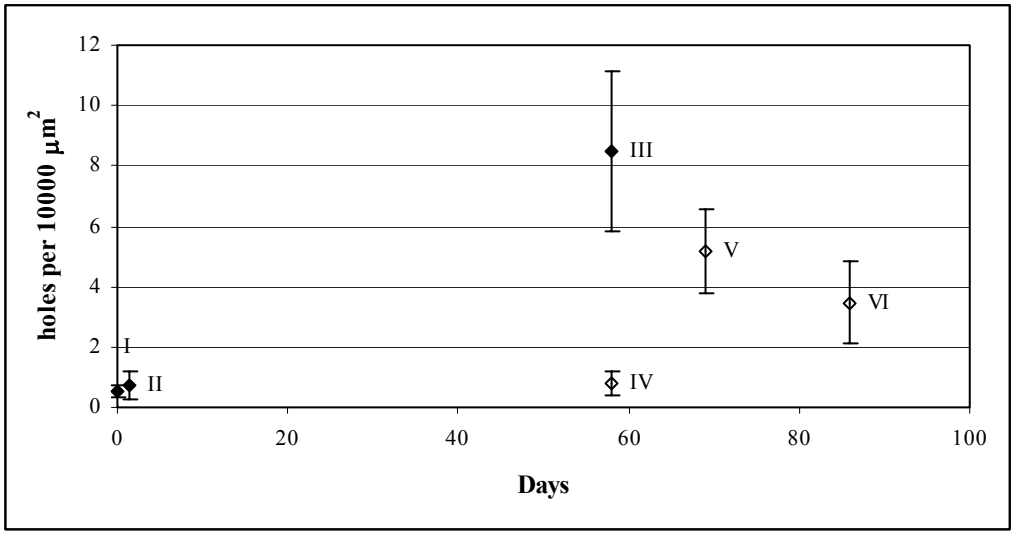

Figure 6.12: Number of holes (mean ± standard error) found in the 3 representative transects (caudal, middle and rostral) for pink snapper in trial 13 (Group I and II) and trial 14 (III – VI). Hollow symbols indicate fish that were exposed to air-gun noise twice.

The number and area of maculae used to quantify the damage to the caudal, middle and rostral transects are outlined in Table 6.5. The number of maculae and area used in quantification of damage due to air-gun noise exposure varied between groups as

preparation damage and the filamentous structure limited the samples available for analysis.

Table 6.5: Details of samples used to quantify damage using the transect method.

Group	No. of macula	No. of images	No. holes	Area (x 10000 μm^2)
I	6	84	58	119.19
II	3	38	39	54.75
III	5	56	665	76.69
IV	4	64	73	91.19
V	4	56	385	72.13
VI	3	28	134	38.94

Using the counts along the 3 transects no significant ($p > 0.05$) damage to the macula was observed in the fish in group II fish when compared to fish in group I (not exposed to air-gun noise). However, the number of holes found in group III fish was significantly higher ($p < 0.05$) than in group I. Group VI fish, sampled 28 days after air-gun exposure in trial 14, had significantly less damage than Group III fish. No significant differences in damage were found between the rostral, middle and caudal transects. Some examples of the extensive damage observed in group III fish, not obscured by the filamentous structure, are shown in figure 6.13. The maculae from group IV fish had significantly lower damage than group III, V and VI.

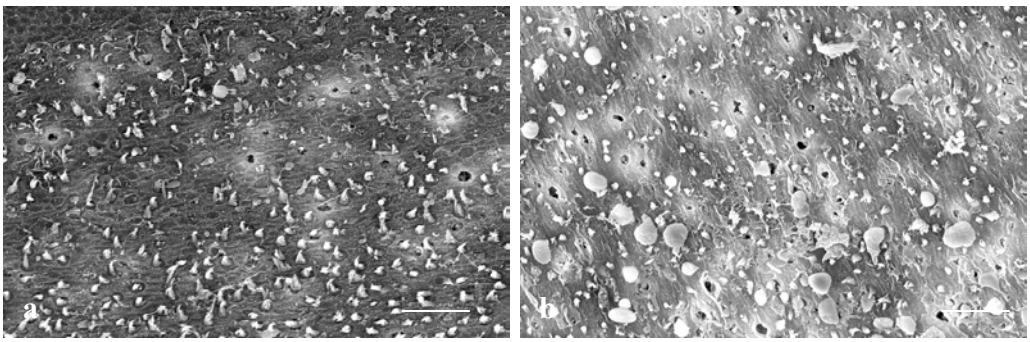

Figure 6.13: Examples of extensive areas of damage to the saccular macula of pink snapper 58 days after (group III) air-gun noise exposure. Scale bars: a, b, 20 μm.

6.3.2 Trial 9 and squid samples

Samples of the saccular macula were taken from two of the species of fish used in the Exmouth trials (*Epinephelus rivulatus* and *Lutjanus carponotatus*). Damage to these samples was not quantified. However, Figures 6.14 and 6.15 show the shape of their saccular macula.

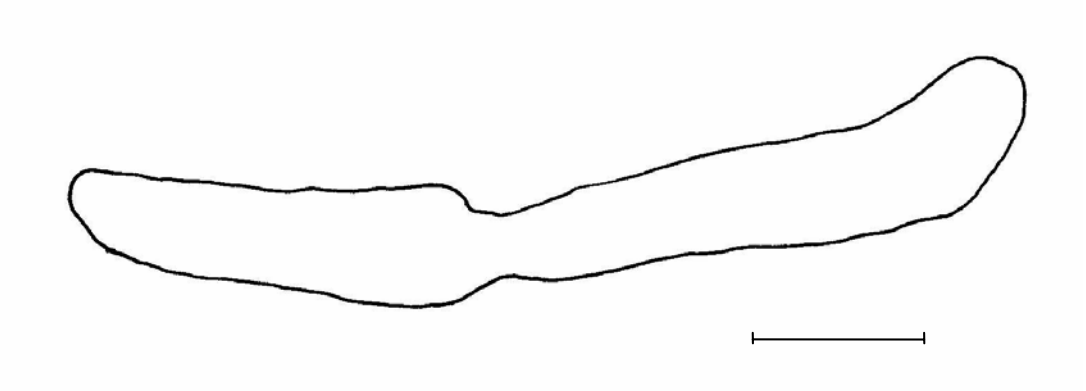

Figure 6.14: Diagram of right saccular macula dissected from a Chinaman rockcod (*Epinephelus rivulatus*) with a standard length of 200 mm. Scale bar: 1 mm

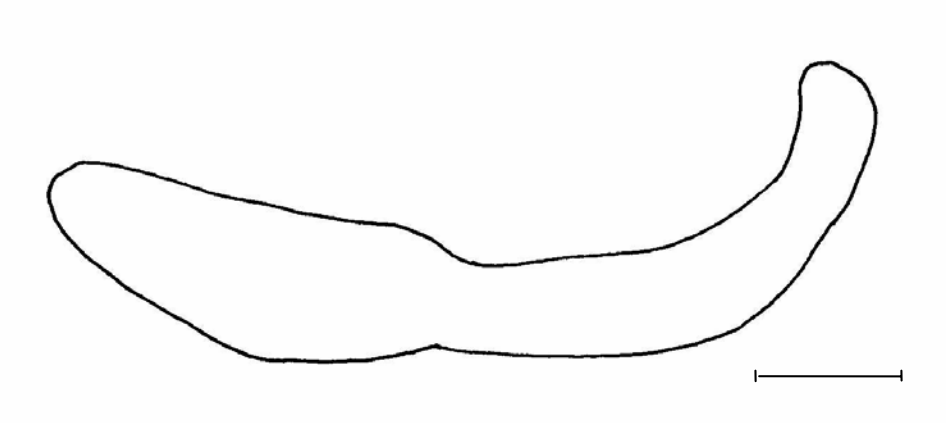

Figure 6.15: Diagram of right saccular macula dissected from a stripy sea perch (*Lutjanus carponotatus*) with a standard length of 215 mm. Scale bar: 1mm

Damage similar in appearance to the damage observed in the pink snapper was observed to the saccular maculae of the Chinaman rockcod exposed to air-gun noise (Fig. 6.16).

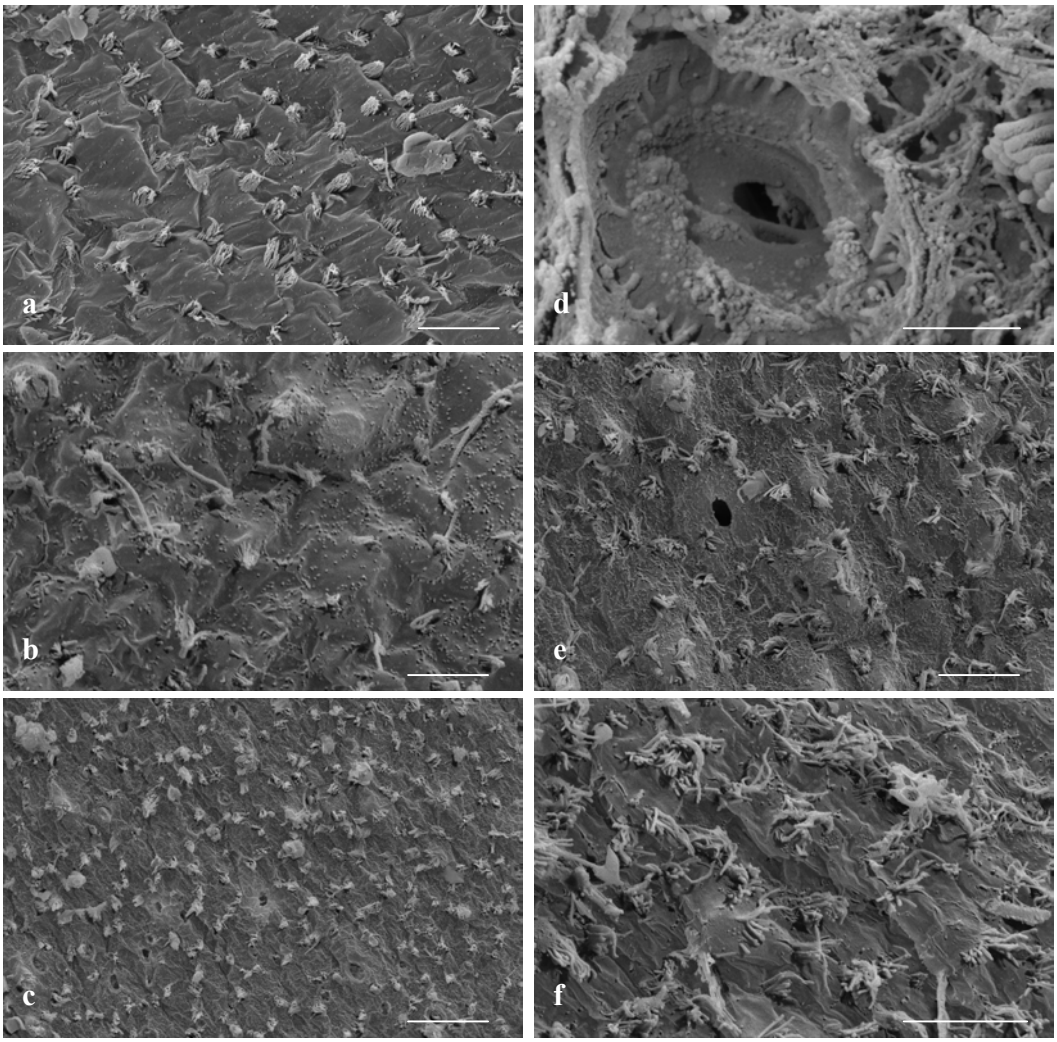

Figure 6.16: Images of the saccular macula of Chinaman rockcod (*Epinephelus rivulatus*) from trial 9. a and b) Maculae from control fish. c, d and e) Maculae of rockcod exposed to air-gun noise showing ablated hair bundles. f) Macula of exposed rockcod showing 'squashed' hair bundles. Scale bars: a, 10 μm; b, 5 μm; c, 20 μm; d, 2 μm; e, 10 μm; f, 10 μm.

The structure of the statocyst of *Sepioteuthis australis* is shown in Figure 6.17. The anticristae (cartilaginous protrusions) that divide the statocyst into channels can be seen. The statolith lies in loose contact with the macula princeps at the anterior end of the statocyst with its long axis approximately in the dorsal-ventral plane of the squid (S). Some common characteristics of the statolith used to identify squid species are shown (Clarke 1978).

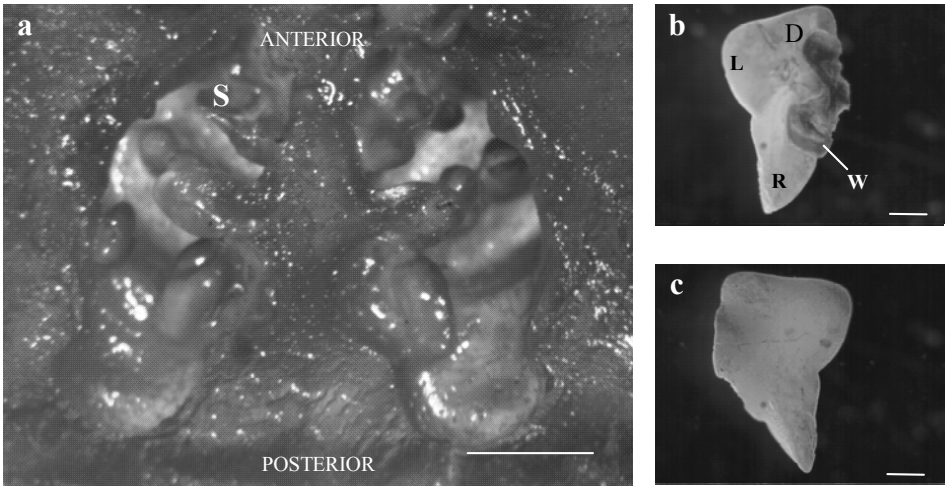

Figure 6.17: Images of squid (*Sepioteuthis australis*) statocysts (mantle length of 190 mm). a) Internal image of the statocysts after removal of the lateral wall. Protrusions into cavity are anticristae (S = position of statolith); b) Anterior view of the right calcareous statolith (D = dorsal dome, L = lateral dome, R = rostrum, W = wing); c) Posterior view of the right statolith. Scale bars: a, 2 mm; b, c, 1mm.

6.4 Discussion

The results suggest that the noise regime used in this investigation did cause damage to the saccular macula of *Pagrus auratas.* Damage appears to be particularly apparent to the middle to caudal region of the macula and seems consistent with the effect of over stimulation of the otolith. Further investigation revealed that at 58 days after exposure to air-gun noise hair bundle damage was significantly greater and that no regeneration of the hair cells was apparent.

Although damage to the sensory epithelium of the saccular macula was observed, the actual number of ablated cells was low, relative to the total number of cells present on the macula. With the low number of ablated hair cells it is tempting to assume that the probable effect that this damage would have on the hearing capabilities of the fish would be minimal. However, the mechanisms fish use to discriminate between noises of varying characteristics is still poorly understood (Hastings et al. 1996; McKibben 1999) and therefore assumptions on the overall effect that such damage, as observed in this investigation, would have on the function of the macula of the fish are speculative. Also, only gross damage to the hair cells was quantified. Tip link damage, which may have rendered the hair cells non functional was not

quantified in these experiments. The gross effects quantified here may indicate widespread loss of hearing capability as discussed below. Interestingly, although the same group of fish was used in trials 13 and 14 different behavioural responses to air-gun noise were observed. In trial 13 significant alterations in the behaviour of pink snapper such as alarm responses, swimming patterns and position in the water column were observed (Chapter 7). However, no behavioural changes were observed in response to air-gun noise in trial 14. As the trials were separated by 58 days it is unlikely that habituation to the air-gun noise caused the lack of behavioural alterations. These results are indicative that the damage to the saccular maculae induced by the air-gun noise exposure in trial 13 resulted in some alteration in the hearing capacity of the pink snapper and therefore their ability to hear the air-gun noise compromised.

When compared with other accounts of damage to the hair cells of the macula induced by intense acoustic signals, the direct damage observed in this investigation, in the form of ablated cells was severe. In previous studies on fish where hair bundle damage has been observed as a result of intense acoustic signals the damage has been limited to the hair bundles only (Hastings et al. 1996). The actual hair cells appear to remain relatively intact. In this study only the 'pits' in the sensory epithelium, resulting from hair cells that had been completely removed or sunken, were recorded as damage. Considering that the electrical potential of the sensory epithelium is maintained by the integrity of the epithelium and transduction channels, which are responsible for the differences in ionic concentration on either side of the membrane, this type of damage, if not rapidly repaired, could have dire consequences on the function of the ear (Hudspeth 1985). Without the potential difference across the membrane an electrical signal will not be generated and therefore, even if hair bundles are in intact, the brain can not detect the acoustic stimulus. To assess the full extent of the damage to the function of the saccular macula and surrounding tissue, observations of the ultrastructure of the saccular epithelium, tip link connections and neural processes using would have to be performed.

The results shown in Figures 6.9 and 6.12 and Table 6.4 represent only the

completely ablated hair cells. The damage to the sensory epithelium could actually be more extensive than indicated by the results presented. Usually the hair bundles of the hair cells form a rigid, uniform structure with each stereocilia being in close proximity to another. When mechanically disturbed each cilia of the hair bundle remains relatively straight along its length, bending from the base of the cell (Hudspeth 1985). However, in this investigation many of the fish exposed to air-gun noise exhibited regions of the macula with hair bundles that appeared flattened and disorganised (Fig 6.1). These regions often surrounded ablated hair cells, which suggest a common mechanism causing these changes in macula appearance that is, over stimulation of the otolith.

It is reasonable to assume that, although not ablated, the function of the 'squashed' hair bundles may be impaired (Pickles et al. 1987; Clarke and Pickles 1996). Hudspeth (1985) put forward a possible model for the mechanicoelectrical transduction that takes place in vertebrate hair cells. This model suggests that the transmembrane pores (transduction channels), responsible for the flow of ionic currents across the hair cell membrane and, therefore the electrical signals to the VIIth nerve, are situated mainly at the distil ends of each stereocilia. In the literature it is reported that a fine filament links the tip of each stereocilia with the side of the adjacent stereocilia (Pickles et al. 1984; Zhao et al. 1996). This model proposes that when stimulated the stereocilia bend, stretching the linking filament which then holds the channel gate open or closed (depending on the direction of the stimulus) for longer than in resting conditions (Hudspeth 1985). In this case, if the model holds true, then it is likely that the linkage filaments between cilia were broken and therefore encoding of acoustic signals would not be possible. Regeneration of these tip links has been reported within several hours of damage and it has been suggested that breakages in these filaments could be associated with a temporary threshold shift in hearing (Zhao et al. 1996)

In this study damage was most apparent to the middle region of the saccular macula. Enger (1981) found that exposing cod to signals 100 – 110 dB re 1μPa above the known auditory threshold at 150 - 250 Hz consistently caused damage to the ciliary

bundles on the saccular macula in a pattern that suggested regionalisation of frequency sensitive hair cells. It is interesting to note that in the in this study the five areas with the greatest number of ablated hair cells, that is bz, bq, cd, bo and be (Fig. 6.9) also possessed a high concentration of type F2 ciliary bundles. It has been suggested that the variation in ciliary bundle length is related to frequency detection properties (Popper and Fay 1993). Therefore, it is tempting to speculate on the possibility that type F2 ciliary bundles could be tuned to the most prevalent frequency in the air-gun signal (10 – 100 Hz, Fig. 4.2). However, the rostral and caudal ends of the macula also possess type F2 ciliary bundles and displayed no significant damage. There is also evidence that the morphological polarity of the sensory hair cells are responsible, at least in part, for the encoding of acoustic directional information (Lu 1998; Lu and Popper 1998). Therefore, it could be possible that the damage observed in this study is related to the direction of the sound source. Another explanation for the pattern of damage observed is that it is a result of the shape and surface topography of the otolith.

The saccular macula of pink snapper fits into a sulcus in the approximate middle of the convex side of the saccular otolith (Fig. 6.8a). According to Gauldie (1993) the pink snapper otolith has a mixed aragonite/calcite/vaterite composition. The otolithic membrane lies between the macula and the otolith, the hair bundles protruding through the otolithic membrane (Dunkelberger 1980; Popper and Fay 1993). It could be assumed that the force of the impacting otolith would be greater on the longer hairs and therefore the greater the potential for damage. The fact that the damage is also most apparent at the maximum angle of curvature is also evidence that it is the impact of the otolith onto the hair cells that is resulting in the type of damage observed in this study. Additionally the layered and jagged surface of the saccular sulcus (Fig 6.8c and d) lends itself to 'ripping' out hair bundles.

Interestingly, low levels of damage to the saccular macula were also apparent in some control fish. It is possible that the observed damage resulted during the dissection of the macula from the otolith membrane or in the preparation for imaging on the SEM. However, as the damage found in fish exposed to air-gun noise was

consistently and significantly higher than in control fish this is unlikely. The results shown in Figure 4.5 indicate that the background noise during acclimation reached relatively high levels (137 dB re 1μPa) at times. It may be that normal hair cell death or low levels of damage are tolerated by the fish under normal conditions/noise exposures. Spontaneous continual loss of individual hair cells through programmed cell death has be reported in normal avian vestibular organs (Gleich et al. 1994; Kil et al. 1997).

Regeneration of vertebrate hair cells after damage by acoustic trauma has been reported. However, in this study damage to the hair cells covering the saccular macula was significantly greater at 58 days than in the fish sampled 18 hours after exposure to air-gun noise. Evidence suggests that damaged hair cells require approximately 7-10 days post treatment with ototoxic drugs and intense sound exposure to regenerate (Corwin and Cotanche 1988; Lombarte et al. 1993; Husbands et al. 1999; Woolley et al. 2001). It is also known that many species of fish continue to produce new hair cells throughout their lives (Corwin 1983; Popper and Hoxter 1984; Lanford et al. 1996). Perhaps the damage to hair cells and the surrounding areas resulting from the noise regime in this study was too severe for the regeneration processes to occur within the time frame of 58 days. Previous research has indicated that the surrounding supporting cells of the sensory epithelium may be precursors for new hair cells, either through mitosis or transdifferation of supporting cells regeneration (Warchol and Corwin 1996; Corwin and Oberholtzert 1997; Berg and Watson 2002). The damage observed in this study appears to include the surrounding supporting cells and therefore this could have prevented or hindered regeneration of the hair bundles.

Hastings et al. (1996) exposed *Astronotus ocellatus* to sounds of frequency 60 and 300 Hz and intensities of 100, 140 and 180 dB re 1μPa with damage resulting in the fish exposed to a continuous signal of 180 dB re 1μPa at 300 Hz for one hour. Damage was restricted to the striola of the lagena and utricle and the results suggest that the damage was not immediate. By sampling two groups of fish at differing time periods following exposure to the same noise regime, different levels of damage

were observed. Fish sacrificed four days post treatment showed macula damage while fish sacrificed within one day of treatment exhibited no significant damage which suggests damage to the macula resulting from acoustic trauma may take some time to manifest so as to be visible (Hastings et al. 1996). The results of trial 14 expand these findings, as significant cell death occurred within 58 days after air-gun exposure.

The nature of the damage in trial 14 was similar to the damage observed in trial 13, except generally more extensive. The presence of blebbing was consistent with expansions of the hair cell ciliary bundle surface causing eventual rupture leading to a hole in the macula replacing the hair bundle (Popper 2002a). It is possible that the filamentous structure observed on the maculae from trial 14 is part of the regenerative process of the damaged macula. Alternatively the observed filaments may be remnants of the otolithic membrane. The position of some of the filaments, for example in samples where holes left by ablated hair bundles were covered with the structure, suggests that this is unlikely. The author is unaware of reports of this filamentous structure in the literature. However, the fish macula damage reported in the literature has been to the ciliary bundles only whereas in this study damage appeared to be more severe with apparent destruction of the hair cell.

There was evidence of a gradual decrease in damage of the pink snapper maculae 11 days after the second exposure (69 days after the first air-gun noise exposure) with significantly less damage to the maculae of the fish sampled 28 days after the second air-gun noise exposure. This group of fish had been exposed to air-gun noise in trial 13, 86 days prior to sampling. This is an interesting observation, as no regeneration had been observed in the maculae of the fish sampled 58 days after air-gun noise exposure. The more rapid onset of regeneration observed in trial 14 could be due to the fact that the regeneration process was already underway when the fish were exposed to noise in trial 14. Alternatively, the fish in trial 14 were exposed to almost half the number of air-gun signals above 171 dB re 1 μ Pa (Table 6.1) as the fish in trial 13 which may have limited the damage to the maculae in trial 14. Models of otolith movement suggest that sound levels above 171 dB re 1μPa result in a

dramatic increase of the response of the macula-otolith system (McCauley et al. 2000).

An anomaly in the results was observed in the fish sampled immediately after the second exposure to air-gun noise (group IV). It would be expected that this group of fish would have, at a minimum, the same amount of damage to the maculae as group III fish. However, the maculae of this group of fish showed a level of damage equivalent to the fish sampled 18 hours after the first air-gun exposure (group II). As mentioned in the methods, maculae from a group of fish were sampled 17 days after group VI fish but the samples were misplaced. It is believed that this group of fish may have been the samples that were labeled as group IV fish. Alternatively, the unusual result may be a product of the transect method used to quantify the damage. Using the entire macula to quantify damage resulted in finding significantly more damage to the fish sampled 18 hours after exposure to air-gun noise (group II) when compared to unexposed fish (group I). However, this was not the case when the transect method was used. The transect method used limited areas to quantify damage and therefore specific areas of damage may not have been quantified using this method.

The actual consequences of hearing damage would depend to what the fish is listening. Many species of fish are known to use acoustic communication in reproductive, social, feeding and territorial behaviour (Blaxter 1988; Hopkins 1988; Popper and Fay 1993). Some fish possess specialised accessory structures (e.g. Weberian ossicles, gas filled structures connected to the inner ear) that enhance the fishes hearing capabilities by transducing the pressure component of the noise to the inner ear (Bone et al. 1995a). Pink snapper do not possess any such structures and are therefore considered non hearing specialists and are likely to be mainly sensitive to particle motion (Popper and Fay 1993). Pink snapper are also not known to 'vocalize'. So what effect could hearing loss have on this species?

Even without anthropogenic noise sources, the marine environment is relatively noisy as a result of noise generated by physical processes and biota (Myrberg 1980).

Being able to intercept and interpret these noises would be of benefit to an underwater inhabitant providing a wealth of environmental cues (Rogers and Cox 1988). The interception and decoding of acoustic communication signals from other marine animals could be useful (Myrberg 1981). However, more generally, hearing can be used to form an 'image' of the underwater environment by locating and identifying sound sources and sound scatterers (Myrberg 1981; Popper and Fay 1993). The water's surface and bottom scatter sound and every moving object underwater produces sound. The ability to receive and decode these signals could mean the difference between survival and death (Myrberg 1981).

Determining the air-gun signal level threshold for damage to the fish is not possible from the data obtained in this study. The trials were primarily designed to ascertain behavioural effects of seismic surveys and thus required an approaching – departing scenario to simulate a real scenario. Therefore, fish were exposed to a wide range of noise levels ranging from, 144 – 191 dB re 1µPa with the majority of the signal energy concentrated between 10-100 Hz. As stated previously hearing capabilities vary widely amongst fish but this range is within the hearing thresholds for most fish that have been studied, including non-hearing specialists (Popper and Fay 1993; McCauley 1994). The time period that fish were exposed to each noise level varied and therefore, as fish were sampled at the conclusion of the entire noise regime, it was not possible to determine at what point the damage to the saccular macula actually occurred. It is tempting to assume that the damage resulted from the shots of higher amplitude. This assumption is supported by models of otolith movement in a sound field (McCauley et al. 2000). However, it is also likely that the shots of lesser intensity but more frequent exposure or even the combination of the entire noise regime were responsible.

Figure 4.2 indicates that the similarity of the acoustic signal from the air-gun used in this study to the signal received from a 'typical' seismic survey enables relatively accurate comparisons from here to real situations. However, it is difficult to define a typical seismic survey as each survey has a sound source designed for the purpose required (McCauley et al. 2000). Due to the complexity of how sound travels in

water, differing factors that seem to be relatively trivial could completely alter the acoustic signals to which the fish are exposed (Rogers and Cox 1988). Nevertheless, as a comparison, signals of greater than 180 dB re 1μPa can be expected at distances within 500 m from a seismic survey array (44L, R.M. data).

It is important to note that in this study fish were held captive whereas in a real situation fish would usually be able to escape from, at least, the higher intensity noise as seismic survey vessels only travel between 4 - 6 knots (Dalen and Raknes 1985; McCauley 1994). Although it is probable that damage to the sensory system could be avoided, changes in behaviour and stress response levels induced by attempting to avoid the noise could be detrimental to the fishes well being (Santulli et al. 1999).

Extrapolation of these results to other species of fish should be approached with caution. The huge variation in the hearing capabilities and mechanisms employed by fish makes assumptions about the effect of sound on fishes hearing, based on data from other species, speculative (Scholik and Yan 2001; Scholik and Yan 2002). Recent evidence suggests that, in some fish, all three otolithic organs are involved in hearing, not just the sacculus (Popper and Fay 1993). Therefore, prior to making an assumption on the effect of damage to the sacculus on overall hearing, the effect of air-gun noise on the other two otolithic organs should be investigated.

Little is known about the hearing capabilities of the pink snapper, including the actual hearing threshold. However, damage was found and therefore it can be assumed that seismic survey noise does have the potential to damage the auditory system of fish. Although not quantified, damage of similar nature to what was observed in pink snapper exposed to air-gun noise was also found in Chinaman cod that were exposed to air-gun noise. Hearing specialists would be more susceptible to damage (Hastings et al. 1996). Also size and design of the actual otolith would effect the mechanical properties of the fishes ear and therefore the probability of mechanical damage to the macula (Popper and Fay 1993; McCauley 1994).

Mathematical models that predict the displacement and recovery time (that is,

returning to rest position) of the otolith when stimulated by particle motion in a sound field have been proposed (Lychakov 2000; McCauley et al. 2000). These models suggest that otoliths of greater mass will have a higher displacement and response time. As it is assumed that it was the otolith that caused the damage observed in this study, this becomes particularly important in fish with larger otoliths. Contributing to the unpredictability, otolith size is not proportional to the size of the fish between species (McCauley 1994).

Results from this investigation suggest that air-gun noise, used in seismic surveys, does induce damage to the hair cells of the sensory epithelium of the sacculus in *Pagrus auratas*. However, the effect that the observed damage has on the function and survival of the fish and whether regeneration of the damaged cells occurs after 58 days is still uncertain. Due to the variation in hearing capabilities, behaviour and habitat of different species of fish and the complexities of the characteristics of sound travelling in water, the relevance of the data when applied to different situations remains to be clarified but suggests caution in the use of intense noise sources in regions heavily populated with, or important to fish.

Chapter 7

Behavioural response of fish and squid to air-gun noise

7.0 BEHAVIOURAL RESPONSE OF FISH AND SQUID TO AIR-GUN NOISE

7.1 Introduction

The effect of noise on behaviour of terrestrial vertebrates has been researched for many decades and is well documented (Fletcher and Busnel 1978). In the past decade, interest has been focussed on the effect of anthropogenic noise on marine animals, with the main focus being on marine mammals. Recent research has determined that sound can be used to control fish behaviour to a certain extent (Kuwada et al. 2000; Popper 2002b; Schmaltz et al. 2002).

Recently the effect of anthropogenic noise sources on the behaviour of fish and invertebrates has become a concern, especially to commercial fisheries (Popper 2002b). In particular, the effect of seismic survey noise on fish populations has come under scrutiny (Rusby 1995; Ketchington 2000; Engas and Lokkeborg 2002). The sound generated by the air-gun arrays used in offshore seismic surveys is usually between 20-500 Hz, which is within the detectable frequency range for fish of known hearing capabilities (Popper and Fay 1993). However, although fish may be able to hear air-gun signals, previous studies have indicated that the sound may have to be well above the detection threshold to elicit a significant change in behaviour (Blaxter et al. 1981b; Knudsen et al. 1992).

Behavioural responses that have been observed in marine finfish in response to noise, include: changes in schooling behaviour (Pearson et al. 1992), changes in positioning in the water column (Dalen and Raknes 1985; Greene 1985; Pearson et al. 1992), reluctance to take baited hooks (Skalski et al. 1992), changes in swimming speeds (Engas and Lokkeborg 2002), migration (Lokkeborg and Soldal 1993; Engas et al. 1996) and startle responses (Blaxter et al. 1981b; Wardle et al. 2001).

There is a dearth of information on the effect of air-gun noise on the behaviour of marine invertebrates. Low frequency noise has reportedly been used to successfully deter barnacle larvae from settling on ship hulls (Branscomb and Rittschof 1984). There is anecdotal evidence of squid being attracted to intermittent low frequency

noise (Maniwa 1976). It has also been shown that cephalopods are capable of 'hearing' in the infrasound range and far field sound (Hanlon and Budelmann 1987; Packard et al. 1990). Wardle et al. (2001) observed little effect on invertebrate (crustaceans, echinoderms and molluscs) populations inhabiting a reef that was exposed to air-gun noise.

From previous research it is apparent that underwater noise from anthropogenic sources does affect the behaviour of fish and, at least some invertebrates. However, behavioural reactions and the noise levels required to induce them need to be characterised so that, if required, effective mitigation techniques can be designed and applied.

The aim of the behavioural section of this study was to determine if exposure to air-gun signals could elicit a change in behaviour in fish and squid and, should changes be observed, to determine at what sound level changes occurred and the nature of the changes.

7.2 Materials and methods

Details of the animals used are given in Table 3.2. Noise exposure regimes are given in Figures 4.6 – 4.9. Experimental site layout for Jervoise Bay trials is shown in Figure 3.1.

Animals housed in the large sea cage were used in behavioural observations. A high resolution black and white video camera (Panasonic 1/3" CCD, WV-BP312 with 4.5 mm focal length lens) was placed in the south-eastern cage corner and a colour, digital, video camera (Sony 1/3" CCD DC10P with 4 mm focal length lens) in the north eastern corner. Cameras had horizontal and vertical fields of view of 114^{o} and 87^{o}, and 132^{o} and 101^{o} for the Panasonic and Sony cameras respectively.

Observations during the acclimation time indicated that different species tended to occupy different vertical sections of the cage, so the camera depth was adjusted to match the depth range of the most abundant species. The cameras cabled back to the

pontoon or breakwater and were logged to tape on Samsung video cassette recorders (VCR). A single monitor which could be switched to either camera was used to view animals during trials. Once activated, the VCR displayed time bases which were checked against a master watch to allow correlation of the air-gun operations with behaviour. For trials conducted with the VCRs on the pontoon, sound was recorded to each VCR from a single microphone suspended on the pontoon (trials 1-5). This allowed verbal notes and the air-gun signal (which could be clearly heard above water) to be logged to video tape. For experiments made with the cameras cabled back to the breakwater and the pontoon ranged over 5-450 m from the sea cage, underwater sound was cabled to the VCR units from a Clevite CH17 hydrophone, through a 40 dB gain impedance matching amplifier. Thus the background noise and air-gun signal were logged to video tape. The VCR units had an automatic gain control (AGC) on the audio input. The AGC resulted in all air-gun signals above a certain level being clipped and therefore, to the listener were of similar loudness. This acted to reduce bias in the behavioural scoring procedure.

The experimental regime for behavioural observations involved a one hour (approximately) pre-exposure observation with the VCRs running, exposure observation and 45-60 minutes of post exposure observation. In later trials (5, 10-14) a second air-gun exposure was carried out 50-100 minutes after the first exposure, and again a 45-60 minutes observation of post exposure behaviour was made. Thus 3-5 hours of footage for each camera were made for each trial.

A series of 72 two-character codes was used to describe behaviour (Appendix 4). Behaviours of groups of animals were the main focus of the study, rather than the behaviour of individuals. To code behaviours from a video the codes were entered directly to computer whilst watching the video. The computer program used was designed to record a time stamp for each carriage return and these were subsequently adjusted to real time to correlate behavioural responses and air-gun noise exposure.

Types of behavioural responses were coded, with the codes falling into general groups:

- position in water column (upper, mid, lower);
- swimming patterns – directional changes (squid can swim forwards and backwards), approaching cameras, circling;
- swimming speed - very fast, fast, slow and idle;
- schooling patterns - loose, tight or no schools;
- animal colouration - light, dark, patterns and observed changes;
- alarm responses - startle responses ('C' turn), parting, darting, flash expansion and jetting and ink sac ejection in squid;
- aggressive interactions.

In addition, house-keeping codes such as indication of numbers of animals involved, air-gun on and off points, passage of boats and animals out of view were recorded.

These codes were available from two cameras at opposite ends of the cage and as the field of view from each camera did not overlap, the data sets could be combined. This was complicated by the differing visibility offered by the black and white versus the colour digital camera. The water quality in Jervoise Bay ranged from poor to medium, with a high loading of small particulates such as salps (Thaliacea) and other matter usually present in the water column. In bright sunlight these small particles tended to produce high amounts of backscatter in the horizontal plane (not in the vertical plane). The colour camera coped poorly with this backscatter, tending to under compensate the brightness giving an over exposed image. This generally resulted in the colour camera being harder to view during analysis and having less depth-of-field than the black and white camera.

7.2.1. Data analysis

Analysis of the data from the behavioural observations was used to show:

i) differences in the animal's responses between the periods before, during and after air-gun noise; and

ii) differences in behavioural responses that occurred at varying levels of air-gun noise.

Graphical displays of each recorded behaviour were constructed to indicate air-gun noise level at each observation (Appendix 5) allowing behavioural trends to be observed. To detect any difference between behaviours before, during and after air-gun exposure, behaviours were divided into two groups, that is, behaviours that could be analysed as counts per period (e.g. alarm responses) and behaviours that involved calculating the time spent actually performing that behaviour (e.g. swimming speed and vertical position in water column).

To analyse differences in frequency of occurrence of a particular behaviour between periods (i.e. air-gun off and air-gun on) the behaviour index (I) for each period was calculated as the ratio of the number of times that particular behaviour was observed (s) to the total number of behavioural counts (S) (Equation 7.1).

$$I = \frac{s}{S} \qquad (7.1)$$

To analyse the relationship between noise level and behavioural response, noise level thresholds were designated. The noise level thresholds (T) chosen were; $113 < T_1 < 158$, $158 < T_2 < 163$, $163 < T_3 < 168$, $168 < T_4 < 173$ and $T_5 > 173$ dB re 1 μPa with 113 dB re 1 μPa corresponding to zero air-gun noise level as air-gun signals were always above this level. A behaviour index was then calculated for each noise level threshold. This type of analysis was not possible for trials using the fixed pontoon / air-gun approach (2, 3 and 5) due to the low range of noise levels achieved by using this exposure method.

The calculated behaviour indices assume that the behavioural responses of fish were induced solely by the air-gun noise. However, observations showed that the same behavioural responses could occur in the absence of the stimuli during the air-gun off periods. To take this into account a behavioural response index (d) was calculated as a difference between the indices I_p and I_n for the air-gun on and off periods (Equation 7.2).

$$d = I_p - I_n \quad (7.2)$$

where:

d = behavioural response index

$I_p = s_p / S_p$

$I_n = s_n / S_n$

s_p = specific behaviour (e.g. alarm responses) counts per period above threshold

S_p = total behavioural counts per period above air-gun threshold

s_n = specific behaviour counts per period with no air-gun noise

S_n = total behavioural counts per period with no air-gun noise

Therefore, a positive behavioural response index indicated that the particular behavioural response was observed more often during air-gun noise exposure at or above the specified air-gun threshold than when the air-gun was turned off.

The relationship between the behavioural response index (d) and the specified air-gun threshold was then analysed using regression models (SPSS release 10.0.5 for Windows).

Changes in swimming behaviour and vertical position were calculated by the same methods, except that the time spent exhibiting the behaviour to be analysed was calculated (d_t) rather than the frequency of observations. The behavioural response index for these responses was calculated as shown in Equation 7.3.

$$d_t = I_p - I_n \quad (7.3)$$

where:

d_t = behavioural response index of behaviours measured in time

$I_p = t_p / T_p$

$I_n = t_n / T_n$

t_p = time spent displaying particular behaviour for period above specified air-gun noise threshold.

T_p = time species in view for period above specified air-gun noise threshold
t_n = time spent displaying specific behaviour for period with no air-gun noise
T_n = time species in view for period with no air-gun noise

Statistically significant differences in behaviours between air-gun on and off periods within each trial could not be calculated due to lack of suitable replication. However, pooled data was subjected to statistical analysis with each species from each trial acting as a replicate.

Where relevant, independent samples t-tests and one-way ANOVAs were applied to the data (SPSS release 10.0.5 for Windows). Prior to applying parametric statistical tests, data was checked for normality and homogeneity of variance using Kolmogorov-Smirnov test for goodness of fit and Levene test respectively. If the assumption for homoscedastic data was not met, then an appropriate data transformation function was applied (Zar 1974). A Mann-Whitney test was applied to the data if transformation was not successful. Differences were considered significantly different if $p < 0.05$.

In trial 2 the proximity of the fish to the camera presented the opportunity to analyse the startle response of the striped trumpeter and therefore different methods of analysis were used and are outlined in section 7.3.1.1.

Decibel statistics for each trial were calculated by converting decibel values to a linear scale, calculating relevant statistics and then converting the values back to a decibel scale.

7.3 Results

Behavioural observations were made for trials 2 - 5 and 8 - 14. Due to equipment failure and poor visibility, no behavioural observations were analysed for trial 4. It became evident after the first few trials that concentrating on one species per trial led to easier and, therefore more accurate observations.

It was also observed that the behaviour of experimental fish altered when the air-gun noise commenced. As the vertical position of each camera was set during the acclimation period according to the vertical position in which the fish spent most of the time, the altered behaviours during air-gun noise exposure would sometimes take the animals out of the field of view of the cameras.

The data obtained from each trial are analysed below.

7.3.1 Trial 2

In trial 2, 12 silver bream and approximately 50 striped trumpeters were present in the sea cage. These fish were exposed to air-gun noise for 00:59:59 hours. The noise level varied between 170 – 176 dB re 1 μPa with a mean level of 174 dB re 1 μPa.

7.3.1.1 The startle response of striped trumpeter *(Pelates sexlineatus)*

The school of juvenile striped trumpeter were taking refuge inside the cage and were in the cage voluntarily. They could have easily escaped through the mesh cage liner at any time. An obvious startle response (characterised by the classic 'C' turn) was displayed by these fish in response to the air-gun shots.

The behaviour of the school of striped trumpeter was observed during the first forty signals of the air-gun. At a known time just prior to each air-gun signal the video was paused and then moved forward frame by frame (each frame represented 1/25 of a second). Each fish was observed for a minimum of five frames. The distance each fish moved was estimated as body lengths per frame. The resulting movement per C turn during air-gun signals, using five fish per signal as a sample, is shown in Figure 7.1. After forty signals from the air-gun (six minutes and forty seconds) the speed at which the fish responded to the air-gun noise had significantly reduced ($p < 0.05$) with a negative linear correlation ($r^2 = 0.71$).

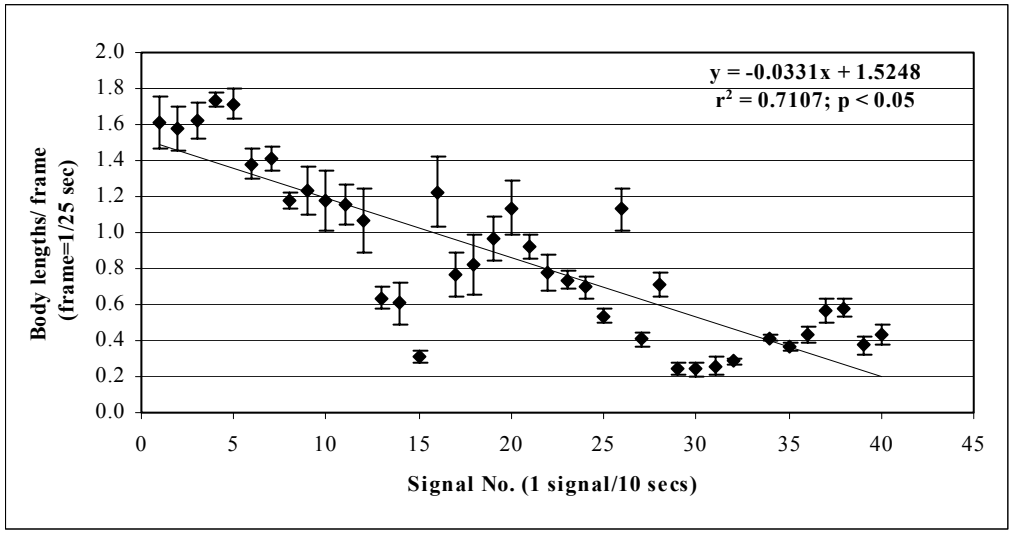

Figure 7.1: Speed travelled (body lengths/frame) by *Pelates sexlineatus* in response to the first forty air-gun shots to which they were exposed (mean ± standard error). Each point represents an air-gun shot and the reaction of five fish. Linear regression line is shown.

This procedure was also used to measure the total distance travelled by each fish at each signal. After forty signals of the air-gun, the distance travelled in response to each signal had significantly reduced ($p < 0.05$) with a negative linear correlation with a coefficient of determination of 0.61 (Fig. 7.2). The relationship was also analysed with a second order polynomial model, which gave a coefficient of determination of 0.70 ($y = 0.0065x^2 – 0.4419 + 10.24$) (Fig.7.2).

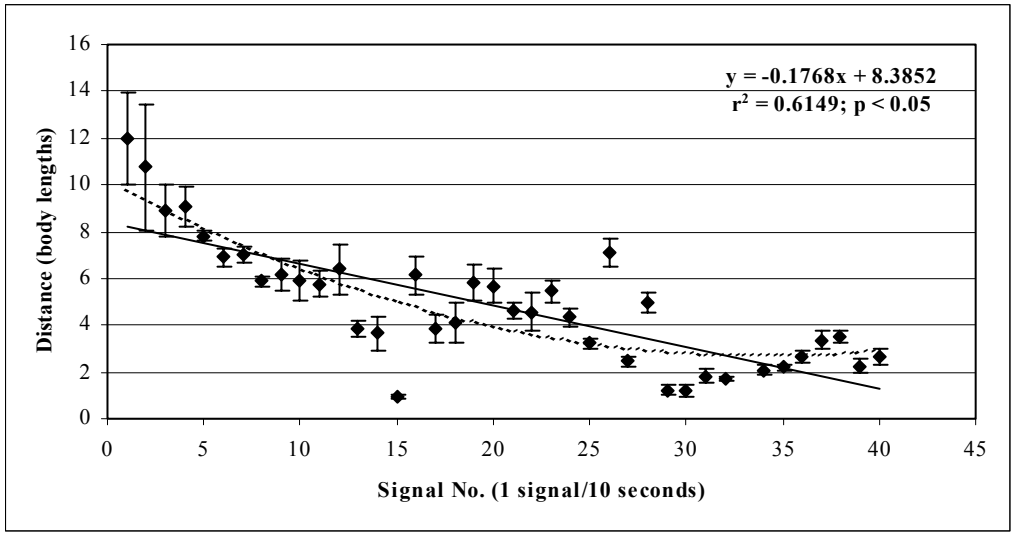

Figure 7.2: Total distance travelled (body lengths) by *Pelates sexlineatus* in response to the first forty air-gun shots to which they were exposed (mean ± standard error). Each point represents an air-gun shot and the mean of the reaction of five fish. Linear and polynomial regression (dashed) lines are shown.

Note that the response was still present but to a lesser extent after the first forty signals. Beyond this point the fish moved to the background of the camera view and therefore accurate measurements were impossible. Some time after this period the fish left the camera field of view and were believed to have fled the cage.

7.3.2 Trial 3

In trial 3, 20 silver bream, approximately 50 striped trumpeter and 9 pink snapper were present in the sea cage. Air-gun noise levels ranged from 167 – 181 dB re 1 μPa with an air-gun noise exposure time of 1:00:33 hours. The mean air-gun noise level was 174 dB re 1 μPa.

A finer mesh was fitted to the cage after trial 2. During deployment of the new cage liner a school of striped trumpeter became trapped in the mesh which resulted in the fish being held captive in the cage (unlike trial 2). It is possible that the school of striped trumpeter observed in trial 3 was the same one as in trial 2 and had therefore been exposed to air-gun noise previously.

The main observation for the striped trumpeter was the increase in the alarm responses of 'darting' (animals swimming at high speeds for short period of time) and 'parting' (groups of fish quickly expanding) during air-gun exposure (Fig. 7.3) (see Appendix 4 for behavioural definitions). The behavioural response index for time spent in the lower portion of the cage revealed that the striped trumpeter spent more time in the lower portion of the cage during air-gun operation ($d_t = 0.217$).

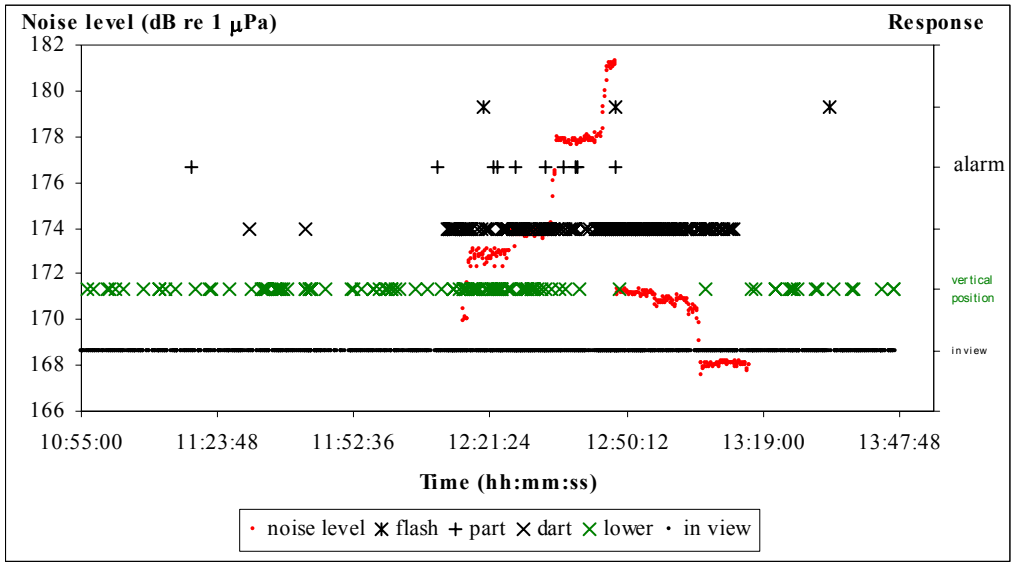

Figure 7.3: Behavioural responses of striped trumpeters (*Pelates* sp.) during trial 3. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are the result of summed data from both cameras. Right y axis indicates the behavioural response group; ticks indicate the individual behaviour. For example, as shown in Fig. 7.3 *Pelates* sp. exhibited three types of alarm responses: flash expansion, parting and darting. Note that only significant behavioural responses are shown (e.g. for vertical position, only the time spent in the lower portion of the cage is displayed). For all recorded behaviours see Appendix 5.

The silver bream in trial 3 exhibited no alarm behaviours in response to air-gun exposure but were sighted more often at the bottom of the cage during air-gun operation ($d_t = 0.386$). During air-gun operation the silver bream were observed swimming in tight groups exclusively. However, when compared with the time spent swimming in tight groups during the periods with no air-gun noise a behavioural response index of -0.022 was obtained. The silver bream swimming in small circles was only observed during the air-gun operation (Fig. 7.4).

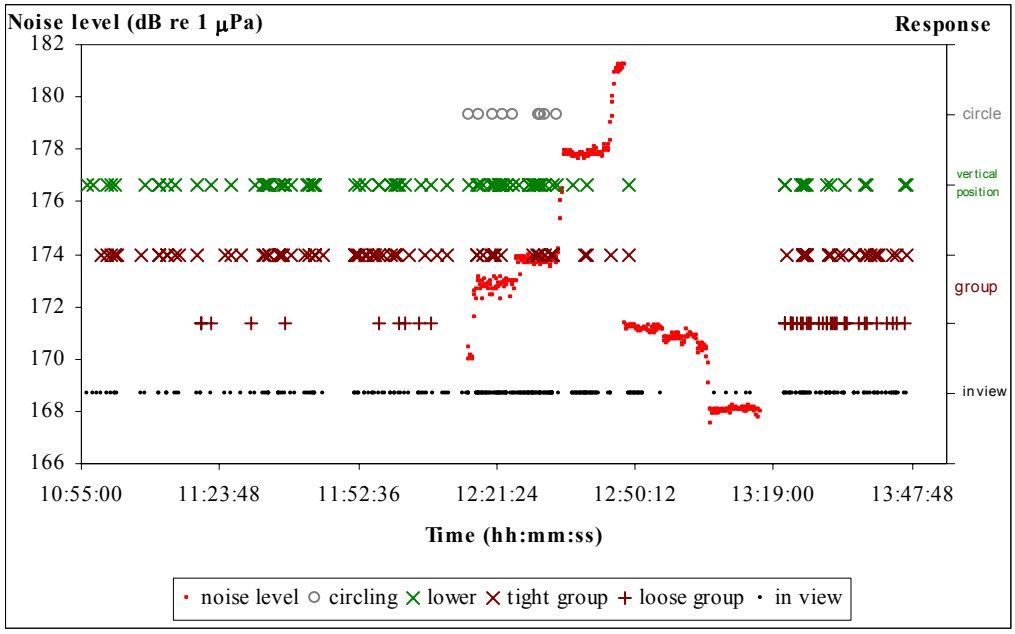

Figure 7.4: Behavioural responses of silver bream during trial 3. Air-gun noise level units are dB re 1 µPa mean squared pressure. No alarm responses were observed. Data displayed are the result of summed data from both cameras.

7.3.3 Trial 5

Nine silver bream, 10 mullet, 23 herring, 12 squid and 2 cuttlefish were in the sea cage during trial 5. These animals were exposed to two periods of air-gun noise separated by 1:26:25 hours. The first period of air-gun noise exposure was 00:58:56 hours in duration and ranged between 171 – 180 with a mean level of 177 dB re 1 µPa. The second period lasted for 1:01:36 with noise levels ranging between 173 – 185 with a mean of 176 dB re 1 µPa.

The visibility in trial 5 was poor, therefore accurate identification of the fish species present was only possible when they were close to the camera (Appendix 5, Fig. 3 – 6). However, the squid were identifiable and therefore analysis of their behaviour was possible.

In trial 5 the squid in view of the camera ejected ink at the first air-gun signal (174 dB re 1 µPa). They were then observed moving backwards, away from the air-gun, that is heading south, at the mid - top of the cage (Fig. 7.5). The backward motion

consisted of a series of jetting motions, each movement corresponding to an air-gun signal. The animals then disappeared from view of the cameras appearing three times at the top, south end of the cage (Fig. 7.5). Observations from the dinghy revealed that the squid were aggregated at the south end of the cage (furthest away from the air-gun) in the top section of the water column for the majority of the first period of air-gun exposure. The squid remained out of view of the cameras until the second exposure where they were observed in the top portion of the cage. The animals were in view of the cameras for 41% of the second air-gun exposure and were sited exclusively in the top portion of the cage.

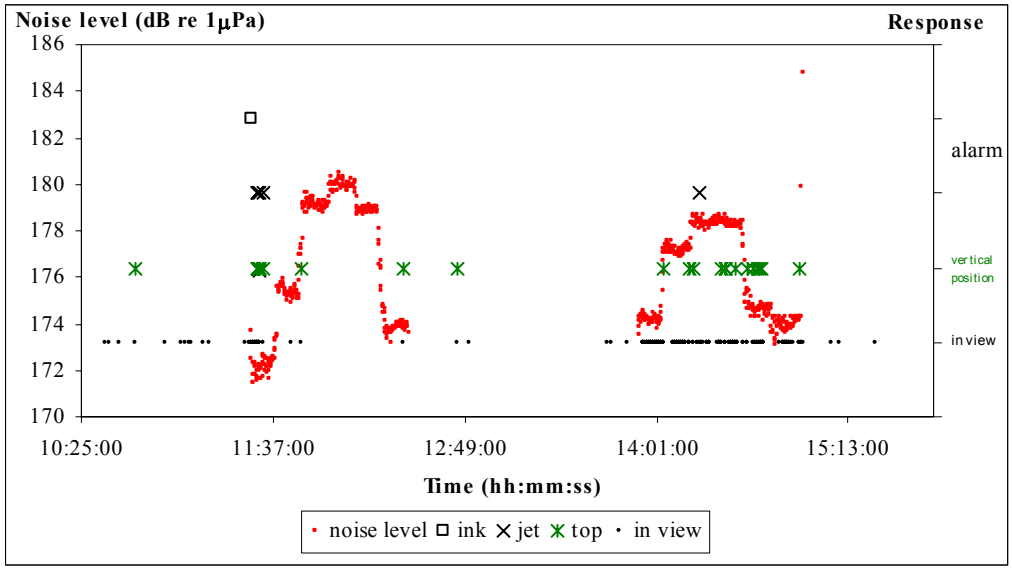

Figure 7.5: Behavioural responses of squid during trial 5. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

7.3.4 Exmouth - trials 8 and 9

During the Exmouth trials, the fish were constantly fighting a strong current (approximately 1.5 knots) which may have influenced behavioural responses. The data analysis for trials 8 and 9 was conducted on all species present in the sea cage, that is, species behaviours were not treated separately.

In trial 8 the fish species (and their numbers) present in the cage were as follows: black tipped cod (3), Chinaman cod (13), western butterfish (20 – 40) and silver streaked wrasse (15 – 20). The fish were exposed to 1:01:55 hours of air-gun noise that ranged between 129 – 182 with a mean level of 167 dB re 1 µPa.

Some observations to note are that in trial 8 all of the fish in view swam very fast from right to left in camera 1 and left to right in camera 2, that is away from the approaching air-gun at the first closest approach (174 dB re 1 µPa) and at the last closest approach (182 dB re 1 µPa) (Appendix 5, Fig. 8).

It also appeared as though the presence of the cameras could have altered the behaviours of the western butterfish as individuals from this species were continually observed approaching camera 2 throughout trial 8 (Fig. 7.6).

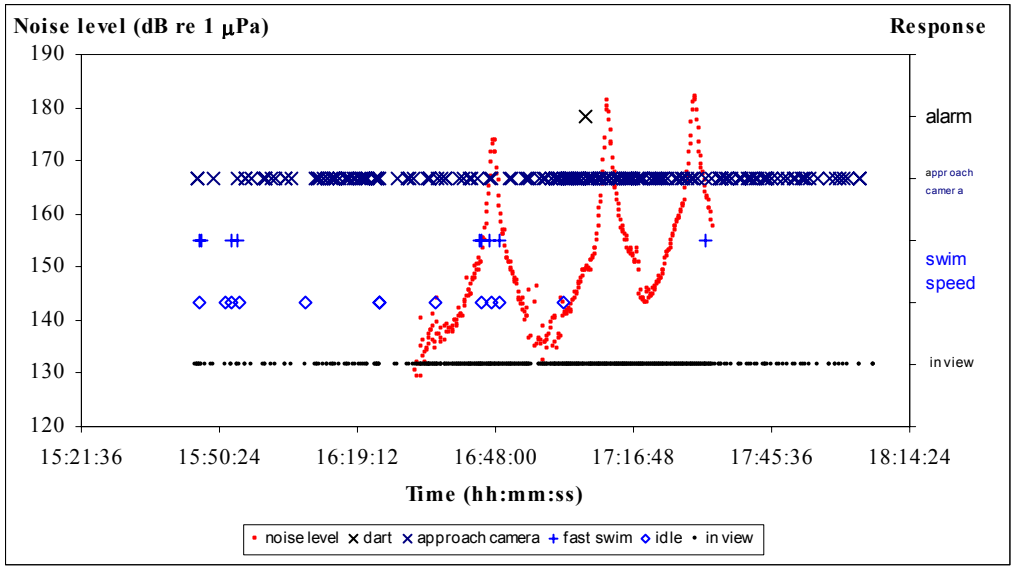

Figure 7.6: Behavioural responses of all fish species in trial 8. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

The species (and their numbers) present in trial 9 were: long finned rock cod (1), Chinaman rock cod (10), blue spotted emperor (3), stripey sea perch (10), western butterfish (20 – 40) and silver streaked wrasse (15 – 20). The air-gun operated for

0:34:04 hours with noise levels ranging between 139 – 178 dB re 1 µPa. The mean air-gun noise level was 169 dB re 1 µPa.

In trial 9 fast swimming away from the approaching air-gun was also observed (Appendix 5, Fig. 9). Also fish were observed 'darting' (extremely quick swim for a short period of time) at the first gun shot of trial 9 (149 dB re 1 µPa) and then again at the next two closest approaches (both 178 dB 1µPa) (Fig. 7.7).

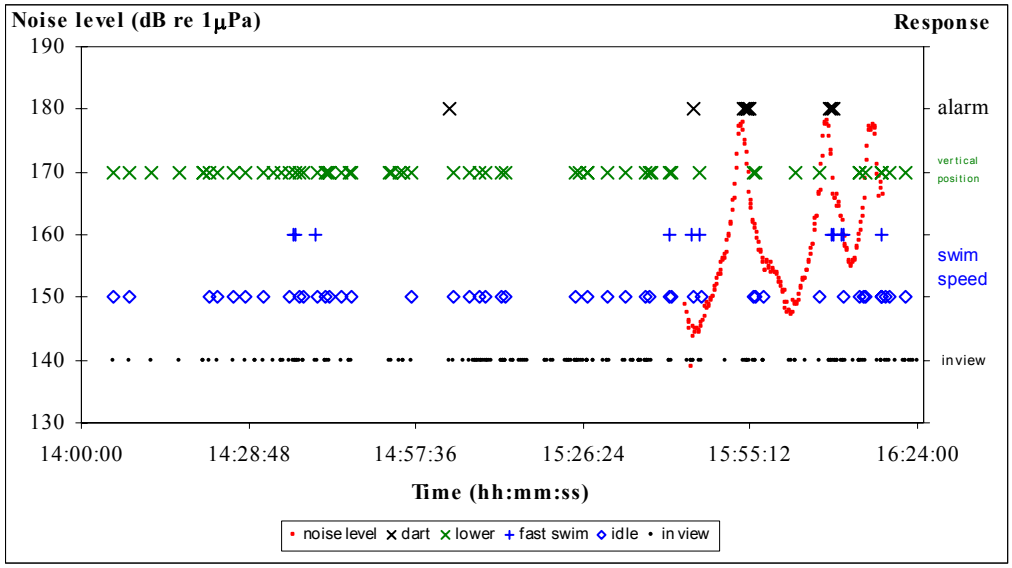

Figure 7.7: Behavioural responses of all fish species during trial 9. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

One count of an alarm response was observed pre air-gun exposure. The behavioural response index (see Equation 7.2) for the alarm responses was more apparent during air-gun operation, particularly at the highest noise levels. This relationship is examined in Figure 7.8.

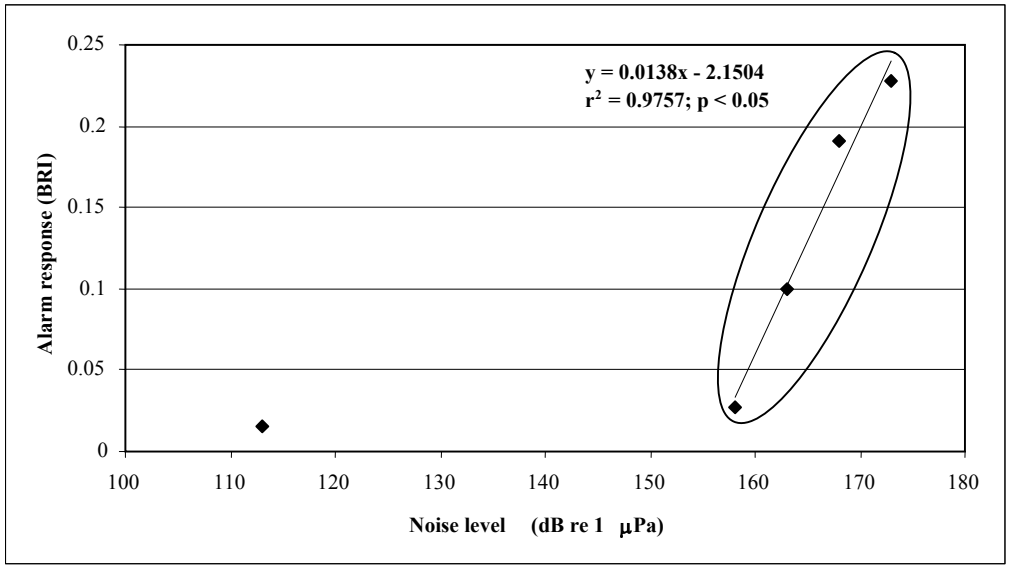

Figure 7.8: Behavioural response index (BRI) for alarm responses of all fish in trials 9 at specified noise levels. Noise level units are dB re 1 µPa mean squared pressure. The points used in the regression analysis are circled.

Little difference was observed in the frequency of alarm responses between the background noise and noise levels of approximately 158 dB re 1 µPa. As noise levels increased above 158 dB re 1 µPa, the frequency of alarm responses also increased. A positive linear correlation (r^2 = 0.98) was observed in the frequency of alarm responses with increasing noise levels (> 158 dB re 1 µPa).

7.3.5 Trial 10

Nineteen squid were present in the sea cage for trial 10. The squid were exposed to two periods of air-gun noise separated by 1:11:17 hours with no air-gun noise. During the first period of air-gun operation, the squid were exposed to air-gun noise levels in the range of 147 – 188 with a mean of 174 dB re 1 µPa. The first air-gun noise exposure was 0:46:57 hours in duration. The second period of exposure lasted for 0:22:04 hours with noise levels ranging between 155 – 188 with a mean of 177 dB re 1 µPa.

During trial 10 the squid displayed what appeared to be aggressive behaviour with much of the interest directed at camera 2 for 26 minutes, 13 minutes after the first period of air-gun noise exposure, and then at camera 1 (south side of cage),

particularly during a period of 7 minutes after the second air-gun exposure (Appendix 5, Fig.10). Colour changes (light to dark colouration) were also observed throughout the trial but particularly during the final hour. Also a white oval patch was clearly visible on the mantle of many of the squid at various times during the experiment (Appendix 5, Fig. 10). At the conclusion of trial 10, a mass of squid eggs were found attached to the moorings of camera 1.

No animals were observed ejecting ink as in trial 5 (Fig. 7.9). This trial involved a gradual increase in air-gun level, unlike trial 5 where the air-gun was started at 30 m from the cage.

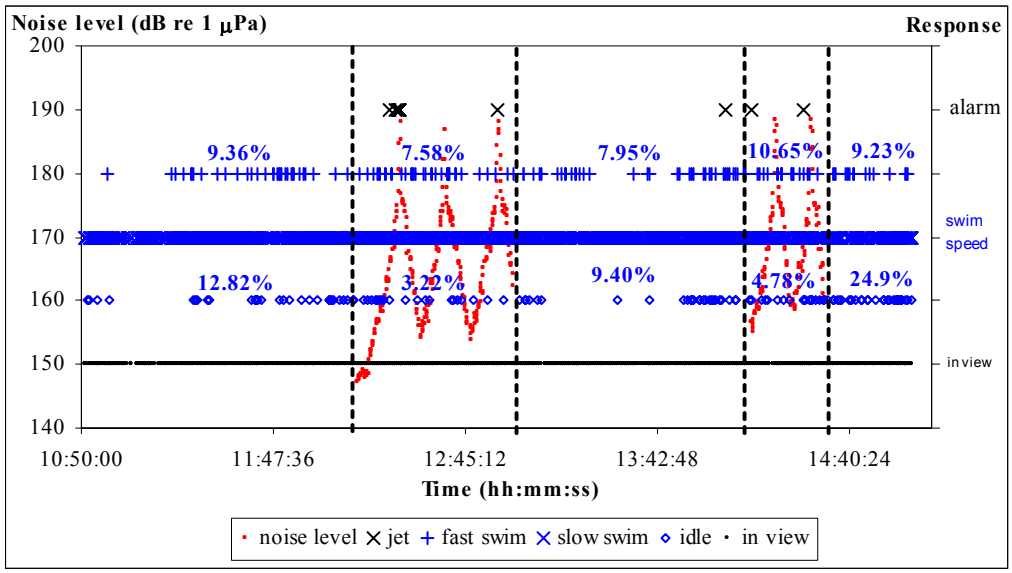

Figure 7.9: Behavioural responses of squid during trial 10. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras. The percentage time that the animals displayed the behaviour during each period is indicated (%).

Except for one event, alarm responses were only observed when the air-gun was in operation. An increase in the frequency of alarm responses (behavioural response index) as noise level increased above 158 dB re 1 µPa was observed (Fig. 7.10). When fitted with a second order polynomial regression model a coefficient of determination 0.96 ($y = 0.0122x^2 - 3.3127 + 224.16$) was obtained. Above noise

levels of 158 dB re 1 μPa the frequency of alarm responses increased exponentially with a coefficient of determination of 0.97 (Fig. 7.10).

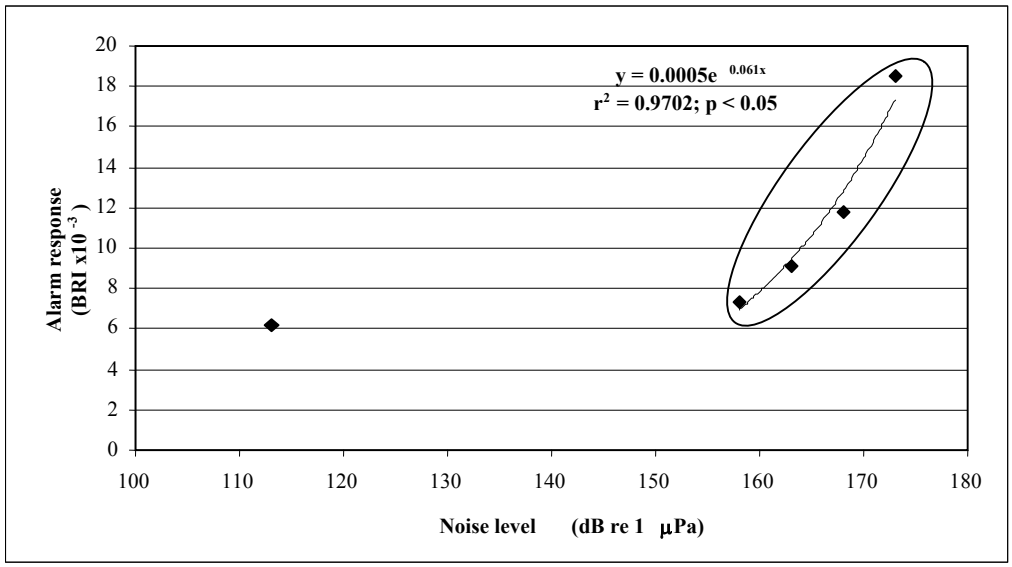

Figure 7.10: Behavioural response index (BRI) for alarm responses of squid in trial 10 at specified noise levels. Noise level units are dB re 1μPa mean squared pressure. Points included in regression analysis are circled.

The squid in trial 10 spent less time idling during periods of air-gun operation (Fig. 7.9) when compared to the time spent idling when the air-gun was not operating. There was no observed difference in swimming speed and vertical position in the water column between periods of air-gun exposure and no air-gun exposure. However, there was a general trend for the squid in trial 10 to increase their swimming speed above noise levels of 158 dB re 1 μPa and then slow their swimming speed or become idle at the surface during the most intense air-gun signals (Fig. 7.11).

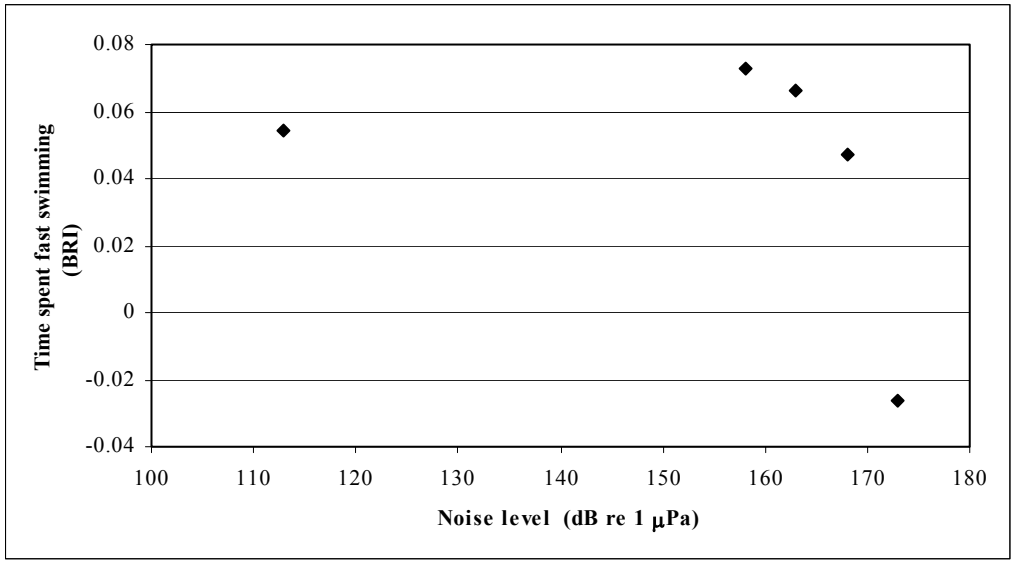

Figure 7.11: Behavioural response index (BRI) for time spent fast swimming by the squid in trial 10 at specified air-gun noise levels. Noise level units are dB re 1μPa mean squared pressure.

7.3.6 Trial 11

The same animals were used in trials 10 and 11. Therefore, the squid in trial 11 had been previously exposed to air-gun noise 5 days prior to trial 11. Trial 11 involved two periods of air-gun exposure separated by 1:12:04 hours. The first exposure was 0:46:37 hours in length with air-gun noise levels ranging from 156 – 190 dB re 1 μ Pa and a mean air-gun noise level of 176 re 1 μPa. The second exposure lasted for 0:39:12 hours with noise levels between 155 – 192 dB re 1 μ Pa and a mean air-gun noise level of 178 re 1 μPa.

In trial 11 the squid were observed 'fast swimming' more often during air-gun operation than they were when the air-gun was off (Fig. 7.12). It is also interesting to note that at each of the highest six levels of exposure either the jetting or flash expansion of school was observed (Fig. 7.12).

No animals were observed ejecting ink as in trial 5.

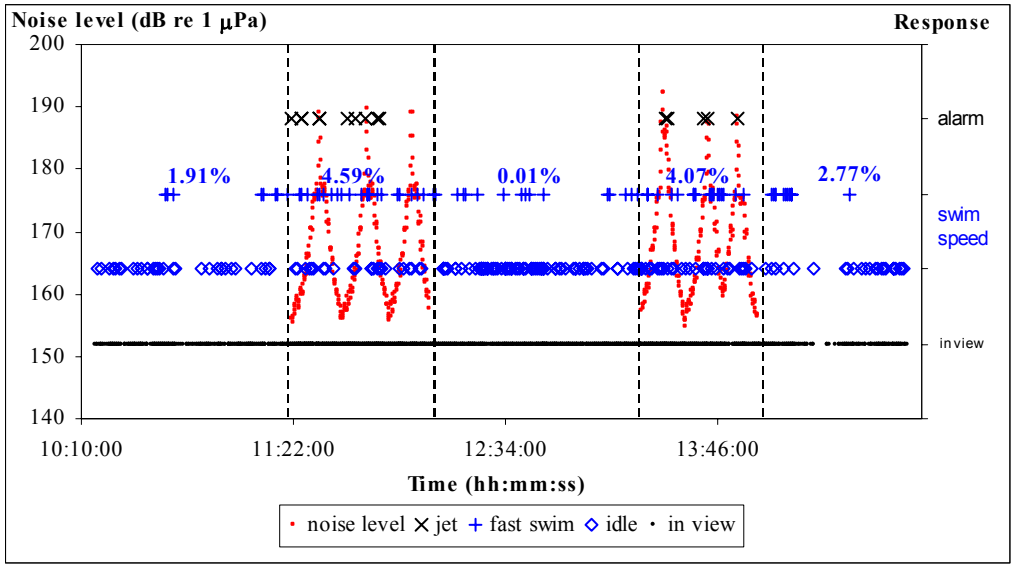

Figure 7.12: Behavioural responses of squid during trial 11. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras. The percentage time that the animals displayed the behaviour during each period is indicated (%).

The relationship between noise level and frequency of alarm response was examined using regression models (Fig. 7.13). Using a polynomial second order regression model, giving a coefficient of determination of 0.9996 ($y = 0.0107x^2 – 2.9072x + 200.11$). As noise levels exceeded 158 dB re 1 µPa the frequency of alarm responses increased with a linear relationship giving a coefficient of determination of 0.996 (Fig. 7.13).

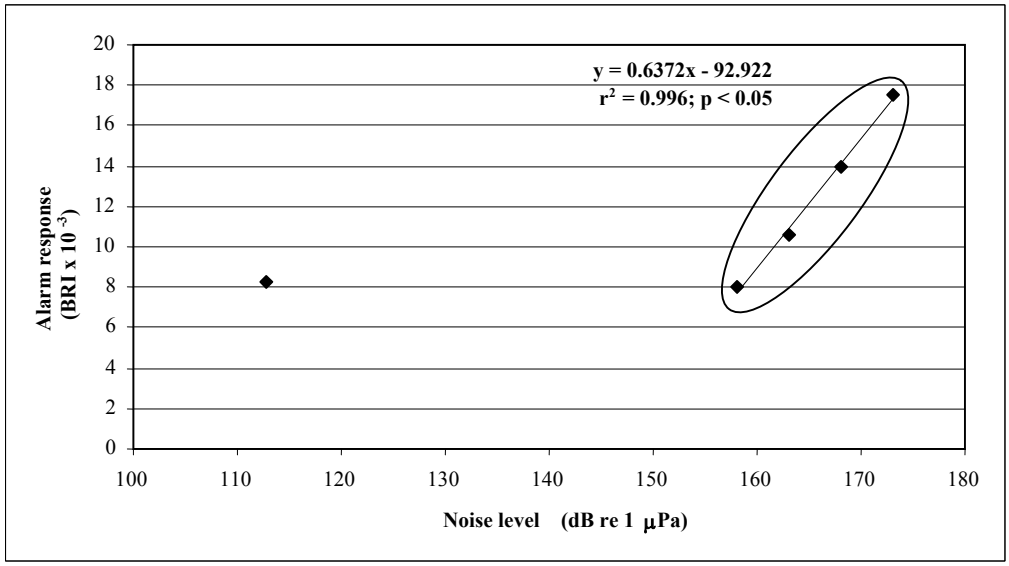

Figure 7.13: Behavioural response index (BRI) for number of alarm responses of squid in trial 11 at specified noise levels. Noise level units are dB re 1µPa mean squared pressure. Points included in regression analysis are circled.

The trend of the squid increasing swimming speed as the air-gun approached and then becoming idle at the highest noise intensities that was observed in trial 10 was also noted in trial 11 (Fig. 7.14).

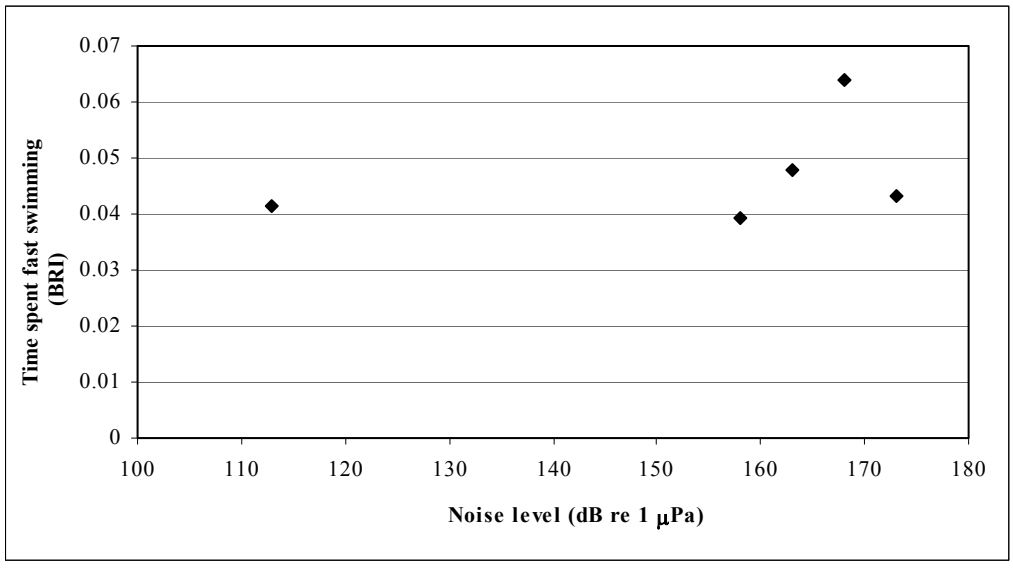

Figure 7.14: Behavioural response index (BRI) for time spent fast swimming by the squid in trial 11 at specified air-gun noise levels. Noise level units are dB re 1µPa mean squared pressure.

7.3.7 Trial 12

The species and their numbers in trial 12 were as follows: trevally (15), dhufish (1), cod (3), goatfish (2), wrasse (3). In trial 12 the noise exposure regime involved two periods of air-gun noise separated by 1:24:16 hours. The air-gun noise levels of the first period of air-gun noise ranged between 149 – 184 dB re 1 μPa with a mean level of 170 dB re 1 μPa. The first air-gun noise exposure was 0:55:57 hours in duration. The second period of air-gun noise was 0:41:57 hours in duration with air-gun noise levels ranging between 136 – 182 dB re 1 μ Pa and a mean level of 168 dB re 1 μPa.

Although five species were present in the cage for trial 12, only observations for trevally were recorded. During trial 12, the trevally were in view of the cameras for the majority of the air-gun exposure periods.

Trevally were observed to swim faster in tighter groups during air-gun exposure. They also changed direction more often during air-gun exposure, particularly during the first air-gun exposure period (Fig. 7.15).

'Darting' was observed only during air-gun operation (Fig. 7.15). During the first exposure more observations of the darting behaviour were recorded at times of higher noise levels (approximately 175 – 180 dB re 1 μPa) (Fig. 7.15). The first exposure resulted in more observations of darting behaviour than in the second exposure. Darting in the second exposure coincided with the commencement of shooting and then again at the higher intensities of noise of the first pass of the air-gun (Fig. 7.15)

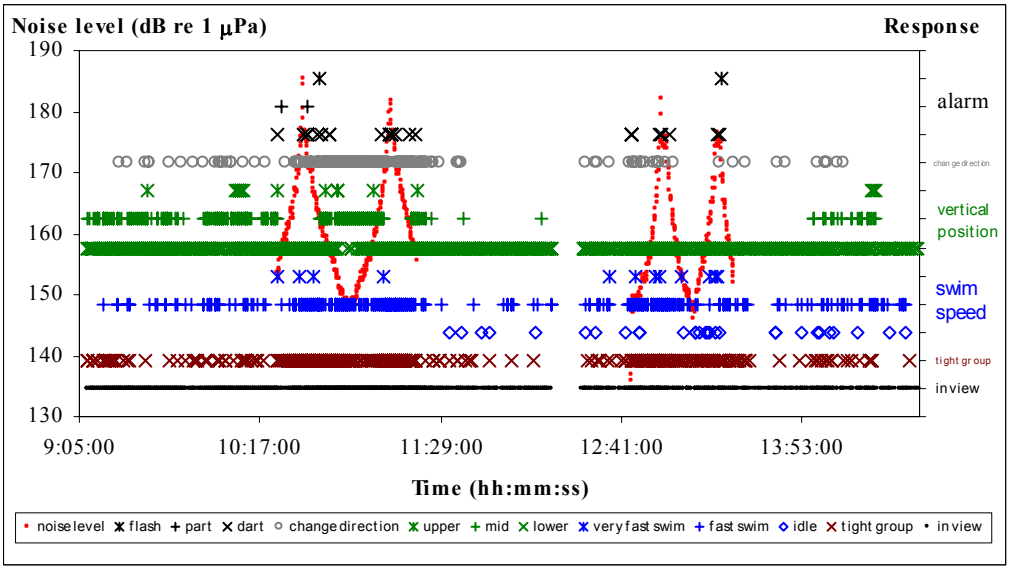

Figure 7.15: Behavioural responses of trevally during trial 12. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

The observations of the school of trevally exhibiting a breakdown in school structure with individuals swimming in all directions and then regrouping (i.e. flash expansion) prior to air-gun exposure (see Appendix 5, Fig. 12) coincided with the presence of divers in the cage. Therefore any data points before the exit of the diver from the cage were not included in the analysis of the results. Figure 7.16 displays the relationship between air-gun noise level and alarm responses of the trevally in trial 12. The number of alarm responses increased exponentially as noise level increased, with a coefficient of determination of 0.996 (Fig. 7.16). The rate of increase in the behavioural response index of alarm responses between 113 and 158 dB was relatively high when compared to other species that were used in this study.

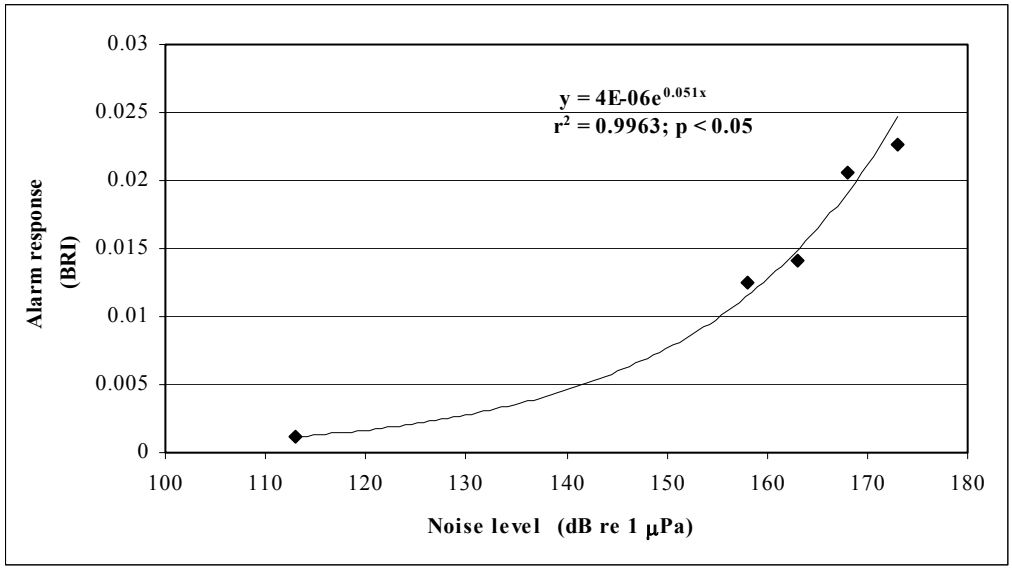

Figure 7.16: Behavioural response index (BRI) for number of alarm responses of fish in trial 12 at specified noise levels. Noise level units are dB re 1 µPa mean squared pressure. All points included in regression analysis.

An increase in the time that the trevally spent fast swimming in a tight group in the lower section of the cage was also observed as noise levels exceeded 158 dB re 1 µPa (Fig. 7.17).

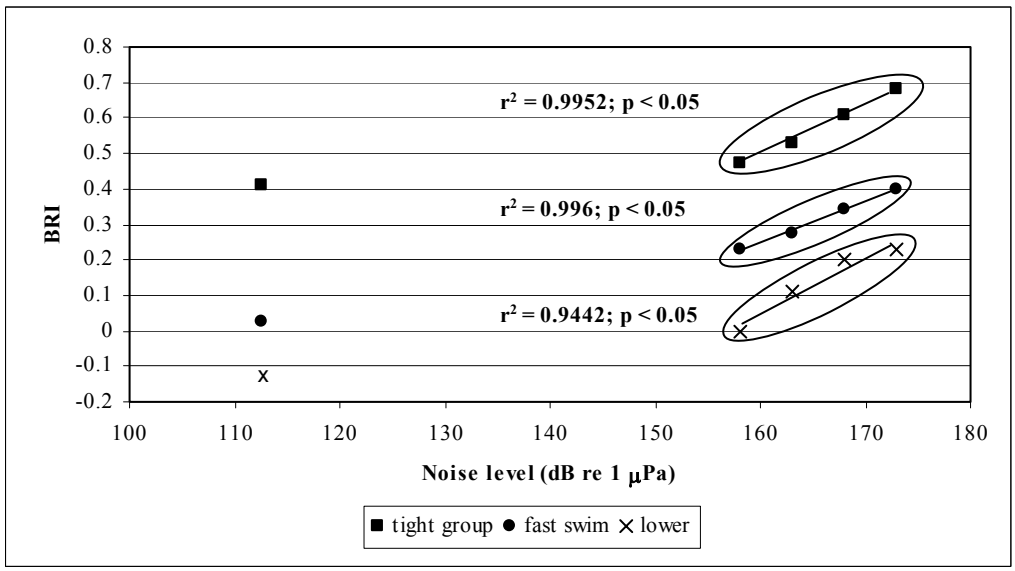

Figure 7.17: Behavioural response index (BRI) for time spent fast swimming in tight groups in the lower section of the cage by the trevally in trial 12 at specified air-gun noise levels. Noise level units are dB re 1µPa mean squared pressure. Points included in regression analysis are circled.

The relationship between the trevally fast swimming in tight groups in the lower section of the cage at noise levels above 158 dB re 1 μPa can be described with a linear relationship with coefficients of determination of 0.996 (y = 0.0116x – 1.6047), 0.995 (y = 0.0139x – 1.7267) and 0.9442 (y = 0.0155x – 2.4274) respectively.

7.3.8 Trial 13

In trial 13, fifty pink snapper were present in the sea cage. These fish were exposed to two periods of air-gun noise separated by 1:12:12 hours. The first period of air-gun noise lasted 1:05:05 hours with noise levels ranging between 144 – 191 dB re 1 μ Pa. The second period of air-gun noise was 0:36:21 hours in duration with air-gun noise levels ranging between 149 – 183 dB re 1 μ Pa. Both exposures resulted in a mean air-gun noise level of 172 dB re 1 μPa

The main observations to note in trial 13 were that on the onset of air-gun exposure (143 dB re 1μPa) the fish in view swam 'very fast' from the top to the bottom of the cage (Fig. 7.18). The fish then remained out of view of camera 1 for the remainder of the first air-gun exposure. However, during the first air-gun exposure the fish were occasionally in view of camera 2. Every time the fish were in view of camera 2 they were observed at the bottom of the cage. For most of the second exposure the fish were in view and were also observed at the bottom of the cage (Fig. 7.18).

Fish were observed swimming 'very fast' almost exclusively during air-gun operation. The only recording of the school of fish parting and then regrouping was during the second air-gun exposure (Fig 7.18).

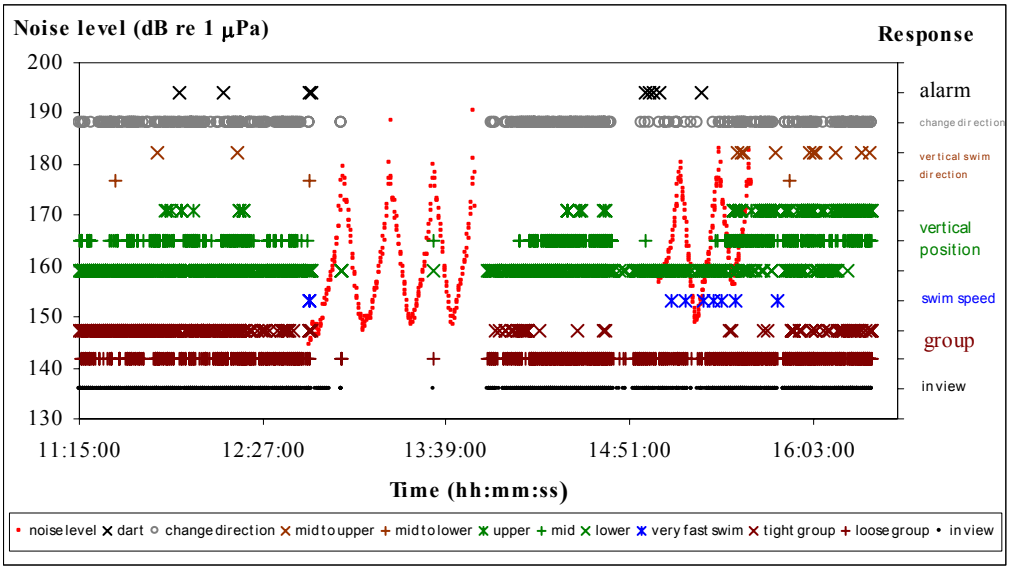

Figure 7.18: Behavioural response of pink snapper to air-gun noise in trial 13. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

The pattern of the fish swimming direction should also be noted. It appears that the fish were swimming around the perimeter of the cage and continually changing direction (Fig. 7.18).

7.3.9 Trial 14

In trial 14 the same fish were used as in trial 13, therefore the fish in trial 14 had been previously exposed to air-gun noise 58 days prior to trial 14. There were 32 pink snapper in the sea cage for trial 14. These fish were exposed to four periods (runs) of air-gun noise. Details of these exposures are outlined in Table 7.1.

Table 7.1: Details of air-gun noise exposure for trial 14. Noise levels are in dB re 1 µPa mean squared pressure.

Run	Noise level		Time of exposure (h:mm:ss)
	range	mean	
1	134 - 179	168	0:23:12
2	129 - 187	170	0:28:50
3	128 - 183	167	0:26:30
4	151 - 180	171	0:09:20

Fish were observed quickly parting and regrouping (flash expansion) during the second half of the trial just prior, during and after air-gun noise exposure (Fig. 7.19). There was no difference observed in the time spent between the different vertical positions of the cage prior, during or after air-gun exposure.

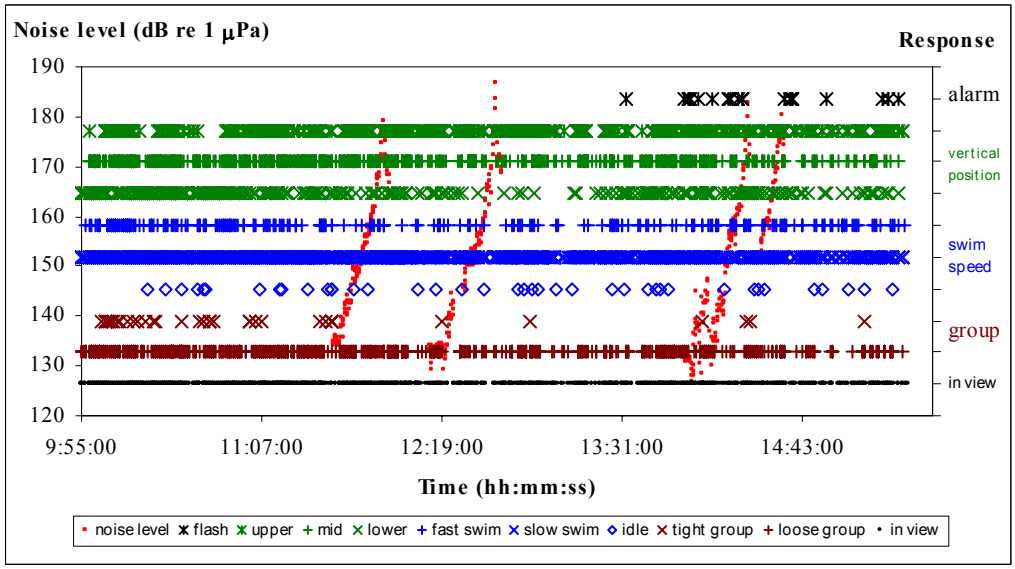

Figure 7.19: Behavioural response of pink snapper to air-gun noise in trial 14. Air-gun noise level units are dB re 1 µPa mean squared pressure. Data displayed are a result of summed data from both cameras.

There were no obvious correlations of the difference ratios of any observed behaviour which could be linked to air-gun noise levels. The behavioural results of this trial need to be considered in conjunction with the analysis of ear damage from their previous exposure in trial 13 (Chapter 6).

7.3.10 Behavioural generalisations

When taking the behavioural observations from all trials into consideration some general conclusions can be drawn about the behavioural changes induced by air-gun noise in this study.

7.3.10.1 Alarm responses

In the fish trials, comparisons of the frequency of alarm responses between the air-gun on and off periods indicated that there was an increase in alarm responses during exposure to air-gun noise (Figure 7.20).

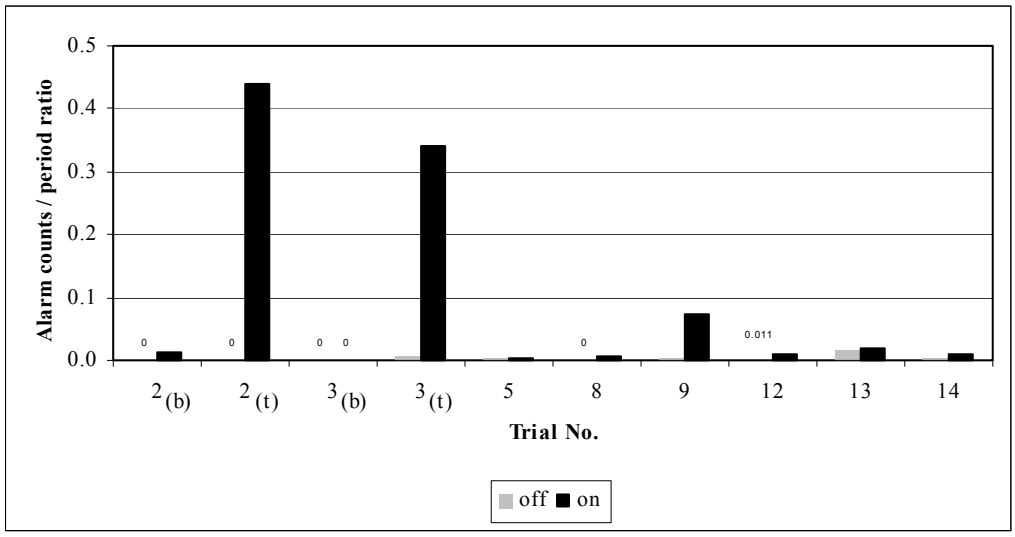

Figure 7.20: Frequency of alarm responses in fish for periods of no air-gun noise compared with periods of air-gun noise. Frequency is calculated as alarm responses per period divided by the total behavioural counts per period. For trials 2 and 3 the frequency of alarm responses is shown for two species (i.e. striped trumpeter (t) and silver bream (b))

This trend is significant ($p < 0.05$) when alarm counts per period were averaged across all trials. Species in some trials appear to have a higher occurrence of alarm responses during air-gun noise exposure, for example trials 2, 3 and 9. In other trials the results may have been affected by some factors. For example, in trial 8 fish were swimming against a strong current and in trial 13 the pink snapper were not in view during the first period of air-gun exposure and therefore no startle responses could be observed for this period. In trial 14 the experimental fish had been previously exposed to air-gun noise which could have affected their hearing and the alarm responses displayed.

When the data for frequency of alarm responses of the squid from trial 5, 10 and 11 are pooled, a significantly ($p < 0.05$) lower frequency of alarm responses is identified for the period when the air-gun was off (Fig. 7.21). The occurrence of alarm responses is also significantly ($p < 0.05$) lower during the second period of air-gun noise than during the first period (Fig. 7.21).

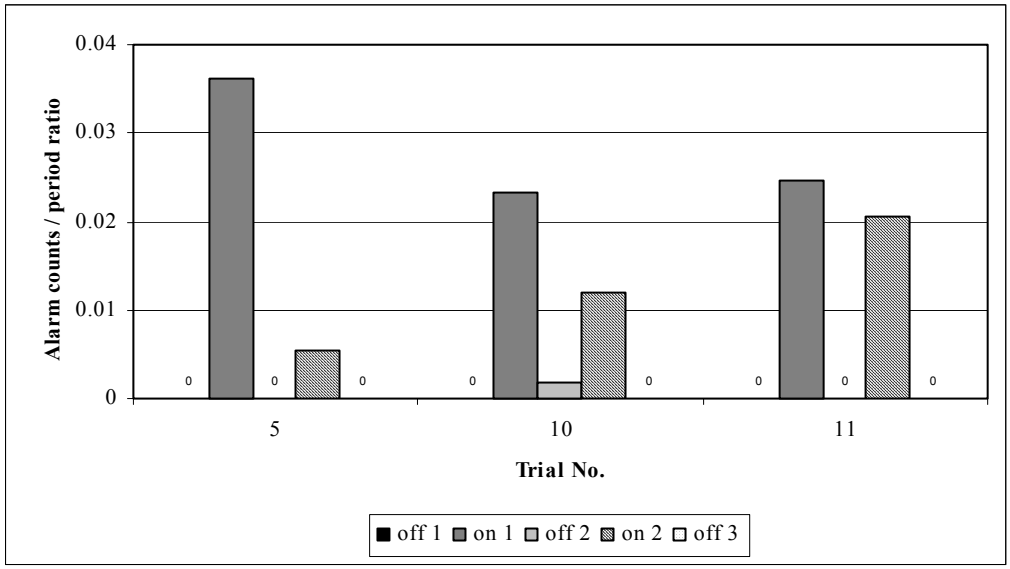

Figure 7.21: Frequency of alarm responses in squid (*Sepioteuthis australis)* for periods of no air-gun noise compared with periods of air-gun noise. Frequency is calculated as alarm responses per period divided by the total behavioural counts per period. Off and on refer to the air-gun status.

A general increase in the frequency of alarm responses in squid was observed when noise levels exceeded 158 - 163 dB re 1 μPa.

7.3.10.2 Swimming patterns and schooling behaviour

When the results for fast swimming and formation of tight groups for each species from trials 3, 12 and 14 at noise levels above 163 dB re 1 μPa were averaged, behavioural response indices of 0.215 ± 0.174 and 0.123 ± 0.100 respectively were obtained (Fig. 7.22). Therefore, there was a trend for experimental fish to swim faster and form tighter groups during exposure to air-gun noise above noise levels of 163 dB re 1 μPa. However, this difference was not statistically significant ($p > 0.05$). The fish in trial 8, 9 and 13 were excluded from the analysis as in trial 8 and 9 the fish were swimming against a strong current with some species taking refuge in the folds in the net and in trial 13 the fish were out of view for the majority of time during air-gun operations. The fish in trial 14 were included but may have been affected by air-gun exposure in trial 13.

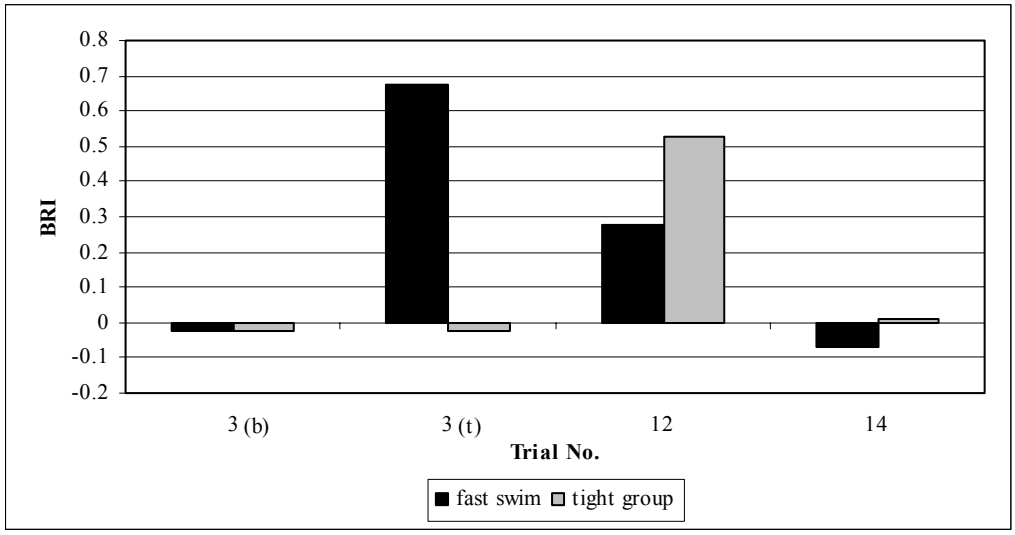

Figure 7.22: Behavioural response index (BRI) for proportion of time that the fish in trial 3, 12 and 14 spent fast swimming in a tight group. For trial 3 these behavioural responses are shown for two species (i.e. silver bream (b) and striped trumpeter (t))

The squid in trial 10 and 11 did show a trend to increase their swimming speed as the air-gun began approaching the cage and then when the noise level exceeded approximately 168 dB re 1 μPa the animals slowed their swimming speed and became idle.

7.3.10.3 Vertical position in water column

When the results for each fish trial (excluding trial 8, 9 and 13 for the reasons outlined above) were averaged, it was found that the fish spent significantly ($p < 0.05$) more time in the lower section of the cage during air-gun operation above 163 dB re 1 μPa compared to when the air-gun signal was below 163 dB re 1 μPa or was turned off, with a behavioural response index of 0.189 ± 0.047.

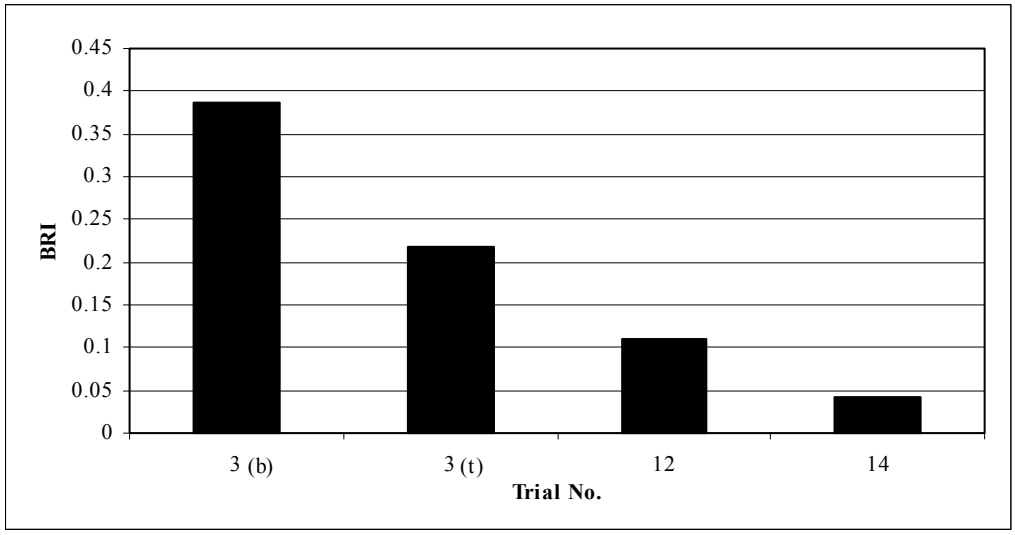

Figure 7.23: Behavioural response index (BRI) for proportion of time that the fish in trial 3, 12 and 14 spent in the lower 1/3 of the cage. For trial 3 the time spent in the lower portion of the cage is shown for two species (i.e. silver bream (b) and striped trumpeter (t))

From observations in the dinghy during the squid trials it was perceived that at the highest intensity noise the squid moved towards the top of the cage, to a depth of approximately 1 m. This observation was reinforced with the behavioural scoring from trial 5 but not in trials 10 and 11. The camera and dinghy observations from trial 10 and 11 confirmed that the squid were scattered in loose groups in the middle to top region of the cage.

7.3.10.4 Recovery

In trials 3, 12 and 13 the time taken for the fish to return to the vertical position that they occupied in the acclimation period could be calculated (Table 7.2). Likewise the fish in these trials spent most of the time during air-gun exposure out of view of the cameras and therefore the time taken to come back into view after the cessation of air-gun noise could be calculated (Table 7.2). The exception to this is the trevally in trial 12 which spent the majority of the duration of air-gun exposure in view of the cameras.

Table 7.2: Time taken for fish to return to the field of view after air-gun noise exposure and resume swimming in the same vertical position in the water column as before air-gun exposure.

Trial	Camera	Pass	Species	Return to view (minutes)	Resume 'normal' vertical position in cage (minutes)
3	2	1	silver bream	4	17
12	1	2	trevally	n/a	31
13	1	1	pink snapper	5	18
		2	pink snapper	4	11
13	2	1	pink snapper	9	29
		2	pink snapper	9	29

The results suggest that the fish in trial 3, 12 and 13 took between 4 and 31 minutes to return to normal behaviour.

7.4 Discussion

The behavioural observations in this study suggest that air-gun noise does result in alterations in fish and squid behaviour. The major findings on the effect that air-gun noise has on the behaviour of fish and squid were: habituation to air-gun noise; an increase in alarm responses during air-gun noise and as noise level increased; some alterations in swimming speeds; and changes in schooling behaviours.

Prior to discussing the results obtained it is important to mention that, as the animals in this study were held captive and two fish species (pink snapper and silver bream) were hatchery reared, the behavioural responses observed may not be the same as for unrestrained, wild fish in their natural environment. Evidence suggests that behavioural responses in fish of the same species will differ depending on whether the individuals were hatchery reared or wild caught (Woodward and Strange 1987; Knudsen et al. 1992).

The hatchery environment, in the majority of cases, is a noisy one. Pumps and air bubbling through air stones contribute significantly to underwater noise and are usually necessary in any hatchery (Bart et al. 2001). Therefore, it can be assumed

that hatchery reared fish would be accustomed and somewhat desensitised to high noise levels.

Hatchery reared fish would also be adapted to the captive environment and could be expected to exhibit different behaviour than wild fish (McDonald et al. 1998; Johnsson et al. 2001). Knudson et al. (1992) found that hatchery reared Atlantic salmon (*Salmo salar*) displayed a different avoidance response to noise than that of wild caught fish. The hatchery reared fish swam away to the point furthest from the noise whereas the wild caught fish swam to the deepest part of the tank, where structure existed on the bottom of the tank even though it was not the furthest point away from the noise. These behavioural differences can be explained by the differences in the two groups of fish's normal habitat. However, it is interesting to note that both groups of fish displayed avoidance reactions at sound levels of identical intensity and frequency.

Behavioural responses of the same species of fish can differ, depending on whether the animals are captive or unrestrained (Noakes and Bayus 1990). Understandably, it is not only being captive but also the lack of normal stimuli surrounding the animal that induces the differences in behavioural response. The presence of another species in a captive environment could also affect behaviour of certain species (Kelsey et al. 2002). In this study trials were often conducted on more than one species at a time and therefore it is possible that some behavioural alterations occurred as a result of inter species interactions.

The pink snapper and silver bream used in this study were hatchery reared fish while all other animals were wild caught using a variety of methods (Table 3.2). The majority of previous research that has been conducted on the effect of seismic survey noise on marine animals has concentrated on the behavioural responses of wild animals.

7.4.1 Fish

The types of behaviour observed in response to noise in the fish are similar to those reported by other researchers including: alarm and startle responses and changes in schooling patterns, position in the water column and swimming speeds. A correlation between behavioural responses and noise level was also demonstrated.

In this study changes in schooling behaviour and vertical position in most species was observed at 158 – 163 dB re 1 μPa. Specifically, at these noise levels the fish began to swim faster, in tighter groups and congregate at the bottom of the cage. Pearson et al. (1992) reported similar behaviours in captive rockfish (*Sebastes* spp.) at 180 dB re 1 μPa mean peak. Converting the mean square pressure values used in this study to mean peak values, using factors derived and outlined in McCauley et al. (2000), places the change in behaviours observed in this study at 168 – 173 dB re 1 μPa mean peak. This is lower than the noise levels required to induce alarm responses in the rockfish used in Pearson et al (1992). However, after extrapolation of their results it was suggested that 'subtle' changes in behaviour, such as the vertical position in water column, could occur at 161 dB re 1 μPa mean peak. The differences in results between studies could be caused by a number of factors such as differences in behavioural definitions, species and acclimation procedures (in Pearson et al. 1992 the experimental fish were wild caught and transferred into the cages one day prior to the trials) or because of the approach-depart air-gun regime used in the present study as opposed to the stationary air-gun used by Pearson et al. (1992).

Similar results have been reported in experiments using wild unrestrained fish being exposed to noise. Fish huddling in groups and swimming towards the lower part of the water column in response to approaching vessel noise (Olsen 1990) and air-gun noise are reported in the literature (Chapman and Hawkins 1969; Dalen and Raknes 1985; Dalen and Knutsen 1987).

Alarm behaviours in fish are common in response to noise and have been reported by many authors (Blaxter and Hoss 1981; Pearson et al. 1992; Wardle et al. 2001).

Sudden fast behavioural alterations such as startle responses and flash expansions of schools (classified as alarm responses in this study) and changes in schooling behaviour such as individuals forming a tight group or huddling are comparable with the behaviour of fish avoiding predators (Shaw 1975; Pitcher and Parish 1993; Godin 1997). It is thought that these cohesive groups confuse the potential predator by overloading the visual sensory channels induced by many moving targets (Milinksi 1990).

The startle response in fish is an involuntarily response mediated by the Mauthner cells, a bilateral pair of brain stem neurons (see section 2.4.3.1.1). Stimulation of a Mauthner cell induces a unilateral contraction of the muscles on the opposite side of the cell that is stimulated and causes bending of the fish into a 'C' shape, usually away from the stimulus (Diamond 1971). This reflex action puts the fish on a trajectory away from the predator and is followed by a period of fast swimming (Eaton and Hackett 1984).

Startle responses, characterised by the classic 'C' turn, were observed in the striped trumpeter and were persistent at noise levels of 170-183 dB re1 μPa mean squared pressure (182 – 195 dB re 1 μPa mean-peak). Pearson et al. (1992) observed startle responses in captive rockfish (*Sebastes* spp.) exposed to air-gun noise at a level of 200-205 dB re 1 μPa mean-peak. The differences in response thresholds between studies could be due to a number of factors. Species differences would be the main factor. Rockfish are a predominately bottom dwelling, territorial, predatory species (Carr 1991; Love et al. 2002) that tend to take refuge when startled, whereas striped trumpeter are a small shoaling pelagic fish that depend on evasion responses such as the startle response for protection (Godin 1997).

Wardle et al. (2001) exposed a small reef system to the noise from three 2.5 L air-guns at constant range. The reef was subjected to eight air-gun exposures over a four day period ranging from 17-86 minutes in length and with the air-gun signalling every 57-188 seconds. The reef was observed with underwater cameras and the movements of five individual fish were tracked with acoustic tags that were attached

to fish that had been caught from the reef and then released prior to the trials. The only significant behavioural change observed was the occurrence of startle responses ('C' turns) displayed by all fish swimming in view of the camera at noise levels of 195-219 dB re 1 μPa peak levels. These levels are consistent with the noise levels found to induce startle responses in the current study (after conversion from mean square pressure to peak levels).

Considering the results of the trials outlined in this document and the behaviours reported in other studies, it is surprising that no significant avoidance responses were observed in Wardle et al. (2001). A significant factor that could have contributed to this result is that the reef system exposed consisted mainly of resident species. The results may have been different for fish not territorial to a specific area. The air-gun being stationary and the longer time elapsed between signals may have also influenced the behavioural reactions of the fish.

In Wardle et al. (2001) the air-gun was fired once per minute. In other studies, and in real seismic surveys, the air-gun/s usually fire every 5-15 seconds (McCauley 1994). The time lapsed between signals may be an important factor in the behavioural response of fish to noise. Also, the stationary air-gun provided no approaching danger signals to the animals inhabiting the reef. Wardle et al. (2001) suggested that this could have also contributed to the lack of directional responses to the noise displayed by the fish. During a real seismic survey the air-guns would be moving and, given the nature of underwater sound transmission, at some point would rapidly begin to increase. The noise of the seismic vessel would also provide a continuous noise that could be used by the surrounding animals to process the direction of the noise source.

Although the same fish were used in trials 13 and 14 each trial resulted in different behavioural responses to air-gun noise. In trial 13 the fish swam to the bottom of the cage where they remained for the duration of noise exposure. In trial 14 the fish did not display a preference for any portion of the cage. There are several possible explanations for this difference in behaviour. For example, the fish may have become

habituated to the noise from trial 13 and did not associate the noise with danger. Also, in trial 14 a different air-gun noise regime was used, with air-gun noise levels beginning 10 dB lower that in trial 13 which may have resulted in the fish becoming habituated to the noise before higher noise levels were reached. Alternatively, the damage that resulted to the ears of the fish in trial 13 compromised hearing ability. Finally, it is possible that the longer acclimation time to the cage (i.e. 70 days) by the time trial 14 had begun, had an effect on fish behaviour.

Fifty eight days separated trial 13 and 14 and, although studies have suggested that fish are capable of long term memory, it is unlikely that the fish would remember the noise without some sort of association, for example with pain or food reward (Gleitman and Rozin 1971). If pain was associated with the air-gun noise then a more pronounced behavioural response would be expected as would a physiological stress response (Schreck 1990b). The acclimation time for the fish in trial 13 was 24 days. This time period is generally accepted as long enough for fish to become acclimated to a new environment, especially as the pink snapper had been reared in a cage environment (Pottinger and Pickering 1992). Therefore, damage to the ears, resulting in altered hearing capabilities is more likely to be responsible for the lack of altered behavioural response (see Chapter 6). However, startle responses and flash expansions were observed in trial 14 in response to the noise. Therefore, it can be assumed that the damage to the ears did not prevent all fish from sensing the air-gun noise at high levels.

The calculation of the recovery time for trials 3, 12 and 13 suggest that the fish return to normal behavioural patterns soon after the cessation of air-gun noise. A quick recovery time has also been reported in the majority of the literature covering studies that have included investigating the behavioural recovery time after exposure to air-gun noise (Skalski et al. 1992; Lokkeborg and Soldal 1993).

As stated above, the behavioural responses observed in this study do not provide conclusive evidence for the effects that air-gun noise from seismic surveys may have on the behaviour of wild fish. However, the consistency between the behaviours

induced by air-gun noise in this study and in other reports suggests that to some level we can predict the behavioural response of fish to air-gun noise.

7.4.2 Squid

The behaviours observed in the squid in response to air-gun exposure could be classified into the same categories as the observed fish behaviour. That is, alarm responses, changes in swimming patterns and vertical position. The response of squid to air-gun noise has not been previously reported in the literature.

The squid in this study were wild caught from the same area as the experimental site. They appeared to readily adapt to captivity and within 4 days associated boat noise with feeding and were observed at the surface of the cage when the dinghy approached the cage.

The squid in trial 5 were observed ejecting ink at the first air-gun signal. The primary function of this response in squid is thought to be predator evasion (Hanlon and Messenger 1996b). The dense cloud of ink can either act as a facade or decoy (pseusdomorphs). Evidence suggests that, as in many cases squid ink contains L-DOPA and dopamine, which are both molecules that act as olfactory stimuli, it may also act as an alarm substance (Lucero et al. 1994). Subsequent to ejecting the ink the animals were observed jetting away from the direction of the air-gun. Jetting in squid is a known escape response usually mediated by the 'giant fibres' (Otis and Gilly 1990; Wells and O'dor 1991; Hanlon and Messenger 1996b). It is assumed that, if the squid had not been held captive, they would have fled the area.

The ejection of ink was not observed in trial 10 or 11. The first air-gun signal in trial 5 was received at the cage at 174 dB re 1 μPa whereas in trial 10 and 11 the air-gun was started further away from the cage and therefore the signal received by the squid was lower at the beginning of the trial that is, 147 dB re 1 μPa and 156 dB re 1 μPa respectively. However, the intensity of the air-gun signal did exceed 174 dB re 1 μPa in both trial 10 and 11 but the squid did not display the inking behaviour. Although this result is only preliminary, it would appear that the responses displayed by the

squid are somewhat dependant on the animals becoming accustomed to the noise at low levels. This effect has been reported in fish and marine mammals (Blaxter and Hoss 1981; McCauley 1994). Blaxter et al. (1981) found that exposing herring (*Clupea harengus*) to a sound signal that took many cycles to reach maximum amplitude increased the threshold for the sound to induce a startle response. It is interesting to note that in trial 5, although the noise level did exceed 174 dB re 1 μPa as the air-gun approached the cage, the inking response was not observed again. The short rise time that Blaxter et al. (1981) suggested was necessary to elicit a startle response would have become shorter as the air-gun came closer to the cage. Either the squid had depleted their ink reserves or it was the habituation to the noise that reduced the startle response. Habituation and sensitisation have been reported in squid (Long et al. 1989) and the pooled results from all squid trials indicate a significant decrease in alarm response in the second exposure to air-gun noise when compared with the first exposure.

Another point that should be mentioned is that in Trial 5 other species were present in the cage, whereas in trial 10 and 11 squid were the only animals present. It is possible that the presence of other animals altered the behaviour of the squid.

In trial 10 the squid were observed displaying what was assumed to be aggressive behaviour towards each other and towards camera 1. At the conclusion of the trial a mass of squid eggs were observed on camera 1. According to Hanlon and Messenger (1996c) the behaviour that was witnessed is classic squid spawning behaviour. The agonistic reactions observed are normal when spawning. The white patch observed on the mantle of several individuals is a result of the neurally controlled chromatophores accentuating the oviducal gland in females and the testis in males and is common in other species of squid during courting rituals, in males and sometimes in females (Boal and Gonzalez 1998). *Sepioteuthis australis* are known to spawn in sea grass meadows, attaching their eggs to blades of seagrass (Moltschaniwskyj and Pecl 2003). Therefore, it is possible that it was the presence of structure that is, the camera, in the cage that stimulated them to spawn rather than being a response induced by the air-gun noise. However, in some animals,

particularly invertebrates, exposure to a stressor stimulates reproductive behaviour (Braley 1989; Pattipeiluhu and Melatunan 1998; Battaglene et al. 2002). Although the reproductive behaviour of *Sepioteuthis australis* is not well documented, it is known that this species of squid usually spawns at night (Edgar 1997) whereas this event occurred during the middle of the day.

The reproductive behaviour observed in trial 10 almost certainly would have affected the scoring of particular behaviours. Behaviours such as vertical position and swimming patterns in particular could have been biased.

In general, the only significant behavioural alteration that the squid displayed in response to air-gun noise was the frequency of alarm responses, particularly at higher noise levels. However, there was a trend for the squid to increase swimming speed as the air-gun approached and then remain idle towards the water surface as the air-gun signal became most intense. There could be a number of different explanations for this behaviour. One is that the animals were 'aware' of the approximately 12 dB difference in noise levels at the waters surface (Table 4.1) compared to levels at depth and therefore remained at the surface while the air-gun signals were most intense. Becoming motionless is a common component of crypsis, a behaviour that squid are renowned for when threatened (Hanlon and Messenger 1996b; Smith 1997).

Another explanation is that the squid 'heard' the approaching dinghy, to which the air-gun was attached, and came to the surface expecting to be fed. As mentioned before, the squid quickly learned to associate the dinghy with being fed and would rise to the surface, approach the dinghy and remain idle until food was thrown into the cage. Almost identical behaviour (the squid did not approach the dinghy while the air-gun was operating) was observed when the air-gun signals were most intense and therefore, when the dinghy was closest to the cage. This explanation is supported by the squid coming to the surface for longer during the second period of air-gun noise exposure than the first in trial 5 (Fig. 7.5) suggesting habituation to the noise resulting from the first period of exposure. If the second explanation is correct then

this could indicate that the air-gun noise did not severely affect the 'hearing' threshold of the squid.

Although some interesting behaviours were observed in the squid in response to air-gun noise in this study, the results are preliminary. From the results it would appear that noise levels greater than 158 dB re 1μPa are required to induce avoidance behaviour in this species. The results also suggest that a ramped air-gun signal and prior exposure to air-gun noise decreases the severity of the alarm responses in this species.

Chapter 8

General Discussion

8.0 GENERAL DISCUSSION

8.1 Introduction

In previous chapters the responses of experimental animals to air-gun noise have been segregated into three areas, that is, behaviour, physiology and morphology and have been discussed in isolation. In reality, each of these responses has the ability to influence the other and to produce some cumulative effects. Also, the noise regimes used in this study are scaled in magnitude to what would be experienced from a real seismic survey. The aim of this chapter is to assemble the results from the entire study, consider the implications of the observed affects in a natural environment and relate them to a real seismic survey.

8.2 Implications of findings

The results of this study have shown that the air-gun noise produced by a seismic survey does have a significant effect on surrounding fish and squid. The implications of these findings are outlined below.

8.2.1 Fish

The majority of studies on the effects of seismic survey noise on fish have been brought about by concern that behaviour altered by seismic survey noise may affect the commercial fishing industry (Holliday et al. 1987; Pearson et al. 1987; Pearson et al. 1992; Skalski et al. 1992; Engas et al. 1993; Lokkeborg and Soldal 1993). Existing research suggests that the noise created by a seismic survey affects catch rates by either inducing the target fish to swim to a lower portion of the water column, out of reach from the trawling nets or induce them to flee from the fishing area. With long line fisheries the noise is also thought to prevent the fish from taking baited hooks. The results of the current study support these previous findings.

However, Wardle et al. (2001) exposed a reef system consisting of a resident fish population to air-gun noise and observed no evidence of fish fleeing the area. Similarly, Pickett et al. (1994) observed no changes in the movement, catch rates or distribution of local bass populations in the vicinity of a seismic survey. These are

examples of how careful one must be when extrapolating the responses observed in one species to another and between seismic surveys of varying characteristics.

It is important to note that if resident fish do not vacate an area to avoid air-gun noise, it does not necessarily indicate that the noise is not affecting the fish. In the current study damage to the ears of fish induced by air-gun noise was found in the absence of a detectable stress response. This suggests that pathological damage can occur to the fish without causing significant discomfort (Schreck 1981). While transient species may flee from the air-gun noise, and therefore avoid pathological damage, territorial species or species that have a reason to stay in the exposed area, for example a spawning aggregation or feeding school, may not be in enough discomfort to move away from the noise. Therefore they may expose themselves to potential ear damage.

Ear damage or habituation to the air-gun noise could be responsible for the difference in behavioural response between the pink snapper in trial 13 and 14. Subsequent to trial 13 and up to trial 14 these fish were feeding and appeared to be interacting normally. However, the captive environment limits the amount of information that can be gained on the real effects of these alterations in the species in terms of fitness in their natural environment. In a real situation evasive behaviours have evolved to protect the animals and therefore, fish with altered evasive behaviours could be at risk of predation or other dangers (Godin 1997). If their lack of response in trial 14 was due to impaired hearing then these fish could also ignore acoustic environmental cues required for survival.

Startle and alarm responses are consistently reported in fish in response to noise (Blaxter and Hoss 1981; Pearson et al. 1992; Wardle et al. 2001). In the captive environment of this study they were observed repeatedly. However, in a real environment it is likely that severe startle / alarm responses would only occur once or twice and then, if possible, the fish would swim to a sufficient distance from the noise source where startle responses no longer occur. Energy expenditure associated with these behaviours could be an issue (Godin 1997). The amount of energy used to

avoid the noise from a seismic survey would be species specific and would also depend on the characteristics of the seismic survey. For example, territorial fish may not swim far away from an area but the energy expended moving away and then back to an area may be quite high. Likewise, surveys that cover a large area may force some fish to swim long distances. The energy used in avoiding a seismic survey could result in less available energy for essential processes such as hunting, evading predators and reproduction.

The curious nature of some fish was seen in this study when a foreign object, that is, the camera, was placed into their environment. Although it is unlikely that this would cause large scale consequences, fish 'investigating' the air-guns when firing begins could experience severe effects, in particular, ear damage.

The length of a seismic survey would have a bearing on the effect on the surrounding environment. Although physiological stress responses were not observed in this study, total noise exposure in each trial never exceeded two hours whereas an actual seismic survey lasts for several days at a minimum, but usually weeks (McCauley 1994). According to the results of this study, it is likely that fish that leave the area when noise reaches a certain level and, therefore avoid the noise, would not experience an increase in physiological stress responses. However, species that have reason to stay in the area under exploration, and therefore, are not inclined to leave the area, would be exposed to the noise for long periods of time. This is perhaps when a detectable physiological response would occur. If this was the case then the effect on the individual would be dependant on the duration of the stress response. It is well documented that chronic stressors can have detrimental effects on fish well being (Pickering 1981). While prolonged stress is known to reduce the immunocompetence, growth and reproductive capacity of fish it can also affect behaviour. It has been reported that stressed fish will take longer to learn and will take longer to avoid danger (Sigismondi and Weber 1988; Olla et al. 1995; Schreck et al. 1997). If aggregating fish species are present in the area then whole fish populations could be put at risk.

The effects of air-gun noise on directly exposed species have been reported in this study. However, the alterations that occurred to these species would undoubtedly have an effect on other species within the ecosystem. Lokkeborg and Soldal (1993) reported that, while long line and trawl catches of cod (*Gadus morhua*) decreased after exposure to noise from an actual seismic survey, the catch of prawns, the natural prey of the cod, increased. Likewise, Engas et al. (1996) observed a greater reduction in the number of large fish than that of small fish in an area exposed to a 5 day seismic survey. A number of explanations for this change in distribution have been put forward, for example, different swimming speeds, differing hearing ability and habituation rates. Whatever the reason, the altered distribution could have significant effects on the entire ecosystem.

The majority of studies that have included an investigation into the recovery time of fish in an area exposed to seismic survey noise have observed a quick recovery (Skalski et al. 1992; Lokkeborg and Soldal 1993). However, Engas et al. (1996) reported that the abundance and catch rates of the cod did not return to pre-seismic survey levels in the five day period following the seismic survey. From observations in the current study it appears that fish return to normal behavioural patterns soon after the cessation of air-gun noise exposure however, no recovery from the ear damage was observed up to 58 days after exposure. It is unknown if these fish would have survived in their natural environment.

8.2.2 Squid

The results of this study suggest that the response of squid to air-gun noise is variable. As alarm responses were observed at approximately 158 dB re 1μPa, it would appear that squid would display avoidance behaviours once seismic survey noise exceeded this level. However, although alarm responses were still observed at higher levels (170 – 180 dB re 1μPa), the swimming speed generally decreased.

These results are difficult to interpret. The squid were held captive which may have influenced their avoidance behaviour. However, even if squid do not avoid air-gun noise in their natural environment by fleeing, the results suggest that alarm responses

(jetting) would still be apparent. As with fish, the expenditure of energy associated with repeated alarm responses could affect the fitness of squid.

In practice seismic survey operators usually begin the survey by turning on each gun separately, starting with the gun of lowest chamber volume (ramping) (McCauley 1994; APPEA 1996). This technique is employed to 'warn' animals in the area that may be sensitive to the noise and give them the opportunity to leave the vicinity prior to the noise from the air-gun array reaching full intensity. Observations from this study suggest that this practice would be of particular benefit to squid.

Although the time required to return to normal behaviours after exposure to air-gun noise is not known, the squid in this study were fed and ate immediately after the cessation of air-gun noise which suggests a rapid recovery.

Squid form an important component of most marine food chains, both as predator and prey (Gales et al. 1993; Hanlon and Messenger 1996a). Although air-gun noise appeared to have had little consistent effect on the behaviour of the squid in this study, little is known about the stress response of squid. If the reproductive behaviour observed in trial 10 was in response to stress induced by the air-gun noise then seismic survey noise could induce these animals to lay their eggs in less than optimum conditions and therefore affect the survival of their young and future generations.

As with fish it is important to note that it is likely that different species of squid will respond differently to seismic survey noise. Their response is also likely to be dependant on their stage of life. As seen in this and other studies, food appears to be a powerful stimulus to these animals (Hanlon et al. 1987; Hanlon and Messenger 1996a). Therefore, the presence of food in an area could override the stimulus to leave an area affected by seismic survey noise.

8.3 Zones of effect

Zones of effect have been used by researchers and are useful to define the distance at which particular impacts of seismic survey noise on the surrounding marine life will occur (Malme et al. 1989; Erbe and Farmer 2000). Relevant zones are thought to be the distance at which (McCauley 1994):

i) the noise is audible to the surrounding animals;
ii) other natural noises of the surrounding area can be masked;
iii) significant behavioural responses occur;
iv) animals begin to exhibit avoidance tactics;
v) pathological and lethal effects occur.

Some of these effects were observed in this study. In section 8.3.1, where possible, the range of each zone is defined according to the results of this study.

8.3.1 Guideline zones of effect from this study

The results of this study have contributed to the existing knowledge on the effects of seismic survey noise on marine animals and can be used as guidelines for zones of effects. A point that must be reiterated is that the animals in this study were held captive and some were hatchery reared. Therefore, care must be taken when applying the zones of effect outlined below to wild stocks.

Figures 8.1 and 8.2 display the observed effects of air-gun noise on the fish and squid used in this study. The noise level known to cause the observed effects is indicated. The distances at which these noise levels could be expected from a real seismic survey air-gun array are also indicated. These distances are based on measurements taken from a 2678 cui (44 L) air-gun array at a depth of 120 m and calculated for a receiver at a depth of 32 m (see McCauley et al. (2000) for details). It must the emphasised that the figures are indicative only. As mentioned above the zones of effect will vary according to the nature of the seismic survey, the area to be explored and the species present in the vicinity.

8.3.1.1 Fish

The zone of audibility for the fish species used in this study is not known. However, the lowest air-gun noise level to which the fish in this study were exposed was 128 dB re 1 μPa (10 – 1000 Hz) which is well above hearing thresholds for fish with known hearing capabilities (Popper and Fay 1993). Therefore it is assumed that all air-gun signals used in this study could be 'heard' by the experimental fish.

The zone at which altered behavioural responses were detected for most fish species in this study was at approximately 158 – 163 dB re 1 μPa or at 2.1 – 5 km distance from a 2678 cui array (Fig. 8.1).

Consistent startle responses were observed in striped trumpeter at noise levels of 167 – 181 dB re 1 μPa or at 0.65 – 2 km from an actual seismic survey air-gun array. Alarm responses such as flash expansion, parting and darting in other species of fish became more frequent at noise levels above 170 dB re 1μPa. Startle / alarm responses are usually associated with the zone of avoidance (Godin 1997). However, as the animals were held captive the zone of avoidance is a point of conjecture. In most fish species it is likely that the inner boundary of the zone of avoidance would be at noise levels lower than required to produce a startle response.

Although damage to the saccular maculae was found in pink snapper exposed to air-gun noise, the actual characteristic of the noise regime required to produce this damage was not determined. Therefore, the only conclusion that can be made about the zone of pathological damage is that it occurs during 1 hour 40 minutes and 37 seconds (Table 3.1) of air-gun noise exposure at levels of 144 – 191 dB re 1 μPa (10 – 1000 Hz).

No mortality due to air-gun noise exposure was observed in this study. Therefore it can be assumed that if lethal effects can be induced by seismic surveys the outer boundary for the zone of lethal effects is closer that 0.2 km from a 2678 cui air-gun array.

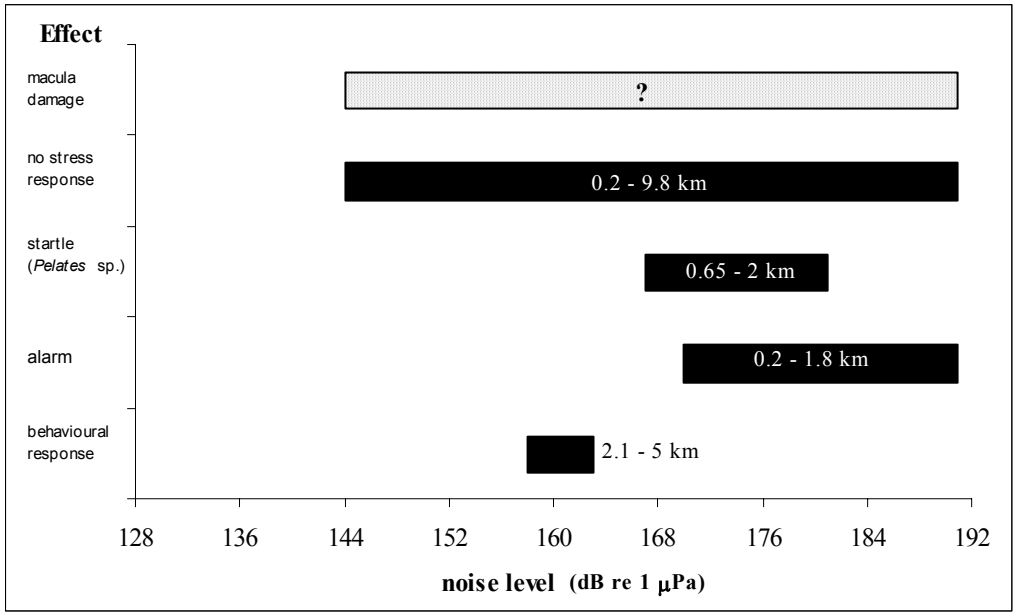

Figure 8.1: Guideline 'zones of effect' for the captive fish in this study. Estimated zones are based on measurements from a 2678 cui air-gun array at a depth of 120 m for a receiver at 32 m. The stippled bar for macula damage indicates that the precise nature of noise to cause observed damage is not known. Noise level units are dB re 1 µPa mean squared pressure.

8.3.1.2 Squid

As with the fish, the zone of audibility for the squid used in this study can not be determined from the results. However, it can be assumed that the squid used in this study were able to detect air-gun noise at approximately 158 dB re 1 µPa or at a distance of 2.1 km from a 2678 cui air-gun array, as it was at this noise level that significant changes in behaviour were observed.

The zone of behavioural response and the zone of avoidance for the squid used in this study appear to be similar. Alarm responses were observed prior to other behavioural changes at noise levels between 158 – 163 dB re 1 µPa or 2.1 – 5 km distance from a 2678 cui air-gun array (Fig. 8.2). At approximately 163 - 168 dB re 1 µPa the swimming speed of the captive squid altered. It is possible that behavioural alterations did occur prior to the alarm responses but were too subtle to be noted by the observer. Therefore, in this study the results suggest that the zone of avoidance is

between 2 – 5 km from a 2678 cui air-gun. However, as the animals were held captive this is speculative.

The squid ejecting ink is considered an avoidance tactic to evade predators (Hanlon and Messenger 1996d), therefore animals displaying this behaviour would be in the zone of avoidance which according to the results of this study is at 174 dB re 1 μPa (0.9 – 1.5 km from a 2678 cui air-gun array). It is important to be aware that this behaviour was a result of directly exposing the squid to this noise level, that is, the result was not repeated when the animals were exposed to a gradual increase in noise to this level (ramped). Therefore animals further than 1.5 km in distance from a seismic survey operating with a 2678 cui air-gun array when the air-guns are started would probably not display this response even when noise levels exceeded 174 dB re 1 μPa.

As no morphological examination was conducted on the squid the zone of pathological damage is unknown. As no squid died as a result of air-gun noise in this study it can be assumed that air-gun noise of up to 192.4 dB re 1 μPa which could be expected approximately 0.2 km from a 2678 cui air-gun array is not lethal for *Sepioteuthis australis*.

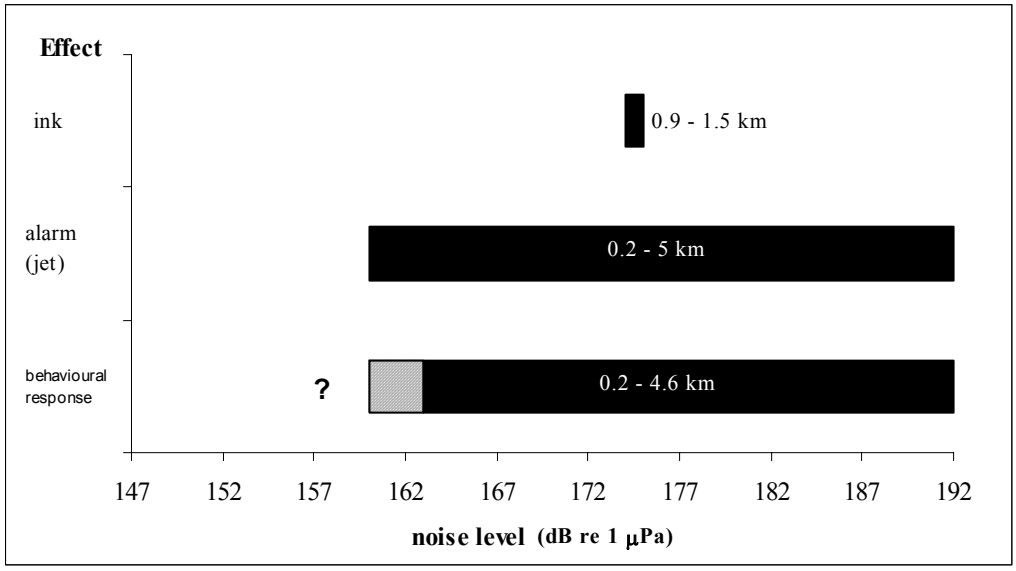

Figure 8.2: Guideline 'zones of effect' for the captive squid in this study. Estimated zones are based on measurements from a 2678 cui air-gun array, a water depth of 120 m for a receiver at 32 m. The lined bar of behavioural response indicates that alarm responses occurred at noise levels lower than 163 dB re 1 μPa and therefore it is possible that subtle behavioural alterations went unnoticed. Noise level units are dB re 1μPa mean squared pressure.

8.4 Conclusions and recommendations for future research

The general conclusions of this study are that the air-gun noise used in the exposure trials induced:

- No detectable significant physiological stress response in *Pagrus auratus* and *Rhabdosargus sarba* (measured by circulating levels of cortisol and glucose);
- Damage to the saccular sensory epithelium of *Pagrus auratus* in the form of ablated hair bundles;
- Alarm responses, faster swimming speeds and tighter groups become more apparent in fish species at noise levels above 158 – 163 dB re 1 μPa;
- A decrease in severity of alarm responses over time of air-gun noise exposure in some fish species and *Sepioteuthis australis*;
- A tendency to occupy the lower portion of the cage in fish species at noise levels greater than 158-163 dB re 1 μPa and,
- An increase in alarm responses in *Sepioteuthis australis* at noise levels greater than 158-163 dB re 1 μPa.

When applying the results of this study to a real situation the major caveat is that the experimental animals were held captive. Although the air-gun noise did induce a significant change in behaviour for the animals in this study, as mentioned, the behavioural and stress response of captive animals are not necessarily identical to animals in their natural environment. Therefore some questions still remain: would the fish and squid species used in this study have tolerated the noise and stayed in the immediate vicinity? Would they stay in the area but at a certain distance from the noise? What would that distance be? If they left the area, would they return once the noise levels were reduced or stopped? These questions should be addressed in future research.

As this study was primarily designed to observe the effect of air-gun noise on fish and squid behaviour the precise nature of noise required to produce the damage to the sensory epithelium of the sacculus of pink snapper could not be determined. Future studies should be specifically designed so that this can be ascertained. Also all three end organs should be examined for damage.

As mentioned, the lack of behavioural responsiveness to repeated air-gun noise exposure observed in trial 14 may have been a result of hearing damage from previous exposure or habituation to air-gun noise. To determine the effect of the damage to fish ears observed in this study on hearing ability, future research should incorporate auditory brainstem response techniques (Kenyon et al. 1998).

Throughout this document comments have been made that factors such as age and sex of the surrounding animals and seasonality could influence the effect that seismic survey noise has on an ecosystem. Future studies should address these issues.

In conclusion, although generalised responses of marine fish and squid can be inferred from this study and other literature, there are many factors that must be considered when deciding on the potential effects of an offshore seismic survey in a specific area. The behavioural responses observed do not necessarily equate to

significant effects on wild populations and commercial fisheries. Mitigation techniques should be developed and proper risk assessment needs to be undertaken before beginning a seismic survey. As research indicates that precise responses to seismic survey noise are species specific, this should include knowledge of the species present in the area and awareness of their biology. Further research into the effects of seismic surveys on marine fish and invertebrates is important so that results can be used to design effective mitigation techniques that benefit wild populations of fish and commercial fisheries, without compromising the economic value of offshore seismic exploration.

References

REFERENCES

Adams, S. M. (1990) Status and use of biological indicators for evaluating the effects of stress on fish. Journal of American Fisheries Society, 8, pp 1-8.

Adler, H. J. and Raphael, Y. (1996) New hair cells arise from supporting cell conversion in the acoustically damaged chick ear. Neuroscience Letters, 205, pp 17-20.

Akamatsu, T., Nanami, A. and Yan, H. Y. (2003) Spotlined sardine *Sardinops melanostictus* listens to 1-kHz sound by using its gas bladder. Fisheries Science, 69, pp 348-354.

Anderson, D. P. (1990) Immunological Indicators: Effects of environmental stress on immune protection and disease outbreaks. American Fisheries Symposium, 8, pp 38-50.

Aota, S., Holgren, K. D., Gallaugher, P. and Randall, D. J. (1990) A plausible role for catecholamines in ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Biology, 151, pp 57-70.

APPEA (Australian Petroleum Production and Exploration Association) (1996) Code of environmental practice - onshore and offshore. APPEA, Sydney, 88 pp.

APPEA (2002) Industry Information: Facts and Figures, http://www.appea.com.au/Industryinformation/statistics/FactsAndFigures/.

Bart, A. N., Clark, J., Young, J. and Zohar, Y. (2001) Underwater ambient noise measurements in aquaculture systems: a survey. Aquaculture Engineering, 25, pp 99-110.

Barton, B., Schreck, C. and Sigismondi, L. (1986) Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society, 115, pp 245-251.

Barton, B. A. and Iwama, G. K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases, pp 3-26.

Battaglene, S. C., Seymore, J. E., Ramofafia, C. and Lane, I. (2002) Spawning induction of three tropical sea cucumbers, *Holothuria scabra, H. fuscogilva* and *Actinopyga mauritiana*. Aquaculture, 207, (1-2): pp 29-47.

Batty, R. S., Blaxter, J. H. S. and Fretwell, K. (1993) Effect of temperature on the escape responses of larval herring, *Clupea harengus*. Marine Biology, 115, (4): pp 523-528.

Berg, A. and Watson, G. M. (2002) Rapid recovery of sensory function in blind cave fish treated with anemone repair proteins. Hearing Research, 174, pp 296-304.

Billard, R., Bry, C. and Gillet, C. (1981) Stress, environment and reproduction in teleost fish. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, Sydney, pp. 185-208.

Blanschko, H. (1939) The specific action of L-DOPA decarboxylase. Journal of Physiology, 96, pp 50-51.

Blaxter, J. H. S. (1988) Sensory performance, behaviour and ecology of fish. In: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (eds.), Sensory Biology of Aquatic Animals, Springer-Verlag, New York, pp. 203-232.

Blaxter, J. H. S., Denton, E. J. and Gray, J. A. B. (1981a) Acousticolateralis system in clupeid fishes. In: Tavolga, W. N., Popper, A. N. and Fay, R. R. (eds.), Hearing and Sound Communication in Fishes, Springer-Verlag, New York, pp. 39-59.

Blaxter, J. H. S., Grey, J. A. B. and Denton, E. J. (1981b) Sound and startle responses in herring shoals. Journal of the Marine Biological Association of the United Kingdom, 61, pp 851-869.

Blaxter, J. H. S. and Hoss, D. E. (1981) Startle response in herring: The effect of sound stimulus frequency, size of fish and selective interference with the acoustico-lateralis system. Journal of the Marine Biological Association of the United Kingdom, 61, pp 871-879.

Boal, J. G. and Gonzalez, S. A. (1998) Social behaviour of individual oval squids (Cephalopoda, Teuthoidea, Loliginidae, *Sepioteuthis lessoniana*) within a captive school. Ethology, 104, (2): pp 161-178.

Bollard, B. A., Pankhurst, N. W. and Wells, R. M. G. (1993) Effects of artificially elevated plasma cortisol levels on blood parameters in the teleost fish *Pagrus auratus* (Sparidae). Comparative Biochemistry and Physiology A, 106, (1): pp 157-162.

Bone, Q., Marshall, N. B. and Blaxter, J. H. S. (1995a) Sensory systems and communication. In: Bone, Q., Marshall, N.B., Blaxter, J.H.S. (eds.), Biology of Fishes (second edition), Blackie Academic and Professional, Melbourne, pp. 219-262.

Bone, Q., N.B., M. and J.H.S., B. (1995b) Endocrine systems. In: Bone, Q., Marshall, N.B., Blaxter, J.H.S. (eds.), Biology of Fishes (second edition), Blackie Academic and Professional, Melbourne, pp. 195-218.

Bonga, S. E. W. (1997) The stress response of fish. Physiological Reviews, 77, (3): pp 591-695.

Braley, H. and Anderson, T. A. (1992) Changes in blood metabolite concentrations in response to repeated capture, anesthesia and blood sampling in the golden perch, *Macquaria ambigua*. Comparative Biochemistry and Physiology, 103A, (3): pp 445-450.

Braley, R. D. (1989) Farming the giant clam. World Aquaculture, 20, (1): pp 6-17.

Branscomb, E. S. and Rittschof, D. (1984) An investigation of low frequency sound waves as a means of inhibiting barnacle settlement. Journal of Experimental Marine Biology and Ecology, 79, pp 149-154.

Brinn, J. E. (1973) The pancreatic islets of bony fish. American Zoologist, 13, pp 653-665.

Budelmann, B. U. (1977) Structure and function of the angular acceleration receptor systems in the statocysts of cephalopods. Symposium of the Zoological Society of London, 38, pp 309- 322.

Budelmann, B. U. (1988) Invertebrate equilibrium receptor systems. In: Atema, J., Fay, R. R., Popper, A. N. and Tavolga, W. N. (eds.), Sensory Biology of Aquatic Animals, Springer-Verlag, New York, pp. 757-782.

Budelmann, B. U. (1990) The statocysts of squid. In: Gilbert, D. L., Adelman, W. J. J. and Arnold, J. M. (eds.), Squid as Experimental Animals, Plenum Press, New York, pp. 421-439.

Budelmann, B. U. (1992a) Hearing in crustacea. In: Webster, D. B., Fay, R. R. and Popper, A. N. (eds.), The Evolutionary Biology of Hearing, Springer-Verlag, New York, pp. 131-139.

Budelmann, B. U. (1992b) Hearing in nonarthropod invertebrates. In: Webster, D. B., Fay, R. R. and Popper, A. N. (eds.), The Evolutionary Biology of Hearing, Springer-Verlag, New York, pp. 141-155.

Budelmann, B. U. and Williamson, R. (1994) Directional sensitivity of hair cell afferents in the octopus statocyst. Journal of Experimental Biology, 187, pp 245-259.

Caldwell, J. and Dragosnet, W. (2000) A brief overview of seismic air-gun arrays. The Leading Edge, 19, (8): pp 898-902.

Carmichael, G. L. (1984) Long distance truck transport of intensively reared largemouth bass. Progressive Fish Culturist, 46, pp 111-115.

Carr, M. H. (1991) Habitat selection and recruitment of an assemblage of temperate zone reef fishes. Journal of Experimental Marine Biology and Ecology, 146, (1): pp 113-137.

Carragher, J., Sumpter, J., Pottinger, T. and Pickering, A. (1989) The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. General and Comparative Endocrinology*,* 76, pp 310-321.

Casagrand, J. L., Guzik, A. L. and Eaton, R. C. (1999) Mauthner and reticulinospinal responses to the onset of acoustic pressure and acceleration stimuli. Journal of Neurophysiology*,* 82, (3): pp 1422-1437.

Casillas, E. and Smith, L. S. (1977) Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). Journal of Fish Biology*,* 10, pp 481-491.

Cato, D. and McCauley, R. D. (2002) Australian research in ambient sea noise. Acoustic Australia*,* 30, (1): pp 13-20.

Caton, A. (2002) Fishery Status Reports 2000-2001: Resource Assessments of Australian Commonwealth Fisheries Bureau of Rural Sciences, Canberra, 252 pp

Chapman, C. J. and Hawkins, A. D. (1969) The importance of sound in fish behaviour in relation to capture trawls. FAO Fisheries Report*,* 3, (162): pp 717-729.

Chen, L., Trautwein, P.G., Shero, M., Salvi, R.J. (1996) Tuning, spontaneous activity and tonotopic map in chicken cochlear ganglion neurons following sound-induced hair cell loss and regeneration. Hearing Research*,* 98, (1-2): pp 152-164.

Chester Jones, D. K. O., Chan, I. W., Henderson, I. W. and Ball, J. N. (1969) The adrencortical steroids, adrenocorticotropin and the corpuscles of Stannius. In: Hoar, W. S. and Randall, A. J. (eds.), Fish Physiology, Academic Press, London, pp. 322-364.

Clarke, J. A. and Pickles, J. O. (1996) The effects of moderate and low levels of acoustic over stimulation on stereocilia and their tip links in the guinea pig. Hearing Research*,* 99, pp 119-128.

Clarke, M. R. (1978) The cephalopod statolith - an introduction to its form. Journal of the Marine Biological Association of the United Kingdom*,* 58, pp 701-712.

Coombs, S. and Fay, R. R. (1989) The temporal evolution of masking and frequency selectivity on the goldfish (*Carassius auratus*). Journal of Acoustical Society of America*,* 86, (3): pp 925-933.

Coombs, S. and Popper, A. N. (1982) Comparative frequency selectivity in fishes: Simultaneously and forward-masked psychophysical tuning curves. Journal of Acoustical Society of America*,* 71, (1): pp 133-141.

Corwin, J. T. (1983) Postembryonic growth of the macula neglecta auditory detector in the ray, *Raja clavata*: Continual increases in hair cell number, neural convergence, and physiological sensitivity. The Journal of Comparative Neurology, 217, pp 315-356.

Corwin, J. T. and Cotanche, D. A. (1988) Regeneration of sensory hair cells after acoustic trauma. Science, 240, pp 1772-1774.

Corwin, J. T. and Oberholtzert, J. C. (1997) Fish n' Chicks: Model recipes for hair-cell regeneration. Neuron, 19, (5): pp 951-954.

Dalen, J. and Knutsen, G. M. (1987) Scaring effects in fish and harmful effects on eggs, larvae and fry by offshore seismic explorations. In: Merklinger, H. M. (ed.), Progress in Underwater Acoustics, Plenum Publishing Corporation, New York, pp. 93-102.

Dalen, J. and Raknes, A. (1985) Scaring effects on fish from three-dimensional seismic surveys, Institute of Marine Research, FO 8504, Bergen, Norway, 22 pp.

Deffenbaugh, M. (2002) Mitigating seismic impact on marine life: Current practice and future technology. Bioacoustics, the International Journal of Animal Sound and its Recording, 12, pp 316-318.

Demski, L. S., Gerald, J. W. and Popper, A. N. (1973) Central and peripheral mechanisms of teleost sound production. American Zoologist, 13, pp 1141-1167.

Denton, E. J., Gray, J. A. B. and Blaxter, J. H. S. (1979) The mechanics of the clupeid acoustico-lateralis system: Frequency responses. Journal of the Marine Biological Association of the United Kingdom, 59, (1): pp 37-47.

Diamond, J. (1971) The Mauthner cell. In: Hoar, W. S. and Randall, D. J. (eds.), Fish Physiology, volume V, Sensory Systems and Electric Organs, Academic Press, New York, pp. 265-346.

Dilly, P. N. (1976) The structure of some cephalopod statoliths. Cell and Tissue Research, 175, (2): pp 147-163.

DME (Department of Minerals and Energy) (2000) Environmental assessment processes for petroleum activities in Western Australia, Petroleum Division, Perth, 46 pp.

Domenici, P. and Blake, R. W. (1993) The effect of size on the kinematics and performance of (*Pterophyllum eimekei*) escape responses. Canadian Journal of Zoology, 71, pp 2319-2326.

Domenici, P. and Blake, R. W. (1997) Review: The kinematics and performance of fish fast-start swimming. The Journal of Experimental Biology, 200, pp 1165-1178.

Donaldson, E. M. (1981) The pituitary-interrenal axis as an indicator of stress in fish, In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, Sydney, pp. 11-47.

Donaldson, E. M. and Fagerlund, U. H. M. (1972) Corticosteroid dynamics on Pacific salmon. General and Comparative Endocrinology, 3, pp 254-265.

Dragosnet, W. (2000) Introduction to air-guns and air-gun arrays. The Leading Edge, 19, (8): pp 892-897.

Duncan, A. and McCauley, R. D. (2000) Characterisation of an air-gun as a sound source for acoustic propagation studies. Paper presented to the Undersea Defence Technology Pacific 2000 Conference, Sydney, Australia, February.

Dunkelberger, D. G., Dean, J.M., Watabe, N. (1980) The ultrastructure of the otolith membrane and otolith in the juvenile mummichog, *Fundulus heteroclitus*. Journal of Morphology, 163, pp 367-377.

Eaton, R. C. and Bombardieri, R. A. (1978) Behavioural function of the Mauthner neuron. In: Faber, D. S. and Korn, H. (eds.), Neurobiology of the Mauthner Cell, Raven Press, New York, pp. 221-244.

Eaton, R. C., Canfield, J. G. and Guzik, A. L. (1995) Left-right discrimination of sound onset by the Mauthner system. Brain, Behaviour and Evolution, 46, pp 165-179.

Eaton, R. C. and Emberley, D. S. (1991) How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. Journal of Experimental Biology, 161, pp 469-487.

Eaton, R. C., Guzik, A. L. and Casagrand, J. L. (1997) Mauthner system discrimination of stimulus direction from the acceleration and pressure components at sound onset. Biological Bulletin, 192, pp 146-149.

Eaton, R. C. and Hackett, J. T. (1984) The role of the Mauthner cell fast starts involving escape in teleost fishes. In: Eaton, R. C. (ed.), Neural mechanisms of startle behaviour, Plenum Press, New York, pp. 213-266.

Eddy, F. B. (1981) Effects of stress on osmotic and ionic regulation in fish. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, Sydney, pp. 77-102.

Edgar, G. J. (1997) Australian marine life. Reed Books, Victoria, 544 pp

Einarsdottir, I. E. and Nilssen, K. J. (1996) Stress responses of Atlantic salmon (*Salmo salar* L.) elicited by water level reduction in rearing tanks. Fish Physiology and Biochemistry, 15, (5): pp 395-400.

Ellis, A. E. (1977) Ontogeny of the immune response in *Salmo salar*. In: Solomon, J. B. and Horton, J. D. (eds.), Developments in Immunology, Elsevier, Amsterdam, North-Holland, pp. 225-231.

Ellis, A. E. (1981) Stress and the modulation of the defence mechanism in fish. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, Sydney, pp. 147-169.

Ellsaesser, C. F. and Clem, L. W. (1986) Haematological and immunological changes in channel catfish stressed by handling and transport. Journal of Fish Biology, 28, pp 511-521.

Engas, A. and Lokkeborg, S. (2002) Effects of seismic shooting and vessel-generated noise on fish behaviour and catch rates. Bioacoustics, the International Journal of Animal Sound and its Recording, 12, pp 313-316.

Engas, A., Lokkeborg, S., Ona, E. and Soldal, A. V. (1993) Effects of seismic shooting on catch and catch availability of cod and haddock. Fisken Og Havet, 9, pp 117.

Engas, A., Lokkeborg, S., Ona, E. and Soldal, A. V. (1996) Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Canadian Journal of Fisheries and Aquatic Sciences, 53, pp 2238-2249.

Epple, A. (1969) The endocrine pancreas. In: Hoar, W. S. and Randall, D. J. (eds.), Fish Physiology, Academic Press, London, pp. 275-319.

Epple, A., Hathoway, C. B. and Nibbio, B. (1989) Circulatory catecholamines in the eel: origins and functions. Fish Physiology and Biochemistry, 7, pp 123-130.

Erbe, C. and Farmer, D. M. (2000) A software model to estimate zones of impact on marine mammals around anthropogenic noise. Journal of the Acoustic Society of America, 108, (3): pp 1327-1331.

Evans, D. H. (1993) Osmotic and ion regulation. In: Evans, D. H. (ed.), The Physiology of Fishes, CRC Press, London, pp. 315-341.

Faber, D. S. and Korn, H. (1978) Electrophysiology of the Mauthner cell: Basic properties, synaptic mechanisms and associated networks. In: Faber, D. S. and H., K. (eds.), Neurobiology of the Mauthner Cell, Raven Press, New York, pp. 47-131.

Falk, M. R. and Lawrence, M. J. (1973) Seismic exploration: its nature and effect on fish, Resource Management Branch, Fisheries Operations Directorate, CEN/T-73-9, Winnepeg, 51pp.

Fay, R. R. (1985) Sound intensity processing by the goldfish. Journal of the Acoustic Society of America, 78, (4): pp 1296-1309.

Fay, R. R. (1988) Peripheral adaptations for spatial hearing in fish. In: Atema, J., Fay, R. R., Popper, A. N. and Tavolga, W. N. (eds.), Sensory Biology of Aquatic Animals, Spinger-Verlag, New York, pp. 711-731.

Fay, R. R. (1992) Structure and function in sound discrimination among vertebrates. In: Webster, D. B., Fay, R. R. and Popper, A. N. (eds.), The Evolutionary Biology of Hearing, Springer-Verlag, New York, pp. 229-263.

Fevolden, S., Refstie, T. and Roed, K. (1991) Selection for high and low cortisol stress response in Atlantic salmon (*Salmo Salar*) and rainbow trout. Aquaculture, 95, pp 53-65.

Fisheries, W. A. (2000) A guide to recreational fishing in Shark Bay. Recreational Fisheries Program, Perth, 5 pp.

Fletcher, J. L. and Busnel, R. G. (1978) Effects of Noise on Wildlife. Academic Press, New York, 305 pp

Flock, A. (1971) Sensory transduction in hair cells. In: Lowenstein, W. R. (ed.), Handbook of Sensory Physiology: Principles of Receptor Physiology, volume I, Springer-Verlag, New York, pp. 369-411.

Flodmark, L. E. W., Urke, H. A., Halleraker, J. W., Arnekleiv, J. V., Vollestad, L. A. and Poleo, A. B. S. (2002) Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. Journal of Fish Biology, 60, pp 238-248.

Foo, J. T. W. and Lam, T. J. (1993) Serum cortisol response to handling stress and the effect of cortisol implantations on testosterone level in tilapia, *Oreochromis mosambicas*. Aquaculture, 115, pp 145-158.

Fox, H. E., White, S. A., Kao, M. H. F. and Fernald, R. D. (1997) Stress and dominance in a social fish. Journal of Neuroscience, 17, (16): pp 6463-6469.

FRDC (Fisheries Research and Development Corporation) (1999-2000) Australian Fishing Industry, http://www.frdc.com.au/industry/recreation.htm.

Fries, C. R. (1986) Effects of environmental stressors and immuno suppressants on immunity in *Fundulus heteroclitus*. American Zoologist, 26, pp 271-282.

Fryer, J. N. and Maler, L. (1980) Identification of putative CRF neurones in the goldfish hypothalamus (Abstract). The Physiologist, 23, pp 145.

Fujii, R. (1993) Coloration and chromatophores. In: Evans, D. H. (ed.), The Physiology of Fishes, CRC Press, London, pp. 535-560.

Gales, R., Pemberton, D., Lu, C. C. and Clarke, M. R. (1993) Cephalopod diet of the Australian fur seal: Variation due to location, season, and sample type. Australian Journal of Marine and Freshwater Research, 44, pp 657-671.

Gauldie, R. W. (1993) Polymorphic crystalline structures of fish otoliths. Journal of Morphology, 218, pp 1-28.

Gausland, I. (1998) Physics of sound in water. Proceedings of the Seismic and Marine Mammals Workshop, London, pp. 204.

Gausland, I. (2000) Impact of seismic surveys on marine life. The Leading Edge, 19, (8): pp 903-905.

George, C. J. (1977) The implication of neuroendocrine mechanisms in the regulation of population characteristics. Fisheries, 2, pp 14-19.

Geoscience Australia (2002) Australian Petroleum Exploration and Development Activity. Canberra, 4 pp

Gisiner, R.C. (1998) Effects of anthropogenic sounds on the hearing of marine animals. Proceedings of the Workshop on the Effects of Anthropogenic Noise in the Marine Environment, Marine Mammal Science Program, Office of Naval Research, February, pp. 19-49.

Gleich, O., Dooling, R. J. and Manley, G. A. (1994) Inner ear abnormalities and their functional consequences in Belgian Waterslager canaries (*Serinus canarius*). Hearing Research, 79, pp 123-136.

Gleitman, H. and Rozin, P. (1971) Learning and memory. In: Hoar, W. S. and Randall, D. J. (eds.), Fish Physiology, volume VI Academic Press, New York, pp. 191-278.

Godin, J. J. (1997) Evading Predators. In: Godin, J. J. (ed.), Behavioural Ecology of Teleost Fishes, Oxford University Press, New York, pp. 191-236.

Greene, C. R. J. (1985) A pilot study of possible effects of marine seismic air-gun array operation on rockfish plumes, Greeneridge Sciences, California, 50 pp.

Greene, C. R. J. and Richardson, W. J. (1988) Characteristics of marine seismic survey sounds in the Beaufort Sea. Journal of the Acoustic Society of America, 83, pp 2246-2254.

Grutter, A. S. and Pankhurst, N. W. (2000) The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish *Hemigymnus melapterus*. Journal of Fish Biology, 57, pp 391-401.

Ha, S. H. (1985) Evidence of temporary hearing loss (temporary threshold shift) in fish subjected to laboratory ambient noise. Proceedings of the Pennsylvania Academy for Science, 59, pp 78.

Hanlon, R. T. (1990) Maintenance, rearing, and culture of Teuthoid and Sepioid squids. In: Gilbert, D. L., Adelman, W. J. J. and Arnold, J.M. (eds.), Squid as Experimental Animals, Plenum Press, New York, pp. 35-62.

Hanlon, R. T. and Budelmann, B. U. (1987) Why cephalods are probably not deaf. The American Naturalist, 129, (2): pp 312- 317.

Hanlon, R. T. and Messenger, J. B. (1996a) Cephalopod Behaviour. Cambridge University Press, Cambridge, 219 pp

Hanlon, R. T. and Messenger, J. B. (1996b) Defence. In: Hanlon, R. T. and Messenger, J. B. (eds.), Cephalopod Behaviour, Cambridge University Press, Cambridge, pp. 66-93.

Hanlon, R. T. and Messenger, J. B. (1996c) Reproductive behaviour. In: Hanlon, R. T. and Messenger, J. B. (eds.), Cephalopod Behaviour, Cambridge University Press, Cambridge, pp. 94-119.

Hanlon, R. T. and Messenger, J. B. (1996d) Senses, effectors and the brain. In: Hanlon, R. T. and Messenger, J. B. (eds.), Cephalopod Behaviour, Cambridge University Press, Cambridge, pp. 12-30.

Hanlon, R. T., Turk, P. E., Lee, P. G. and Yang, W. T. (1987) Laboratory rearing of the squid *Loligo pealaei* to the juvenile stage: growth comparisons with fishery data. Fishery Bulletin, 85, pp 163-167.

Hastings, M. C., Popper, A. N., Finneran, J. J. and Lanford, P. J. (1996) Effects of low -frequency underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus ocellatus*. Journal of the Acoustic Society of America, 99, (3): pp 1759-1766.

Hawkins, A. D. (1981) The hearing abilities of fish. In: Tavolga, W. N., Popper, A. N. and Fay, R. R. (eds.), Hearing and Sound Communication in Fishes, Springer-Verlag, New York, pp. 109-133.

Hawkins, A. D. (1986) Underwater sound and fish behaviour. In: Pitcher, T. J. (ed.), The Behaviour of Teleost Fish, Chapman and Hall, London, pp. 114-151.

Henderson, I. W. and Kime, D. E. (1987) The adrenal cortical steroids, In: Pang, P. K. T. and Schreibman, M. P. (eds.), Vertebrate Endocrinology: Fundamentals and Biomedical Implications, volume 2, Academic Press, Orlando, pp. 121-130.

Hendrickson, W. B. (1977) Voices in the deep. Sea Frontiers, 23, (4): pp 234-241.

Holliday, D. V., Pieper, R. E., Clarke, M. E. and Greenlaw, C. F. (1987) The effects of air-gun energy releases on the eggs, larvae, and adults of the northern anchovy (*Engraulis mordax*). The American Petroleum Institute, T-86-06-7001-U, Washington D.C., 108 pp.

Holzbauer, M. and Sharman, D. F. (1972) The distribution of catecholamines in vertebrates. In: Blaschko, H. and Mushcholls, E. (eds.), Catecholamines, Springer, Berlin, pp. 110-185.

Hopkins, C. D. (1988) Social communication in the aquatic environment. In: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (eds.), Sensory Biology of Aquatic Animals, Springer-Verlag, New York, pp. 233-268.

Hubbard, S. J. (1960) Hearing and the octopus statocyst. Journal of Experimental Biology, 37, pp 845-853.

Hudspeth, A. J. (1985) The cellular basis of hearing: the biophysics of hair cells. Science, 230, pp 745-752.

Hudspeth, A. J. (1989) How the ear works. Nature, 404, pp 341-397.

Husbands, J. M., Steinberg, S. A., Kurian, R. and Saunders, J. C. (1999) Tip link integrity on chick tall hair cell stereocilia following intense sound exposure. Hearing Research, 135, pp 135-145.

Idler, D. R. and Truscott, B. (1972) Corticosteroids in fish. In: Idler, D. R. (ed.), Steroids in Non-Mammalian Vertebrates, Academic Press, New York, pp. 127-252.

Johnsson, J. I., Hojesjo, J. and Fleming, I. A. (2001) Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. Canadian Journal of Fisheries and Aquatic Science, 58, (4): pp 788-794.

Kawaski, K., Morita, M., Maejima, T. and Oda, Y. (1996) Long-lasting habituation of Mauthner cell-initiated escape response in goldfish. Neuroscience Research, 24-26, (Supplement 21): pp S244.

Kelsey, D. A., Schreck, C. B., Congleton, J. L. and Davis, L. E. (2002) Effects of juvenile steelhead on juvenile chinook salmon behaviour and physiology. Transactions of the American Fisheries Society, 131, (4): pp 676-689.

Kenyon, T.N., Ladich, F. and Yan, H.Y. 1998. A comparative study of hearing ability in fishes: The auditory brainstem response approach. Journal of Comparative Physiology A, 182, pp 307-318.

Ketchington, T. J. (2000) Impacts of seismic surveys on fish behaviour and fisheries catch rates on Georges Bank, Gadus Associates, Halifax, Nova Scotia, 24 pp.

Kil, J., Warchol, M. E. and Corwin, J. T. (1997) Cell death, cell proliferation and estimates of hair cell life spans in the vestibular organs of chicks. Hearing Research, 114, pp 117-126.

Knudsen, F. R., Enger and Sand, O. (1992) Awareness reactions and avoidance responses to sound in juvenile Atlantic salmon. Fish Biology, 40, (4): pp 523-534.

Kostyuchenko, L. P. (1971) Effects of elastic waves generated in marine seismic prospecting on fish eggs in the Black Sea. Hydrobiologia, 9, (5): pp 45-48.

Kryter, K. D. (1985) The effects of noise on man (second edition), Academic Press, Florida, 688 pp

Kuwada, H., Masuda, R., Shiozawa, S., Kogane, T., Imaizumi, K. and Tsukamoto, K. (2000) Effect of fish size, handling stresses and train procedure on the swimming behaviour of hatchery reared striped jack: Implications for stock enhancement. Aquaculture, 185, pp 245-256.

Lagardere, J. P. (1982) Effects of noise on growth and reproduction of *Crangon crangon* in rearing tanks. Marine Biology, 71, pp 177-185.

Lanford, P. J., Presson, J. C. and Popper, A. N. (1996) Cell proliferation and hair cell addition in the ear of the goldfish, *Carassius auratus*. Hearing Research, 100, pp 1-9.

Laurent, P. and Perry, S. F. (1990) Effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout, *Salmo gairdneri*. Cell Tissue Reserve, 259, pp 429-435.

Lokkeborg, S. and Soldal, A. V. (1993) The influence of seismic exploration with air-guns on cod (*Gadus morhus*) behaviour and catch rates. International Council for Exploration of the Sea Marine Science Symposium, 196, pp 62-67.

Lombarte, A., Yan, H. Y., Popper, A. N., Chang, J. S. and Platt, C. (1993) Damage and regeneration of hair cell ciliary bundles in a fish ear following treatment with gentamicin. Hearing Research, 64, pp 166-174.

Long, T. M., Hanlon, R. T., Ter Maat, A. and Pinsker, H. M. (1989) Non-associative learning in the squid *Lolliguncula brevis* (Mollusca, Cephalopoda). Marine Behaviour and Physiology, 16, pp 1-9.

Love, M. S., Yoklavich, M. and Thorsteinson, L. (2002) The rockfishes of the northeast Pacific University of California Press, California, 390 pp

Lowenstein, O. (1971) The labyrinth. In: Hoar, W. S. and Randall, D. J. (eds.), Fish Physiology, volume V, Academic Press, New York, pp. 207-240.

Lu, Z. and Popper, A. N. (1998) Morphological polarizations of the sensory hair cells in the three otolithic organs of a teleost fish: Flourescent imaging of ciliary bundles. Hearing Research, 126, (1-2): pp 47-57.

Lu, Z., Song, J., Popper, A.N. (1998) Encoding of the acoustic directional information by saccular afferents of the sleeper goby, *Dormitator latifrons*. Journal of Comparative Physiology A, 182, pp 805-815.

Lucero, M. T., Farrington, H. and Gilly, W. F. (1994) Quantification of L-DOPA and dopamine in squid ink: Implications for chemoreception. Biological Bulletin, 187, pp 55-63.

Lychakov, D. V., Rebane, Y.T. (2000) Otolith regularities. Hearing Research, 143, (1-2): pp 83-102.

Malme, C. I., Miles, P. R., Miller, G. W., Richardson, W. J., Roseneau, D. G., Thomson, D. H. and Greene, C. R. J. (1989) Analysis and ranking of the acoustic disturbance potential of petroleum industry activities and other sources of noise in the environment of marine mammals in Alaska. Bolt Beranek and Newman Systems and Technologies Corporation, 6945, Cambridge, Massachusetts, 307 pp.

Maniwa, Y. (1976) Attraction of bony fish, squid and crab by sound. In: Schuijf, A. and Hawkins, A. D. (eds.), Sound Reception in Fish, Elsevier, Amsterdam, pp. 271-283.

Mann, D. A., Lu, Z. and Popper, A. N. (1997) Ultrasound detection by a teleost fish. Nature, 389, pp 341.

Matishov, G. G. (1990) The Barents Sea on the verge of devastation. Priroda, 3, pp 30-37.

Matsui, H., Kawasaki, K. and Oda, Y. (1997) Long-lasting depression of Mauthner cell-initiated escape response produced by inhibitory long-term potentiation. Neuroscience Research, 27-29, (Supplement 21): pp S264.

Mayer-Gostan, N., Wendelaar-Bonga, S. E. and Balm, P. H. M. (1987) Mechanisms of hormone actions on gill transport. In: Pang, P. K. T. and Schreibman, M. P. (eds.), Vertebrate Endocrinology: Fundamentals and Biomedical Implications, volume 2, Academic Press, Orlando, pp. 121-130.

Mazeaud, M. M. and Mazeaud, F. (1981) Adrenergic responses to stress in fish. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, Sydney, pp. 49-75.

Mazeaud, M. M., Mazeaud, F. and Donaldson, E. M. (1977) Primary and secondary effects of stress in fish: Some new data with a general review. Transactions of the American Fisheries Society, 106, pp 201-212.

McCauley, R. D. (1992) Aspects of marine biological sound in northern Australia II: Temporal and spatial variability in observed calling patterns of some acoustically dominant inter reefal, inshore fishes. James Cook University, Marine Biology Department, Townsville, Queensland, 51 pp.

McCauley, R. D. (1994) Environmental implications of offshore oil and gas development in Australia - seismic surveys. In: Swan, J. M., Neff, J.M., Young, P.C. (eds.), Environmental Implications of Offshore Oil and Gas Development in Australia, APEA, Sydney, pp. 20-121.

McCauley, R. D. (2001) Biological sea noise in northern Australia: patterns of fish calling. PhD Thesis, James Cook University, Townsville, Queensland, pp. 250.

McCauley, R. D. (2003) *Personal Communication.* Centre for Marine Science and Technology, Curtin University, GPO Box U 1987, Perth 6845, Western Australia.

McCauley, R. D., Fewtrell, J., Duncan, A. J., Jenner, C., Jenner, M.-N., Penrose, J. D., Prince, R. I. T., Adhitya, A., Murdoch, J. and McCabe, K. (2000) Marine seismic surveys: Analysis and propagation of air-gun signals; and the effects of exposure on humpback whales, sea turtles, fishes and squid. Centre for Marine Science and Technology, Curtin University, R99-15, Perth, Western Australia, 185 pp.

McCormick, M. I. (1998) Behaviourally induced maternal stress in a fish influences progeny quality by hormonal mechanism. Ecology, 79, (6): pp 1873-1883.

McCormick, S. D., Shrimpton, J. M., Carey, J. B., O'Dea, M. F., Sloan, K. E., Moriyama, S. and Bjornsson, B. T. (1998) Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth factor I and cortisol. Aquaculture, 168, (1-4): pp 221-235.

McDonald, D. G., Milligan, C. L., McFarlane, W. J., Croke, S., Currie, S., Hooke, B., Angus, R. B., Tufts, B. L. and Davidson, K. (1998) Condition and performance of juvenile Atlantic salmon (*Salmo salar*): Effects of rearing practices on hatchery fish and comparison with wild fish. Canadian Journal of Fisheries and Aquatic Science, 55, (5): pp 1208-1219.

McKibben, J. R., Bass, A.H. (1999) Peripheral encoding of behaviourally relevant acoustic signals in a vocal fish: Single tones. Journal of Comparative Physiology A, 184, (6): pp 563-576.

Meites, S. and Bohman, N. (1963) In vitro stabilisation of blood glucose with water. Clinical Chemistry, 9, pp 594.

Meyers, J. R., Copanus, E. H. and Zottoli, S. J. (1998) Comparison of the fast startle responses between two elongate bony fish with an Anguilliform type of

locomotion and the implications for the underlying neuronal basis of escape behaviour. Brain, Behaviour and Evolution, 52, pp 7-22.

Milinksi, M. (1990) Information overload and food selection, In: Hughes, R. N. (ed.), Behavioural Mechanisms of Food Selection, Vol. G20, Springer-Verlag, Berlin, pp. 721-736.

Moltschaniwskyj, N. A. and Pecl, G. T. (2003) Small-scale spatial and temporal patterns of egg production by the temperate loliginid squid *Sepioteuthis australis*. Marine Biology, 142, pp 509-516.

Mommsen, T. P., Vijayan, M. M. and Moon, T. W. (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, 9, pp 211-268.

Moynihan, M. (1985) Why are cephalopods deaf? The American Naturalist, 125, pp 465-469.

Myrberg, A. A., Jr. (1981) Sound communication and interception in fishes. In: Tavolga, W. N., Popper, A.N., Fay, R.R. (eds.), Hearing and Sound Communication in Fishes, Springer, New York, pp. 395-426.

Myrberg, A. A. J. (1978) Ocean noise and the behaviour of marine animals: Relationships and implications. In: Fletcher, J. L. and Busnel, R. G. (eds.), Effects of Noise on Wildlife, Academic Press, London, pp. 169-208.

Myrberg, A. A. J. (1980) Fish bio-acoustics: its relevance to the 'not so silent world'. Environmental Biology of Fishes, 5, (4): pp 297-304.

Myrberg, A. A. J. (1990) The effects of man-made noise on the behaviour of marine animals. Environment International, 16, pp 575-86.

Nakajima, Y. and Kohno, K. (1978) Fine structure of the Mauthner cell: Synaptic topography and comparative study. In: Faber, D. and Korn, H. (eds.), Neurobiology of the Mauthner Cell, Raven Press, New York, pp. 133-166.

Nandi, J. (1962) The structure of the interrenal gland in teleost fishes. University of California Published Zoology, 65, pp 129-211.

Nilsson, S. and Holmgren, S. (1993) Autonomic nerve functions. In: Evans, D. H. (ed.), The Physiology of Fishes, CRC Press, London, pp. 279-313.

Noakes, D. L. and Bayus, J. R. (1990) Behavior. In: Schreck, C. B. and Moyle, P. B. (eds.), Methods for Fish Biology, American Fisheries Society, Bethesuda, Maryland, pp. 555-583.

Oda, Y., Kawasaski, K., Morita, M. and Matsui, H. (1996) Inhibitory long-term potentiation underlying long-lasting habituation of goldfish escape response. Journal of Neuroscience, 20, pp S244.

Olla, B. L., Davis, M. W. and Schreck, C. B. (1995) Stress induced impairment of predator evasion and non-predator mortality in Pacific salmon. Aquaculture Research, 26, (6): pp 393-398.

Olsen, K. (1990) Fish behaviour and acoustic sampling. Rapports et Proces - Verbaux des Reunions du Conseil International pour l'Exploration de la Mer, 189, pp 147-158.

Otis, T. S. and Gilly, W. F. (1990) Jet-propelled escape in the squid *Loligo opalescens*: concerted control by giant and non-giant motor axon pathways. Proceedings of the National Academy of Science U.S.A., 87, pp 2911-2915.

Packard, A., Karlsen, H. E. and Sand, O. (1990) Low frequency hearing in cephalopods. Journal of Comparative Physiology A, 166, pp 501-505.

Pankhurst, N. W. and Sharples, D. F. (1992) Effects of capture and confinement on plasma cortisol concentrations in the snapper *Pagrus auratus*. Australian Journal of Marine and Freshwater Research, 43, pp 345-356.

Pattipeiluhu, S. M. and Melatunan, S. (1998) Induced spawning of abalone, *Haloitis asinina*. Workshop of the Tropical Marine Mollusc Programme, Lombok, pp. 248.

Pearson, W. H., Skalski, J. R. and Malme, C. I. (1987) Effects of sounds from a geophysical survey device on fishing success. Mineral Management Service, 14-12-0001-30273, California, 293 pp.

Pearson, W. H., Skalski, J. R. and Malme, C. I. (1992) Effects of sounds from a geophysical survey device on behaviour of captive rockfish (*Sebates* spp.). Canadian Journal of Aquatic Science, 49, pp 1343-1356.

Peter, R. E. and Fryer, J. N. (1981) Endocrine functions of the hypothalamus of actinopterygians. In: Davis, R. E. and Northcutt, R. G. (eds.), Fish Neurobiology and Behaviour, University of Michigan Press, Ann Arbor, pp. 221-240.

Petrie, E. and others (2001) Oil and gas resources of Australia 2000. Geoscience Australia, Canberra, 245 pp.

Petrie, E. and others (2002) Oil and gas resources of Australia 2001. Geoscience Australia, Canberra, 256 pp.

Pickering, A. D. (1981) Stress and Fish. Academic Press, London, 429 pp

Pickering, A. D. (1989a) Environmental stress and the survival of brown trout, *Salmo trutta*. Freshwater Biology, 21, pp 47-55.

Pickering, A. D. (1989b) Factors affecting the susceptibility of salmonid fish to disease. Reports of the Freshwater Biological Association, 57, pp 61-80.

Pickering, A. D. (1992) Rainbow trout husbandry: management of the stress response. Aquaculture, 100, pp 125-139.

Pickering, A. D. and Pottinger, T. G. (1983) Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. General and Comparative Endocrinology, 49, pp 232-239.

Pickett, G. D., Eaton, D. R., Seaby, R. M. H. and Arnold, G. P. (1994) Results of bass tagging in Poole Bay during 1992. Laboratory Leaflet No. 74, Ministry of Agriculture, Fisheries and Food, Lowestoft, 12 pp.

Pickles, J. O., Comis, S. D. and Osbourne, M. P. (1984) Crosslinks between stereocilia in the guinea pig organ of corti, and their possible relation to sensory transduction. Hearing Research, 15, (2): pp 95-195.

Pickles, J. O., Osbourne, M. P. and Comis, S. D. (1987) Vulnerability of tip links between stereocilia to acoustic trauma in the guinea pig. Hearing Research, 25, (2-3): pp 173-183.

Pitcher, T. J. and Parish, J. K. (1993) Functions of shoaling behaviour in teleosts. In: Pitcher, T. J. (ed.), Behaviour of Teleost Fishes, Chapman and Hall, London, pp. 363-439.

Popper, A. N. (1981) Comparative scanning electron microscopic investigations of the sensory epithelia in the teleost sacculus and lagena. The Journal of Comparative Neurology, 200, pp 357-374.

Popper, A. N. (2000) Hair cell heterogeneity and ultrasonic hearing: recent advances in understanding fish hearing. Philosophical Transactions of the Royal Society of London, Biological Sciences, 355, (1401): pp 1277-1280.

Popper, A. N. (2002a) *Personal Communication.* Department of Biology and Neuroscience and Cognitive Science Program, University of Maryland, College Park, Maryland 20742.

Popper, A. N. (2002b) An overview of the applied use of sound in fisheries and fish biology. Bioacoustics, the International Journal of Animal Sound and its Recording, 12, pp 303-305.

Popper, A. N. and Clarke, N. L. (1976) The auditory system of the goldfish (*Carassius auratus*): Effects of intense acoustic stimulation. Comparative Biochemistry and Physiology A, 53, pp 11-18.

Popper, A. N. and Coombs, S. (1980) Auditory mechanisms in teleost fishes. American Scientist, 68, pp 429-440.

Popper, A. N. and Edds-Walton, P. L. (1995) Structural diversity in the inner ear of teleost fishes: Implications for connections to the Mauthner cell. Brain, Behaviour and Evolution, 46, pp 131-140.

Popper, A. N. and Fay, R. R. (1993) Sound detection and processing by fish: Critical review and major research questions. Brain Behaviour Evolution, 41, pp 14-38.

Popper, A. N. and Fay, R. R. (1999) The auditory periphery in fishes. In: Fay, R. R. and Popper, A. N. (eds.), Comparative Hearing: Fish and Amphibians, Spinger-Verlag, New York, pp. 43-100.

Popper, A. N. and Hoxter, B. (1981) The fine structure of sacculus and lagena of a teleost fish. Hearing Research, 5, pp 245-263.

Popper, A. N. and Hoxter, B. (1984) Growth of the fish ear: 1. Quantitative analysis of hair cell and ganglion cell proliferation. Hearing Research, 15, pp 133-142.

Popper, A. N., Rogers, P. H., Saidel, W. M. and Cox, M. (1988) Role of the fish ear in sound processing. In: Atema, J., Fay, R. R., Popper, A. N. and Tavolga, W. N. (eds.), Sensory Biology of Aquatic Animals, Springer-Verlag, New York, pp. 687-710.

Popper, A. N., Salmon, M. and Horch, K. (2001) Acoustic detection and communication by decapod crustaceans. Journal of Comparative Physiology A, 187, pp 83-89.

Pottinger, T. G. and Carrick, T. R. (1999) A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress responsiveness in female rainbow trout. Aquaculture, 175, (3-4): pp 351-363.

Pottinger, T. G. and Pickering, A. D. (1992) The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaun) to chronic stress. Journal of Fish Biology, 41, pp 435-447.

Power, M. (1997) Assessing the effects of environmental stressors on fish populations. Aquatic Toxicology, 39, (2): pp 151-169.

Pulsford, A. L., Thomas, M. E., Lemaire-Gony, S., Holden, J., Fossato, V. U. and Pipe, R. K. (1994) Studies on the immune system of the goby, *Zosteriessin ophiocephalus*, from a Venice lagoon. Marine Pollution Bulletin, pp 50-54.

Rance, T., Baker, B. and Webley, G. (1982) Variations in plasma cortisol concentrations over a 24-hour period in the rainbow trout *Salmo gairdneri*. General and Comparative Endocrinology, 48, pp 269-274.

Randall, D. J. and Perry, S. F. (1992) Catecholamines. In: Hoar, W. S., Randall, D. J. and Farrell, A. P. (eds.), Fish Physiology, The Cardiovascular System, part B, volume XII, Academic Press, Sydney, pp. 255-300.

Raphael, Y. (1992) Evidence for supporting cell mitosis in response to acoustic trauma in the avian inner ear. Jounal of Neurocytology, 21, pp 663-671.

Reid, S. G., Bernier, N. J. and Perry, S. F. (1998) The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochemistry and Physiology Part C: Pharmacology, toxicology and endocrinology, 120, (1): pp 1-27.

Richardson, W. J., Greene, C. R. J., Malme, C. I. and Thomson, D. H. (1995) Marine Mammals and Noise. Academic Press, San Diego, 576 pp

Robertson, C. L. (1988) Australia's petroleum prospects: changing perceptions since the beginning of the century. APEA Journal 1988, pp 190-207.

Rogers, P. H. and Cox, M. (1988) Underwater sound as a biological stimulus. In: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (eds.), Sensory Biology of Aquatic Animals, Springer-Verlag, New York, pp. 131-149.

Rogers, P. H., Popper, A. N., Hastings, M. C. and Saidel, W. M. (1988) Processing of acoustic signals in the auditory system of bony fish. Journal of the Acoustic Society of America, 83, (1): pp 338-349.

Rusby, R. I. (1995) Effects of seismic surveys on marine fish and mammals. Shell International Petroleum, EP 95-1405, Hague, 15 pp.

Saidel, W. M. and Popper, A. N. (1983) The saccule may be the transducer for directional hearing of nonostariophysine teleosts. Experimental Brain Research, 50, pp 149-152.

Sand, O. and Karlsen, H. E. (1986) Detection of infrasound by the Atlantic cod. Journal of Experimental Biology, 125, pp 197-204.

Sandler, T. and Idler, D. R. (1972) Steroid methodology. In: Idler, D. R. (ed.), Steroids in Non-Mammalian Vertebrates, Academic Press, New York, pp. 6-36.

Santulli, A., Modica, A., Messina, C., Ceffa, L., Curatolo, A., Rivas, G., Fabi, G. and D'Amelio, V. (1999) Biochemical responses of European Sea Bass (*Dicentrachus labrax* L.) to the stress induced by offshore experimental seismic prospecting. Marine Pollution Bulletin, 38, (12): pp 1105 - 1114.

Schellart, N. A. M. and Popper, A. N. (1992) Functional aspects of the evolution of the auditory system of Actinopterygian fish. In: Webster, D. B., Fay, R.R., and Popper, A. N. (eds.), The Evolutionary Biology of Hearing, Springer-Verlag, New York, pp. 295-322.

Schmaltz, W., Siegesmund, M., Thuermer, K., Kranawettreiser, J. and Hack, H. P. (2002) A new method to investigate the downstream migration of fishes within a hydropower plant area in a middle European river - A possibility to evaluate the effectiveness of behaviour barriers. Bioacoustics, the International Journal of Animal Sound and its Recording, 12, pp 308-310.

Scholik, A. R. and Yan, H. Y. (2001) The effects of underwater noise on auditory sensitivity of a cyprinid fish. Hearing Research, 152, pp 17-24.

Scholik, A. R. and Yan, H. Y. (2002) The effects of noise on the auditory sensitivity of the bluegill sunfish, *Lepomis macrochirus*. Comparative Biochemistry and Physiology A, 133, pp 43-52.

Schreck, C. B. (1981) Stress and compensation in teleostean fishes: response to social and physical factors. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, London, pp. 295-321.

Schreck, C. B. (1990a) Physiological, behavioral, and performance indicators of stress. American Fisheries Symposium, 8, pp 29-37.

Schreck, C. B. (1990b) Physiological, behavioral, and performance indicators of stress. American Fisheries Society Symposium, 8, pp 29 - 37.

Schreck, C. B., Contreras-Sanchez, W. and Fitzpatrick, M. S. (2001) Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture, 197, pp 3-24.

Schreck, C. B., Olla, B. L. and Davis, M. W. (1997) Behavioral responses to stress. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P. and Schreck, C. B. (eds.), Cambridge University Press, Cambridge, pp. 145-170.

Schuijf, A. (1976) The phase model of directional hearing in fish. In: Schuijf, A. and Hawkins, A. D. (eds.), Sound Perception in Fishes, Elsevier, Amsterdam, pp. 63-86.

Schuijf, A. and Buwalda, R. A. (1980) Sound localization: A major problem in fish acoustics. In: Popper, A. N. and Fay, R. R. (eds.), Comparative Studies of Hearing in Vertebrates, Springer-Verlag, New York, pp. 43-78.

Schwalme, K. and MacKay, W. (1991) Mechanisms that elevate the glucose concentrations of muscle and liver in yellow perch (*Perca flavescens* Mitchill) after exercise-handling stress. Canadian Journal of Zoology, 69, pp 456-461.

Seow, J., Betts, D. and Batini, F. (1993) Environmental approval information for petroleum operation in Western Australia. Department of Minerals and Energy, Petroleum Division, Perth, 48 pp.

Shaw, E. (1975) Fish in schools. Natural History, 84, (8): pp 40-46.

Shuter, B. J. (1990) Population-level indicators of stress. American Fisheries Society Symposium, 8, pp 145-166.

Sigismondi, L. A. and Weber, L. J. (1988) Changes in avoidance response time of juvenile chinook salmon exposed to multiple acute handling stresses. Transactions of the American Fisheries Society, 117, pp 196-201.

Skalski, J. R., Pearson, W. H. and Malme, C. I. (1992) Effects of sounds from a geophysical survey device on catch-per-unit-effort in a hook-and-line fishery for rockfish (*Sebastes* spp.). Canadian Journal of Aquatic Science, 49, pp 1357-1365.

Smit, G. L. and Schoonbee, H. J. (1988) Blood coagulation factors in the freshwater fish *Oreochromis mossambicus*. Journal of Fish Biology, 32, pp 673-677.

Smith, J.G. and Jenkerson, M.R. (1998) Acquiring and processing marine vibrator data in the transition zone. Society of Exploration Geophysicists, Expanded Abstracts, pp 136-139.

Smith, R. J. F. (1997) Avoiding and deterring predators. In: Godin, J. J. (ed.), Behavioural Ecology of Teleost Fish, Oxford University Press, New York, pp 162-190.

Snieszko, S. F. (1974) The effects of environmental stress on outbreaks of infectious diseases in fishes. Journal of Fish Biology, 6, pp 197-208.

Sopinska, A. (1983) Effect of physiological factors, stress, and disease on hematological parameters of carp, with particular reference to leukocyte pattern. Acta Ichyological Pisces, 8, pp 59-81.

Stephens, P. R. and Young, J. Z. (1978) Semicircular canals in squids. Nature, 271, pp 444-445.

Stephens, P. R. and Young, J. Z. (1982) The statocyst of the squid *Loligo*. Journal of Zoology, London, 197, pp 241-266.

Stone, D. A. and Forteath, N. (1994) Stress associated with the capture and handling of broodstock. Austasia Aquaculture, 8, pp 48-50.

Strange, R. J., Schreck, C. B. and Golden, J. T. (1977) Corticoid stress responses to handling and temperature in salmonids. Transactions of the American Fisheries Society, 106, pp 213-217.

Sumpter, J. P. (1997) The endocrinology of stress. In: Iwama, G. K., Pickering, A. D., Sumpter, J. P. and Schreck, C. B. (eds.), Fish Stress and Health in Aquaculture, Cambridge University Press, Cambridge, pp. 95-118.

Sverdrup, A., Kjellsby, E., Kruger, P. G., Floysand, R., Knudsen, F. R., Enger, P. S., Serck-Hanssen, G. and Helle, K. B. (1994) Effects of experimental seismic shock on the vascoactivity of arteries, integrity of the vascular endothelium and on the primary stress hormones of the Atlantic salmon. Journal of Fish Biology, 45, pp 973-995.

Swift, D. J. (1983) Blood component value changes in the Atlantic mackerel (*Scomber scombrus* L.) subjected to capture, handling and confinement. Comparative Biochemistry and Physiology A, 76, pp 795-802.

Tavolga, W. N. (1971) Sound Production and Detection. In: Hoar, W. S. and Randall, D. J. (eds.), Fish Physiology, volume V, Academic Press, New York, pp. 135-205.

Temple, G. and Johnston, I. A. (1997) The thermal dependence of fast-start performance in fish. Journal of Thermal Biology, 22, (6): pp 391-401.

Thomas, P. and Robertson, L. (1991) Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anaesthesia with MS-222, quinaldine sulphate and metomidate. Aquaculture, 96, pp 69-86.

Turner, D. and Newton, G. (1992) Spawning roughy in the wild west. Australian Fisheries, November, (51): pp 8-10.

Ungell, A., Kiessling, A. and Nilsson, S. (1984) Transfer changes in fish gills during stress. In: Bolis, L., Ladunaisky, J. and Gilles, R. (eds.), Toxins, Drugs and Pollutants in Marine Animals (Proceedings in Life Sciences), pp. 114-121.

Urick, R. J. (1983a) The nature of sonar. In: Urick, R. J. (ed.), Principles of Underwater Sound (third edition), Peninsula Publishing, California, pp. 1-16.

Urick, R. J. (1983b) Principles of underwater sound (third edition), Peninsula Publishing, California, 423 pp

Urick, R. J. (1983c) Propagation of sound in the sea: Transmission loss, I. In: Urick, R. J. (ed.), Principles of underwater sound (third edition), Peninsula Publishing, California, pp. 99-146.

van der Boon, J., van den Thillart, G. E. and Addink, A. D. F. (1991) The effects of cortisol administration on intermediary metabolism in teleost fish. Journal of Comparative Biochemistry and Physiology A, 100, pp 47-53.

van Weerd, J. H., Komen, J. (1998) The effects of chronic stress on growth in fish: a critical appraisal. Comparative Biochemistry and Physiology: Part A: Molecular and Integrative Physiology, 120, (1): pp 107-112.

Vernon, J. E. N. (1995) Australia's Ocean Age: Science and technology for managing our ocean territory, Prime Minister's Science and Engineering Council, Canberra, 48 pp.

Vijayan, M. and Leatherland, J. (1989) Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch* Walbaum). Canadian Journal of Zoology, 67, pp 2746-2750.

Warchol, M. E. and Corwin, J. T. (1996) Regenerative proliferation in organ cultures of the avian cochlea: Identification of the initial progenitors and determination of the latency of the proliferative response. Journal of Neuroscience, 16, (17): pp 5466-5477.

Wardle, C. S., Carter, T. J., Urquhart, G. G., Johnstone, A. D. F., Ziolkowski, A. M., Hampson, G. and Mackie, D. (2001) The sound of a triple G seismic air-gun and it's effects on the behaviour of marine fish. Continental Shelf Research, 21, (8-10): pp 1005-1027.

Waring, C. P., Stagg, R. M. and Poxton, M. G. (1992) The effects of handling on flounder (*Platichthys flesus* L.) and Atlantic Salmon (*Salmo salar* L.). Journal of Fish Biology, 41, pp 131-144.

Wedemeyer, G. and McLeay, D. (1981) Methods for determining the tolerance of fishes to environmental stressors. In: Pickering, A. D. (ed.), Stress and Fish, Academic Press, London, pp. 247-275.

Wedemeyer, G. A., Barton, B. A. and D.J., M. (1990) Stress and acclimation. In: Schreck, C. B. and Moyle, P. B. (eds.), Methods for Fish Biology, American Fisheries Society, Bethesda, M.D., pp. 451-489.

Wells, M. J. and O'dor, R. K. (1991) Jet propulsion and the evolution of cephalopods. Bulletin of Marine Science, 49, (1-2): pp 419-432.

Wendelaar Bonga, S. E. (1993) Endocrinology. In: Evans, D. H. (ed.), The Physiology of Fishes, CRC Press, London, pp. 469-497.

Weytes, F. A. A., Cohen, N., Flik, G. and Verburg-van Kemenade, B. M. L. (1999) Interactions between the immune system and the hypothalamo-pituitary-interrenal axis in fish. Fish and Shellfish Immunology, 9, pp 1-20.

Williamson, R. (1988) Vibration sensitivity in the statocyst of the northern octopus, *Eledone cirrosa*. Journal of Experimental Biology, 134, pp 451-454.

Williamson, R. (1989) Electrical coupling between secondary hair cells in the statocyst of the squid *Alloteuthis subulata*. Brain research, 486, (1): pp 67-72.

Winberg, S. and Nilsson, S. (1993) Roles of brain monamine neurotransmitters in agnostic behaviour and stress reactions, with particular reference to fish. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology and Toxicology, 106, (3): pp 597-614.

Woodward, C. C. and Strange, R. J. (1987) Physiological stress responses in wild and hatchery reared rainbow trout. Transactions of the American Fisheries Society, 116, pp 574-579.

Woolley, S. M. N., Wissman, A. M. and Rubel, E. W. (2001) Hair cell regeneration and recovery of auditory thresholds following amonoglycoside otoxicology on Bengalese finches. Hearing Research, 153, (1-2): pp 181-195.

Wydoski, R. S., Wedemeyer, G. A. and Nelson, N. C. (1976) Physiological response to hooking stress in hatchery and wild rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society, 5, pp 601-606.

Yan, H. Y. (1998) Auditory role of the suprabrachial chamber in gourami fish. Journal of Comparative Physiology A, 183, pp 325-333.

Yan, H. Y. and Curtsinger, W. S. (2000) The otic gasbladder as an ancillary auditory structure in a mormyrid fish. Journal of Comaparative Physiology A, 186, pp 595-602.

Yoakim, E. G. and Grizzle, J. M. (1980) Histological, histochemical and ultrastructural studies on the interrenal and chromaffin cells of the flathead minnow, *Pimephales promelas* Ratinesque. Journal of Fish Biology, 17, pp 477-494.

Yokote, M. (1982) Islets of Langerhans. In: Hibiya, T. (ed.), An Atlas of Fish Histology, Normal and Pathological Features, Kodansha LTD, Japan, pp. 126-130.

Zar, J. H. (1974) Biostatistical Analysis. Prentice-Hall, London, pp 620.

Zhao, Y., Yamoah, E. N. and Gillespie, P. G. (1996) Regeneration of broken tip links and restoration of mechanical transduction in hair cells. Proceedings of the National Academy of Science U.S.A., 94, pp 15469-15474.

Zottoli, S. J., Bentley, A. P., Prendergast, B. J. and Rieff, H. I. (1995) Comparative studies on the Mauthner cell of teleost fish in relation to sensory input. Brain, Behavior and Evolution, 46, pp 151-164.

Zottoli, S. J., Hordes, A. R. and Faber, D. S. (1987) Localization of optic tectal input to the ventral dendrite of the goldfish Mauthner cell. Brain research, 401, pp 113-121.

Appendices

Appendix 1: Scientific names, water quality, date and time of day for each trial

Table 1: Common name and scientific name for animals in each trial

Trial	Common name	Scientific name
1-5	silver bream	*Rhabdosargus sarba*
2	striped trumpeter	*Pelates sexlineatus*
3, 13-14	pink snapper	*Pagrus auratus*
4-5	mullet	*Mugil cephalus*
4-5	herring	*Nematalosa vlaminghi*
5	cuttlefish	*Sepia apama*
8	black tipped cod	*Epinephelus fasicatus*
8-9	Chinaman rockcod	*Epinephelus rivaltus*
8-9	western butterfish	*Pentapodus vitta*
8-9	silver streaked wrasse (wrasse [1])	*Stethojulis strigiventer*
9	stripy sea perch	*Lutjanus carponotatus*
9	blue spotted emperor	*Lethrinus laticaudis*
9	long finned rock cod	*Epinephelus quoyanus*
5, 10-11	squid	*Sepioteuthis australis*
12	trevally	*Pseudocaranx dentex*
12	black spot goatfish	*Parupeneus signatus*
12	jewfish	*Glaucosoma herbraicum*
12	break sea cod	*Epinephelus aramatus*
12	western king wrasse (wrasse[2])	*Coris auricularis*

Table 2: Water quality parameters of Jervoise Bay and Exmouth experimental sites. Date and time of day of each trial is also indicated.

Trial	Date (dd/mm/yy)	Time (hh:mm:ss)	Temperature (^{0}C)		Salinity (ppt)	DO_2 (mg/L)	
			*S	B		S	B
1	17/2/97	11:10:44 – 12:11:04	22.4	22.0	37.1	6.5	5.9
2	4/3/97	9:57:50 – 12:23:00	22.9	22.7	37	6.7	5.8
3	9/4/97	10:56:00 – 13:46:58	21.8	21.4	-	-	-
4	29/5/97	10:42:55 – 13:24:14	21.5	21.5	-	-	-
5	4/7/97	10:33:49 – 15:23:31	16.5	16.5	-	-	-
8	22/10/97	15:45:17 – 18:07:00	24.5	23.9	-	-	-
9	24/10/97	14:05:27 – 16:28:10	24.5	23.9	-	-	-
10	17/4/98	10:51:15 – 14:59:16	21	20.9	-	-	-
11	21/4/98	10:14:27 – 14:50:21	21.5	21.0	-	-	-
12	15/6/98	9:08:20 – 14:39:42	17.9	17.9	-	-	-
13	19/9/98	11:15:10 – 16:26:05	19	18.7	-	-	-
14	16/11/98	9:55:00 – 15:25:01	21.9	21.9	36.1	7.7	7.8

* S = surface; B = bottom

Appendix 2: Recording equipment specifications

Table 1: Specifications of recording gear used. All tapes were used in long play mode giving four hour tapes. Sensitivity is given as dB re 1 $V^2/\mu Pa^2$. RANRL was the Royal Australian Navy Research Laboratories. Serial numbers of the GEC hydrophones are given in brackets.

Equipment	Model	No.	Code	Specifications
hydrophone	Clevite CH17	1	-	sensitivity = -204.7; capacitance = 1.8 nF; cable length = 35 m
hydrophone	GEC Marconi SH 101X	4	-	sensitivity = (080) -204, (081) -203.5, (082) -203.5, (083) -206; capacitance = 9.4 nF; cable length = all 45 m
preamplifier	RANRL type	4	UPMP, DPMP (comprising separate split channel amps) CPA6 & CPA7	low noise; input impedance = 1 MΩ; linear frequency response < 4 Hz - >20 kHz; gain 20 or 40 dB
tape deck	Sony DAT D8	4	-	32 kHz sample rate; 4 hour tape; linear response 20 Hz – 14 kHz

Table 2: Designation and combination of recording gear used. Specifications of equipment are given in Table 1.

Designation	Hydrophone	Preamps	Tape Decks	Timers
portable	GEC – Marconi	DPMP	D8	free run
portable	Clevite CH17	Video cassette recorder		

Appendix 3: 1/3 octave band limits used in analysis of air-gun signal

Centre Frequency (Hz)	Lower Frequency (Hz)	Upper Frequency (Hz)	Bandwidth Correction (dB)
0.49	0.44	0.55	-9.47
0.62	0.55	0.69	-8.46
0.78	0.69	0.87	-7.46
0.98	0.87	1.10	-6.46
1.23	1.10	1.38	-5.45
1.55	1.38	1.74	-4.45
1.95	1.74	2.19	-3.45
2.46	2.19	2.76	-2.44
3.10	2.76	3.48	-1.44
3.91	3.48	4.38	-0.44
4.92	4.38	5.52	0.57
6.20	5.52	6.96	1.57
7.81	6.96	8.77	2.57
9.84	8.77	11.05	3.58
12.40	11.05	13.92	4.58
15.63	13.92	17.54	5.58
19.69	17.54	22.10	6.59
24.80	22.10	27.84	7.59
31.25	27.84	35.08	8.60
39.37	35.08	44.19	9.60
49.61	44.19	55.68	10.60
62.50	55.68	70.15	11.61
78.75	70.15	88.39	12.61
99.21	88.39	111.36	13.61
125.00	111.36	140.31	14.62
157.49	140.31	176.78	15.62
198.43	176.78	222.72	16.62
250.00	222.72	280.62	17.63
314.98	280.62	353.55	18.63
396.85	353.55	445.45	19.63
500.00	445.45	561.23	20.64
629.96	561.23	707.11	21.64
793.70	707.11	890.90	22.64
1000.00	890.90	1122.46	23.65
1259.92	1122.46	1414.21	24.65
1587.40	1414.21	1781.80	25.65
2000.00	1781.80	2244.92	26.66
2519.84	2244.92	2828.43	27.66
3174.80	2828.43	3563.59	28.66
4000.00	3563.59	4489.85	29.67
5039.68	4489.85	5656.85	30.67
6349.60	5656.85	7127.19	31.67
8000.00	7127.19	8979.70	32.68
10079.37	8979.70	11313.71	33.68

Appendix 4: Codes used in scoring behaviour of fish and squid

BEHAVIOUR	CODE	DESCRIPTION
Species		
break sea cod	BS	*Epinephelus aramatus*
butter fish	BA	*Pentapodus vitta*
Charlie Court	CT	*Epinphelus fasicatus, E. rivaltus, E. quoyanus*
cuttlefish	CF	*Sepia apama*
goat fish	GT	*Parupeneus signatus*
herring	HE	*Nematalosa vlaminghi*
jewfish	JW	*Glaucosoma herbraicum*
mullet	LL	*Mugil cephalus*
silver bream	SB	*Rhabdosargus sarba*
trevally	SK	*Pseudocaranx dentex*
blue spotted emperor	SN	*Lethrinus laticaudis*
stripy sea perch	SF	*Lutjanus carponotatus*
squid	SQ	*Sepioteuthis australis*
striped trumpeter	BF	*Pelates sexlineatus*
wrasse	WR	*Coris auricularis;Stethojulis strigiventer*
unidentified fish	UF	used when fish behaviour was observed but species could not be identified
School/group		
loose group	LG	animals in group but not in close proximity to each other
tight group	TG	close association with each other i.e. < 2 body lengths
animals in all directions	AD	no order to group observed, no uiniform direction
majority of animals	MA	most of animals displaying a particular behaviour
some animals	SE	some animals (i.e. < 50% displaying a particular behaviour)
Alarm		
flash expansion	FL	tight groups quickly separating & then reforming a tight group
parting	PA	groups of animals quickly expanding
darting	DA	animals swimming very fast for a short period of time
Swimming speed		
idle	ID	animals displaying no detectable horizontal or vertical movement
slow swimming	SS	animals swimming non-purposefully; aimlessly
fast swimming	FS	animals swimming at faster than normal speed; purposefully
very fast swimming	VF	animals swimming much faster than normal; purposefully
Horizontal movement/position		
right to left of screen	RL	horizontal swimming direction
left to right of screen	LR	
circling	CC	animals changing direction > 4 times in the field of view
animals on left hand side	LH	behaviours observed on left hand side of screen
animals on right hand side	RH	behaviours observed on right hand side of screed
change direction	CD	all individuals in group changing direction at same time

Appendix 4: Codes used in scoring the behaviour of fish & squid

Vertical position		
upper	UP	animals observed in upper 1/3 of cage
mid	MD	animals observed in middle of cage
lower	LO	animals observed in lower 1/3 of cage
Vertical movements		
top to lower	UL	vertical swimming direction
top to mid	UM	
lower to top	LU	
lower to mid	LM	
mid to lower	ML	
mid to top	MU	
General behaviour		
approach camera	CM	animals observed approaching the camera i.e. not just swimming past camera but approaching with 'curiosity'
unsure of numbers	UN	some factor (eg poor visibility) resulting in uncertainty of number of animals exhibiting behaviour
fish in centre of cage	CN	behaviours observed in the centre of cage
fish being fed	FD	at the conclusion of some trials the animals were fed while cameras were operating
away from camera	AW	animals swimming away from camera
towards camera	TW	animals swimming towards camera
on side	SD	animals swimming on side; disorientated
Squid specific		
ink ejected	IK	squid observed ejecting ink
jetting	JE	squid observed moving quickly backwards in 'jerking' motion
backwards	BW	squid observed swimming backwards i.e. body first
forwards	FW	squid observed swimming forward i.e. head / tentacles & arms first
body pointed at surface	BP	body directed at surface, head directed to lower section of cage
dark colouration	DK	noticeable colour change to darker shade
light colouration	LT	noticeable colour change to lighter shade
white spot	WS	large white spot observed on squid mantle
attack	AK	animals show 'aggressive' behaviour
House keeping		
in view	IV	animals in view
out of view	OV	animals out of view
poor visibility	VB	observations difficult as a result of poor visibility
boat engine	XX	audible boat engine noise
gun on	GN	audible air-gun noise
gun off	GF	no air-gun noise
diver in cage	DV	diver in cage at the beginning & end of trials i.e. only observations recorded when the diver was out of the cage were analysed
diver out of cage	DO	
video stop/start	??	faulty equipment
observation from dinghy	DG	animal behaviour observed from dinghy
stop clock	ZZ	video paused
moving camera	MC	camera moving as a result of current
moving cage	MG	cage moving as a result of current

Appendix 5: Graphical representations of all observed behaviours of fish and squid in each trial

Figures 1 - 14 are graphical representations of the behavioural observations from trials 3, 5, 8 - 14. Axes at bottom of page represent the air-gun exposure levels and time of day. The legend for figures 1 - 14 is given in Table 1. Species represented are given at each figure. Due to the large quantity of data recorded in each trial, the observations from each camera are displayed separately.

Table 1: Legend for Figures 1 - 14.

Group	Behaviour	Symbol
Vertical position	Top, mid or lower	dots
Vertical movements	Top to bottom Top to mid Bottom to top Bottom to mid	arrows
Swimming speed	idle	Dot
	Slow swim	Small arrow
	Fast swim	Larger arrow 2 feathers
	Very fast swim	Largest arrow 3 feathers
Horizontal swim direction	Swim left of screen	Left slant arrow
	Swim right of screen	Right slant arrow
	Swimming in circles	Open circle
Field of view	LHS	Square
	RHS	diamond
Startle responses	Dart	Small one feather arrow
	School part	Larger 2 feathered arrow
	Flash expansion of school	Larger 3 feathered arrow
	Change direction	cross
School formation	Loose school	Circle
	Tight school	Plus sign
	Most animals	dot
	Some animals	Dot below
Specific behaviours	Approach camera	Arrow with one feather
Time in view	Horizontal bars	
Air-gun levels	Given for each shot fired during trial	
*Startle responses	Eject ink	Square
	Jerk	circle
*Specific behaviours	Dark colouration	Large dot
	Light colouration	circle
	White spot on mantle	Cross in circle
	Squid 'attacking' each other	diamond

* Indicates behaviour specific to squid.

Trial 3

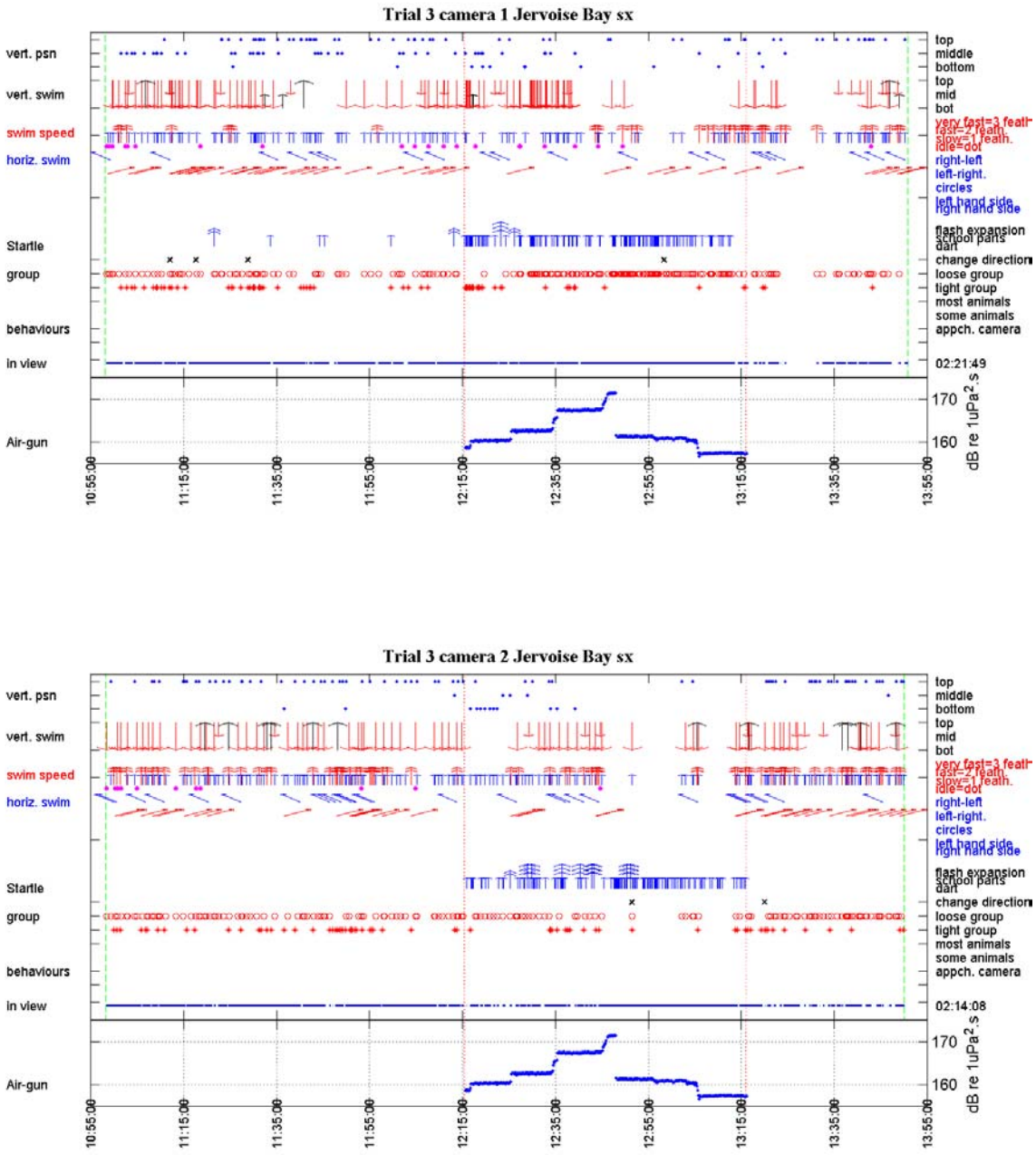

Figure 1: Behavioural observations for juvenile striped trumpeter (*Pelates sexlineatus*) in trial 3.

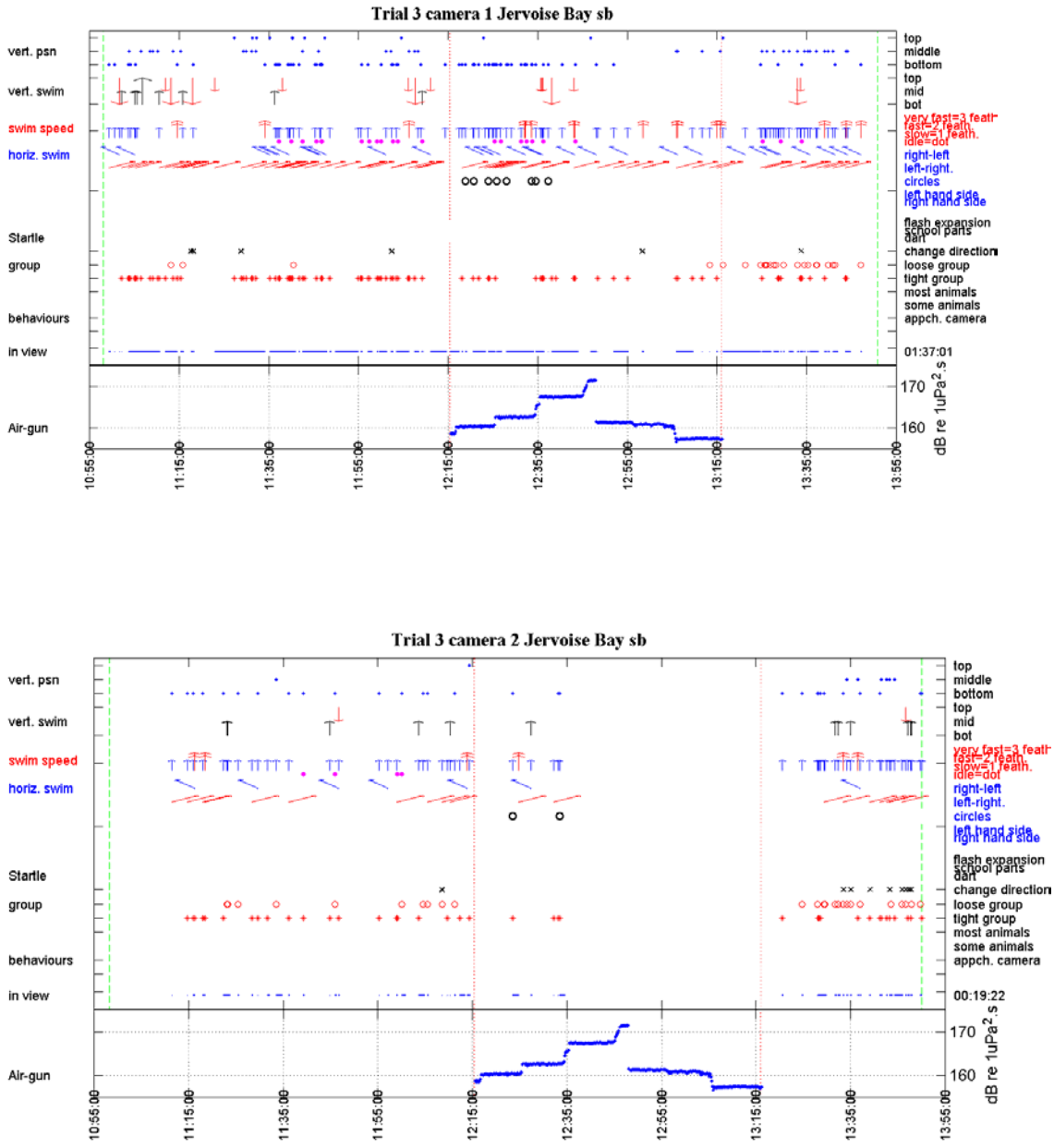

Figure 2: Behavioural observations for silver bream (*Rhabdosargus sarba*) in trial 3.

Trial 5

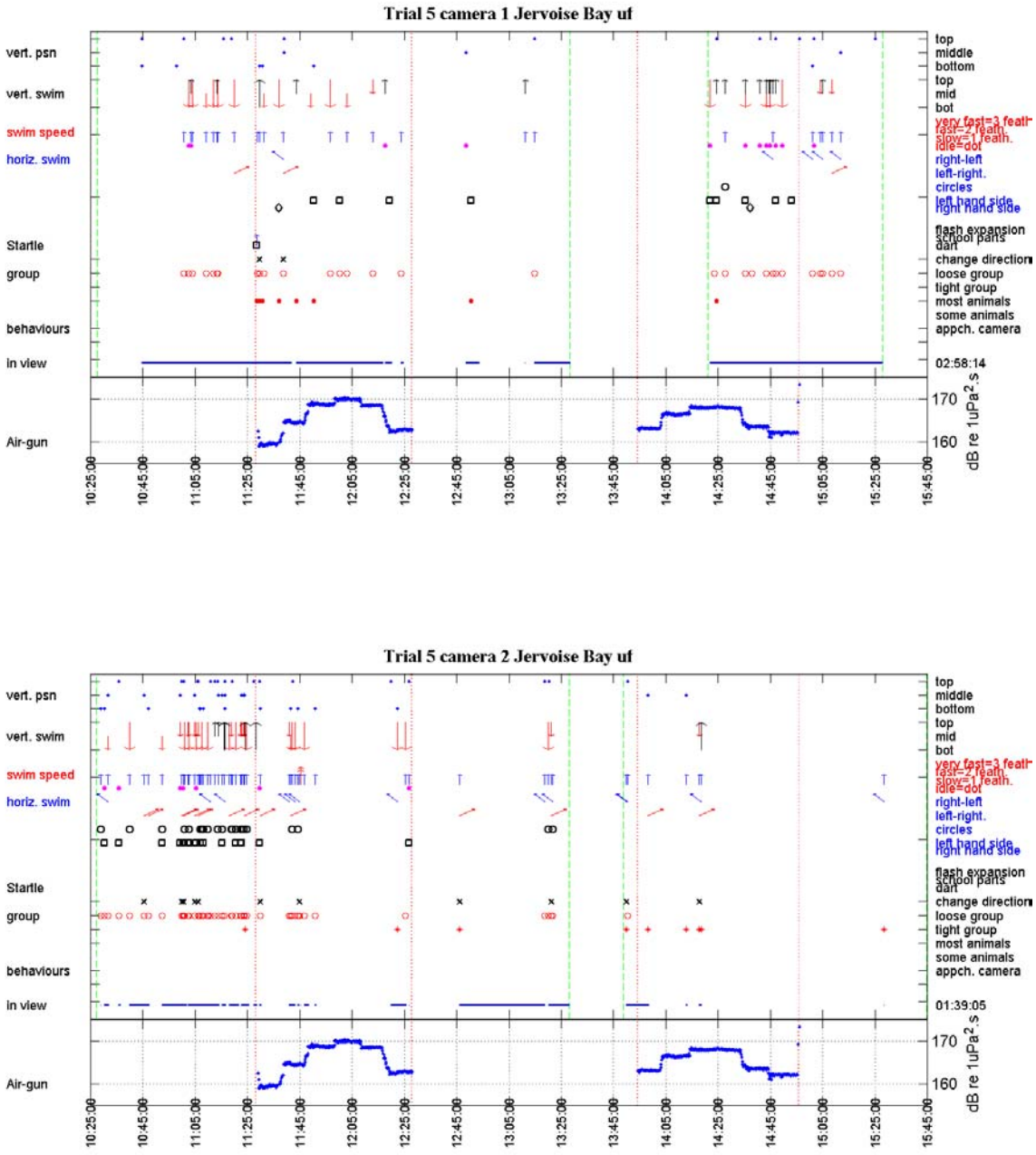

Figure 3: Behavioural observations for unidentified fish in trial 5.

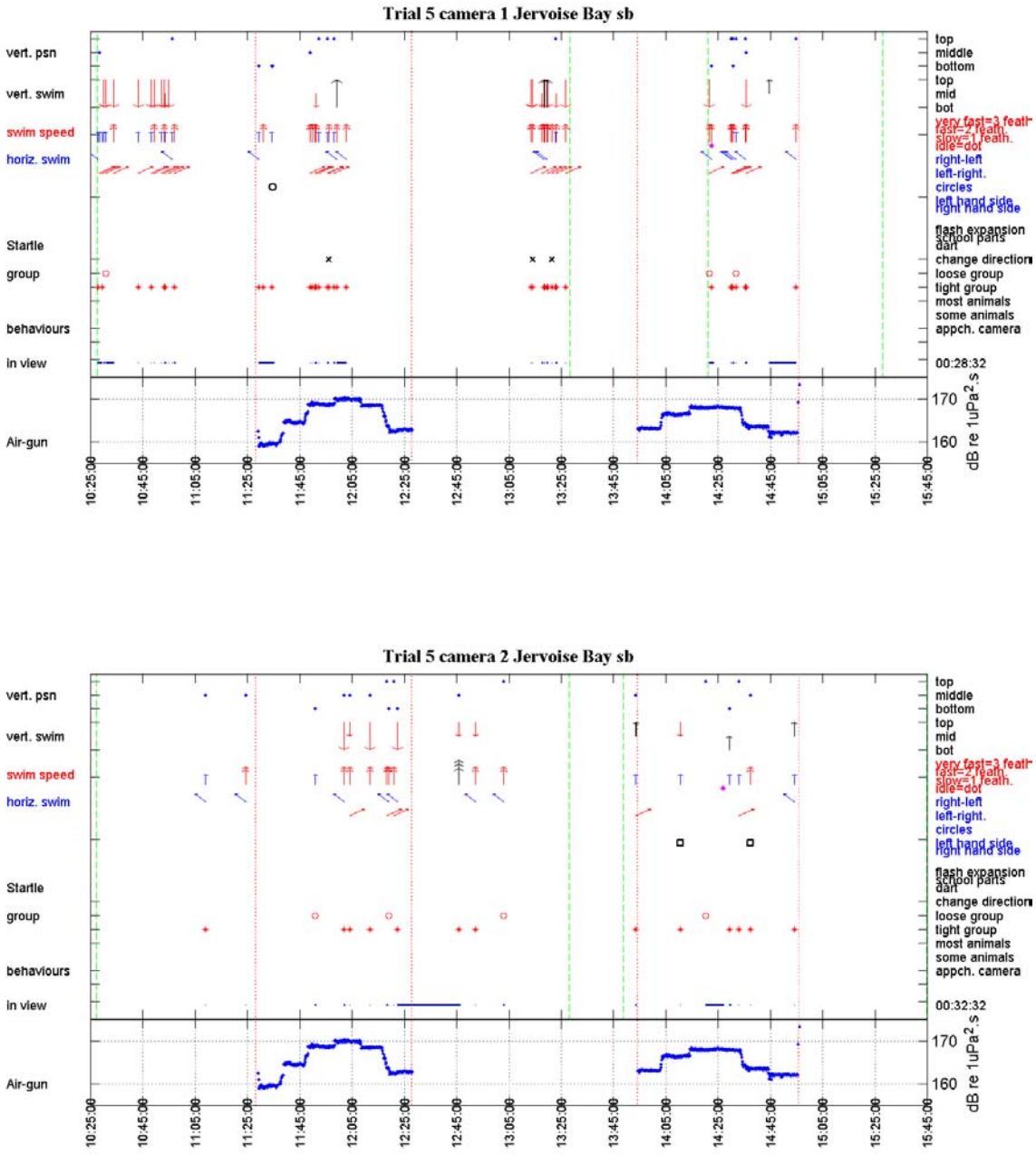

Figure 4: Behavioural observations for silver bream (*Rhabdosargus sarba*) in trial 5**.**

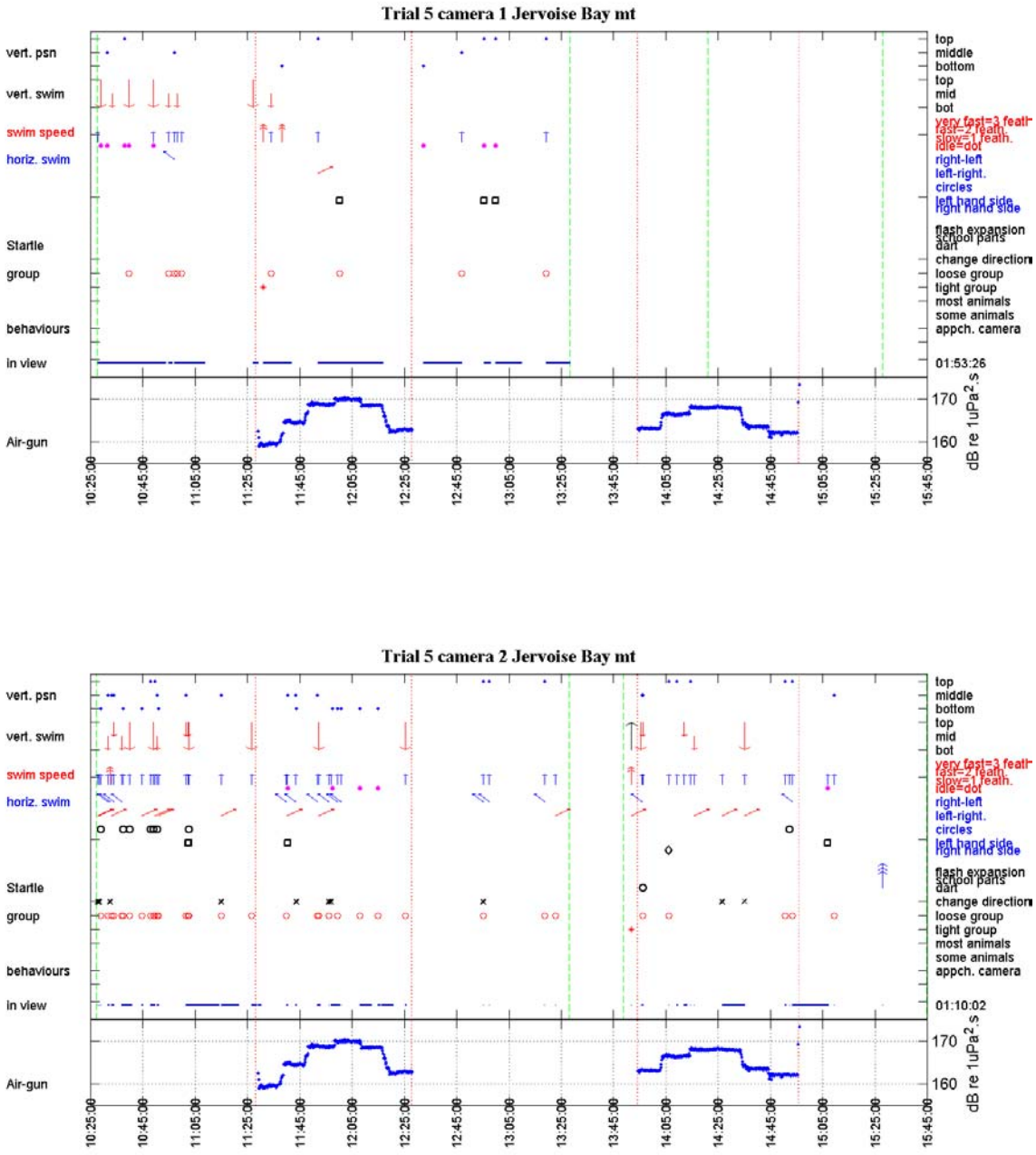

Figure 5: Behavioural observations for mullet (*Mugil cephalus*) in trial 5.

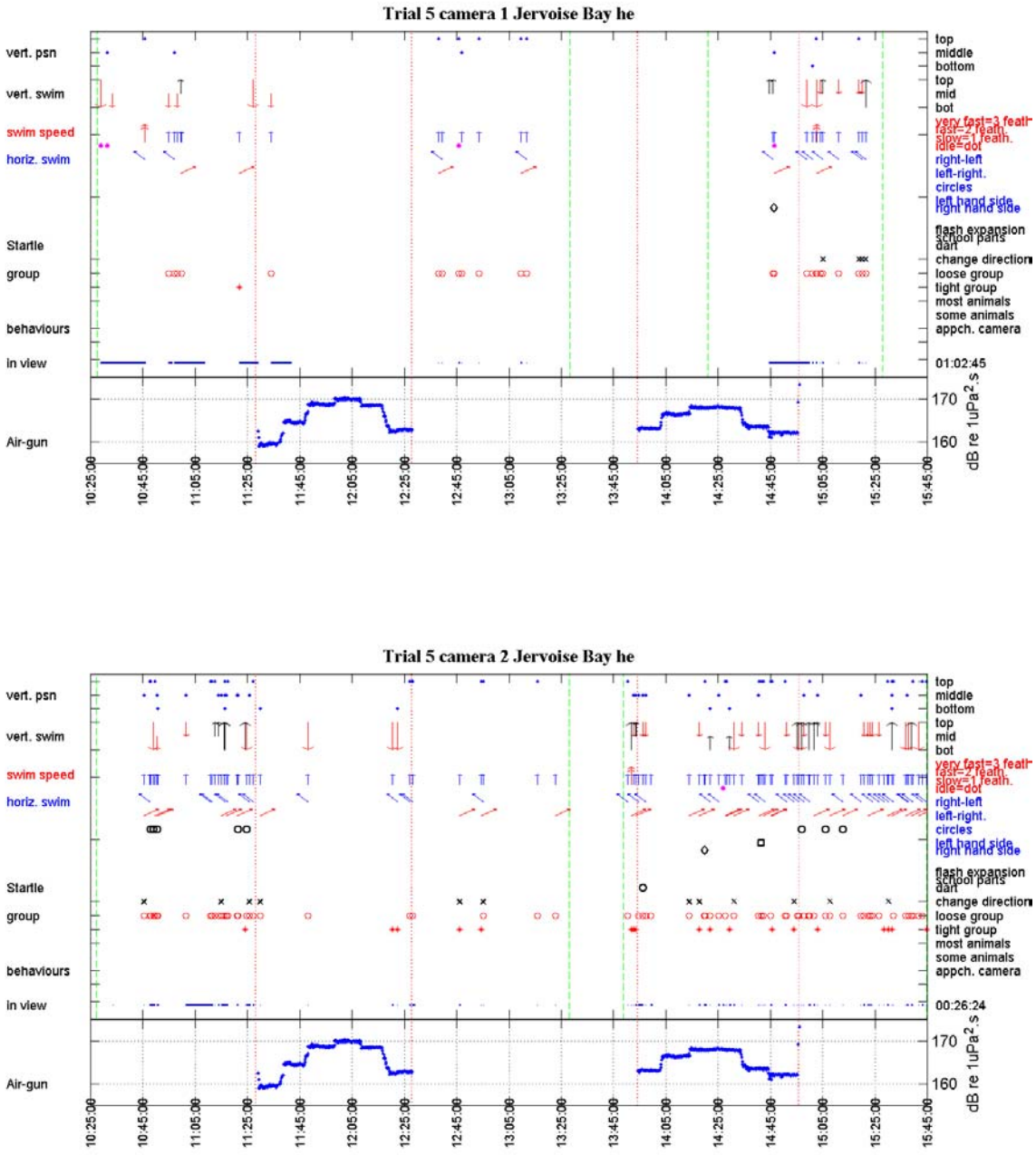

Figure 6: Behavioural observations for herring (*Nematalosa vlaminghi*) in trial 5.

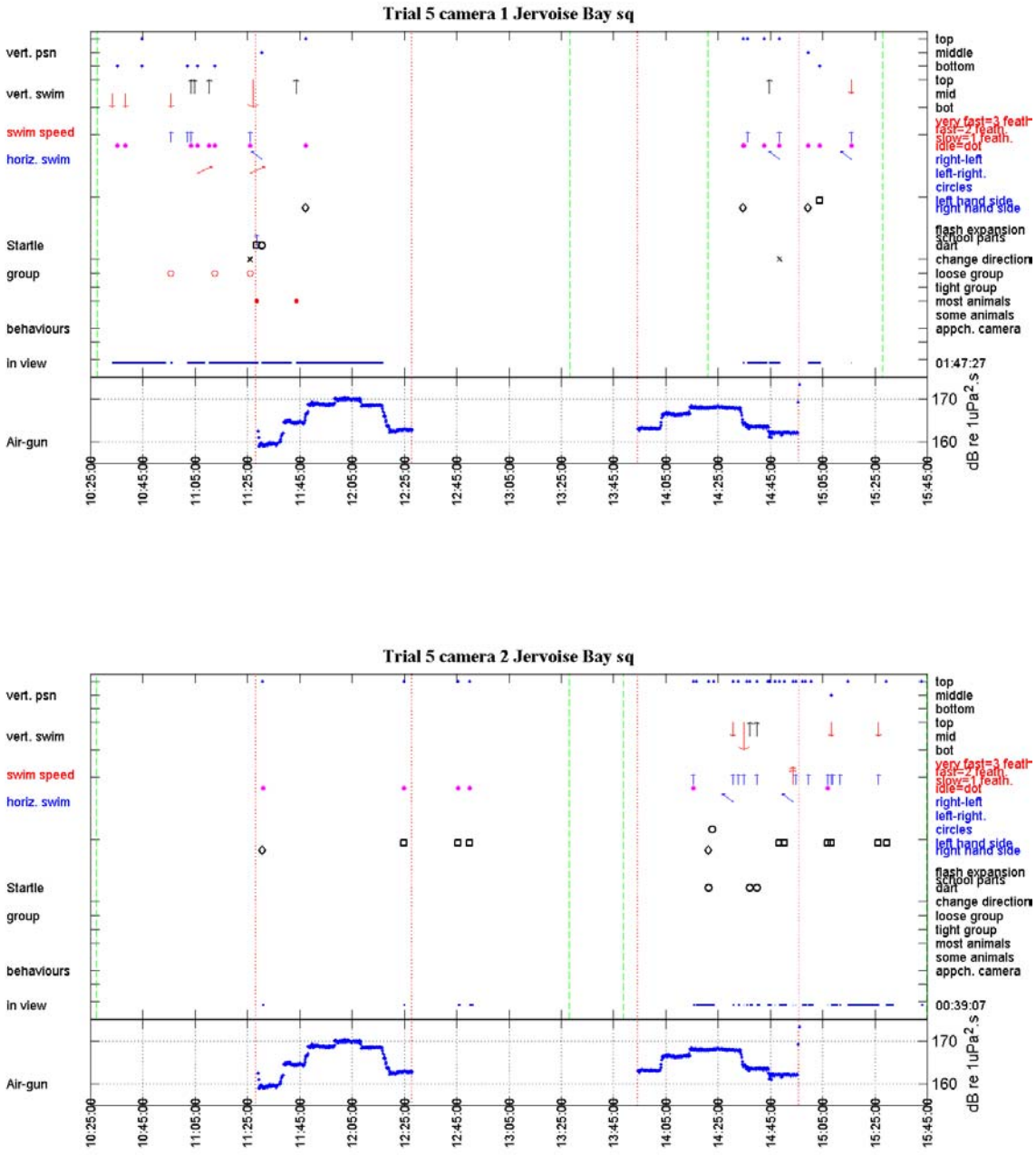

Figure 7: Behavioural observations for squid (*Sepioteuthis australis*) in trial 5.

Exmouth - trials 8 and 9

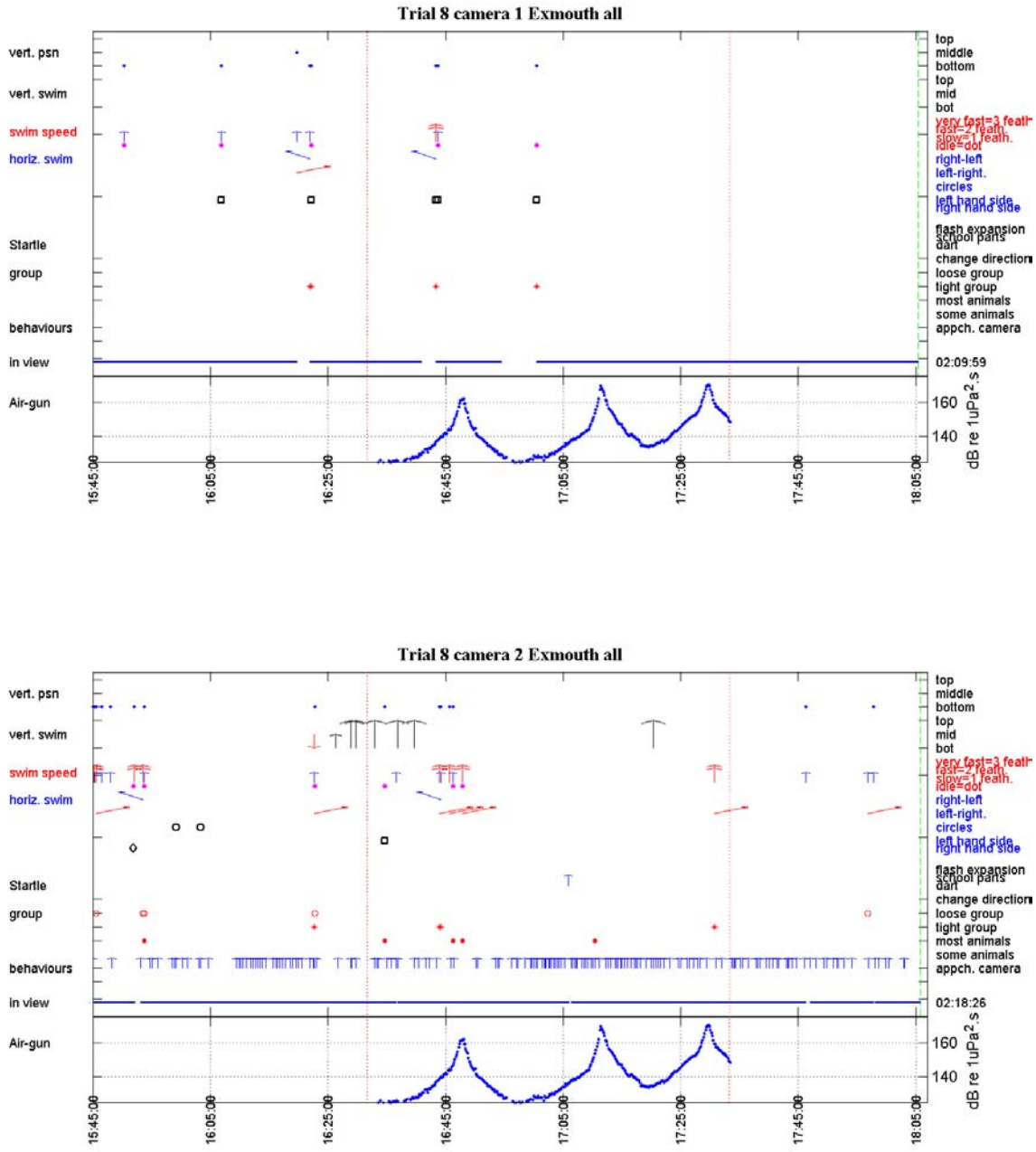

Figure 8: Behavioural observations for all species held in the cage at Exmouth in trial 8.

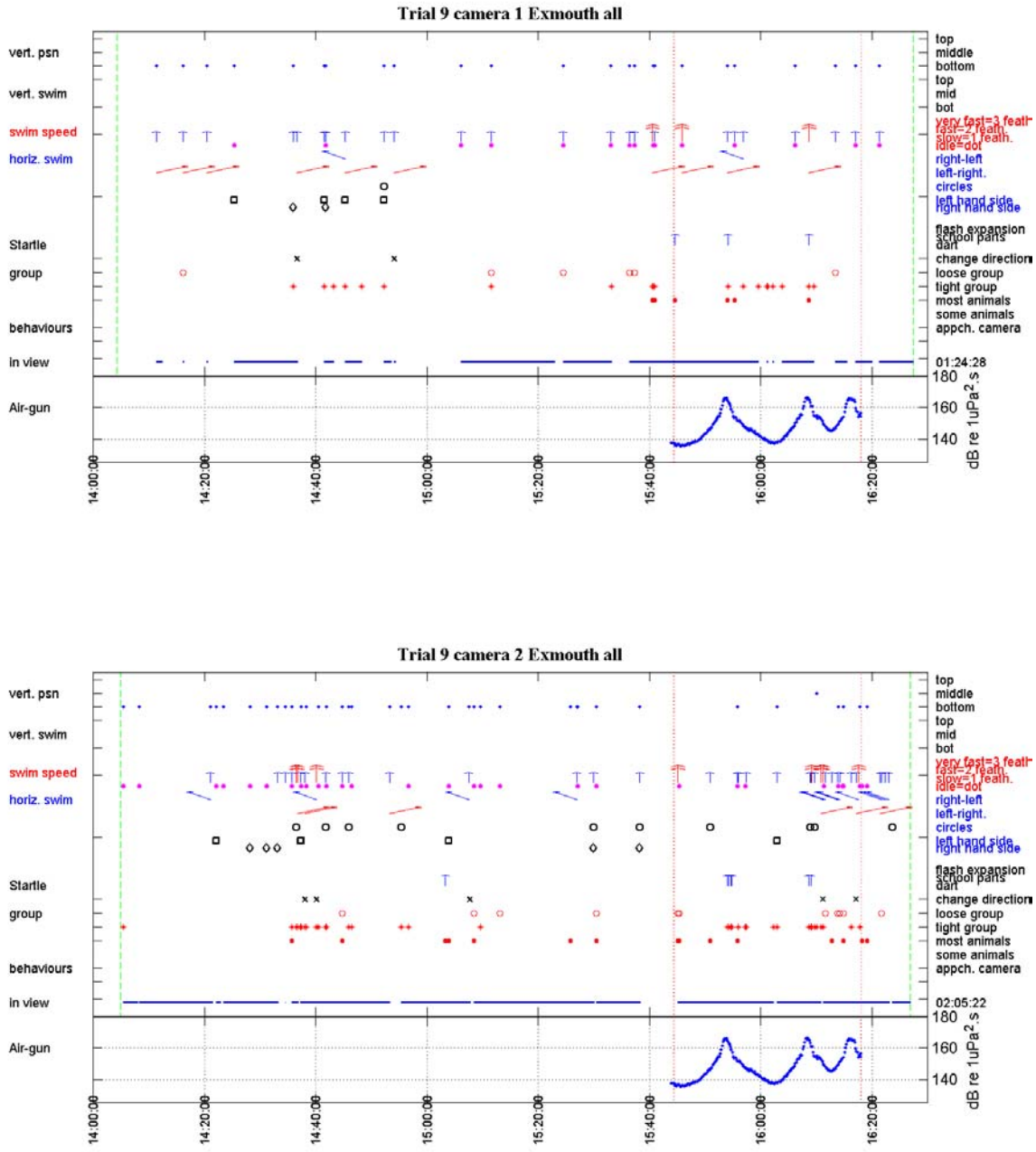

Figure 9: Behavioural observations for all species held in the cage at Exmouth in trial 9.

Trial 10

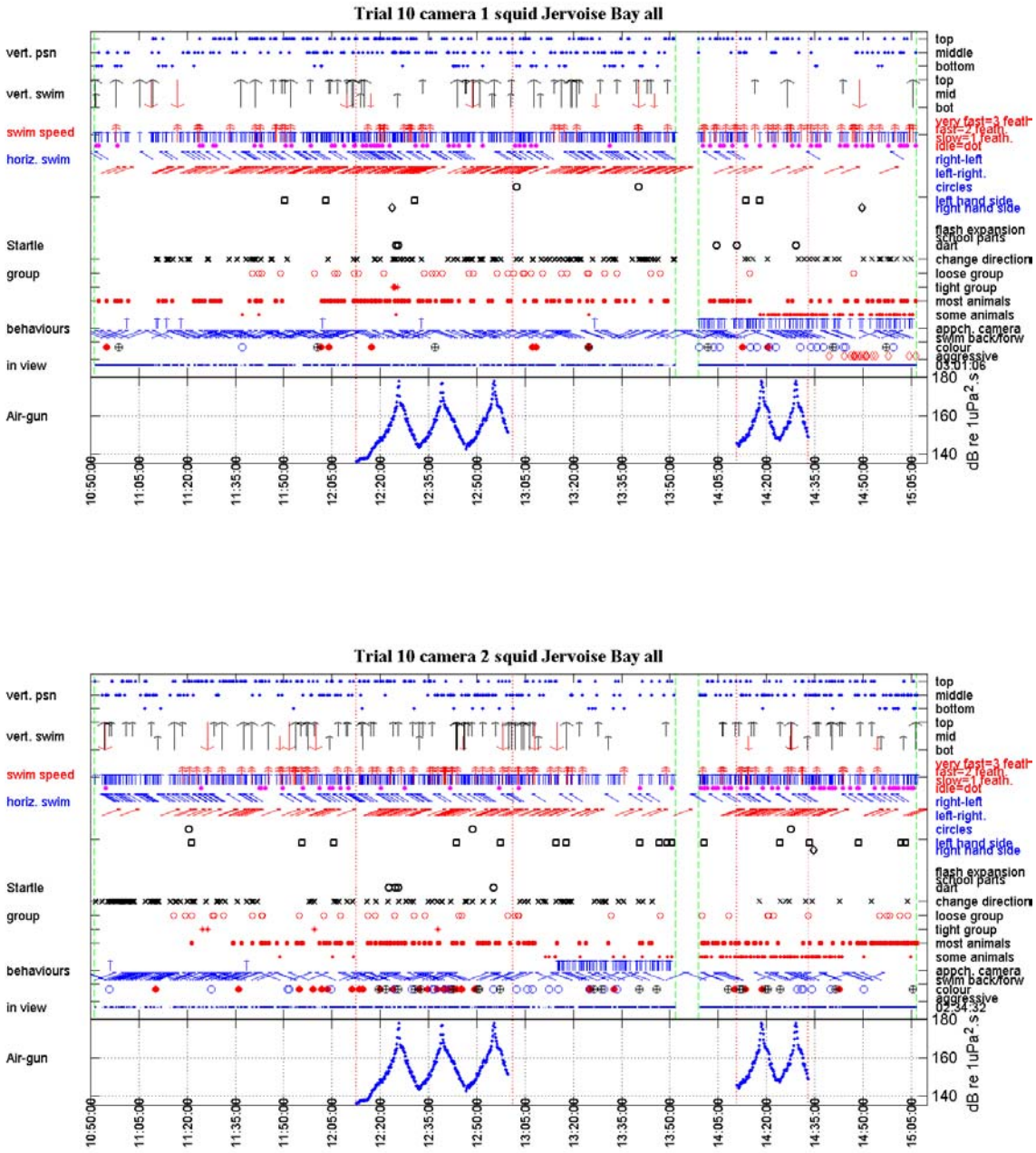

Figure 10: Behavioural observations for squid (*Sepioteuthis australis*) in trial 10.

Trial 11

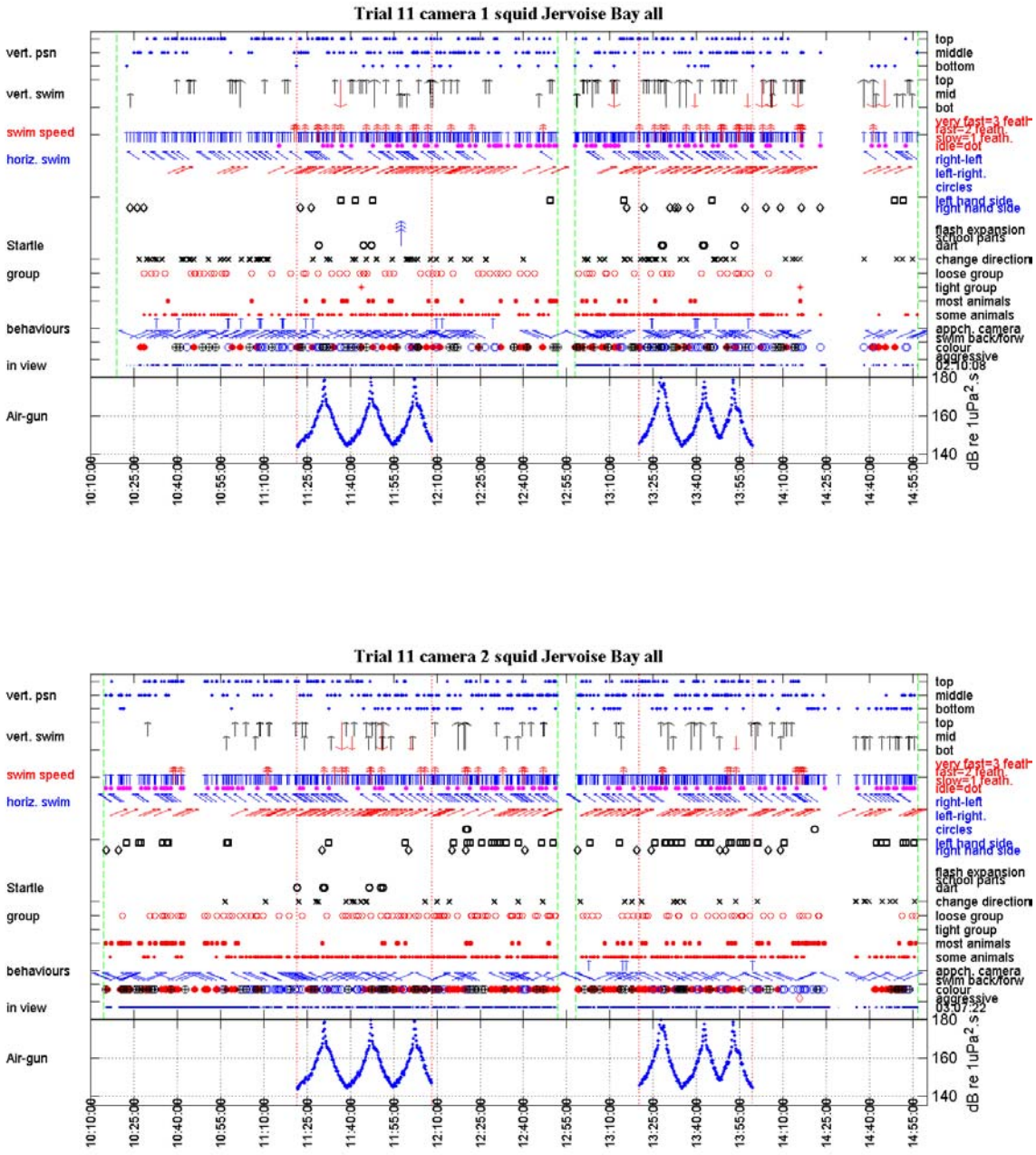

Figure 11: Behavioural observations for squid (*Sepioteuthis australis*) in trial 11.

Trial 12

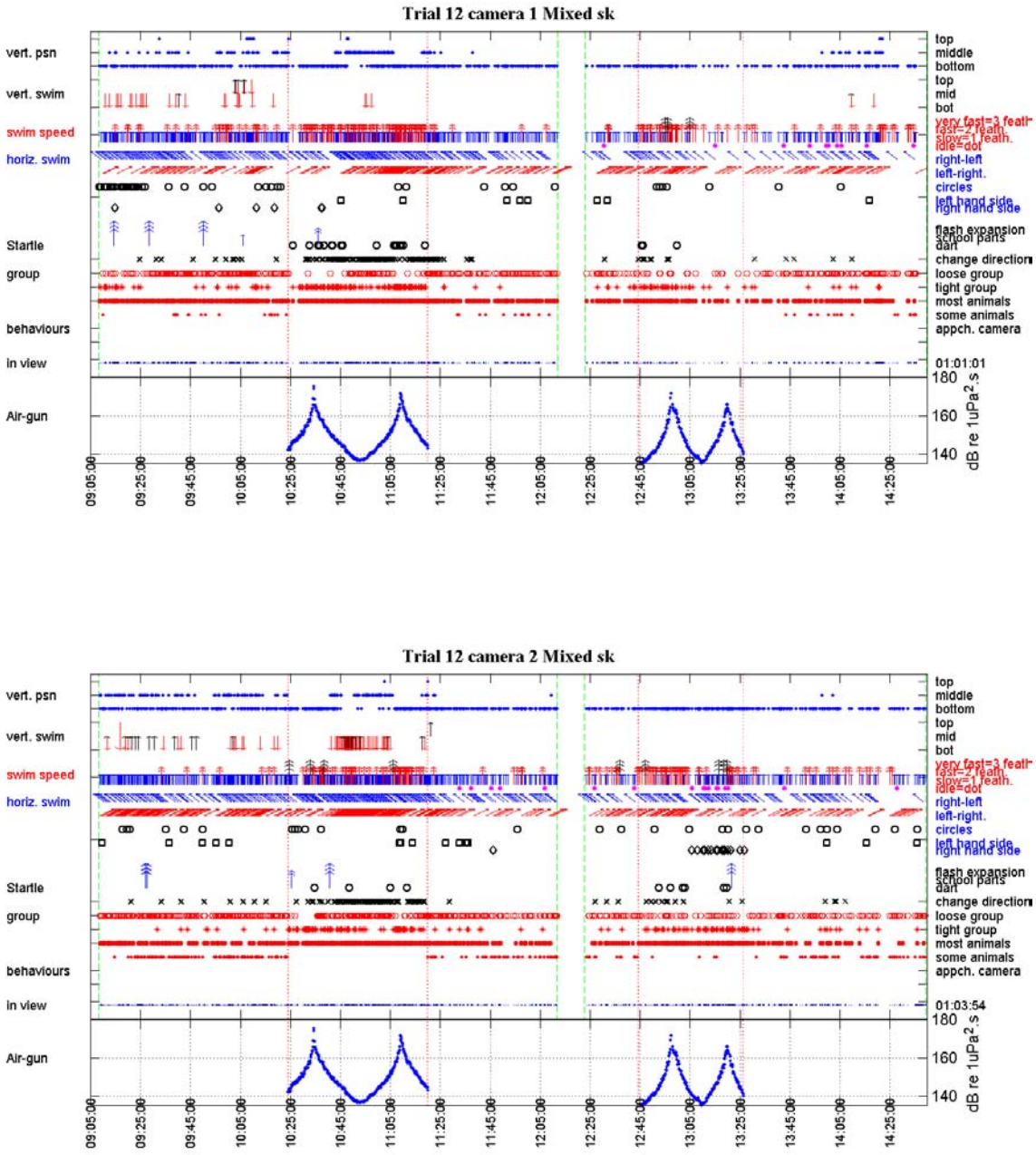

Figure 12: Behavioural observations for trevally (*Pseudocaranax dentex*) in trial 12.

Trial 13

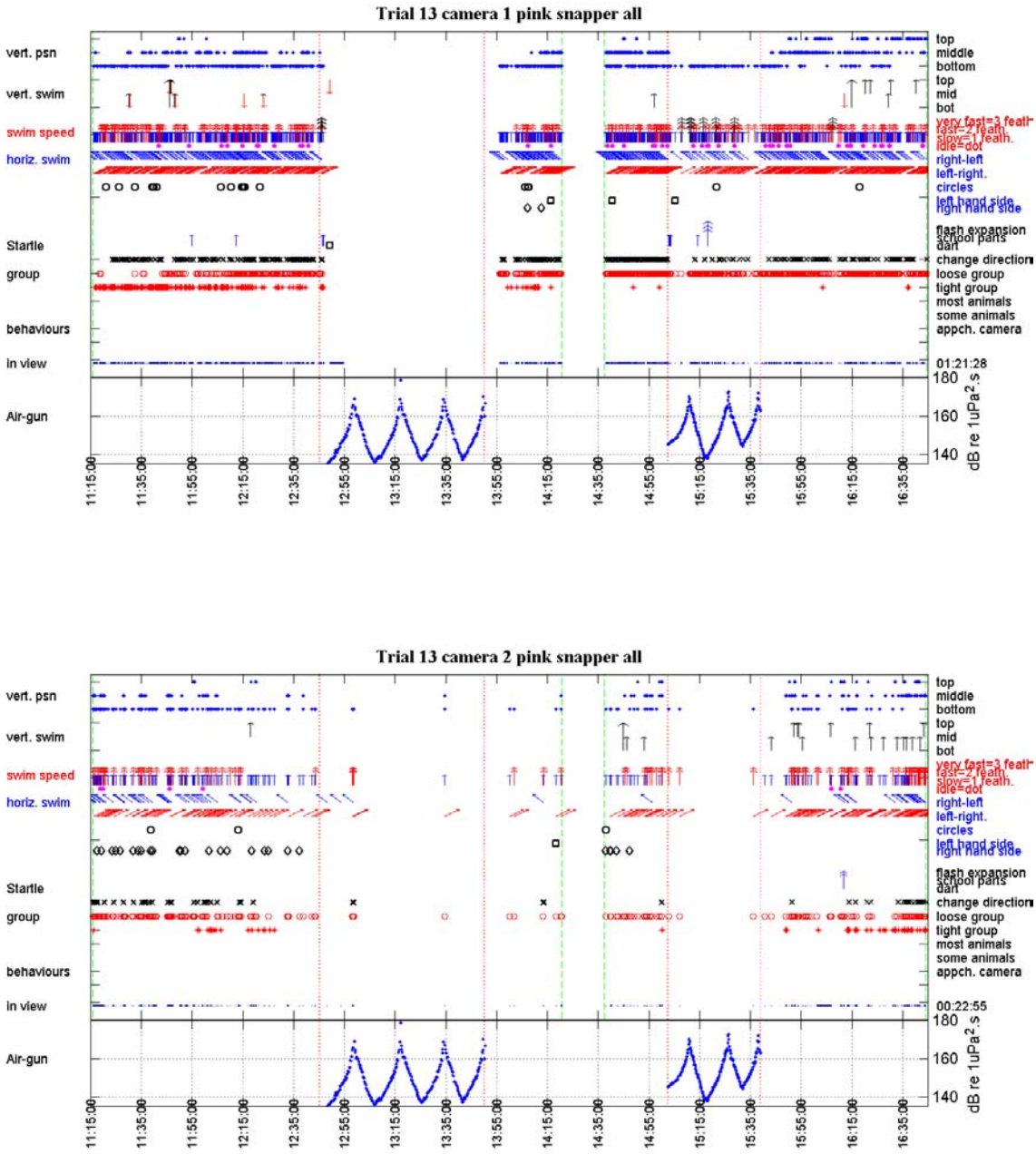

Figure 13: Behavioural observations for pink snapper (*Pagrus auratus*) in trial 13.

Trial 14

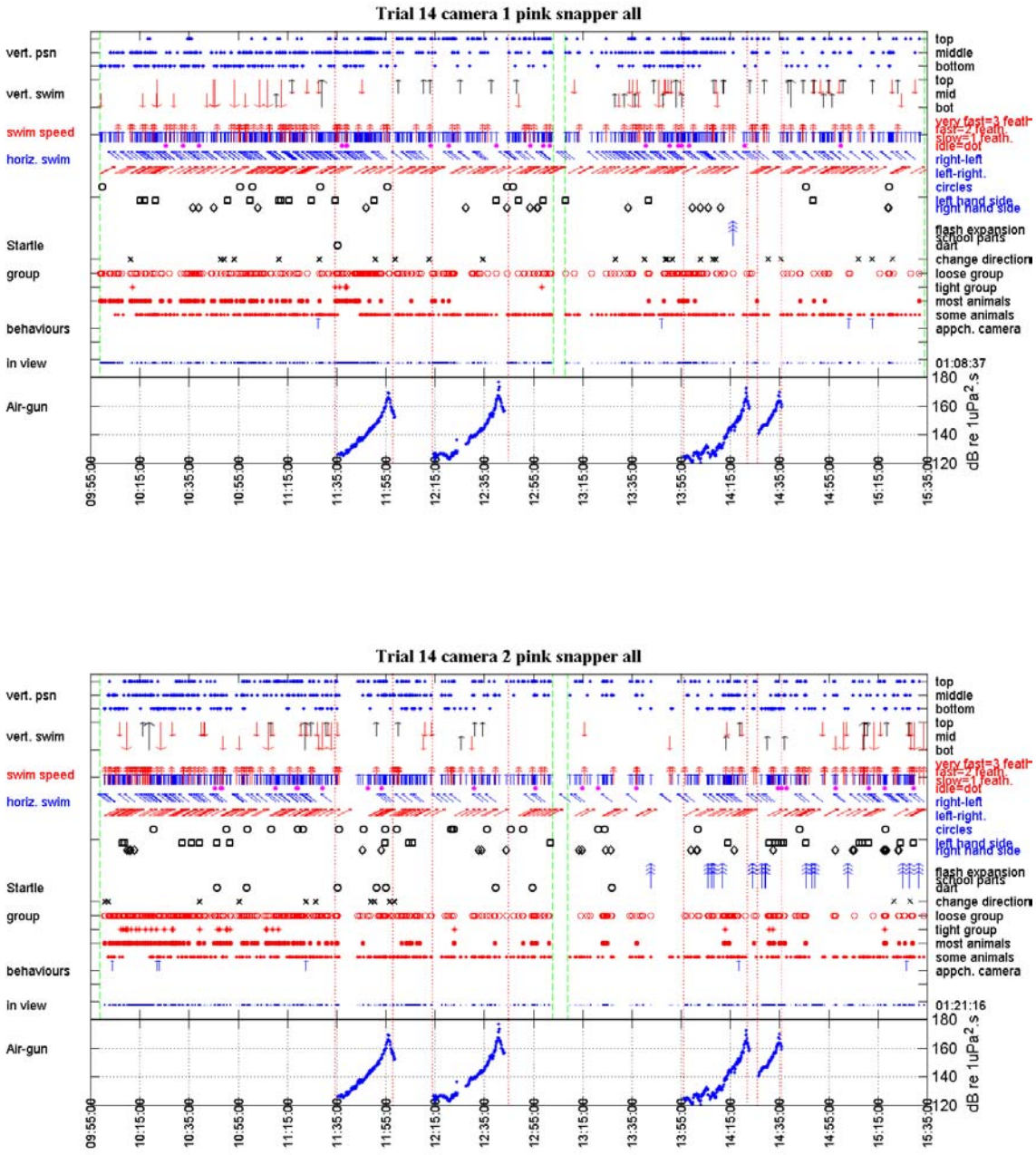

Figure 14: Behavioural observations for pink snapper (*Pagrus auratus*) in trial 14.