

School of Molecular and Life Sciences

**An Assessment of the Optimal Techniques for Sampling Shallow Water
Reef Fishes**

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**This thesis is presented for the degree of
Master of Research
of
Curtin University**

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8th edition (2013). Data was collected in accordance with the animal ethics approval from the Curtin University Animal Ethics Committee (ARE2020-26 and ARE2021-3).

Signature: 

Date: 01/10/2022

Abstract

Marine Protected Areas (MPAs) are a popular conservation strategy aimed at managing anthropogenic pressures and protecting habitats and the diversity of ocean flora and fauna. Robust, cost-effective sampling of fish assemblages is important for understanding the effects of these management strategies on ocean ecosystems and assessing changes in fish assemblages over time. The development of methods to sample fish assemblages has progressed greatly from destructive methods such as toxins, dynamite, and trawl nets, which do not align with the conservation objectives of MPAs to non-destructive methods. These non-destructive methods include underwater visual census (UVC) and stereo-video methods. Diver operated stereo-video (stereo-DOV) and baited remote underwater stereo-video systems (stereo-BRUVs) are widely used methods that have well documented biases and advantages. The use of stereo-video fitted to a micro remote operated vehicle (stereo-ROV) is an emerging technology that is undergoing testing and development to validate its use for marine research. When selecting a sampling method in a monitoring program, it is important to understand the advantages and biases associated with each method. These advantages and biases may affect the assemblage composition sampled as well as logistical, financial, and safety considerations of the monitoring program.

I compared the sampling effectiveness and efficiencies of three commonly used methods of sampling fish assemblages (UVC, stereo-BRUVs, stereo-DOV) and one emerging method (stereo-ROV). I assessed the assemblage composition, numbers of species and individuals, and the statistical power to detect hypothetical changes for each method. I also assessed the ability to measure length, the length frequency distribution sampled by stereo-DOV and stereo-ROV and the behaviour of fishes towards each technique. I found that stereo-BRUVs sampled a distinctive assemblage compared to UVC and stereo-ROV and that stereo-ROV and UVC sampled significantly different assemblages. Stereo-DOV and stereo-ROV sampled comparable assemblages with some site level differences driven by schooling species. Small differences in the number of individuals and length frequency distribution sampled by stereo-DOV and stereo-ROV were observed. Overall, stereo-BRUVs sampled more mobile, predatory species, and the transect methods sampled more site attached species. After removing schools of fish that were disproportionately affecting the data, the differences in length frequency distribution sampled by stereo-DOV and stereo-ROV were unlikely to be biologically significant. The behavioural differences of fishes towards SCUBA divers and the ROV appeared to be due to life history traits of specific species and levels of fishing pressure. Species in the family *Pomacentridae* showed more aggressive behaviour towards divers due to their feeding regime, and Baldchin Grouper (*Choerodon rubescens*) which is a targeted species in the Jurien Bay Marine Park, had a weaker and shallower relationship between fork length and minimum approach distance (MAD) with the ROV compared to divers. This indicated that individuals from this targeted species were less wary of the ROV compared to divers, especially larger individuals. This pattern has the potential to improve measurement accuracy of species that are less wary of the ROV because measurement accuracy increases as fish get closer to the stereo-video cameras.

I concluded that to effectively monitor MPAs a combination of stereo-BRUVs, which more effectively sampled mobile, predatory fisheries indicator species, and one of the transect based methods, which more effectively sampled site attached species should be used. Due to

the advantages of stereo-video based methods including the accuracy and precision of length measurements, accurate definition of transect area, and ability to undertake reviews of species identification, I recommend a stereo-video transect technique over UVC as a complementary method to stereo-BRUVs. Finally, due to the similarities in assemblage composition, number of species and length frequency distribution, and the behavioural effect of targeted species towards the micro-ROV, along with the logistical and safety advantages of stereo-ROV over stereo-DOV, I recommend using a combination of stereo-ROV and stereo-BRUVs for sampling fish inside and outside MPAs.

Acknowledgement of Country

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions' values and commitment to our role as leaders in the Reconciliation space in Australia.

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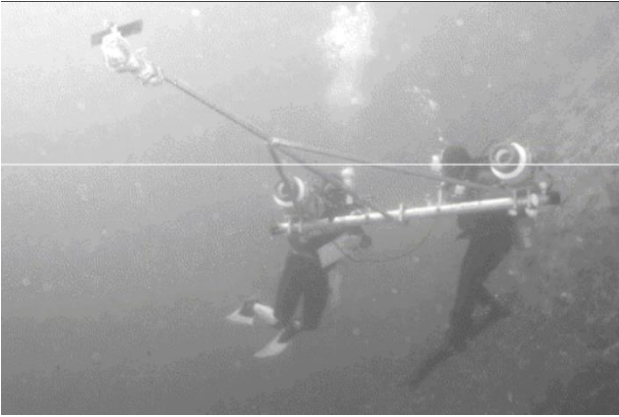
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1. CHAPTER 1: GENERAL INTRODUCTION



Harvey & Shortis, 1998



Goetze et al., 2019



Langlois et al., 2020



Harvey, 2018

Photographs: Representative images of stereo-video technologies and applications over time

1.1 Background and Rationale

Marine protected areas (MPAs) aim to reduce anthropogenic pressures by creating areas in the sea where extractive activities such as recreational and commercial fishing are limited (Claudet & Guidetti, 2010). The establishment of MPAs has increased in popularity as a method to protect the biodiversity of fish stocks and habitats (Côté et al., 2001; Mora & Sale, 2011; Ward & Hegerl, 2003). MPA effectiveness varies with factors including illegal harvesting, migration of fauna outside protected areas (Bergseth et al., 2017; Edgar et al., 2014), and design aspects such as depth, range, and connectivity among protected areas (Goetze et al., 2021). This variation in effectiveness highlights the importance of monitoring fish assemblages to assess changes over time to understand the effects of particular management strategies.

The methods used to undertake reef fish monitoring have progressed from destructive methods (Stephan, 1904), to underwater visual census (UVC) (Brock, 1954) and stereo-video sampling which has undergone thorough and extensive testing and validation in the last 30 years (Goetze et al., 2019; Harvey & Shortis, 1995, 1998; Langlois et al., 2020; Whitmarsh et al., 2017). An understanding of the advantages and biases associated with ecological data collection is important to incorporate into experimental design and for inferring patterns in biological data over time (Harmelin-Vivien & Francour, 1992). All methods of collecting ecological data have inherent advantages and biases (MacNeil et al., 2008a). For sampling fish assemblages, some of these advantages and biases include variation in a methods ability to collect robust data on a number of indicators including abundance, biomass and behaviour (Bach et al., 2020; Goetze et al., 2017; Nash & Graham, 2016) which may vary in ecosystems with differing levels of habitat complexity (Holmes et al., 2013). Methods also vary in the time taken to collect this data and the logistical constraints such as the need for taxonomic expertise or consideration for diver decompression tables. Evaluation and comparison of these methods is important for optimising the effectiveness and efficiency of monitoring programs, especially with the development of new technologies. In this introduction, I briefly analyse the advantages and biases of four methods used for assessing fish assemblage composition that are investigated throughout this thesis. These methods include three commonly used methods (baited remote underwater stereo-video systems (stereo-BRUVs), diver operated stereo-video (stereo-DOV), and UVC) and one emerging method (micro remote operated vehicle with attached stereo-video system (stereo-ROV)).

1.1.1 Underwater visual census

The growing concerns about the effects of destructive sampling on the marine environment led to a transition from destructive methods of assessing fish population dynamics such as dynamite, toxins, and trawl nets to non-destructive methods such as UVC (Harmelin-Vivien & Francour, 1992; Mallet & Pelletier, 2014; Murphy & Jenkins, 2010). UVC was developed as a method for quantifying fish assemblage compositions using SCUBA diver observations (Brock, 1954). The development and validation of UVC led to it emerging as the predominant method to survey shallow water temperate and tropical reefs (Cappo et al., 2003). Different methods of UVC have been utilised, including transects and point counts

(see review by Murphy and Jenkins (2010)), however, all UVC methods rely on trained scientific divers which can introduce bias (Bach et al., 2020; Harvey et al., 2002a; MacNeil et al., 2008b). These biases may include effects of fish behaviour towards SCUBA divers (Chapman et al., 1974; Lindfield et al., 2014; Watson & Harvey, 2007) and inter and intra-observer error in estimating fish length and sampling area (Harvey et al., 2002a; Harvey et al., 2004; Prato et al., 2017). UVC is limited by time and depth constraints associated with SCUBA diving no-decompression limits (Andradi-Brown et al., 2016). Technical solutions to some limitations of UVC include the use of closed circuit rebreathers (CCR) to dampen the effect of fish behaviour towards divers (Lindfield et al., 2014) and transitioning to stereo-video systems (introduced by Harvey and Shortis (1995)) to collect robust length data (see reviews in: Cappo et al., 2003; Cappo et al., 2006; Cappo et al., 2001; Harvey & Mladenov, 2001; Mallet & Pelletier, 2014; Murphy & Jenkins, 2010).

1.1.2 Diver operated stereo-video

Stereo-video technology was first tested for reef fish surveys by Harvey and Shortis (1995). A stereo-video system consists of two inward converging cameras mounted on a base bar to measure fish size, abundance and minimum approach distance (MAD – used as a proxy for behaviour). Since this development, stereo-DOVs have gained popularity as a sampling method as they produce accurate and precise measures of fish counts and lengths (Goetze et al., 2015; Holmes et al., 2013; Watson et al., 2010). Along with the high degree of accuracy, there are standardised protocols for calibration, field deployment, and video analysis available (Goetze et al., 2019; Harvey & Shortis, 1998). This standardisation is essential for reducing methodological variation and allowing data synthesis for both spatial and temporal comparisons (Harvey et al., 2021). Furthermore, stereo-DOVs (amongst other stereo-video systems) provide a permanent record of observations which decreases the reliance on taxonomic expertise in the field (Goetze et al., 2015; Holmes et al., 2013; Langlois et al., 2010). They also allow accurate definition of transect area which helps overcome intra-observer bias (Cappo et al., 2003). Despite these advantages, stereo-DOVs detect fewer cryptic species than UVC (Holmes et al., 2013) and remain affected by the depth and time limitation of SCUBA diving (Cappo et al., 2003; Harvey et al., 2001b; Lindfield et al., 2014), and fish behavioural responses (Chapman et al., 1974; Watson & Harvey, 2007). The use of CCR has been suggested to dampen fish behavioural avoidance (Lindfield et al., 2014), however this introduces further safety, training and financial considerations (Norro, 2016). These limitations have acted as a catalyst in the development and use of remotely operated sampling methods (Logan et al., 2017; Seiler et al., 2012; Sward et al., 2019; Warnock et al., 2016).

1.1.3 Baited remote underwater stereo-video systems

Stereo-BRUVs are used widely for collecting fish assemblage data as they eliminate depth and behavioural limitations of diver-based methods (Cappo et al., 2006; Harvey et al., 2012), and increase the proportion of predatory species sampled (Harvey et al., 2018). Stereo-BRUVs also offer the advantage of published standardised operating procedures (Harvey et al., 2013; Langlois et al., 2020), and an ability to sample a unique assemblage of fishes due to

attraction of fish towards the bait (Harvey et al., 2007; Harvey et al., 2018). Bait use increases the abundance counts of carnivorous species without impacting the counts of most herbivores (Harvey et al., 2007; Langlois et al., 2010; Watson et al., 2005; Watson et al., 2010). It also has been shown to attract herbivores which demonstrate interest in the increased fish activity in the area (Cappo et al., 2006). This sampling of carnivorous species is unique to stereo-BRUVs due to the mobile nature, relatively low density and patchy distribution of many species in this trophic group (Harvey et al., 2007). This is an important consideration due to the commercial value of many carnivorous fish species (Harvey et al., 2012). The advantages gained from bait attraction introduce the limitation of the inability to define the sampling area due to the uncertainty of the bait plume dispersal (Cappo et al., 2003; Cappo et al., 2006; Harvey et al., 2007). For this reason, stereo-BRUVs data is recorded as relative abundance estimates (e.g., using MaxN – maximum number of individuals in one frame for each species) rather than density per unit area (Ellis & DeMartini, 1995; Logan et al., 2017). Due to the unique assemblage composition sampled by stereo-BRUVs, various studies have suggested their use in conjunction with a transect method to gather robust, representative data (Colton & Swearer, 2010; Langlois et al., 2010; Schramm et al., 2020a; Schramm et al., 2020b). This combination also allows sampling of site attached species which are more accurately sampled with transect based methods (Schramm et al., 2020a). Watson et al. (2010) emphasised the importance of critically considering the goals of the research, in particular the species of interest, when choosing a sampling method. Both the higher trophic groups, and site attached species are of interest when sampling MPAs as each group can reflect different pressures. Using stereo-BRUVs in a monitoring program increases sampling of these higher trophic groups (Harvey et al., 2018) which improves the ability to assess responses to fishing pressures.

1.1.4 Micro remote operated vehicle with attached stereo-video system

The use of ROVs for sampling fish has undergone development and testing over the last decade (Ajemian et al., 2015; Andaloro et al., 2013; Consoli et al., 2016; Sward et al., 2019; Trenkel et al., 2004). ROVs overcome safety considerations of operating with SCUBA divers and reduces depth and time limitations (Parry et al., 2003; Smolowitz et al., 2015). ROVs may also eliminate some of the biases of fish behaviour (Stamoulis et al., 2020), however the evidence is limited to a few species-specific studies (Laidig et al., 2013; Lorange & Trenkel, 2006; McLean et al., 2017; Trenkel et al., 2004). Most of these studies used industrial sized ROVs which emit artificial light and high amplitude, low frequency sound (Sward et al., 2019). Testing of micro ROVs which are physically smaller, and quieter has been a focus of recent research which suggests their use may dampen fish behavioural responses (Schramm et al., 2020a; Schramm et al., 2020b; Warnock et al., 2016). A micro stereo-ROV unit consists of a stereo-video unit, conceptually the same as that developed by Harvey and Shortis (1995), except smaller, attached to a micro ROV which is deployed from a boat and controlled remotely via an umbilical cord (Schramm et al., 2020a; Schramm et al., 2020b). This combination of technologies allows ROVs to accurately gather abundance, biomass, and behavioural data. The umbilical cord poses challenges in complex reefs due to possible entanglement (Pacuneski et al., 2008). Furthermore, ocean currents can pose challenges in accurately controlling the system (Ajemian et al., 2015). Comparisons of stereo-ROV

sampling with stereo-DOV sampling have produced mixed results with some differences in the assemblage and numbers of species and individuals sampled which has been hypothesised to be due to difference in operating altitude between methods (Schramm et al., 2020a). With the standardisation of methodology, stereo-ROVs have potential to be successfully implemented as a fish monitoring tool due to their ability to gather robust data on a wide range of indicators (abundance, length, biomass, and behaviour) with increased safety when compared to diver-based methods.

1.2 Study area

The data presented in my thesis was collected in the Jurien Bay Marine Park (JBMP) which is adjacent to the central west coast of Western Australia. The JBMP was gazetted in 2003 and covers over 82,000 hectares of nearshore ocean and hosts a unique assemblage of tropical and temperate marine fish species. This unique assemblage results from the system being in a transitional zone between sub-tropical and temperate habitats with warm water being pushed poleward by the Leeuwin Current. The southern-most coral reef in WA lies to the north of the JBMP is the Houtman Abrolhos Islands. Regime shifts in the macroalgae, and fish community compositions have been observed in the JBMP following the 2011 marine heatwave (Wernberg et al., 2016) and coral cover at some sites has been estimated at up to 30% (Ross et al., 2021). The fish assemblage in the JBMP also reflects the unique location and transitional zone with both tropical and temperate species being present (Department of Biodiversity Conservation and Attractions., In Prep) Schooling kyphosids, large territorial pomacentrids, carnivorous labrids (such as Western King Wrasse and Baldchin Grouper), and other carnivores (such as Pink Snapper, Trevally, and Samson Fish) are a few of the fish species often of interest in sampling (Cundy et al., 2017). These species have unique traits that warrant consideration when designing sampling programs such as the territorial nature of pomacentrids possibly resulting in them approaching divers and skewing results, or the mobile and relatively low density of Samson Fish potentially making them difficult to sample with transect methods.

Both recreational and commercial fishing are of social value in the JBMP. Line fishing and rock lobster pots are the main extractive activity with diving making up a smaller proportion of fishing effort (Ryan et al., 2019). Commercial fishing in the region is dominated by rock lobster fishing, however fishes including sharks, West Australian Dhufish, Pink Snapper, Baldchin Grouper, and Emperors are also fished with demersal gillnets and longlines (Gaughan & Santoro, 2018). Fine scale information on historical fishing effort in the JBMP in published literature is largely lacking.

Zoning in the JBMP includes general use; which allows both recreational and commercial fishing, sanctuary zones (fully protected areas); where all extractive activities are prohibited, special purpose (scientific reference and aquaculture zones); that allow commercial and recreational fishing for rock lobster only, as well as shore-based fishing. Fish monitoring has consisted of UVC with focus on indicators including abundance, biomass, community composition and species richness. This has been done across a range of the zones in the park with a focus on shallow (2 – 15 m) rocky reef areas (Department of Biodiversity Conservation and Attractions, In Prep).

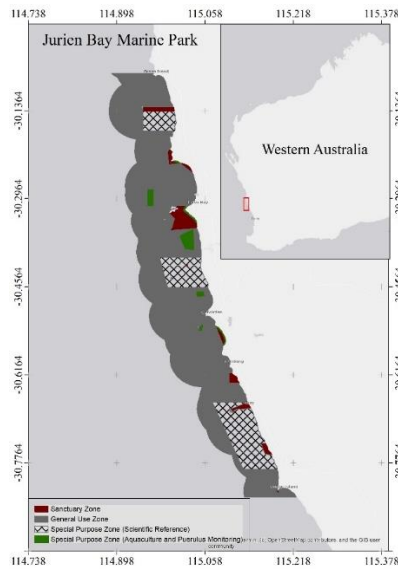


Figure 1.1 Map showing the location of the Jurien Bay Marine Park on the Western Australian coast.

1.3 Aims and objectives

The primary aim of my research was to investigate what the optimal techniques for sampling shallow water reef fishes are. I compared three commonly used methods: stereo-BRUVs, stereo-DOV and UVC, and one emerging method: stereo-ROV. The indicators compared included: the assemblage composition, number of species, the number of individuals, the statistical power to detect change, the length frequency distribution and fish behaviour. I aimed to address the following research questions:

- 1) How do the four sampling methods compare in relation to (1) the assemblage composition sampled (2) the number of species and number of individuals sampled and (3) their statistical power to detect hypothetical changes in the number of species and number of individuals sampled.

Based on the findings from this research question, I developed another research question.

- 2) How do stereo-DOV and stereo-ROV compare in relation to (1) their ability to measure fish length, (2) the length frequency distribution of fish measured and (3) the behaviour of fish towards each system.

1.4 A comparison of underwater visual census, baited, diver operated and remotely operated stereo-video for sampling shallow water reef fishes (chapter 2)

In chapter two I compared the sampling ability of three commonly used methods of sampling fish populations and one emerging method. Three of the methods were transect based (stereo-DOV, stereo-ROV and UVC) for which a total of 44 transects across 11 sites were collected. For the fourth method (stereo-BRUVs), 44 replicates, each of 60-minute duration were collected. All sites were in water up to 10 m deep on rocky reef habitat. Each method was compared in their assemblage sampled, number of species and individuals sampled and their

ability to detect hypothetical changes. The methods ability to sample within and beyond marine reserves that are protected from fishing was also assessed.

1.5 A comparison of the length frequency distribution and behavioural response of fishes to remote and diver-based stereo-video sampling (chapter 3)

Based on the findings in chapter two, I further investigated the sampling abilities of stereo-DOV and stereo-ROV. This comparison consisted of 54 replicate transects for each method across nine sites. The ability of the two methods to collect length measurements and the length frequency distribution of measurements gathered was compared. The behaviour of fishes towards SCUBA divers and the ROV was investigated using MAD. The levels of fishing pressure and life history traits of species was considered in the interpretation of the observed behaviour towards the two methods.

1.6 Thesis structure

I have structured this thesis into four chapters: a general introduction, two data chapters and a general discussion. This structure is outlined in Figure 1.2. Data chapter one has been published in *Estuarine, Coastal and Shelf Sciences* (Jessop et al., 2022) and data chapter 2 has been formatted for publication in the *Journal of Experimental Marine Biology and Ecology*. The two data chapters have been written as stand-alone chapters for publication. Consequently, there is some repetition through the thesis.

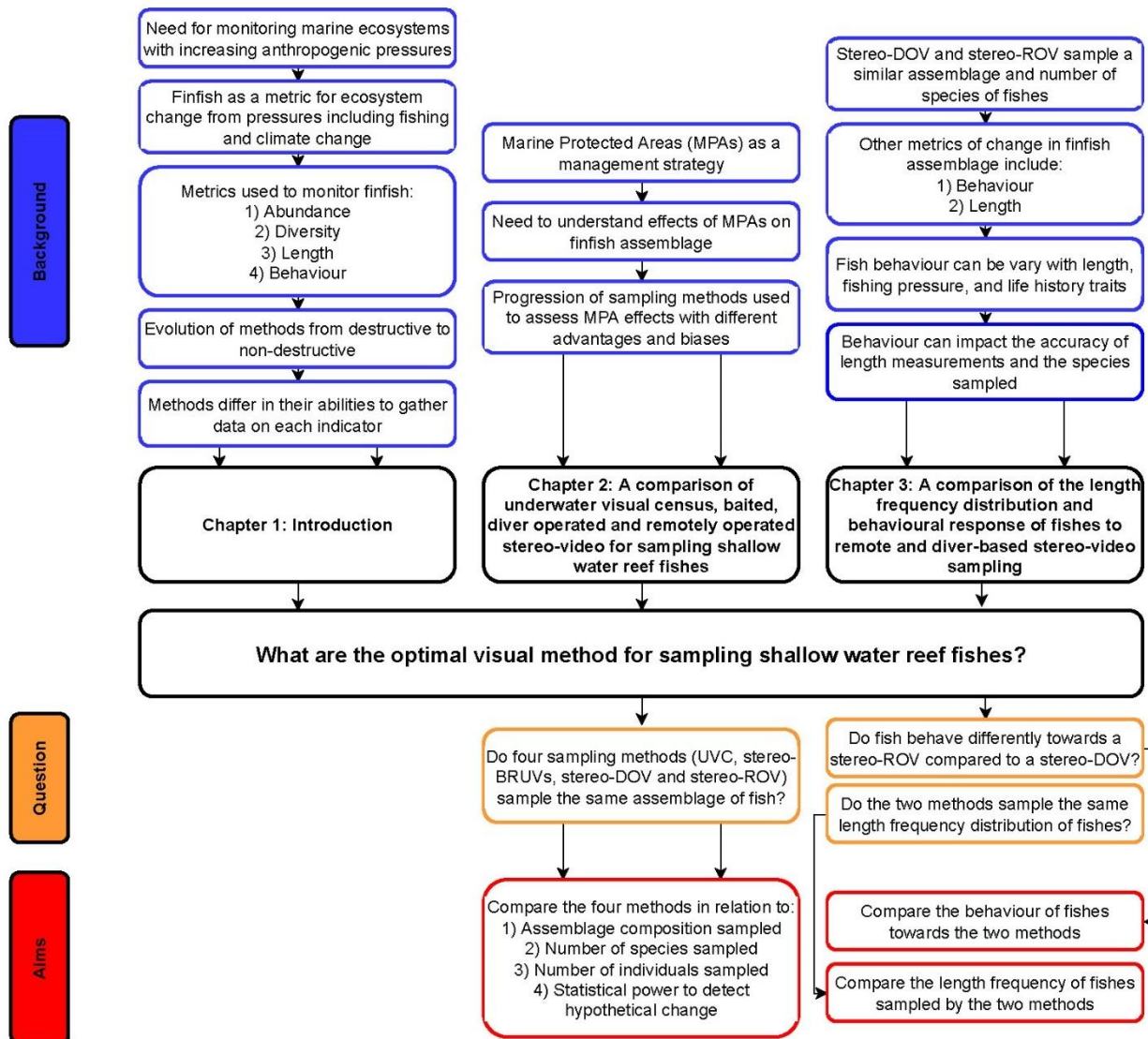


Figure 1.2 Thesis flow diagram outlining structure with background, research questions and aims.

**2. CHAPTER 2: A COMPARISON OF UNDERWATER VISUAL CENSUS,
BAITED, DIVER OPERATED AND REMOTELY OPERATED STEREO-
VIDEO FOR SAMPLING SHALLOW WATER REEF FISHES**



Photograph: Banded sweep (*Scorpiis georgiana*) observed with a stereo-DOV in the Jurien Bay Marine Park

2.1 Abstract

Marine Protected Areas (MPAs) are a popular conservation strategy aimed at managing anthropogenic pressures and protecting habitats and the diversity of ocean flora and fauna. Robust, cost-effective sampling of fish assemblages is important in understanding the effects of these management strategies on ocean ecosystems. We compared the sampling effectiveness and efficiencies of three commonly used methods of sampling fish assemblages (underwater visual census (UVC), baited remote underwater stereo-video systems (stereo-BRUVs), and diver operated stereo-video (stereo-DOV) and one emerging method (remotely operated vehicle with stereo-video system (stereo-ROV)). We assessed the assemblage composition, numbers of species and individuals, and the statistical power to detect hypothetical changes in the number of species and individuals for each method.

Stereo-BRUVs sampled a distinctive assemblage compared to all transect-based methods, with more individuals, total species, and predatory fishes from higher trophic groups that are targeted by commercial and recreational fishers. UVC also sampled a distinctive assemblage compared to stereo-ROV and stereo-BRUVs (comparisons with stereo-DOV were not possible due to sampling restrictions). The fish assemblage sampled by UVC consisted of more species and individuals than stereo-ROV and small bodied or cryptic species that were not detected by the video methods. Although stereo-DOV sampled more individuals than stereo-ROV with differences in small schooling species at a few sites, the assemblage composition was broadly comparable. To effectively monitor MPAs a combination of BRUVs, which more effectively sampled fisheries indicator species, and one of the transect based methods should be used. Given the similarities in the assemblages sampled by the stereo-video transect based methods and the advantages associated with health and safety, logistics and field efficiency with remote methods, we recommend stereo-ROV and stereo-BRUVs.

Keywords: Temperate Fish; Marine Park Monitoring; Diver operated stereo-video; Stereo-ROV; Stereo-BRUVs; Underwater visual census

2.2 Introduction

Marine ecosystems are sensitive to anthropogenic pressures such as overfishing and pollution which can result in biodiversity declines and ecosystem collapses (Worm et al., 2006). Marine Protected Areas (MPAs) are spatially defined areas of ocean with biodiversity management objectives, often aimed at managing pressures such as fishing (Claudet & Guidetti, 2010). The implementation of MPAs is an increasingly popular means of managing and conserving marine ecosystems by protecting fish stocks and essential fish habitats (Ballantine, 2014; Côté et al., 2001). While well managed MPAs often have a high fish biomass, species richness and abundance within, and sometimes beyond their boundaries (Babcock et al., 1999; Ballantine, 2014; Colléter et al., 2014; Côté et al., 2001; Cresswell et al., 2019; Denny et al., 2019; Goetze et al., 2021; Halpern, 2003; Lester & Halpern, 2008; Mosquera et al., 2000; Westera et al., 2003; Worm et al., 2009) the magnitude of these increases is dependent on a number of factors, including the degree of protection within the zoned area (Edgar et al., 2014; Lester & Halpern, 2008). The degree of protection that individual MPAs afford is highly variable, with fully protected areas that exclude all extractive and destructive activities providing the greatest conservation benefits (Gorud-Colvert et al., 2021). Fully protected areas that are connected, large, old, enforced and extend from shallow to deeper waters can provide increased conservation benefits (Edgar et al., 2014; Goetze et al., 2021). When combined with traditional fisheries management approaches (e.g. catch quotas), fully protected areas may be an optimal conservation approach in rebuilding and protecting marine ecosystems (Worm et al., 2009), as well as providing an invaluable scientific tool for assessing human impacts (Ballantine, 2014). Due to the increasing uptake of ocean zoning for conservation (Côté et al., 2001; Ward & Hergerl, 2003), the social and ecological benefits of MPAs (Ballantine, 2014), and the subsequent need to robustly demonstrate the effect of fully protected areas (Edgar et al., 2014), it is essential they are subject to rigorous and standardised monitoring to determine their effectiveness relative to management objectives.

Effective monitoring and management of MPAs requires robust data on fish population demographics including diversity, abundance, length, and biomass (Bach et al., 2020; Cinner et al., 2016; Jennings et al., 2014; McClanahan et al., 2011; Nash & Graham, 2016). The methods for gathering fish data have transitioned from destructive methods such as the use of trawl nets, toxins, and dynamite, to non-extractive observational methods such as underwater visual census (UVC) (Brock, 1954; Harmelin-Vivien & Francour, 1992; Mallet & Pelletier, 2014; Murphy & Jenkins, 2010). Non-extractive sampling methods represent a more desirable option that aligns with the conservation and management objectives of MPAs and allows sampling to take place within fully protected areas (Cappo et al., 2003; Cappo et al., 2006). Historically, UVC has been one of the predominant methods used to survey shallow water temperate and tropical reefs (Cappo et al., 2003; Hill & Wilkinson, 2004). UVC allows SCUBA divers to quantify fish assemblage composition, size structure and biomass using *in-situ* observations (Brock, 1954). While UVC requires a high degree of training and taxonomic expertise, its simplicity means that under strict supervision it can also be adopted to citizen science projects to further reduce costs and increase survey capacity (Edgar & Stuart-Smith, 2014; Lamine et al., 2018). Despite this, the reliance on divers who are highly competent in taxonomic classification and in counting and estimating the lengths of fish

remains a limitation (Bach et al., 2020; Harvey et al., 2002a; MacNeil et al., 2008a; MacNeil et al., 2008b). Additionally, sources of bias exist which include intra-observer error in estimating the numbers and lengths of fish, and the transect area (Harvey et al., 2004; Harvey et al., 2002b; Prato et al., 2017; Watson et al., 1995). There are also concerns about the attraction and repulsion of fish to SCUBA divers which may be associated with the noise of the bubbles they exhale (Gray et al., 2016; Lindfield et al., 2014; Watson & Harvey, 2007). The reliance of UVC on SCUBA divers also limits the time and depth of surveys due to decompression limits (Andradi-Brown et al., 2016).

Some of the limitations and biases associated with the *in-situ* estimates of fish by divers have been overcome with the development and transition to diver operated stereo-video (stereo-DOV) (Cappo et al., 2003; Cappo et al., 2006; Cappo et al., 2001; Harvey & Mladenov, 2001; Harvey & Shortis, 1995; Mallet & Pelletier, 2014; Murphy & Jenkins, 2010). The initial prototype stereo-DOV developed by Harvey and Shortis (1995) has formed the basis of the stereo-video technology used today. Since their development, stereo-DOVs have become a popular alternative to UVC because of their efficiency in the field and ability to provide a permanent record that can be reviewed in the laboratory, reducing inter observer variability and reliance on highly trained field personnel (Cappo et al., 2003; Goetze et al., 2015; Harvey et al., 2004; Holmes et al., 2013; Watson et al., 2010). The stereo component facilitates accurate definition of transect area (Harvey et al., 2004), highly accurate measurements of fish length (Harvey et al., 2002a), and measurement of distance-based behavioural metrics (Goetze et al., 2017; Lindfield et al., 2014). The methodological standardisation of stereo-DOVs (Goetze et al., 2019) is also beneficial in temporal and cross-system comparisons (Bax et al., 2019). Despite these advantages, stereo-DOVs remain limited by the time and depth constraints of SCUBA diving and are less effective at sampling cryptic species when compared to UVC (Holmes et al., 2013). Consideration of the time needed to complete video analysis post field work is also important (Holmes et al., 2013). The diver associated limitations of both UVC and stereo-DOVs can be overcome with remote sampling.

Over the last two decades remotely operated vehicles (ROVs) have undergone development and testing as an alternative approach for assessing fish population dynamics (Ajemian et al., 2015; Andaloro et al., 2013; Consoli et al., 2016; Trenkel et al., 2004). As ROVs are a relatively new technology, an understanding of the behavioural response of fishes toward them is limited to a few species-specific studies involving industrial sized ROVs (Laidig et al., 2013; Lorange & Trenkel, 2006; McLean et al., 2017; Ryer et al., 2009; Stoner et al., 2008; Sward et al., 2019; Trenkel et al., 2004). The use of micro ROVs, which are physically smaller and emit less noise may dampen behavioural effects (Sward et al., 2019). Strong ocean currents and complex reefs can pose challenges with ROV surveys due to their remotely operated nature and the need for an attached umbilical control cord (Ajemian et al., 2015; Pacuneski et al., 2008). A combination of micro ROV technology with stereo-video (stereo-ROV) has been tested for assessing fish assemblage parameters including abundance, length, and biomass (Schramm et al., 2020a; Schramm et al., 2020b). This combination of technology brings the precision and accuracy advantages of stereo-video and combines them with the advantages of remote sampling to reach deeper water with added safety advantages (Parry et al., 2003; Schramm et al., 2020a; Schramm et al., 2020b; Smolowitz et al., 2015; Sward et al., 2019; Warnock et al., 2016). As stereo-ROV and stereo-DOV are both transect

based stereo-video methods, they may be able to sample fish populations with similar results if the biases are understood and overcome (Schramm et al., 2020a).

Transect methods generally sample a low proportion of predatory species of higher trophic groups due to their relative low abundances, patchy distribution and mobile nature. These species are often highly targeted species making them susceptible to fishing pressure (Harvey et al., 2007). To help increase encounter rates of these groups, baited remote underwater stereo-video systems (stereo-BRUVs) have been increasingly adopted over the past two decades (Whitmarsh et al., 2017). They provide a standardised technique (Cappo et al., 2001; Langlois et al., 2020), that can also overcome the depth constraints of SCUBA diving (Cappo et al., 2006; Harvey et al., 2012). Stereo-BRUVs sample a unique assemblage including a higher proportion of predatory fishes which are attracted to the bait (Harvey et al., 2007; Harvey et al., 2018; Langlois et al., 2010; MacNeil et al., 2008b; Watson et al., 2010), but are also effective at counting non predatory fishes such as herbivores, corallivores, and planktivores (Harvey et al., 2007; Watson et al., 2005). The use of bait also decreases the variance in the resulting data (Cappo et al., 2003; Harvey et al., 2007), especially compared to transect methods which sample areas of high habitat heterogeneity (McCormick & Choat, 1987; Schramm et al., 2020a). The unique assemblage sampled by stereo-BRUVs is advantageous in monitoring programs because of the high commercial and social value associated with predatory fish. However, uncertainty of the area covered by the bait plume prevents the definition of the sampling area (Cappo et al., 2003; Cappo et al., 2006; Harvey et al., 2007; Schramm et al., 2020b) and limits the method to relative estimates of abundance rather than density per unit area (Cappo et al., 2003; Ellis & DeMartini, 1995; Logan et al., 2017; Priede et al., 1994). While the relative biases and differences between baited video and transect based methods are well understood, the combination of methods facilitates an increased understanding of the fish assemblage, and the relative abundance and density of fish associated with various habitats and spatial zoning strategies (Logan et al., 2017; Schramm et al., 2020a; Schramm et al., 2020b; Watson et al., 2010; Willis et al., 2000).

As new methodologies arise (e.g. stereo-ROV), it is important to compare and contrast to existing methods to ensure time-series data and comprehensive monitoring approaches can be achieved. We aimed to assess the differences between stereo-DOV, stereo-ROV, UVC, and stereo-BRUVs within a shallow water MPA (marine park) in relation to (1) the assemblage composition (2) the number of species and number of individuals sampled, and (3) the power of each method to detect hypothetical change in the number of species and individuals, both inside and outside of fully/highly protected areas (no-take for finfish). It was hypothesised that stereo-DOV and stereo-ROV would sample a similar assemblage of fishes and that UVC and stereo-BRUVs would each sample a unique assemblage, with UVC sampling more small bodied cryptic species and stereo-BRUVs sampling more predatory species with higher statistical power driven by the lower variance in the data.

2.3 Materials & Methods

2.3.1 Study area

Sampling was conducted in the Jurien Bay Marine Park (JBMP) during April and May of 2021. The JBMP covers over 82,000 hectares of the nearshore ocean, adjacent to the central coast of Western Australia. (Figure 2.1). All sampling took place in the lagoon area at depths ranging between four and ten meters. The area lies within the largest limestone reef system in Australia, known as the Great Southern Reef (Bennett et al., 2015) and hosts a unique assemblage of tropical and temperate marine species (Department of Conservation and Land Management, 2005). The marine flora in the JBMP is historically dominated by *Ecklonia radiata* and a diverse assemblage of foliose algae in reef areas intertwined with seagrass meadows (Wernberg et al., 2006). Zoning in the JBMP includes general use; which allows both recreational and commercial fishing, sanctuary zones (fully protected areas); where all extractive activities are prohibited, special purpose (scientific reference and aquaculture zones); that allow commercial and recreational fishing for rock lobster only, as well as shore-based fishing (Figure 2.1).

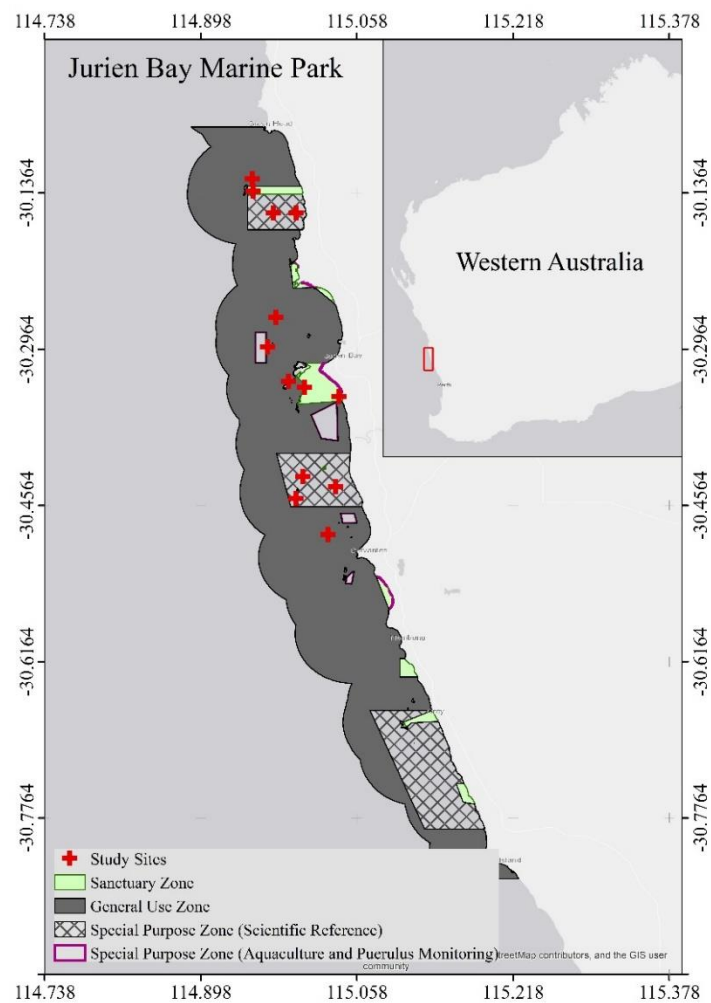


Figure 2.1 Map of the survey location, highlighting sample sites along the central west coast of Western Australia in the Jurien Bay Marine Park.

2.3.2 Experimental design

Poor weather conditions and variation in water visibility prevented all methods from being completed across all sites within the same timeframe. To account for this, two separate experimental designs were used. A comparison of UVC, stereo-ROV and stereo-BRUVs was completed at 11 sites (three in fished areas and eight in special purpose zones and sanctuary zones), and a comparison of stereo-DOV and stereo-ROV at 9 sites (four in fished areas and five in special purpose zones and sanctuary zones). For UVC a total of 48 belt transects (four replicates per site), 5 m wide by 5 m high and 50 m long were completed following the standard reef life survey (RLS) survey methods (Edgar & Stuart-Smith, 2014). The same transect size and replication was completed for stereo-ROV. The stereo-BRUVs sampling used four replicate samples per site with a total of 48 samples, each 60 minutes in duration. To compare stereo-ROV to stereo-DOV, 54 belt transects were collected for each method (six replicates per site). Transects were the same size and length as UVC transects. All transects had a separation of $\geq 10\text{m}$.

2.3.3 Underwater visual census (UVC)

UVC surveys were completed by two SCUBA divers trained in local fish taxonomy, following the RLS method outlined by Edgar and Stuart-Smith (2014). Divers laid a 50 m long transect tape and recorded length and abundance data of all fishes encountered within five meters either side of the tape. This was done in two blocks (one each side of the tape) by two divers. The same one block which was completed by the same divers across all sites was used for comparison to the other transect techniques. The time taken to complete a 50 m UVC transect was estimated at approximately seven and a half minutes based off dive times.

2.3.4 Stereo-video systems

The stereo-video systems consisted of two cameras, mounted on a base bar at a slight inward converging angle, as described by Harvey and Shortis (1995, 1998). The separation of the cameras was 700 mm with an inward converging angle of 8° for the stereo-DOV and stereo-BRUVs and 595 mm with an inward converging angle of 5° for the stereo-ROV. Differences in the time taken to complete stereo-video transects between methods was tested with a t-test after testing for homogeneity of variance with an F-test.

2.3.4.1 Diver operated stereo-video (stereo-DOV)

Stereo-DOV surveys were completed with two SCUBA divers following the method outlined by Goetze et al. (2019). 50 m transects were completed in an average time of 1 minute and 44 seconds ± 0.041 (1SE). The cameras used on the stereo-DOV system were Sony FDR X3000 action cameras recording at 1080p, 60 fps, and a medium field of view to reduce motion parallax associated with the stereo video system and fish moving simultaneously.

2.3.4.2 Stereo-video remotely operated vehicle (stereo-ROV)

The ROV used was a Blue Robotics BlueROV 2 (457 × 330 × 254 mm) that was fitted with a stereo-video system with two Sony RX0 II video cameras recording at 1080p and 50fps. The stereo-ROV unit was operated approximately 50 m ahead of a surface vessel and attached by an umbilical control cord. The vessel maintained this distance while the stereo-ROV moved along the transect. Its location was tracked using a Seatrac X150 USBL Beacon at the boat and X010 Modem Beacon attached to the stereo-ROV. The USBL system had a range resolution of ± 0.1 and angular resolution of approximately two percent of the acoustic range (approximately one meter at the range used in this study). The USBL facilitated navigation, but also allowed us to calculate transect length. Where possible, the stereo-ROV was flown approximately 50 cm above the benthos to match the stereo-DOV procedure (Goetze et al., 2019). This was not always possible in complex reef where sudden changes in topography occurred. The footage from each site was separated into transects using the tracking data from the USBL, on average each 50 m transect took 1 minute and 21 seconds $\pm 2.44 \times 10^{-5}$ (1SE).

2.3.4.3 Baited remote underwater stereo-video systems (stereo-BRUVs)

Stereo-BRUVs sampling was completed using eight systems, each equipped with stereo-video using the same cameras and camera settings as the stereo-DOV system. The methods followed those outlined by Langlois et al. (2020). Stereo-BRUVs sampling did not take place on the same day as any transect method sampling to avoid any confounding influence of bait attraction.

2.3.5 Calibration and image analysis

Calibration of each stereo-video system was completed before and after the sampling. The software 'CAL' (<https://www.seagis.com.au/bundle.html>) was used, following the methods outlined by Harvey and Shortis (1998). Video analysis was completed using the software 'EventMeasure Stereo' (<https://www.seagis.com.au/event.html>). For the transect methods, all fish within transects were identified to species level (where possible). Where a species level identification was not possible, individuals were pooled to genus. For the stereo-DOV and stereo-ROV analysis, all fish within transects were recorded when they were closest to the camera system, following the protocol outlined by Goetze et al. (2019). For stereo-BRUVs image analysis, the maximum number of fish from one species present (MaxN; Priede et al., 1994) was used as a measure of relative abundance and 3D points were used to determine whether fishes were within the sampling area. This indicator of relative abundance was used to avoid multiple observations from the same system of the same individuals taking place (Cappo et al., 2003; Cappo et al., 2001; Willis et al., 2000). All observations were limited to a range of seven meters away from the cameras to maintain accuracy and precision (Harvey et al., 2010).

2.3.6 Statistical analysis

2.3.6.1 Assemblage composition

To test for differences in the multivariate assemblage sampled by stereo-DOV, stereo-ROV, stereo-BRUVs and UVC a three factor permutational multivariate analysis of variance (PERMANOVA, 9999 permutations $\alpha = 0.05$) was done using Primer 7 with the PERMANOVA+ add on (Anderson et al., 2008). The three factors were (1) method (fixed), (2) protection status (fixed, two levels (fished and no-take)), and (3) site (random, nested within status). Visibility was included in the design as a covariate. This was performed a total of three times on a Bray Curtis resemblance matrix to compare the assemblage sampled by (1) stereo-BRUVs, UVC and stereo-ROV with presence/absence data, (2) stereo-DOV and stereo-ROV with untransformed data, and (3) UVC and stereo-ROV with fourth root transformed data.

To compare stereo-BRUVs to stereo-ROV, and UVC a presence/absence transformation was applied to allow comparison between methods with fundamentally different sampling units. For the other two comparisons transformations were applied based on the variance shown by a permutational analysis of multivariate dispersion (PERMDISP) (Brückner & Heethoff, 2017). Where status was highly non-significant ($p > 0.250$), it was pooled and the PERMANOVA was done with two factors (method and site). Differences in the assemblages sampled by methods at each site were explored with post-hoc pairwise comparisons of the method \times site interaction to determine the location of significant differences. In the comparisons including UVC, interaction terms that involved both site and visibility were removed from the model because the UVC visibility data was collected at a site level, rather than transect level. The effect of visibility on the variation in assemblage compositions sampled by each method was explored with distance based linear models (DistLM) (Legendre & Anderson, 1999; McArdle & Anderson, 2001). The effect of the method was explored graphically with non-metric multidimensional scaling plots (nMDS) and canonical analysis of principal coordinates (CAP) (Anderson & Willis, 2003). Leave one out allocation tests were performed to analyse whether any distinct groups were formed by method. Pearson's correlations were also used to determine the species having the most impact on differences in the samples. Species with a Pearson's value of > 0.35 , 0.30 , and 0.40 for stereo-BRUVs, versus stereo-ROV and UVC, stereo-DOV versus stereo-ROV, and stereo-ROV versus UVC respectively, were overlaid as vectors on the CAP and considered as focal species. Species that are subject to fishing pressure and climate change as defined by the state government agency responsible for managing and monitoring marine parks in W.A. (herein referred to as indicator species; Table A.2) were also overlaid as vectors (Department of Biodiversity Conservation and Attractions, In Prep). All species in overlays were tested for significant differences with PERMANOVAs on the untransformed univariate Euclidean distance resemblance matrix using the design outlined above.

2.3.6.2 Number of individuals and species

To test for significant differences in the number of individuals and number of species sampled by stereo-DOV, stereo-ROV, and UVC the same three factor design as in the

assemblage analysis was used and visibility was also included as a covariate. This was performed twice to compare stereo-DOV to stereo-ROV, and UVC to stereo-ROV. PERMANOVAs were done on a Euclidean Distance resemblance matrix of the number of species and number of individuals sampled by each method ($\alpha = 0.05$). A square root transformation was applied to the number of species sampled by stereo-ROV and UVC to meet assumptions of variance, all other univariate analysis was done on untransformed data. Pooling of the factor of status was also done when $P > 0.25$. Once again, interactions with both visibility and site were removed from the comparisons including UVC. DistLM analysis was done to explore the effect of visibility on the variation in samples by each method. A species accumulation curve for each method was also plotted. For all PERMANOVAs, Monte Carlo bootstrapping was done where the number of unique permutations was low (< 100) (Anderson et al., 2008).

2.3.6.3 Post-hoc power analysis

To assess the ability of the four methods to detect hypothetical change in the assemblage composition, post-hoc power analysis using the calculated effect sizes from our data and based upon one-way fixed effect ANOVAs were performed in G*Power (Faul et al., 2007). This was done for 20% and 50% changes in the number of species and number of individuals.

2.4 Results

A total of 19,500 individuals, from 102 species were counted across the four methods. Stereo-BRUVs sampled a total of 64 species, 18 of which were only sampled by stereo-BRUVs. Of these 18 species, 12 were carnivores, five were omnivores and one was a herbivore. Stereo-BRUVs were the only method to sample the targeted species *Chrysophrys auratus* and *Seriola hippos*. Stereo-ROV sampled 59 species and UVC and stereo-DOV sampled 54 and 51 species respectively. UVC sampled eight species that no other method sampled, four of which were classified as small bodied or cryptic species (*Plagiotremus rhinorhynchus*, *Cirripectes hutchinsi*, *Ostorhinchus doederleini*, and *Helcogramma decurrens*). Stereo-ROV sampled 17 species that were not sampled by stereo-DOV and stereo-DOV sampled ten species that were not sampled by stereo-ROV. The average speed of stereo-DOV and stereo-ROV transects was not significantly different ($t_{(12)} = 1.45$, $p = 0.174$) and the estimated time for UVC transects was greater at approximately seven and a half minutes.

2.4.1 Assemblage composition

2.4.1.1 Stereo-BRUVs, stereo-ROV, and UVC

There was no significant effect of protection status on the presence/absence assemblage composition. With status pooled, there was a significant interaction between method and site, but no significant interaction between visibility and method (Table 2.1). When the covariate visibility was excluded from the analysis, the post-hoc tests indicated that at five of the

eleven sites, stereo-BRUVs sampled a different assemblage composition to UVC and at seven sites, stereo-BRUVs and stereo-ROV sampled different assemblages. At ten of the eleven sites there was no difference between UVC and stereo-ROV ($P > 0.05$). Overall, there was a significant difference in the presence/absence assemblage composition sampled between methods with stereo-BRUVs being significantly different to stereo-ROV and UVC ($P < 0.001$), but UVC and stereo-ROV were not significantly different ($P = 0.121$). Stereo-BRUVs sampled a unique assemblage compared to UVC and stereo-ROV, as displayed by the clustering of samples by method in the nMDS (Figure 2.2). The separation of stereo-BRUVs samples from UVC and stereo-ROV was driven by carnivores and invertivores such as *Pseudocaranx* species, *Pentapodus vitta*, *C. auratus*, *S. hippos*, *Labracinus lineatus* and *Parupeneus spilurus* (Figure 2.2 A). Some of the indicator species also appeared to be correlated with the assemblage sampled by stereo-BRUVs, such as *S. hippos*, *C. auratus*, and *Epinephelides armatus* (Figure 2.2 B). *Pomacentrus milleri* was correlated toward UVC and stereo-ROV samples, whereas *Choerodon rubescens* was correlated away from UVC and stereo-ROV (Figure 2.2 B). The CAP showed a difference in stereo-BRUVs samples from the two transect methods, and stereo-ROV and UVC lacked a clear separation of samples (Figure 2.3). The leave-one-out allocation success test supported the separation of stereo-BRUVs from the two transect methods with 83.8% of stereo-BRUVs samples being successfully allocated. There was less separation between stereo-ROV and UVC with 59.1% and 70.5% of samples being successfully allocated respectively.

2.4.1.2 Stereo-DOV and stereo-ROV

There was no significant effect of protection status on the assemblage composition from samples collected by stereo-DOV and stereo-ROV. With status pooled, there was no significant interaction between visibility and method, but there was a significant interaction between method and site. A post-hoc pairwise test for the method by site interaction showed that at three of the nine sites, stereo-DOV and stereo-ROV sampled significantly different assemblages ($p < 0.019$). These differences were due to the presence of several large schools of fish including *Neatypus obliquus* and *Schuettea woodwardi* which were sampled by stereo-DOV, but not stereo-ROV at two sites, and *Chromis westaustralis* sampled by stereo-ROV and not stereo-DOV at the third site. Overall, there was no significant difference in the assemblage composition sampled by the two methods (Table 2.1). This was supported by the lack of distinction between the stereo-DOV and stereo-ROV data points in the nMDS (Figure 2.2), and the CAP (Figure 2.3). The overlays of species with Pearson's correlations to the data of > 0.3 (Figure 2.2 C) and indicator species (Figure 2.2 D) did not show any species highly correlated with either method. Post-hoc tests on the number of individuals of each of these species indicated that the only species which differed in the number of fish observed by stereo-DOV and stereo-ROV were *Kyphosus cornelii*, *P. milleri* and *C. rubescens* ($P < 0.020$). Stereo-DOV sampled significantly more *K. cornelii* ($\bar{x} = 5.07 \pm 2.73$ (1SE)), *P. milleri* ($\bar{x} = 11.5 \pm 4.35$ (1SE)), and *C. rubescens* ($\bar{x} = 1.20 \pm 0.300$ (1SE)) than stereo-ROV (*K. cornelii* $\bar{x} = 2.30 \pm 1.37$ (1SE), *P. milleri* $\bar{x} = 7.41 \pm 4.87$ (1SE), *C. rubescens* $\bar{x} = 0.52 \pm 0.171$ (1SE)). The leave-one-out allocation success test also supported the similarity between the techniques, with 63.0% of both stereo-DOV and stereo-ROV observations being correctly allocated.

2.4.1.3 Stereo-ROV and UVC

There was no significant effect of protection status on the assemblage composition sampled by stereo-ROV and UVC. With status pooled, there was a significant interaction between method and site and a significant effect of method. There was no significant interaction between method and visibility. A pairwise PERMANOVA on the method by site interaction indicated that there were two sites in which the two methods sampled significantly different assemblages ($P < 0.05$). The main effect of method indicated a significant difference in the assemblage composition sampled by the stereo-ROV and UVC (Table 2.1). The n-MDS (Figure 2.2) and CAP (Figure 2.3) visualisation of the assemblage composition sampled by each method showed a large amount of overlap between the methods with some slight clustering of the UVC samples. The overlay of species with Pearson's correlations to the data > 0.4 showed associations between several of these commonly occurring species and UVC (Figure 2.2 E). *P. milleri* was the only indicator species that was correlated with UVC and no species appeared to have a strong correlation with stereo-ROV (Figure 2.2 F). Post-hoc tests on the number of individuals of each of these species recorded by each method showed that only *C. rubescens*, *C. auricularis* and *Bodianus frenchii* ($P < 0.007$) differed. UVC sampled significantly more *C. rubescens* ($\bar{x} = 1.36 \pm 1.70$ (1SE)), *C. auricularis* ($\bar{x} = 60.3 \pm 43.0$ (1SE)), and *B. frenchii* ($\bar{x} = 0.136 \pm 0.409$ (1SE)) than stereo-ROV (*C. rubescens* $\bar{x} = 0.636 \pm 0.178$ (1SE), *C. auricularis* $\bar{x} = 34.5 \pm 9.45$ (1SE), *B. frenchii* $\bar{x} = 0 \pm 0$ (1SE)). The leave-one-out allocation success test resulted in 65.9% of UVC samples being correctly allocated and 61.4% of stereo-ROV samples being correctly allocated.

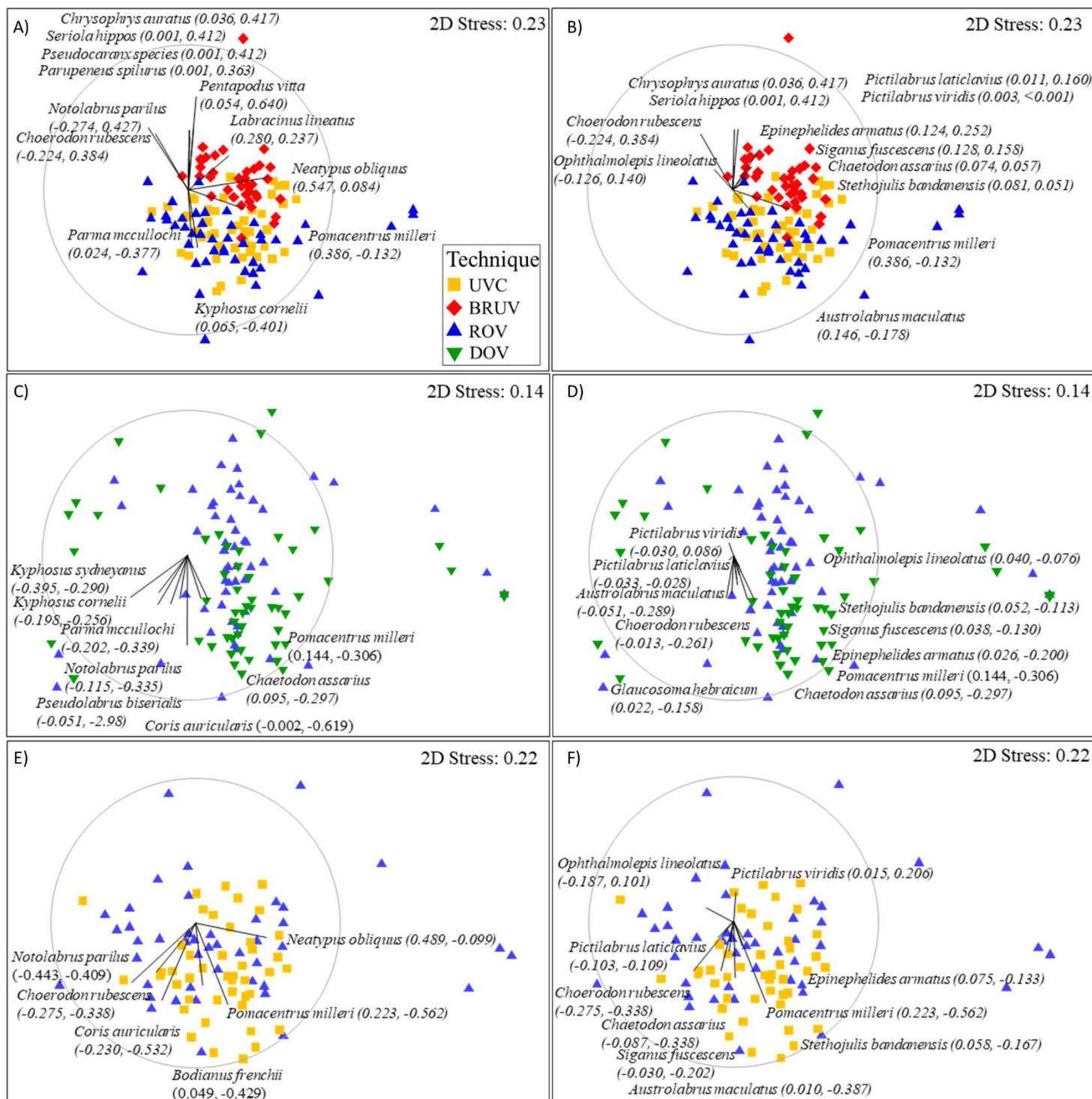


Figure 2.2 Non-metric multidimensional scaling plots of fish assemblages sampled by four methods (stereo-BRUV, stereo-DOV, stereo-ROV, and UVC). A and B are stereo-BRUV, stereo-ROV, and UVC with vectors overlaid as defined by Pearson's correlation = 0.35 and indicator species, C and D are stereo-DOV and stereo-ROV only with vectors overlaid as defined by Pearson's correlation = 0.30 and indicator species, E and F are stereo-ROV and UVC with vectors overlaid as defined by Pearson's correlation = 0.40 and indicator species. Numbers in brackets show correlation values (x, y).

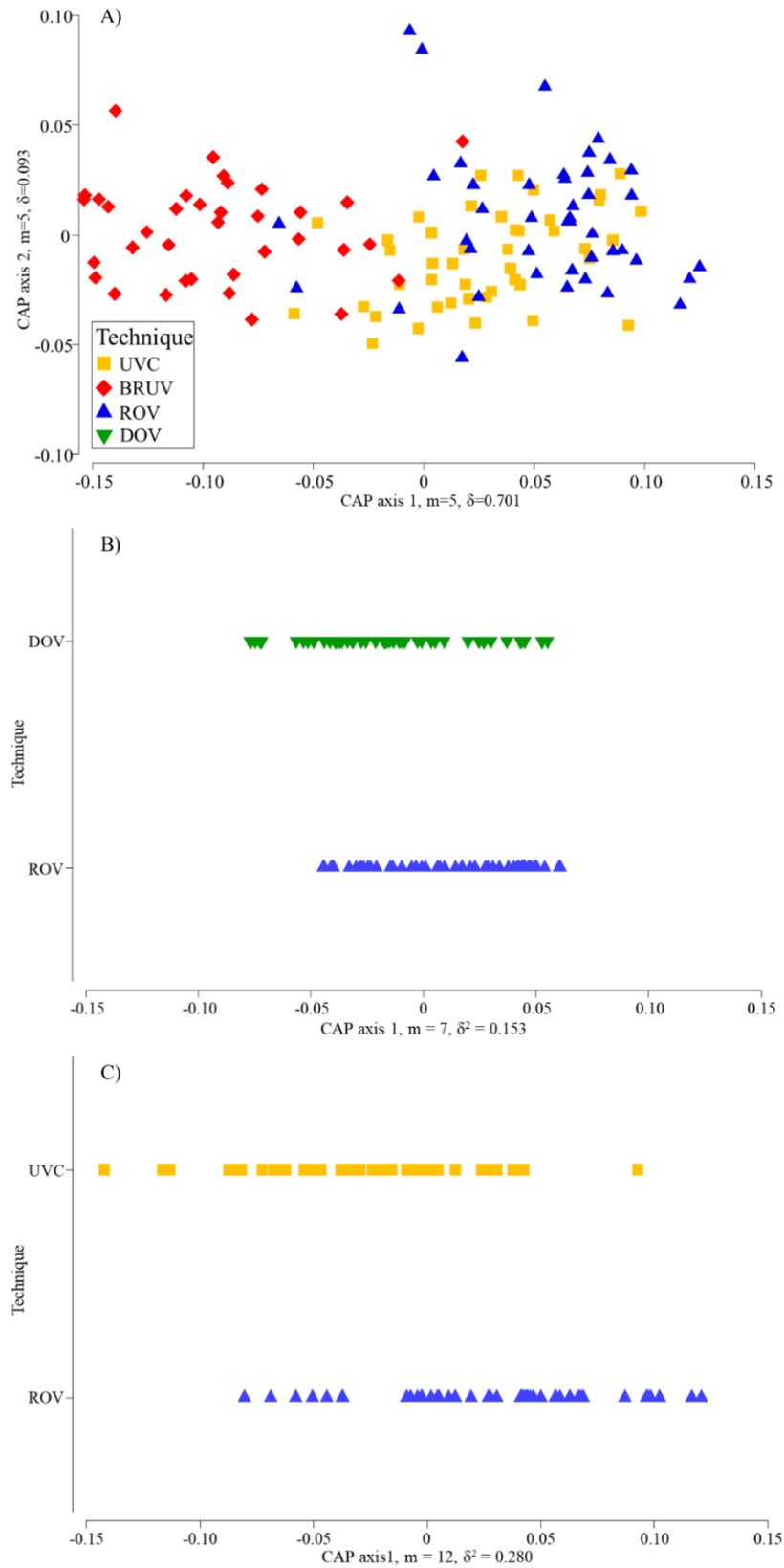


Figure 2.3 Canonical Analysis of principal coordinates (CAP) plots of the fish assemblage sampled by different combinations of underwater visual census (UVC), baited underwater stereo-video (BRUV), a remote operated vehicle with a stereo video attachment (ROV), and diver operated stereo-video (DOV) in the Jurien Bay Marine Park.

Table 2.1 Results of six permutational multivariate analysis of variance on transformed fish assemblage observed by a remotely operated vehicle with a stereo-video attachment (stereo-ROV), diver operated stereo-video (stereo-DOV), baited underwater stereo-videos (stereo-BRUVs) and underwater visual census (UVC) in the Jurien Bay Marine Park. PERMANOVA designs had three factors (a, b, and c) and two factors (d, e, and f). Values in bold show statistical significance at $\alpha = 0.05$. “N/A” indicates that this term was not tested, as UVC visibility estimates were made at the site level.

Source	a) Stereo-BRUVs, stereo-ROV, UVC assemblage				b) Stereo-DOV and stereo-ROV assemblage				c) Stereo-ROV and UVC assemblage			
	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)
Visibility	1	19111	5.75	<0.001	1	20833	2.77	0.003	1	10004	2.08	0.042
Method	2	12950	3.52	<0.001	1	9600	1.61	0.201	1	6512	2.30	0.054
Status	1	3135	0.440	0.838	1	7714	0.642	0.846	1	2545	0.367	0.918
Site (Status)	9	6859	5.15	<0.001	7	11349	5.27	<0.001	9	6738	5.02	<0.001
Visibility x Method	2	3583	1.30	0.224	1	6231	1.43	0.126	1	5884	2.09	0.055
Visibility x Status	1	2569	1.929	0.055	1	1438	0.668	0.788	1	2860	2.13	0.035
Method x Status	2	3490	1.2384	0.256	1	3545	0.791	0.528	1	2908	1.03	0.416
Visibility x Site (Status)	N/A	N/A	N/A	N/A	7	3396	1.58	0.003	N/A	N/A	N/A	N/A
Method x Site (Status)	14	2725	2.046	<0.001	7	3796	1.76	<0.001	6	2821	2.10	<0.001
Visibility x Method x Status	N/A	N/A	N/A	N/A	1	3722	1.73	0.060	N/A	N/A	N/A	N/A
Visibility x Method x Site (Status)	N/A	N/A	N/A	N/A	7	2238	1.04	0.392	N/A	N/A	N/A	N/A
Residual	92	1332			72	2152			66	1343		
Total	124				107				87			
Status pooled	d) Stereo-BRUVs, stereo-ROV, UVC assemblage				e) Stereo-DOV and stereo-ROV assemblage				f) Stereo-ROV and UVC assemblage			
Source	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)
Visibility	1	19111	6.02	<0.001	1	20833	2.89	0.002	1	10004	2.21	0.033
Method	2	12950	3.51	<0.001	1	9600	1.75	0.138	1	6512	2.29	0.048
Site	10	6486	4.87	<0.001	8	10894	5.06	<0.001	10	6318	4.70	<0.001
Visibility x Method	2	3583	1.27	0.236	1	6231	1.55	0.096	1	5884	2.07	0.052
Visibility x Site	N/A	N/A	N/A	N/A	8	3330	1.55	0.003	N/A	N/A	N/A	N/A
Method x Site	17	2806	2.11	<0.001	8	3586	1.67	0.001	8	2837	2.11	<0.001
Visibility x Method x Site	N/A	N/A	N/A	N/A	8	2423	1.13	0.226	N/A	N/A	N/A	N/A
Residual	92	1332			72	2152			66	1343		
Total	124				107				87			

2.4.2 Number of individuals and species

2.4.2.1 Stereo-BRUVs, stereo-ROV, and UVC

The species accumulation curves showed that stereo-BRUVs sampled a higher number of species at any given sample size than both stereo-ROV and UVC, and that UVC sampled a higher number of species at any given sample size than stereo-ROV (Figure 2.4). Stereo-BRUVs also had the highest mean percentage occurrence across samples of all species at $22.0 \pm 2.87\%$ (1SE), followed by UVC at $18.0 \pm 2.81\%$ (1SE) and stereo-ROV at $10.8 \pm 2.17\%$ (1SE) (Table A.1). Stereo-BRUVs achieved the highest power to detect hypothetical change in the number of species and number of individuals at both 20% and 50% change. This was followed by UVC which achieved higher power than stereo-ROV. Stereo-BRUVs achieved a power of $\beta = 0.8$ to detect a 20% change in the number of species within 55 samples whereas UVC and stereo-ROV did not achieve this until 95 and 105 samples respectively. The same pattern was true for detecting a 20% change in the number of

individuals. However, a larger sample size was needed across all methods. Stereo-BRUVs achieved $\beta = 0.8$ power within 90 samples, whereas UVC took 320 samples and stereo-ROV only achieved $\beta = 0.52$ power within the computed maximum sample size of 400 (Figure 2.5).

2.4.2.2 Stereo-DOV and stereo-ROV

There was no significant effect of protection status on the number of species sampled by stereo-DOV and stereo-ROV. With status pooled, there was a significant interaction between method and site, and method and visibility. A post-hoc pairwise test on the method by site interaction showed that at three of the nine sites, stereo-DOV and stereo-ROV sampled a significantly different number of species. At two of the sites, stereo-ROV sampled significantly more species than stereo-DOV, and at one site stereo-DOV sampled significantly more species than stereo-ROV ($P < 0.047$). Overall, there was no significant difference between the methods (Table 2.2, Figure 2.6). A PERMANOVA on the visibility estimates also indicated that there was a significant interaction between method and site (Method \times Site (8, 107), Pseudo-F = 6.86, $P < 0.001$) and pairwise post-hoc tests shows that there were significant differences in visibility between methods at five of the nine sites ($P < 0.032$). However, overall, there was no significant difference in the visibility between methods (Method (1, 107), Pseudo-F = 0.032, $P = 0.86$), indicating that visibility was not having a confounding influence on the test for number of species. The species accumulation curve showed that stereo-DOV and stereo-ROV sampled a similar number of species at any given sample size. Stereo-ROV achieved the highest power to detect hypothetical change in the number of species at both 20% and 50% change, reaching a power of $\beta = 0.8$ to detect a 20% change in the number of species within 120 samples, whereas stereo-DOV took 255 samples to achieve this power (Figure 2.5).

There was no significant effect of protection status on the number of individuals sampled by stereo-DOV and stereo-ROV. With status pooled, there was no significant interaction between method and site. However, there was a significant interaction between visibility and method. A DistLM showed that there was a significant effect of visibility on the number of individuals sampled using stereo-DOV and stereo-ROV ($P < 0.007$) that accounted for 13.2% and 18.8% of the variation in the number of individuals respectively. The mean visibility for stereo-DOV and stereo-ROV samples was 6.97 ± 0.243 (1SE), and 7.06 ± 0.269 (1SE) respectively. Overall, there was a significant difference between the methods (Table 2.2). Stereo-DOV sampled a mean of 91.9 ± 11.7 (1SE) individuals which was significantly higher than stereo-ROV which sampled a mean of 64.5 ± 12.3 (1SE) individuals (Figure 2.6). Stereo-DOV also achieved greater power than stereo-ROV to detect hypothetical change in the number of individuals at both 20% and 50%. Stereo-ROV did not achieve sufficient power ($\beta = 0.8$) to detect 20% change in the number of individuals within the computed sample size of 400, and stereo-DOV took a sample size of 365 to achieve this. To achieve sufficient power to detect a 50% change in the number of individuals, a sample size of 70 was needed for stereo-DOV and 140 for stereo-ROV.

2.4.2.3 Stereo-ROV and UVC

There was no significant effect of protection status on the number of species sampled by stereo-ROV and UVC. With status pooled, there was a significant interaction between method and visibility, but no significant interaction between method and site. A DistLM indicated that visibility was not significantly influencing the variation in the number of species observed by UVC ($P = 0.288$) however, it was significantly affecting the variation in the number of species observed by ROV ($P = 0.005$). 17.6% of the variation in the number of species observed by stereo-ROV could be explained by visibility. A PERMANOVA on the visibility estimates indicated that there was no significant difference in the visibility between the two methods (Method $_{1,87}$, $MS = 1.73$, Pseudo- $F = 0.701$, $P = 0.401$). The mean visibility was 6.79 ± 0.272 m (1SE) and 6.61 ± 0.194 m (1SE) for stereo-ROV and UVC respectively. Overall, there was a significant main effect of method on the number of species recorded (Table 2.2). Stereo-ROV sampled a mean of 6.43 ± 0.460 (1SE) species which was fewer than UVC which sampled a mean of 9.70 ± 0.662 (1SE) species (Figure 2.6). These results should be interpreted with caution due to the potential confounding influence of visibility. The species accumulation curve supported the pattern of UVC sampling more species than stereo-ROV, with this occurring at any given sample size, and UVC levelling off at a higher number of species than stereo-ROV (Figure 2.4). Again, this was consistent with the post-hoc power analysis, with UVC having more power to detect hypothetical change in the number of species than stereo-ROV at any given sample size (Figure 2.5).

There was no significant effect of protection status on the number of individuals sampled by stereo-ROV and UVC. With status pooled, there was no significant interaction between visibility and method or method and site, but there was a significant main effect of method (Table 2.2). The mean number of individuals sampled by stereo-ROV was 77.4 ± 15.0 (1SE) which was significantly fewer than UVC with a mean of 127 ± 16.7 (1SE) (Figure 2.6). Once again, the post-hoc power analysis supported the results of the PERMANOVAs, as UVC achieved a higher power to detect hypothetical change in the number of individuals than stereo-ROV (Figure 2.5). UVC achieved sufficient power to detect a 20% change in the number of individuals within 320 samples and stereo-ROVs did not achieve sufficient power within the computed 400 samples. Stereo-ROV required 120 samples for sufficient power to detect a 50% change in the number of individuals which was double the required sample size for UVC, which achieved sufficient power within 60 samples.

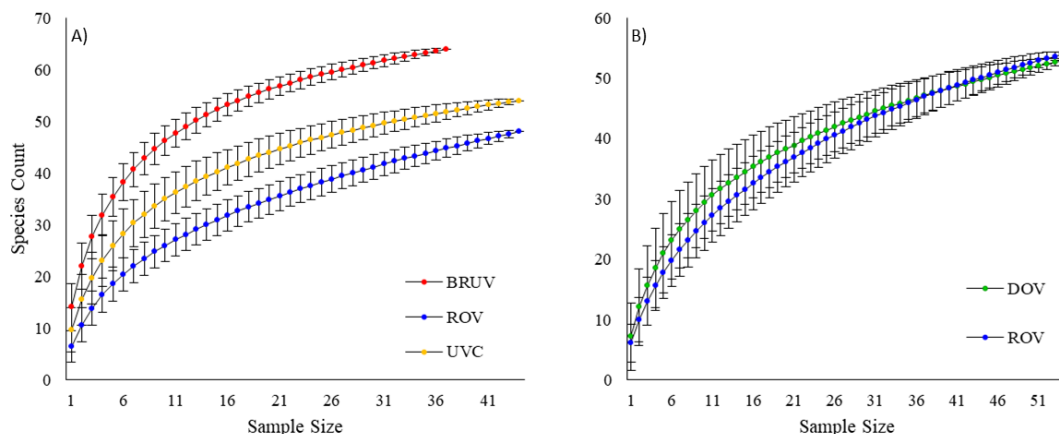


Figure 2.4 Species accumulation curves with an increasing sample size as sampled by (a) baited remote underwater stereo-video (BRUV), ROV, and underwater visual census (UVC), and (b) diver operated stereo-video (DOV) and a remotely operated vehicle with a stereo-video attachment (ROV) in the Jurien Bay Marine Park. Error bars show one standard deviation.

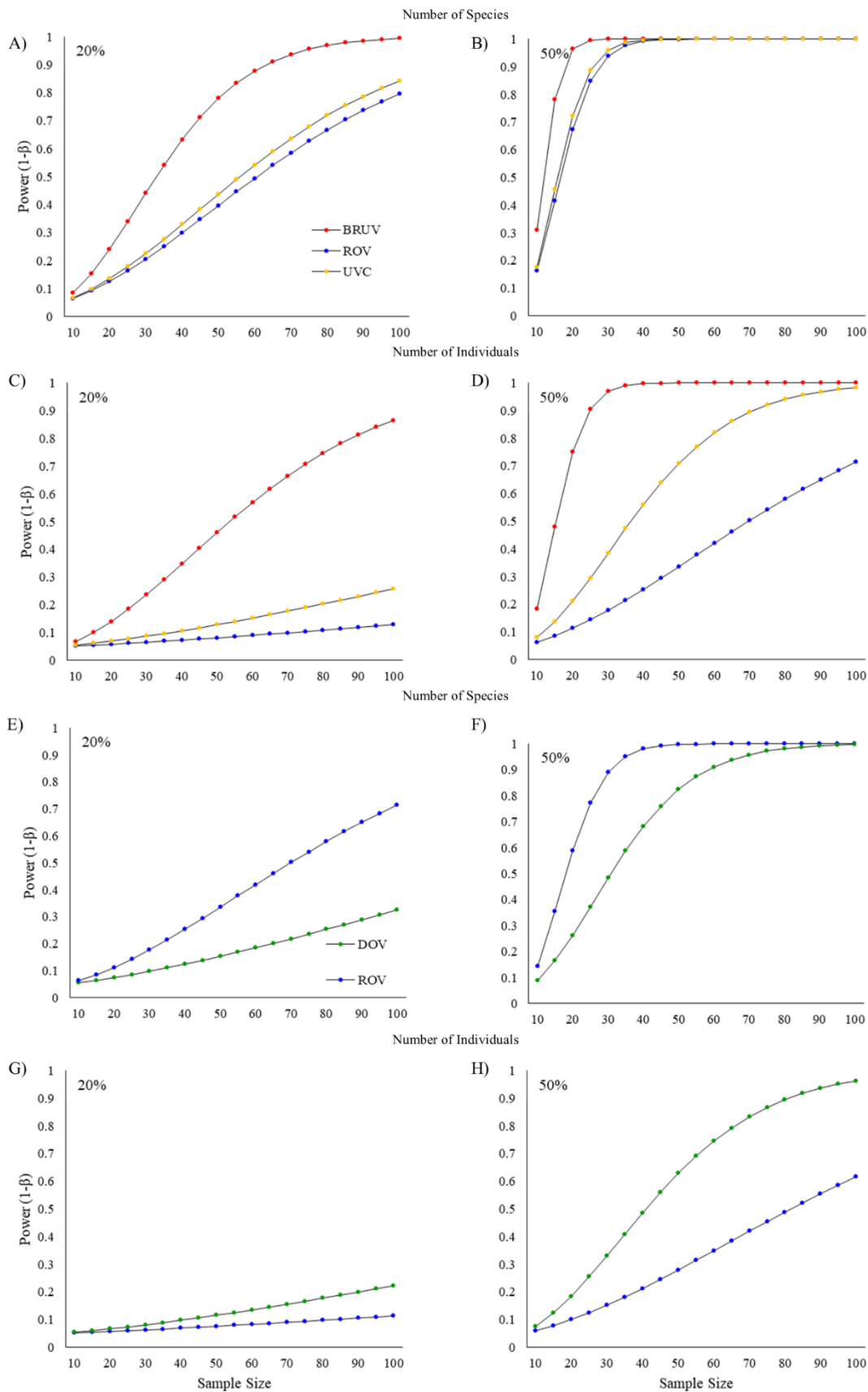


Figure 2.5 Power of baited remote underwater stereo-video (BRUV), a remotely operated vehicle with a stereo-video attachment (ROV), underwater visual census (UVC), and diver operated stereo-video (DOV) to detect hypothetical change in (a, b, e, f) the mean number of species and (c, d, g, h) the mean number of individuals with increasing number of samples.

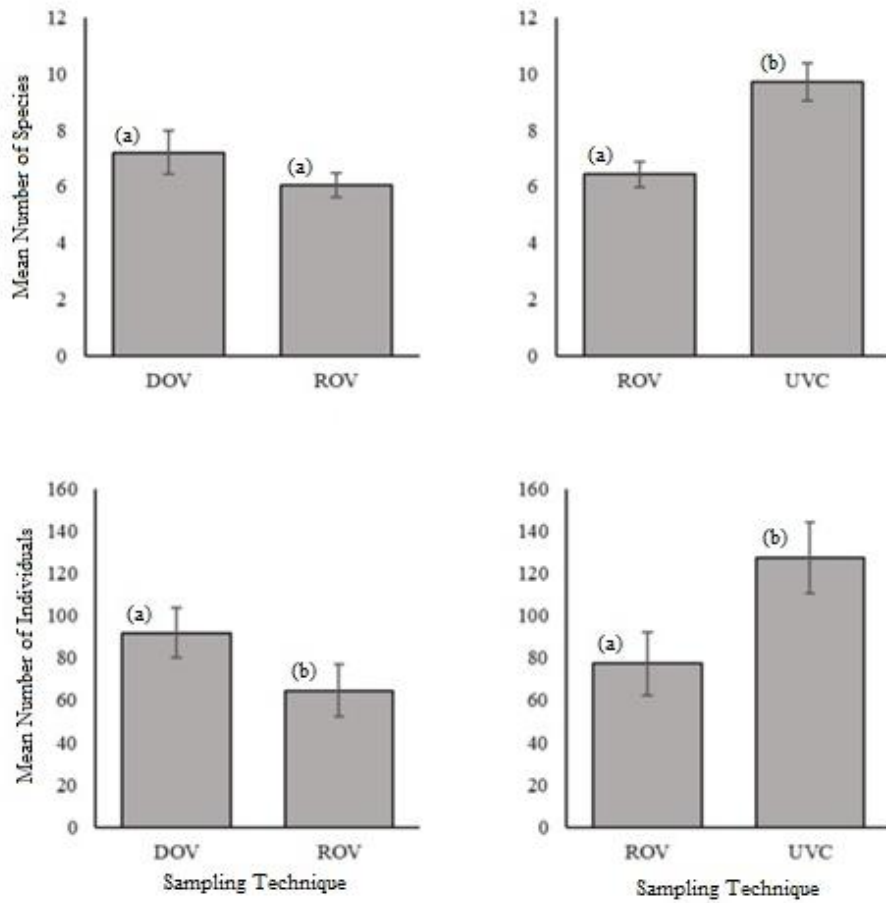


Figure 2.6 Mean ($\pm 1SE$) number of individual and species recorded by diver operated stereo-video (DOV), a remotely operated vehicle with a stereo-video attachment, baited remote underwater stereo-video (BRUV), and underwater visual census (UVC) in the Jurien Bay Marine Park. Similar letters above the bars indicate statistically similar means.

Table 2.2 Results of eight permutational multivariate analysis of variance on counts of species (a, b, e, f) and counts of individuals observed by a remotely operated vehicle with a stereo-video attachment (stereo-ROV), diver operated stereo-video (stereo-DOV), and underwater visual census (UVC) in the Jurien Bay Marine Park. PERMANOVA designs had three factors (a, b, c, d) and two factors (e, f, g, h). Values in bold show statistical significance at $\alpha = 0.05$. “N/A” indicates that this term was not tested, as UVC visibility estimates were made at the site level.

Source	a) Stereo-DOV, stereo-ROV number of species				b) Stereo-ROV, UVC number of species				c) Stereo-DOV, stereo-ROV number of individuals				d) Stereo-ROV, UVC number of individuals			
	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)
Visibility	1	198	2.31	0.144	1	0.956	0.803	0.375	1	1.30E+05	5.86	0.022	1	66071	2.27	0.141
Method	1	40.2	0.875	0.380	1	8.08	45.3	0.001	1	23121	5.45	0.060	1	62520	9.78	0.019
Status	1	142	0.923	0.370	1	0.466	0.260	0.634	1	11563	0.315	0.818	1	8.20	0.010	0.918
Site (Status)	7	142	23.5	<0.001	9	1.73	8.21	<0.001	7	34961	8.07	<0.001	9	41508	6.16	<0.001
Visibility x Method	1	23.8	0.737	0.462	1	6.31	36.1	<0.001	1	15033	4.94	0.056	1	3958	0.628	0.467
Visibility x Status	1	17.4	2.88	0.090	1	1.28	6.06	0.016	1	47.5	0.011	0.915	1	13210	1.96	0.168
Method x Status	1	24.0	0.615	0.461	1	0.263	1.51	0.264	1	1950	1.09	0.333	1	4715	0.748	0.421
Visibility x Site (Status)	7	14.5	2.41	0.033	N/A	N/A	N/A	N/A	7	7839	1.81	0.116	N/A	N/A	N/A	N/A
Method x Site (Status)	7	27.7	4.58	<0.001	6	0.175	0.826	0.550	7	2626	0.606	0.719	6	6306	0.936	0.486
Visibility x Method x Status	1	0.936	0.155	0.693	N/A	N/A	N/A	N/A	1	1200	0.277	0.603	N/A	N/A	N/A	N/A
Visibility x Method x Site (Status)	7	5.00	0.829	0.545	N/A	N/A	N/A	N/A	7	4883	1.13	0.335	N/A	N/A	N/A	N/A
Residual	72	6.04			66	0.211			72	4334			66	6738		
Total	107				87				107				87			
Source	e) Stereo-DOV, stereo-ROV number of species				f) Stereo-ROV, UVC number of species				g) Stereo-DOV, stereo-ROV number of individuals				h) Stereo-ROV, UVC number of individuals			
	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)	df	MS	Pseudo-F	P (perm)
Visibility	1	198	2.33	0.138	1	0.956	0.867	0.370	1	1.30E+05	6.40	0.015	1	66071	2.51	0.130
Method	1	40.2	0.990	0.351	1	8.08	24.7	<0.001	1	23121	5.86	0.047	1	62520	8.87	0.018
Site	8	142	23.5	<0.001	10	1.61	7.60	<0.001	8	32036	7.39	<0.001	10	37358	5.54	<0.001
Visibility x Method	1	23.8	0.833	0.425	1	6.31	19.5	0.002	1	15033	5.31	0.048	1	3958	0.568	0.475
Visibility x Site	8	17.0	2.81	0.012	N/A	N/A	N/A	N/A	8	6902	1.59	0.169	N/A	N/A	N/A	N/A
Method x Site	8	25.1	4.16	0.001	8	0.324	1.53	0.165	8	2505	0.578	0.743	8	6970	1.03	0.426
Visibility x Method x Site	8	4.49	0.744	0.639	N/A	N/A	N/A	N/A	8	4423	1.02	0.400	N/A	N/A	N/A	N/A
Residual	72	6.04			66	0.211			72	4334			66	6738		
Total	107				87				107				87			

2.5 Discussion

We compared the fish assemblage, number of species and number of individuals sample by three commonly used (UVC, stereo-BRUVs and DOVs) and one emerging method (stereo-ROV). Stereo-BRUVs sampled a distinctive assemblage compared to all transect-based methods, with more individuals, total species, and predatory species from higher trophic groups that are targeted by commercial and recreational fishers (Pink Snapper (*Chrysophrys auratus*) and Samson Fish (*Seriola hippos*)). The distinctive assemblage sampled by stereo-BRUVs compared to transect methods suggests that a combination of stereo-BRUVs with a transect method may be an optimal approach to achieve a robust and ecosystem level of sampling for fishes (Logan et al., 2017; Schramm et al., 2020a; Schramm et al., 2020b; Watson et al., 2010; Willis et al., 2000). UVC also sampled a distinctive assemblage that was made up of more individuals and species than other transect methods, including some small bodied cryptic species and species that are difficult to identify in video footage. UVC may therefore be a more suitable method when species diversity estimates are a primary objective.

Notably, stereo-DOV and stereo-ROV were broadly comparable, with the exception of relatively small differences in the number of individuals and observations of schooling species at select sites. Given the consideration of these differences, stereo-ROV could be used to replace or complement monitoring or sampling programs based on stereo-DOV sampling. This creates an opportunity for stereo-video transect sampling in water that is too deep for SCUBA divers, or where the risks of having SCUBA divers in the water are unacceptably high.

Stereo-BRUVs, stereo-ROV and UVC all sampled a different assemblage of fishes. Similar to other studies, stereo-BRUVs sampled the greatest number of species (Goetze et al., 2015; Langlois et al., 2010; Logan et al., 2017; Schramm et al., 2020a; Watson et al., 2010; Willis et al., 2000). The unique species sampled by stereo-BRUVs were mostly carnivores which was also consistent with previous studies that found the use of bait increased the proportion of species from higher trophic groups (Cappo et al., 2003; Cappo et al., 2006; Harvey et al., 2007). These species are often targeted by fishers and the greater ability of stereo-BRUVs to sample Pink Snapper (*C. auratus*), Samson Fish (*S. hippos*), and Silver Trevally (*Pseudocaranx* species) highlights the importance of stereo-BRUVs for sampling indicator species to reflect fishing pressure. In contrast, herbivorous species such as *K. cornelii* and the site attached *Parma mccullochi*, and *P. milleri* (Allen, 1991) are not attracted to bait and were better sampled by the transect methods. Stereo-BRUVs often sample site attached species in lower abundance when compared to transect methods (Langlois et al., 2010; Schramm et al., 2020b; Watson et al., 2010). These non-target species are often important in ecosystem-based fisheries and biodiversity management (Hall & Mainprize, 2004). For example, *C. auricularis*, *N. obliquus* and *Enoplosus armatus* have been defined as indicator species to reflect fishing pressure of western rock lobster in the Jurien Bay Marine Park (Metcalf et al., 2011). Sampling of a suite of both non-target and fisheries indicator species is important to understand and monitor the effects of reduced anthropogenic influences in MPAs (Claudet & Guidetti, 2010), and therefore stereo-BRUVs should be combined with a transect based approach where possible.

Despite differences in some schooling species at select sites, stereo-DOV and stereo-ROV sampled statistically similar assemblages and numbers of species. This contrasts with Schramm et al. (2020a), where stereo-ROV sampled a different assemblage, number of individuals, and number of species to stereo-DOV, which was thought to be driven by the height of operation of the stereo-ROV above the substrate (Schramm et al., 2020a). Schramm et al. (2020a) hypothesised that if the stereo-ROV was operated at a similar height above substrate to stereo-DOV, the methods would record similar assemblages. In our study we attempted to achieve this with ROV pilot training and briefing related to operating height. While stereo-DOV and stereo-ROV observed a similar assemblage and number of species, stereo-DOV observed significantly more individuals. Similar to Schramm et al. (2020a) we propose that this may be due to difficulty in always maintaining the desired height above the substrate. Here operation height varied within a transect due to with ocean currents (Ajemian et al., 2015) and the entanglement hazard of the umbilical cord when operating in complex habitat (Pacuneski et al., 2008) and areas where sudden changes in topography occurred. This may have resulted in the stereo-ROV operating at a higher average height than other transect methods (although this was not measured). Sonar technology could provide an accurate means of measuring operating height of stereo-ROV in real time. Variation in operation

height may also explain the significantly higher number of individuals of other species including *C. rubescens* and *P. milleri* sampled by stereo-DOV. *P. milleri* are small (max fork length < 75mm; Allen, 1991) making observation of them more likely to be affected by changes in the height above the substrate. The increased time taken to complete a stereo-DOV transect compared to stereo-ROV is also likely to have contributed to the increased abundance. Increasing survey time can cause overestimation of fish abundance on transects, particularly with highly mobile species (Pelletier et al., 2011; Smith; Ward-Paige et al., 2010; Watson et al., 1995). The only other species where stereo-DOV sampled significantly more fish than stereo-ROV was *K. cornelii*. *K. cornelii* is a schooling fish (Swainston, 2011) and it is likely this result was driven by chance encounters with schools when sampling with stereo-DOV. These were the only species among those investigated from the Pearson's and indicator species overlays that showed significant differences between methods. None of the indicator species had strong correlations toward either technique. This finding supports the suggestion that a transition from stereo-DOV to remotely operated sampling in the form of stereo-ROV is possible without jeopardising time series comparisons for most species.

It is also important to consider the time and resources necessary to complete a monitoring program with sufficient power to detect changes. Differences in power to detect change between methods may also affect the ability to assess the effectiveness of zoning within MPAs, especially in cases where there is variation in the effectiveness of highly protected areas (Goetze et al., 2021). Stereo-BRUVs collected data with lower variance because of the bait attraction which results in higher overall counts (Cappo et al., 2003; Harvey et al., 2007). Transect methods had less power to detect hypothetical change than stereo-BRUVs, which is consistent with other studies (Cundy et al., 2017; Harvey et al., 2007; Harvey et al., 2012; Schramm et al., 2020a; Schramm et al., 2020b). This is due to the higher variance between samples gathered by transect methods. The higher variance in transect methods has been attributed to high spatial heterogeneity in the habitat area surveyed, which emphasises the importance of selecting an optimal transect length and width for the location to be surveyed (McCormick & Choat, 1987; Schramm et al., 2020a 2020). These power differences mean a higher sample size is necessary for transect methods compared to stereo-BRUVs, which may be costly in both field and laboratory resources. Consideration not only of the number of samples required, but also the resource cost per sample is important. In this study, we required three people and a small boat to complete stereo-ROV surveys, whereas a dive crew of at least three a skipper and a larger vessel was needed for diver-based methods to operate under live-boating conditions. A greater number of stereo-ROV transects could be completed in a day than both stereo-DOV and UVC due to the lower time taken per site, and avoidance of surface intervals as a requirement of dive decompression tables. UVC efficiency is affected by time taken in the field, including surface intervals and time in water, however, requires very little laboratory time. Both the power to detect change and efficiency in both field and laboratory environments should be taken into account when deciding on the appropriate methodology.

The distinct assemblage sampled by UVC compared to stereo-ROV was primarily driven by UVC sampling more individuals of commonly occurring species such as *Notolabrus parilus*, *C. rubescens*, *C. auricularis*, *B. frenchii*, *P. milleri*, and *N. obliquus*. The sampling of a higher number of individuals by UVC has previously been attributed to UVC divers taking longer to complete each transect than the stereo-video transects and potentially

overestimating abundance (Goetze et al., 2015; Pelletier et al., 2011; Smith, 1998; Ward-Paige et al., 2010; Watson et al., 1995). The difference in time taken to complete transects is due to a fundamental difference in the two sampling methods as UVC requires observations to be recorded while completing the transect whereas all observations for stereo-ROV sampling occur in post processing. Further investigation into optimal speed for transect methods may be useful in refining operations of both stereo-video transect sampling and UVC. The inter-observer error of divers estimating transect area, and hence which fish to count within that area may also contribute to UVC sampling more individuals. Experienced scientific divers have been shown to estimate a 7 m point count anywhere between 6.3 and 9.7m (Harvey et al., 2004). They also tend to include larger fish in transects when they were outside of the sampling area (Choat & Bellwood, 1985). In contrast, UVC allows for observations of small bodied cryptic species to be recorded due to the advantage of the human eye over cameras (Holmes et al., 2013) and divers being able to closely assess and follow individual fish to assist in identification (Bortone et al., 1991). Overall, UVC sampled eight species that no other method detected, four of which were small bodied, site attached species from the families Tripterygiidae, Apogonidae, and Blenniidae. The trend of UVC sampling more of these small-bodied site attached species is consistent with other studies (Colton & Swearer, 2010; Holmes et al., 2013; Lowry et al., 2012). The other three species were also small bodied species including *Stegastes obreptus*, *Leptoscarus vaigiensis*, and *Dascyllus trimaculatus*, of which *S. obreptus* was grouped with *P. milleri* in stereo-video methods due to difficulty in consistent species level identification. This difficulty in identification with video was due to limitations in the resolution, exposure, and frame rate of cameras (Cappo et al., 2003). Of these species observed only by UVC, none were indicator species. Further, of the species correlated with UVC over stereo-ROV, only three were significantly different between the two methods and *C. rubescens*, which was recorded in greater numbers using UVC, was the only indicator species. The ability of UVC to sample a higher number of species in comparison to stereo-video transect methods is consistent with other studies (Holmes et al., 2013; Pelletier et al., 2011; Tessier et al., 2013), and therefore may be more suitable when assessing species diversity is a priority.

All methods of gathering ecological data have inherent biases and limitations which must be considered in experimental design (Harmelin-Vivien & Francour, 1992; MacNeil et al., 2008a). To facilitate a transition to remotely operated transect sampling with stereo-ROV and allow sampling of areas inaccessible by SCUBA divers, an understanding of the biases and limitations of each technique is needed. A further consideration is that visibility appeared to account for more variation in the stereo-ROV data compared to the UVC data, even when visibility was not significantly different between the two methods. This indicated that UVC may be a better sampling tool in conditions where visibility is highly variable, or with poor visibility (Holmes et al., 2013). A consideration in facilitating the transition to remotely operated sampling may be that field studies need to be more flexible in their approach and work around environmental conditions such as visibility, wind, currents, and swell. To transition from UVC to stereo-ROV in shallow temperate ecosystems, it would be important to consider the differences that our study highlights in the ability to sample the number of species and individuals as well as cryptic species and species of interest such as *C. rubescens*. This would be especially important when interpreting long term trends in data collected

across methods and may be necessary to develop conversion factors between the methods (Wilson et al., 2018) for some species or groups.

Remote sampling offers advantages of reduced field time and negates the depth and safety limitations that are associated with SCUBA diving (Parry et al., 2003; Smolowitz et al., 2015; Sward et al., 2019). The ability to sample deeper offshore habitats with remote sampling is important as there is often a different assemblage of fishes in these locations and difference responses to MPA management (Goetze et al., 2021). Deeper water continental shelf fish assemblages generally consist of more mobile carnivores as well as larger individuals (Asher et al., 2017; Bach et al., 2019; Fitzpatrick et al., 2012; Wellington et al., 2018). Such species are often of high management importance due to their commercial and recreational value (Harvey et al., 2012). The unique advantages of stereo-BRUVs include the sampling of mobile species of a higher trophic group as well as their ability to sample with lower variance and therefore to achieve high statistical power with relatively fewer samples. These advantages pair well with the ability of transect methods to sample site attached species that are important non-target indicator species. For monitoring of fishes, the benefits of stereo-BRUVs are well established (Logan et al., 2017; Schramm et al., 2020a; Schramm et al., 2020b; Watson et al., 2010; Willis et al., 2000). While further research across different locations is needed, we show that stereo-ROV can provide a complementary transect based method that performs similarly to stereo-DOV, and offers additional benefits associated with field logistics, efficiency and health and safety.

**3. CHAPTER 3: A COMPARISON OF THE LENGTH FREQUENCY
DISTRIBUTION AND BEHAVIOURAL RESPONSE OF FISHES TO A
REMOTE OPERATED VEHICLE AND DIVER-BASED STEREO-VIDEO
SAMPLING**



Photograph: Baldchin groper (*Choreodon rubescens*) amongst macroalgae and rocky reef habitat in the Jurien Bay Marine Park

3.1 Abstract

Successful monitoring of fishes to inform adaptive management relies on accurate and robust data on a range of indicators including diversity, abundance and length-based metrics. The methods for gathering this data have evolved from destructive techniques to underwater visual census (UVC), and now emergent methods such as divers or remote operated vehicles (ROVs) equipped with stereo-video systems. Stereo-video techniques can provide accurate information on traditional metrics such as abundance, length and biomass, as well as metrics on fish behaviour. Fish wariness, measured by the minimum distance between a fish and an observer or stereo-video system before the fish moves away (minimum approach distance; MAD) is an emerging metric for assessing changes in fishing pressure that can be more sensitive than traditional metrics. Differences in fish behaviour across methods can bias results and influence measurement accuracy and precision of stereo-video systems, because accuracy decreases as the distance of the fish increases away from the camera. We assessed the behaviour of six focal species towards a diver operated stereo-video system (stereo-DOV) and a stereo-video system mounted on a remotely operated vehicle (stereo-ROV). We also compared the fish lengths and the length frequency distribution of measurements from the two methods. We aimed to increase the understanding of the relative abilities of the two methods at gathering length-based data and the differences in fish behavioural responses towards the methods. There were no differences in the proportion of length measurements or the mean length, and small differences in the length frequency distribution sampled by the two methods. We found that differences in the MAD between the two methods varied, likely due to different trophic traits such as foraging modes and behavioural patterns between the focal species and potentially adaptations resulting from fishing pressure. Species such as those in the *Pomacentridae* family showed more aggressive behaviour towards SCUBA divers possibly due to their feeding regime while fisheries targeted species such as *Choerodon rubescens* showed more wariness towards divers as a possible adaptation to fishing pressure. At the assemblage level, the relationship between MAD and fish length was weaker with the stereo-ROV, indicating that larger fish showed more wariness towards SCUBA divers than the stereo-ROV system. The decreased wariness of larger fish and fisheries targeted species towards the stereo-ROV compared to stereo-DOV, as well as the similarities in the length frequency distributions sampled by the two methods suggest that stereo-ROV may provide a suitable alternative to stereo-DOV.

Keywords: Temperate Fish; Diver operated stereo-video; Stereo-ROV; Length frequency distribution; Minimum approach distance; Fish behaviour

3.2 Introduction

Monitoring marine fishes relies on a robust assessment of fish diversity, abundance, size structure and biomass (Jennings, 2004; Jennings & Kaiser, 1998; Schramm et al., 2020b). Changes in technology have facilitated the development of new video-based methods for collecting biological data on fish assemblage composition (Cappo et al., 2003; Cappo et al., 2006; Harvey & Shortis, 1995; Mallet & Pelletier, 2014; Schramm et al., 2020a; Schramm et al., 2020b; Sward et al., 2019). These methods have been designed to overcome limitations such as depth constraints of diver-based methods or the need for in-situ taxonomic expertise with underwater visual census (UVC). When designing monitoring or research programs for fish, it is important to understand the limitations and biases associated with the sampling methods available (Harmelin-Vivien & Francour, 1992; MacNeil et al., 2008b). For example, behavioural and physiological traits of different fishes such as trophic level and migratory habits may mean that different methods sample the same species with different efficiencies (Cappo et al., 2006; Langlois et al., 2010; Schramm et al., 2020b; Watson et al., 2010). Methods may also differ in their efficiency and cost in field and laboratory time (Goetze et al., 2015; Langlois et al., 2010), health and safety considerations, and their suitability to sample within no-take marine reserves (Cappo et al., 2003; Cappo et al., 2006). Since the development of the initial diver operated stereo-video (stereo-DOV) prototype by Harvey and Shortis (1995), stereo-video methods have become widely used to gather data on fish assemblage compositions. The use of stereo-video facilitates accurate definition of transect area (Harvey et al., 2004), provides accurate measurements of fish length (Harvey, Fletcher, et al., 2002) and quantitative counts of abundance (Harvey et al., 2007; Watson et al., 2005) with the ability for experts to review identifications and measurements during post processing of imagery (Cappo et al., 2006). Another advantage of stereo-video sampling is its ability to quantify fish behaviour using minimum approach distance (MAD) as a proxy for wariness towards divers (Lindfield et al., 2014; Stamoulis et al., 2020). This has been shown to be a sensitive indicator of fishing pressure in areas where spearfishing is common (Goetze et al., 2017).

While consideration for a variety of assessment metrics is essential in designing monitoring programs (Nash & Graham, 2016), length-based metrics (including biomass) can be more sensitive indicators to changes in fishing pressure than abundance (Claudet et al., 2006; Taylor et al., 2014). Length data can provide insight into fishing pressures that are size selective (Nash & Graham, 2016), and non-fishing related changes such as recruitment events (Shin et al., 2005). It can also add context to the interpretation of abundance data as the functional impact of larger individuals can be greater and more widespread than smaller individuals. For example, larger grazing parrotfishes can remove more algae per unit of body mass than smaller parrotfish, and therefore a reduction in mean body length may lead to a disproportionate loss in ecosystem function (Lokrantz et al., 2008). Furthermore, length-based metrics can be a predictor for trophic levels which could provide data to detect changes in trophic structure as a result of disturbance such as fishing pressure (Jennings et al., 2002) and more complex influences such as habitat degradation from multiple human pressures

(Taylor et al., 2022). These length-based indicators are important considerations for adding context to abundance and species richness data and moving to an ecosystem approach to fisheries management by interpreting functional ecological effects of changes in community structure (Jennings et al., 1995; Jennings & Polunin, 1997; Shin et al., 2005).

Remote operated vehicles (ROVs) are an emerging technology that have been tested as an alternative sampling technique for assessing fish assemblage composition (Ajemian et al., 2015; Andaloro et al., 2013; Consoli et al., 2016; Trenkel et al., 2004). A combination of ROV technology and stereo-video technology has been developed to assess fish assemblage parameters including abundance, length, and biomass (Jessop et al., 2022; Schramm et al., 2020a; Schramm et al., 2020b; Sward et al., 2019). This combination of technologies benefits from both the accuracy and precision of stereo-video and the advantages of remote sampling including health and safety and the ability to sample deeper waters (Parry et al., 2003; Schramm et al., 2020a; Schramm et al., 2020b; Smolowitz et al., 2015; Sward et al., 2019; Warnock et al., 2016). Data collected by stereo-ROVs has been compared to other methods including stereo-DOVs, stereo-BRUVs, underwater visual census (UVC) and slow towed stereo-video with varying results (Jessop et al., 2022; Schramm et al., 2020a; Schramm et al., 2020b). Differences in assemblage composition sampled by stereo-ROV and stereo-DOV have been attributed to differences in the height of operation above the substrate (altitude) with stereo-DOV sampling more individuals and species than stereo-ROV (Schramm et al., 2020a). When attempting to pilot the stereo-ROV at the same altitude as the standardised method for stereo-DOV (~50 cm above substrate) (Goetze et al., 2019), the two methods sampled a statistically similar assemblage and number of species. However, stereo-DOV still sampled more individuals than stereo-ROV (Jessop et al., 2022). This was attributed to the shorter time taken to complete transects with stereo-ROV and difficulty maintaining this altitude with challenges such as ocean currents, swell, and highly complex reef topography (Jessop et al., 2022; Schramm et al., 2020a). Further investigation into the abilities of stereo-DOV and stereo-ROV to sample a variety of population dynamics including length, biomass and behaviour is important to understand the advantages and biases of the two methods.

An understanding of the behaviour of fishes towards ROVs is limited to a few species-specific studies involving industrial sized ROVs (Laidig et al., 2013; Lorange & Trenkel, 2006; McLean et al., 2017; Ryer et al., 2009; Stoner et al., 2008; Trenkel et al., 2004), which emit more light and sound than micro-ROVs, potentially inflating behavioural effects (Sward et al., 2019). An understanding of these behavioural effects is important when using both stereo-DOV and stereo-ROV across gradients of fishing pressure and different levels of protection from fishing. An increase in fishing pressure can affect fish behaviour with wariness of more highly targeted and larger species increasing in highly fished areas (Alós et al., 2012; Alós et al., 2015; Januchowski-Hartley et al., 2015; Kulbicki, 1998). In some cases, this behavioural effect can change the outcome of a marine reserve assessment when using diver-based sampling techniques (Lindfield et al., 2014). These behavioural effects vary with trophic traits such as foraging mode and individual traits such as movement around a fish's home range, and aggressiveness, as well as the types of fishing taking place (Alós et al., 2012). Species-specific responses to stationary SCUBA divers have also been recorded with

some species showing avoidance of divers with observed fish density declining by up to 77% in the presence of divers (Stanley & Wilson, 1995). Other species, such as *Coris auricularis* show attraction to divers (Watson & Harvey, 2007), possibly due to an association of diver presence with food availability from seabed disturbance (Chapman et al., 1974). The noise emitted by SCUBA divers has been quantified with results suggesting the low frequency sound waves can be detected by fishes up to 200 m away (Radford et al., 2005). Fish behaviour has also been shown to vary across different life stages, with larger individuals showing more wariness than smaller individuals towards divers (Goetze et al., 2017). In areas with high spearfishing pressure, MAD has been shown to be a more sensitive indicator of fishing pressure than biomass, length and abundance (Goetze et al., 2017). Behaviour is also a useful metric in comparing sampling methods as a reduction in behavioural avoidance may allow for a higher proportion of more accurate length measurements, because measurement accuracy increases as range decreases (Harvey et al., 2010). The use of micro-ROV technology for sampling fish may reduce some of the behavioural bias that is exhibited by fish towards divers, because micro-ROVs are small and do not produce bubbles (Stamoulis et al., 2020).

This study assessed differences in length data and the behavioural interactions of fish with SCUBA divers and micro-ROVs. Specifically, we aimed to compare and contrast (1) the number of length measurements that could be made from footage collected using stereo-DOV and stereo-ROV, (2) the length frequency distribution of the fish measurements gathered by each method, (3) the wariness of fish (MAD) to each method, and (4) the relationship between MAD and fish length with each method.

3.3 Materials & Methods

3.3.1 Study area

Sampling was conducted in the Jurien Bay Marine Park (JBMP) during April and May of 2021. The JBMP covers over 82,000 hectares of the nearshore ocean, adjacent to the central west coast of Western Australia (Figure 3.1). All sampling took place in the lagoon area at depths ranging between four and ten meters. The area lies within a limestone reef system in part of the Great Southern Reef (Bennett et al., 2015). It hosts a unique assemblage of tropical and temperate marine species (Department of Conservation and Land Management, 2005). The marine flora in the JBMP is historically dominated by *Ecklonia radiata* and a diverse assemblage of foliose algae in reef areas intertwined with seagrass meadows (Wernberg et al., 2006). The JBMP also hosts a community of coral species supported by warm water flowing poleward with the Leeuwn Current, coral cover has been estimated at up to 30% benthic cover in some areas (Ross et al., 2021). The fish assemblage in this reef system is also unique and this study investigates the behaviour of the abundant groups in this system including the large schooling *kyphosids*, carnivorous *labrids*, and large territorial *pomacentrids*. Zoning in the JBMP includes general use; which allows both recreational and commercial fishing, sanctuary zones (fully protected areas); where all extractive activities are prohibited, special purpose (scientific reference and aquaculture zones); that allow

commercial and recreational fishing for rock lobster only, as well as shore-based fishing (Figure 3.1).

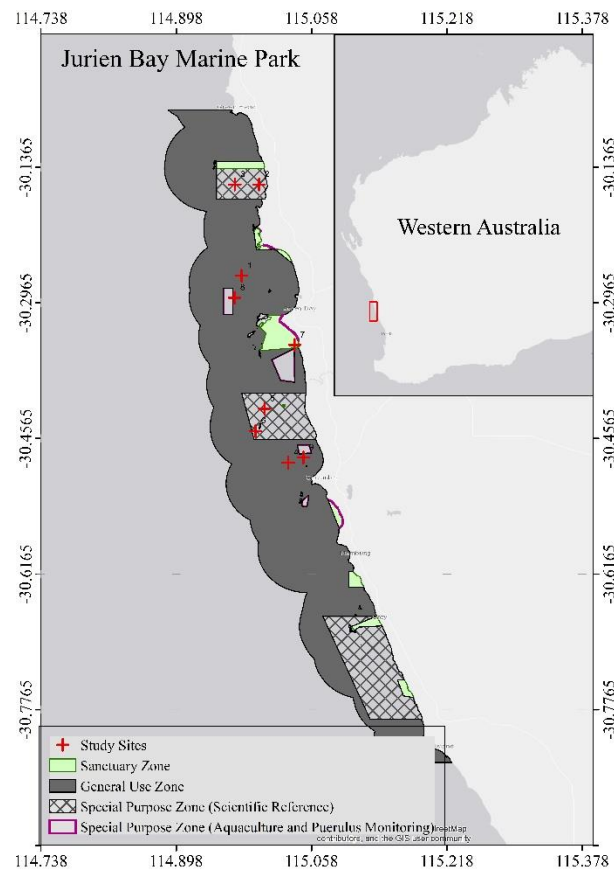


Figure 3.1 Map of survey location, highlighting sample sites along the central west coast of Western Australia in the Jurien Bay Marine Park.

3.3.2 Experimental Design

A comparison of stereo-DOV and stereo-ROV was completed at nine sites (Figure 3.1). At each site, six 50 m belt transects were completed for each method. All transects were five meters wide and five meters high and had a separation of ≥ 10 m. Zoning was not considered as a factor in the analysis as previous studies detected no difference in finfish assemblage across zones in the JBMP (Jessop et al., 2022) and we were unable to sample across the zones with sufficient balance and power due to time and budget constraints.

3.3.3 Stereo-video systems

The stereo-video systems consisted of two cameras, mounted on a base bar at a slight inward converging angle, as described by Harvey and Shortis (1995, 1998). The separation of the cameras was 700 mm with an inward converging angle of 8° for the stereo-DOV and 595 mm with an inward converging angle of 5° for the stereo-ROV.

3.3.3.1 Diver operated stereo-video (stereo-DOV)

Stereo-DOV surveys were completed with two SCUBA divers following the method outlined by Goetze et al. (2019). On average, transects were completed in 1 minute and 44 seconds \pm 0.041 (1SE). The cameras used on the stereo-DOV system were Sony FDR X3000 action cameras recording at 1080p, 60 fps, and a medium field of view to reduce motion parallax associated with the stereo video system and fish moving simultaneously.

3.3.3.2 Stereo-video remotely operated vehicle (stereo-ROV)

The stereo-ROV used was a Blue Robotics BlueROV 2 (457 \times 330 \times 254 mm) that was fitted with a stereo-video system with two Sony RX0 II video cameras recording at 1080p and 50 fps. The stereo-ROV unit was operated approximately 50 m ahead of a surface vessel and attached by an umbilical control cord. The vessel maintained this distance while the stereo-ROV moved along the transect. Its location was tracked using a Seatrac X150 USBL Beacon at the boat and X010 Modem Beacon attached to the stereo-ROV. The USBL system had a range resolution of \pm 0.1 m and angular resolution of approximately two percent of the acoustic range (approximately one meter at the range used in this study). The USBL facilitated navigation, but also allowed us to calculate transect length. Where possible, the stereo-ROV was flown approximately 50 cm above the benthos to match the stereo-DOV procedure (Goetze et al., 2019). This was not always possible in complex reef where sudden changes in topography occurred. The footage from each site was separated into transects from the tracking data from the USBL, on average each transect took 1 minute and 26 seconds \pm 0.041 (1SE).

3.3.4 Calibration and image analysis

Calibration of each stereo-video system was completed before and after the sampling. The software 'CAL' (<https://www.seagis.com.au/bundle.html>) was used, following the methods outlined by Harvey and Shortis (1998). Video analysis was completed using the software 'EventMeasure Stereo' (<https://www.seagis.com.au/event.html>). For the transect methods, all fish within transects were identified to the lowest taxonomic level (usually species) and fork length (from the tip of the snout to the fork of the caudal fin) was measured. Where a length measurement was not able to be recorded due to part of the fish being obscured in the field of view, a 3D point was used to record abundance and MAD data. Where a species level identification was not possible, individuals were identified to genus. For MAD analysis, all fish within transects were measured when they were closest to the camera system, following the protocol outlined by Goetze et al. (2019). All observations were limited to a range of 7 m away from the cameras to maintain accuracy and precision (Harvey et al., 2010). Any measurements with a RMS greater than 20 mm or precision greater than 10% of the fork length were rejected as per guidelines (Harvey et al., 2010).

3.3.5 Statistical analysis

Statistical analysis took place using the programming language R in R studio (Version: RStudio 2022.07.1+554 "Spotted Wakerobin" Release, R Core Team, 2022). Analysis was done at an assemblage level and on focal species. These focal species were defined by species which had a sample size of greater than 25 for both stereo-DOV and stereo-ROV to ensure confidence in the regression model fit (Jenkins & Quintana-Ascencio, 2020). For the MAD and length frequency analysis, stereo-DOV and stereo-ROV models were computed as separate models but displayed on the same axis to allow for comparisons.

3.3.5.1 Length frequency analysis

The ability of stereo-DOV and stereo-ROV to measure fish was compared using a two-sample t-test assuming equal variance (R Core Team, 2022) on the percentage of observations where a length measurement was made. Homogeneity of variance was tested using a Levene's test. A permutational analysis of variance (PERMANOVA) was used to test for differences in the mean length between the methods using Primer 7 with the PERMANOVA + add on (Anderson et al., 2008). This test was used because the length data violated the assumption of normality.

Kernel density estimates (KDE) were constructed for the whole assemblage following the methods published by Langlois et al. (2012) using the packages KernSmooth (Wand, 2021) and sm (Bowman & Azzalini, 2021). Tests for both shape and location of the distributions were done by comparing the area between the KDEs of the two methods to the area resulting from permutations of the data into random pairs. This was represented graphically with a grey band centred on the mean KDE ± 1 SE and based on a null model of no difference (Langlois et al., 2012). Assemblage level comparison of length frequency distribution was justified because the species composition of the assemblage sampled by stereo-DOV and stereo-ROV was not significantly different (Jessop et al., 2022). Schools of fish that were having a disproportionate effect on the data were identified by investigating areas where the distributions of each method were outside the null model of no difference. Large schools of *Neatypus obliquus* and *Kyphosus sydneyanus* that were encountered each at one site with stereo-DOV and not with the stereo-ROV were removed from subsequent analysis due to their disproportionate effect on the data. Separate KDE plots were constructed both with and without these schools of fish to demonstrate the effect of their removal.

3.3.5.2 Minimum approach distance

To assess the relationship between MAD and fork length, linear models were created using R base statistics (R Core Team, 2022) and plotted using ggplot2 (Wickham, 2016). This was done at an assemblage level (with *N. obliquus* and *K. sydneyanus* removed) and for focal species. Fish less than 5 cm were removed from the analysis as they could not be measured with a high degree of accuracy across the full range (distance from camera out to 7 m) (Goetze et al., 2017; Harvey et al., 2010). Regression lines were plotted with confidence intervals and adjusted R^2 values were calculated. An ANOVA design consisting of one factor

with two levels (method, stereo-DOV and stereo-ROV) and length as a covariate with MAD as the response variable was used to test the relationship between MAD and method and MAD and fish length. Pirate plots were created in the package ggplot2 (Wickham, 2016) to visualise the relationship between MAD and method at an assemblage and species-specific level.

3.4 Results

A total of 8,399 observations of fish were made across the two methods. Of these observations 4,933 were made by stereo-DOV and 3,466 were made by stereo-ROV. A total of 1,536 length measurements and 3,397 3D points were recorded by stereo-DOV and a total of 2,091 length measurements and 1,375 3D points were recorded by stereo-ROV. *Coris auricularis* was the most abundant species with 2,229 observations by stereo-DOV and 1,799 observations by stereo-ROV. Six species had greater than 25 observations by both methods and were used as focal species.

3.4.1 Length frequency analysis

At the assemblage level there was no significant difference in the proportion of length measurements to total observations collected by stereo-DOV and stereo-ROV ($t = -1.61$, $df = 16$, $p = 0.127$). The mean proportion of length measurements for stereo-DOV and stereo-ROV was $27.0\% \pm 4.23$ and $36.1\% \pm 3.77$ (1SE) respectively. There was also no significant difference in the mean length of fish observed by the two methods (Method $(1, 2623)$, Pseudo-F = 0.659, $P = 0.428$) with stereo-DOV recording a mean length of $113 \pm 2.40\text{mm}$ (1SE) and stereo-ROV $128 \pm 2.20\text{mm}$ (1SE).

The length frequency distributions observed by the two methods were both positively skewed with similar modes. The shape differed as stereo-DOV had a bimodal distribution and stereo-ROV had a higher probability of observing individuals at the lower mode than stereo-DOV (Figure 3.2). The areas where the two methods significantly differed were illustrated by the areas where the distributions fell outside of the null model of no difference represented by the grey shaded area (Figure 3.2). The second mode in the stereo-DOV data consisted largely of observations of a school of *Neatypus obliquus* at one site. Stereo-DOV also observed a group of fishes with a fork length between 600 mm and 800 mm that was absent in stereo-ROV observations. These individuals were all made up of a school of *Kyphosus sydneyanus* which was observed at one site. The statistical test for location of the two distributions also showed a significant difference in the length frequency distribution observed by both stereo-DOV and stereo-ROV (Figure 3.2). Removal of the schools of both *K. sydneyanus* and *N. obliquus* increased the similarity of the shape and location of the length frequency distribution observed by the two methods (Figure 3.2). The higher probability of stereo-ROV observing individuals at the lower mode and the bimodal distribution of stereo-DOV remained but was less evident. The shape and location of the distributions remained statistically different between the two methods (Figure 3.2).

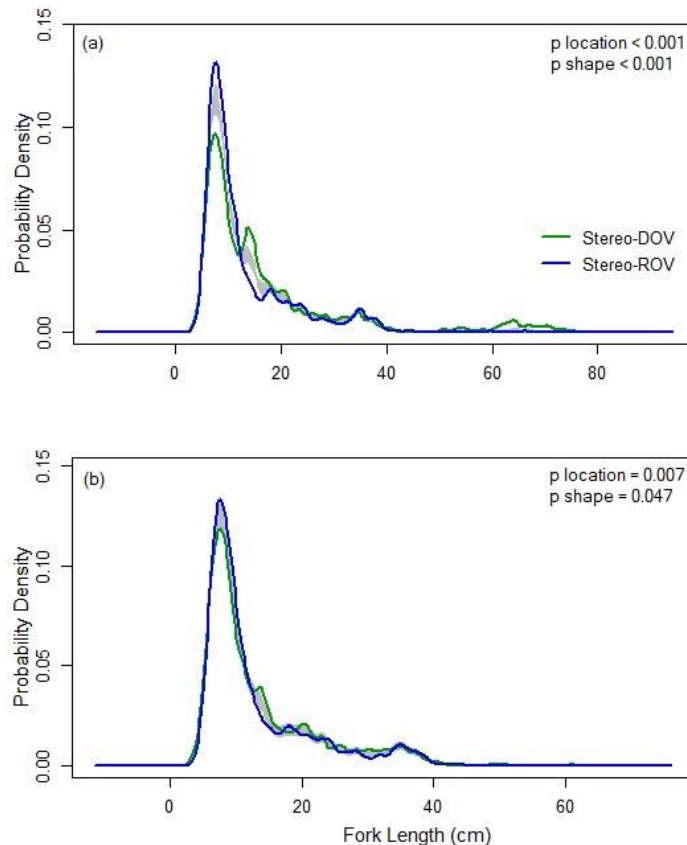


Figure 3.2 Comparison of kernel density estimate probability functions for the (a) assemblage of fishes and (b) the assemblage of fishes minus schools of *Kyphosus cornelii* and *Neatypus obliquus* sampled by stereo-DOV and stereo-ROV. Grey band shows the null model of no difference between the two functions plus or minus one standard error. P values are shown for the significance tests assessing similarity between the shape and location of models for each method.

3.4.2 Minimum approach distance

Linear regression plots were used to test if length was a predictor of MAD for the whole assemblage and chosen focal species for both stereo-DOV and stereo-ROV. At the assemblage level, both methods had a positive linear relationship with MAD increasing as fish length increased. This relationship was stronger with stereo-DOV, which had a higher adjusted R^2 value and steeper gradient than stereo-ROV (Figure 3.3). The analysis of variance showed there was a significant interaction between length and method and significant main effects of both length and method on MAD (Table 3.1). Stereo-DOV sampled individuals at a closer distance (mean MAD = $1,980 \pm 18.5$ mm (1SE)) than stereo-ROV (mean MAD = $2,190 \pm 18.9$ mm (1SE)) and MAD increased with fork length for both methods.

3.4.2.1 Focal species

Minimum approach distance was highly variable across the different focal species with all species except *Kyphosus cornelii* showing an increase in MAD with an increase in fork

length (Table 3.1). Stereo-ROV sampled *K. cornelii* and *Choerodon rubescens* at a significantly lower MAD than stereo-DOV. Stereo-DOV sampled all other focal species (*Coris auricularis*, *Notolabrus parilus*, *Parma mccullochi* and *Pomacentrus milleri*) at a significantly lower MAD than stereo-ROV (Figure 3.3, Table 3.1). *Coris auricularis* and *C. rubescens* had smaller adjusted R² values and shallower slopes of the regression lines fitted to fork length versus MAD for stereo-ROV compared to stereo-DOV (Figure 3.3). *Kyphosus cornelii* and the two *Pomacentridae* species (*P. mccullochi* and *P. milleri*) all had low adjusted R² values fitted to the regression line of fork length versus MAD (≤ 0.082). *Notolabrus parilus* had similar slopes and adjusted R² values between the two methods (Figure 3.3) and no significant interaction between length and method on the MAD (Table 3.1).

Table 3.1 Analysis of co-variance assessing the effect of fish length and method on the minimum approach distance of fish. Values in bold show statistical significance at $\alpha = 0.05$.

Assemblage						<i>Choerodon rubescens</i>					
	df	Sum Sq	Mean Sq	F value	p		df	Sum Sq	Mean Sq	F value	p
Length	1	157.08	157.08	405.41	< 0.001	Length	1	7.05	7.05	20.13	< 0.001
Method	1	34.65	34.65	89.43	< 0.001	Method	1	4.02	4.02	11.47	0.001
Length × Method	1	14.64	14.64	37.78	< 0.001	Length × Method	1	1.14	1.14	3.26	0.076
Residuals	2584	1001.20	0.39			Residuals	55	19.27	0.35		
<i>Coris auricularis</i>						<i>Kyphosus cornelii</i>					
	df	Sum Sq	Mean Sq	F value	p		df	Sum Sq	Mean Sq	F value	p
Length	1	77.19	77.19	240.48	< 0.001	Length	1	0.17	0.17	0.25	0.619
Method	1	30.02	30.02	93.52	< 0.001	Method	1	3.17	3.17	4.69	0.032
Length × Method	1	1.78	1.78	5.53	0.019	Length × Method	1	0.25	0.25	0.37	0.545
Residuals	1610	516.82	0.32			Residuals	143	96.50	0.67		
<i>Notolabrus parilus</i>						<i>Parma mccullochi</i>					
	df	Sum Sq	Mean Sq	F value	p		df	Sum Sq	Mean Sq	F value	p
Length	1	4.38	4.38	16.11	< 0.001	Length	1	6.47	6.47	7.88	0.006
Method	1	1.15	1.15	4.24	0.042	Method	1	3.33	3.33	4.06	0.047
Length × Method	1	0.01	0.01	0.04	0.850	Length × Method	1	1.21	1.21	1.48	0.227
Residuals	95	25.82	0.27			Residuals	87	71.41	0.82		
<i>Pomacentrus milleri</i>											
	df	Sum Sq	Mean Sq	F value	p		df	Sum Sq	Mean Sq	F value	p
Length	1	3.61	3.61	12.04	0.001						
Method	1	12.87	12.87	42.92	< 0.001						
Length × Method	1	0.21	0.21	0.71	0.401						
Residuals	224	67.19	0.30								

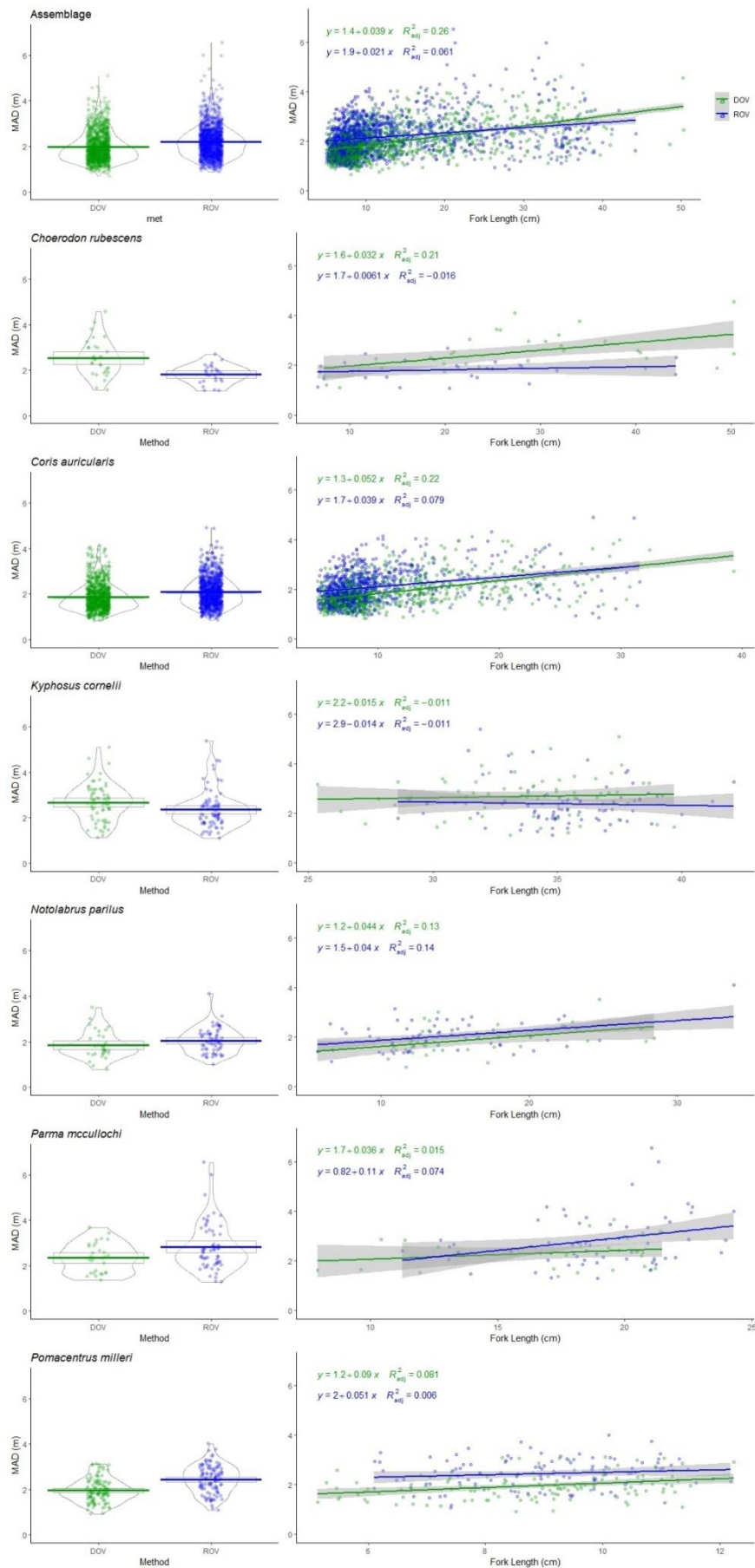


Figure 3.3 Assemblage level and species-specific pirate plots (column 1) and linear regression plots (column 2) illustrating the effect of method on MAD. Pirate plots show data as points, a horizontal-coloured line indicating mean, boxes indicating 95% confidence intervals, and violins indicating density distributions. Linear regression plots show data as points with a fitted linear regression line and 95% confidence interval.

3.5 Discussion

In our comparison of stereo-DOV and stereo-ROV, we found that they provided broadly similar results, although did differ at times at the individual and species level. We found that at the assemblage level, larger fish were less wary of the stereo-ROV than the stereo-DOV. Previous studies have observed a consistent relationship with larger individuals being more wary of divers than smaller individuals (Goetze et al., 2017; Januchowski-Hartley et al., 2014; Januchowski-Hartley et al., 2015). The reduced wariness of fish towards the stereo-ROV may increase the measurement accuracy for larger fish when compared to stereo-DOV. This is because measurement accuracy decreases with increasing distance (Harvey et al., 2010). The length frequency distribution of fishes measured by stereo-DOV and stereo-ROV were broadly comparable, and the number of successful length measurements of fish and the mean lengths were also similar between methods. While we observed differences in the length frequency distribution sampled by the two methods, they were primarily driven by chance encounters with large schools and were negligible once these schools were removed from the analysis. Overall, the number of length measurements was low due to low visibility in the water at the time of the survey and kelp obscuring fish in the field of view of the cameras. Jessop et al. (2022) found that stereo-DOV and stereo-ROV were also comparable in the assemblage composition and number of species sampled with some differences in the number of individuals sampled. The data presented in this study provides further evidence to support the use of stereo-ROV for reef fish sampling in monitoring programs where length data is required and indicates that stereo-ROVs may outperform stereo-DOVs where measuring large fish and targeted species is important.

The technological differences between stereo-DOV and stereo-ROV including emission of artificial noise by the thrusters or electronics pod on the ROV, and bubbles emitted by SCUBA divers provide a possible explanation for the observed differences in MAD. We found species specific patterns of fish behaviour towards divers and the stereo-ROV with species such as the Western King Wrasse (*Coris auricularis*) and the Brownspotted Wrasse (*Notolabrus parilus*) being attracted towards divers. The attraction towards divers may be due to the presence of bubbles, noise, or the association of divers with food available from disturbing the seabed (Chapman et al., 1974). These two labrid species are carnivorous and feed on benthic inverts (Vanderkluft et al., 2007) which supports the hypothesis that they are attracted to divers due to food availability associated with divers disturbing the seabed. This pattern is likely to vary across locations which have been subject to different human impacts over time. Fish may be attracted to or avoid the bubbles and noise of SCUBA divers (Gray et al., 2016; Lindfield et al., 2014; Watson & Harvey, 2007), and noise and light associated with ROVs (Logan et al., 2017; Ryer et al., 2009; Stoner et al., 2008). The noise levels have been quantified for SCUBA divers (Radford et al., 2005), however the noise range of micro-ROVs has not been measured.

Other species-specific behavioural response were observed, which could be related to the trophic traits and foraging mode of the species, or levels of fishing pressure (Alós et al., 2012; Alós et al., 2015; Januchowski-Hartley et al., 2014). For example, Baldchin Groper (*Choerodon rubescens*) is a highly targeted species in the JBMP (Ryan et al., 2019) and an indicator species for the region, which showed less wariness towards the stereo-ROV compared to divers, as well as a weaker relationship between length and MAD observed by

stereo-ROV. This indicates that larger Baldchin Groper were less wary of the stereo-ROV which could improve the measurement accuracy of these fish. Conversely, the Western Buffalo Bream (*Kyphosus cornelii*) which is not targeted by fishers, and had very weak, shallow relationship between fork length and MAD for both stereo-ROV and stereo-DOV. It is possible that the differences in Baldchin Groper behaviour between the methods may be due to fishing pressure which has been shown to influence targeted fish behaviour (Bergseth et al., 2016; Goetze et al., 2017; Januchowski-Hartley et al., 2014). The JBMP has relatively low levels of spearfishing pressure compared to line fishing (Department of Conservation and Land Management, 2005) however, anecdotally spearfishing of species including Baldchin Grouper does occur (Harvey, pers. ob.). The influence of zoning to protect areas from fishing was not accounted for in this study due to insufficient replication inside and outside of protected areas, however this should be further investigated to test the hypothesis that spearfishing pressure is influencing fish behaviour towards SCUBA divers and the ROV differently. In areas of higher spearfishing pressure, the behavioural responses of targeted species could be amplified and use of a stereo-ROV could increase the number of larger individuals being observed and improve both the accuracy of length measurements and the proportion of length measurements to 3D point observations. Previous studies found the wariness of targeted fish towards divers increased because of spearfishing fishing pressure (Bergseth et al., 2016; Goetze et al., 2017; Januchowski-Hartley et al., 2014). This behavioural avoidance may result in difficulty measuring targeted species with stereo-DOV in areas of high spearfishing pressure. In contrast, the dampened effect of this fish behaviour with micro-ROV sampling may reduce the ability to use MAD as an indicator for spearfishing pressure when sampling with a stereo-ROV.

We also observed differences in behaviour of fishes that appeared to be due to their foraging mode. Both the McCulloch's Scalyfin (*Parma mccullochi*) and Miller's Damsel fish (*Pomacentrus milleri*) are members of the *Pomacentridae* family and had a smaller MAD with stereo-DOV than stereo-ROV as well as weak relationships between fork length and MAD. Like other territorial damselfish, these species maintain a patch of food algae by weeding undesirable algae species and agonistically excluding other herbivores from their patch (Ceccarelli et al., 2001; Saunders et al., 2015; Shalders et al., 2018). As a result, these species approach and demonstrate aggressive behaviour towards divers as they defend their territory. The larger MAD observed with stereo-ROV may be a result of these species not perceiving the ROV as a threat to their territory, and therefore not exhibiting defensive behaviours. This finding supports other literature that has found behaviour of fishes towards ROVs to be species specific (Laidig et al., 2013; Lorange & Trenkel, 2006) and highlights the importance of understanding the advantages and biases of ecological sampling techniques which may vary by geographical location and ecosystem type.

The ability of a method to gather accurate data on fish length is important because length-based metrics are considered to be a sensitive indicator to changes in fishing pressure (Claudet et al., 2006). Length data also provides insight into size selective fishing pressures (Nash & Graham, 2016), adds context to interpretation of abundance data (Lokrantz et al., 2008), and reflects more complex stressors such as habitat degradation and multiple human pressures (Taylor et al., 2022). Another application of this length data is the use of size-spectrum models which can be used to predict changes in size-spectra through time and to

compare this among areas subject to different levels of stressors such as fishing, climate disturbances, and pollution. This may assist in understanding how fishing pressure is affecting ecosystem function in conjunction with various co-occurring environmental changes (Blanchard et al., 2017). The use of stereo-ROV to gather accurate length data with a standardised methodology may allow this modelling to occur at a greater spatial scale. The proportion of length measurements gathered in this study was not significantly different between stereo-DOV and stereo-ROV at 27% and 36% respectively, but it was lower than in other studies. For example, Wilson et al. (2018) gathered ~40% with stereo-DOV and Helmrich et al., (in prep) measured between ~50% and ~80% of fish counted with stereo-DOV and stereo-ROV. This was likely due to the complex canopy of kelp present in the habitat in the JBMP which sometimes obscured fish in the cameras field of view, as well as low visibility presenting a challenge in obtaining successful length measurements. This highlights the need to consider the advantages and biases of methods that may differ by geographical location and ecosystem type, and the importance of monitoring programs to be flexible around environmental conditions such as visibility, swell, and wind (Jessop et al., 2022).

Overall, the similarities in sampling abilities of stereo-ROV to the widely used and accepted stereo-DOV (Goetze et al., 2015; Holmes et al., 2013; Watson et al., 2010), indicates that stereo-ROV is a viable alternative to stereo-DOV for sampling shallow water reef fishes. This study investigated these trends in a unique and transitional reef system and investigation should be done across a range of tropical and temperate ecosystems to expand our understanding of the abilities and biases of the two methods. Also, consideration should be given to the unique characteristics of fishes within a specific ecosystem such as foraging modes and potential attraction or repulsion to divers from fishing pressure, feeding of fishes, or a fish's foraging mode when selecting and interpreting data from both stereo-ROV and stereo-DOV. Due to the logistical, safety and financial considerations of stereo-ROV over stereo-DOV and the similarities in assemblage, number of species (Jessop et al., 2022) and length frequency sampled by each method, we recommend the use of stereo-ROVs as a transect sampling method for monitoring fish assemblages. The potential dampened behavioural responses of targeted fish towards the micro ROV compared to SCUBA divers should be further investigated, however the initial findings in this paper suggest an increased ability to accurately measure and identify these fish. This presents further support for the adoption of the stereo-ROV sampling method.

4. CHAPTER 4: GENERAL DISCUSSION



Photograph: Redband wrasse (*Pseudolabrus biserialis*) and a variety of other temperate reef fish around a stereo-BRUV in the Jurien Bay Marine Park

4.1 Summary of findings

The aim of my research was to investigate the optimal techniques for sampling shallow water reef fishes in a temperate MPA. I focused on the assemblage composition sampled, the number of species and individuals sampled and the power of each method to detect hypothetical change. I then narrowed the focus to two transect based stereo-video methods (stereo-DOV and stereo-ROV) and investigated the ability of these methods to measure the length of fish, compared the length frequency distribution of the fish measured, and the behavioural response of fish towards the ROV and the SCUBA divers.

All ecological sampling methods have inherent biases, advantages and disadvantages. In this thesis, I aimed to highlight the importance of understanding the biases associated with different techniques when choosing methods for sampling and monitoring fish populations inside and outside shallow water MPAs. These biases may vary depending on geographical location, ecosystem type and consideration of financial, logistical and safety constraints.

In **chapter two**, I investigated the differences and similarities in the assemblage composition and the number of individuals and species sampled by four methods. I also assessed the statistical power of these methods to detect hypothetical changes in the number of individuals and species in a population. I used three widely accepted methods (stereo-BRUVs, stereo-DOVs and UVC) and one emerging method (stereo-ROV). The data showed that stereo-BRUVs sampled a distinctive assemblage that consisted of more mobile and predatory species compared to the transect methods. The sampling of these species is important due to their value to recreational and commercial fishers and being target species, they are important indicators of the fish assemblage. The assemblage sampled by the transect methods, consisted of higher numbers of site attached species. These species are also important to capture in monitoring programs as they can be used as indicators of other pressures such as regime shifts resulting from climate change. Furthermore, I found that UVC sampled a higher number of species and a distinctive assemblages compared to stereo-ROV. This assemblage consisted of more small bodied and cryptic species and this finding suggests that UVC may be a better sampling tool when species diversity assessment is the priority. A key finding was the similarity in the assemblage composition and number of species sampled by stereo-DOV and stereo-ROV. This finding differed from other comparisons including that of Schramm et al. (2020a) which was done in similar habitat and geographical area to my study. I hypothesised that the reason for this difference was the altitude of operation of the ROV, with the Jurien Bay study placing emphasis on operating the ROV at the same altitude as the stereo-DOV. The recommendations from this study were that a combination stereo-BRUVs and a transect are the optimal methods for sampling shallow water reef fishes, and that stereo-ROV performs similarly to stereo-DOV with added safety and logistical advantages.

In **chapter three**, I took a deeper look at the similarities and differences of the sampling ability of stereo-DOV and stereo-ROV focussing on length-based indicators and fish behaviour towards the two methods. The two methods did not differ in their ability to gather length measurements. After inspection of the data and removal of schools of fish that were disproportionately affecting patterns in the data, the length frequency distributions were broadly comparable. Fish behaviour was investigated at an assemblage level and on focal

species using minimum approach distance (MAD) as a proxy for behaviour. The data indicated that larger fish were less wary of the ROV compared to SCUBA divers. The focal species exhibited different patterns that may have been due to factors such as levels of fishing pressure and life history traits such as feeding regimes. Baldchin Grouper is a targeted species and had less of a behavioural response to stereo-ROV compared to stereo-DOV and allowed the ROV to approach closer than SCUBA divers on average. The regression analysis for this species indicated that larger individuals were less wary of the ROV than divers. Conversely, the Western Buffalo Bream had only weak relationships between fork length and MAD for both methods, possibly because it is not a targeted species and has not developed a behavioural response to SCUBA divers. The two focal damselfish species appeared to show defensive behaviour towards divers and not the ROV which may have been a result of their feeding regime which involves being protective of a patch of territory. This was reflected in the data with a smaller average MAD for this species with stereo-DOV compared to stereo-ROV. These behavioural responses may have been associated with differing amplitudes and frequencies of sound produced from the two methods and quantification of the sound emitted by the micro ROV would be useful to compare to the sound emitted by SCUBA divers which has previously been quantified. From this chapter, I concluded that stereo-ROV may outperform stereo-DOV at obtaining accurate length measurements of large fish and targeted species and recommended this pattern be further investigated across a range of different spearfishing levels.

Below, I critically analyse the limitations of my research and summarise my recommendations for future research in this area (Figure 4.1).

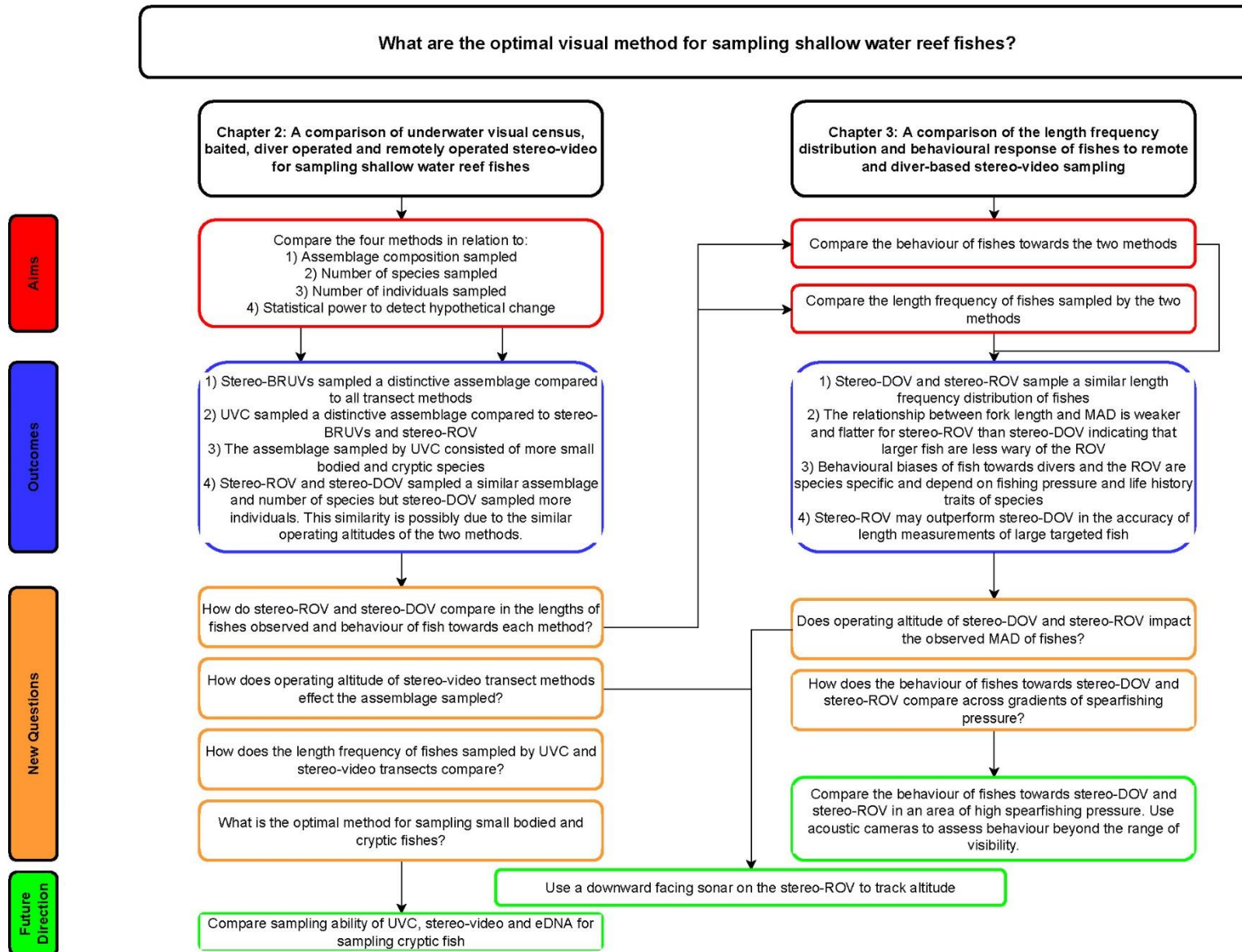


Figure 4.1 Flow diagram summarising the aims, outcomes, new questions and future direction identified from the two data chapters

4.2 Limitations of research

Micro stereo-ROV is an emerging technology which is currently undergoing testing, development and validation for sampling fish assemblages. The technology has the potential to overcome some behavioural responses of fish towards divers and to reduce the safety and logistical constraints associated with diver-based sampling methods. My research contributes to the growing body of literature which focuses on the testing and validation of this method in comparison to other sampling methods. This presented some challenges and limitations that raise further questions and highlight possible future research directions.

The main limitation encountered in chapter two was the inability to compare all four methods directly due to poor weather and water visibility during the time period where the trained UVC divers were available. This limitation was somewhat overcome in the statistical analysis by separating the experimental design and comparing stereo-ROV to stereo-BRUVs and UVC then comparing stereo-ROV and stereo-DOV. This limitation reflected the need of UVC for scientific divers with a high degree of taxonomic expertise which is not needed for the field component of the stereo-video methods.

The sampling of cryptic species was limited in all methods with UVC sampling the most of these species. Inter-observer bias may impact the ability to complete robust time series comparisons on the abundance and diversity of cryptic species. This highlights the use of eDNA to sample cryptic fish species as a future research direction. The inter-observer bias evident with UVC may also impact the length measurements in the data, which also should be a focus of future research.

The similarity observed between stereo-DOV and stereo-ROV in chapter two was hypothesised to be due to maintaining the same operating altitude with the two methods, however this was not measured which limited our ability to provide quantitative evidence supporting the importance of operation altitude. This also presented a possible confounding influence on MAD observed in chapter 3. A degree of the observed differences in MAD between the two methods could have been due to difficulty in maintaining the desired altitude with the ROV in complex reef topography. A downward facing sonar on the ROV should be used to assess the affect of altitude in future research.

The unknown levels of sound emitted by each method was also a limitation. Frequency and amplitude of sound emitted by SCUBA divers has been quantified however the sound produced by the micro-ROV has not been measured to my knowledge. Quantification of sound produced by the micro-ROV in future research would increase our understanding of behavioural responses of fishes.

The spatial replication was limited as sampling was only completed in the JBMP which is subject to relatively low levels of spearfishing pressure, with line fishing being the dominant documented fishing method. This limited my ability to understand how the biases of each method may vary across a spatial scale with different levels of fishing pressure as well as varying complexity and types of ecosystems. Testing of behavioural responses of fishes across a gradient of spearfishing pressure should be done in future research to understand how behaviour towards the ROV varies with fishing pressure. The use of multibeam sonar

acoustic cameras would allow testing of behavioural responses of fishes beyond the range of visibility that is limited when using video cameras.

We were limited by time and budget constraints of a Masters by Research thesis. Emphasis was placed on investigating the methods with consideration for fishing pressure within the JBMP. It is important to note that there are other factors that were not considered that were likely influencing fish assemblages within and between sites. These factors include but are not limited to environmental influences such as temperature and nutrient availability which could impact fish recruitment and therefore length frequencies. It is important that these factors are considered in experimental design when sampling highly heterogeneous environments such as shallow water reefs in the future.

4.3 Future directions

4.3.1 eDNA for sampling cryptic species

UVC has been shown to sample more small bodied and cryptic species and higher species richness than stereo-video methods which are limited by frame rate and camera resolution (Holmes et al., 2013). Despite this, the inability of visual surveys to robustly sample cryptic species had been widely documented (Ackerman & Bellwood, 2000; Robertson & Smith-Vaniz, 2008; Stat et al., 2019). The use of eDNA may present a non-destructive sampling approach to overcome this. eDNA has been used and tested as a method for gathering data on fish population dynamics (Jerde, 2021; Lacoursière-Roussel et al., 2016; Valdivia-Carrillo et al., 2021; Wang et al., 2021). Applications where eDNA may overcome biases of visual sampling include the sampling cryptic species (Westhoff et al., 2022), deep sea fishes (McClenaghan et al., 2020), nocturnal species (Stat et al., 2019) and pelagic fishes (Valdivia-Carrillo et al., 2021). Comparisons of eDNA with UVC have found that eDNA sampled greater species diversity than UVC and that eDNA sampling complements visual sampling (Valdivia-Carrillo et al., 2021). Comparisons with eDNA and stereo-BRUVs have suggested a combined approach to sampling and indicated the potential of eDNA to differentiate between assemblages across habitats with relatively small spatial separation (100s of meters) (Stat et al., 2019). Comparisons with eDNA and stereo-ROVs on offshore oil and gas platforms found that eDNA sampled a different assemblage largely comprised of small or rare fishes not observed by the ROV (Alexander et al., 2022). Future research should focus on the possibility of combining eDNA and stereo-ROV technologies in natural habitats across temperate and tropical ecosystems. This may improve the ability to conduct robust time series comparisons of cryptic species and increase our understanding of the response of these fishes to environmental stressors induced from climate change. However, for eDNA to be useful we will need comprehensive genetic sequences for all species of fish to be incorporated into reference databases.

4.3.2 Comparison of length measurements

Concern for the accuracy and precision of the length estimates gathered by UVC has been expressed in the literature (Harvey et al., 2001a; Harvey et al., 2001b, 2002a; Harvey &

Shortis, 1995). Comparisons between trained and novice divers with stereo-video systems (Harvey et al., 2001b) and comparison of stereo-video and diver measurements of real fish (Harvey et al., 2002a) have supported the accuracy and precision of stereo-video measurements and highlighted the large variance in measurements obtained by SCUBA divers. The data collected during my thesis could be used to compare the length estimates gathered by stereo-DOV to those gathered by UVC at a species-specific level. Kernel Density Estimates would be a useful statistical approach for this. This comparison would provide evidence for the accuracy and precision of length measurements under real operating conditions where swell, currents and visibility all present challenges. Future research could include a direct comparison of stereo-video length estimates with those made by a diver doing UVC. This should be done simultaneously with the SCUBA diver using a full-face mask and recording verbal length estimates on the video soundtrack to enable a direct comparison (Harvey et al., 2002a).

4.3.3 Sonar technology to track altitude

The similarities observed in the sampling ability of stereo-DOV and stereo-ROV in chapter two differed from previous comparisons of these two methods (Schramm et al., 2020a). The hypothesised reason for this was the emphasis we placed on maintaining the same operating altitude for the two methods whereas a difference in altitude was hypothesised to be a cause for differences observed by Schramm et al. (2020a). Slight differences in the operating altitude between the methods could also present a confounding influence on the MAD data collected in chapter 3. The optimal altitude is more difficult to maintain in areas of complex topography with a ROV compared to when diving. Blue Robotics (manufacturer of the ROV used throughout this thesis) manufacture a sonar unit called 'Ping Sonar Altimeter and Echosounder' (<https://bluerobotics.com/store/sensors-sonars-cameras/sonar/ping-sonar-r2-rp/>) which can be mounted on the BlueROV. This unit can be mounted facing downward allowing it to sense the seabed and provide a measurement of operating altitude. This would allow for testing of the effect of altitude on the assemblage, number of species and individuals, and MAD measured by stereo-ROV, and hence validation of the hypothesised effects of altitude. The use of this technology to provide a live feed of altitude to the ROV pilot would also assist in the standardisation of this method and allow cross system comparisons that have previously been limited by differences in altitude (Sward et al., 2019). Another benefit to utilising this technology is the ability to gain a proxy measurement of substrate rugosity by analysing the sonar imaging across space with an acoustic positioning system to measure distance.

4.3.4 Fish behaviour across a gradient of spearfishing pressure

Consistent linear relationships between fork length and MAD have been observed for several species when using stereo-DOV to sample fishes on Pacific Islands subject to pulse harvesting events by spearfishing (Goetze et al., 2017). Goetze et al. (2017) suggested that MAD may be useful as an indicator that is sensitive to detecting changes in fishing pressure in areas with high spearfishing pressure. This highlights the importance of understanding behavioural responses of fishes towards sampling methods. The use of micro stereo-ROVs

which are smaller in size may reduce behavioural responses of fishes (Stamoulis et al., 2020). My research assessed the behavioural biases of six focal species towards SCUBA divers and the ROV with patterns emerging that suggested behavioural responses of targeted species may be dampened when using an ROV compared to divers. This reduced bias may be of benefit when using stereo-ROV because measurement accuracy increases with decreased MAD (Harvey et al., 2010) and it may increase the sampling of large and targeted fishes in areas of high spearfishing pressure. Further testing of the fish behaviour towards the ROV across a gradient of spearfishing pressures should be the focus of future research aimed at the testing and validation of this emerging technology. Pacific Island nations which implement periodically harvested closures subject to pulse spearfishing harvests aimed at reducing fish wariness to increase fishing efficiency (Cinner et al., 2005) would be an optimal location for investigating these trends.

4.3.5 Furthering our understanding of fish behaviour towards ROVs

Stereo-video technology is limited to observing fish within the bounds of water visibility. This limits our understanding of fish behavioural responses to within this range. Fish are able to detect sound emitted by SCUBA divers up to 200 m away (Radford et al., 2005) which indicates that behavioural responses beyond the range of visibility are likely. Measurement of sound emitted by micro ROVs should be undertaken to quantify the range where fish are likely to detect the sound waves. Further investigation of behaviour beyond the visibility range could be done with an ROV using a combination of stereo-video and multibeam sonar. Blueprint Subsea developed a product called the ‘Oculus’ (<https://www.blueprintsubsea.com/oculus/>) that is designed to be mounted on an ROV and can gather imagery out to 40 m. This combination of technology would be a unique and valuable approach to furthering our understanding of fish behavioural responses towards both ROVs and divers.

4.3.6 Standard operating procedure for stereo-ROV

Development and implementation of standard operating procedures (SOP) works to increase the ability to make robust spatial and temporal comparisons and combine data in a standardised way. Other comparisons with data collected by ROVs have been limited by factors such as differences in operating altitude (Sward et al., 2019). A SOP for using ROVs to gather image based benthic data has been developed (Monk et al., 2020) however, investigation of the areas of future research outlined above would increase our understanding of the advantages and biases of stereo-ROV as a tool for sampling fish assemblage composition. With this understanding, a SOP could be developed specifically for using micro stereo-ROVs for sampling fish assemblages in monitoring programs. The development of a SOP would increase standardisation of sampling and make stereo-ROVs a more appealing tool to implement in monitoring programs as it would provide a comprehensive guide to its use.

4.3.7 Consideration of factors driving change

Although this thesis focused on fishing pressure as a main driver of changes to fish assemblages, it is important that experimental design considers how a variety of natural and human pressure can drive change. An example of this is the potential for Baldchin Grouper recruitment events to alter length frequency distribution of this species, which without careful consideration, such changes could be falsely attributed to fishing pressure. Recruitment events could be investigated with stereo-video systems with targeted experimental designs aimed at investigating when and where such events occur. This would require repeated sampling within identified recruitment habitat and habitat where recruits transition. It will also require the development of stereo-video systems capable of targeting smaller fish in shallower habitats. Statistical modelling can also be used to attribute changes to different pressures, such as that done by Taylor et al. (2022). This can be used to better inform management strategies.

4.4 Conclusions

Sampling of fish assemblages in MPAs is important to assess their impact as a conservation strategy and to monitor the responses of fish to key pressures. A progression of technologies has overcome many sampling limitations and provided new indicators for assessing change in fish populations. The ability of certain methods to provide robust data on a range of indicators varies by their inherent capabilities as well as across a range of environments. For example, diver-based methods are limited by depth accessible to SCUBA divers, whereas remote methods such as stereo-BRUVs and stereo-ROV sampling overcomes this depth limitation. Furthermore, diver-based methods may result in a larger behavioural response of fish in areas with high spearfishing pressure and remote methods may dampen this behavioural effect. My research compared the effectiveness of four methods with regards to their ability to collect robust data on a range of indicators. Emphasis was also placed on the logistical considerations and time taken to complete a sampling program with each method because this is an important consideration for managers when designing a monitoring program. I found that a combination of stereo-BRUVs and a transect method sampled both site attached species and mobile, predatory species. The stereo-ROV method may reduce the behavioural responses of some fish and provide improved logistics and fewer safety concerns in comparison to diver-based methods. I therefore recommend a combination of stereo-BRUVs and stereo-ROV to monitor fish populations within and beyond MPAs.

Appendix – A: Supplementary material for Chapter 2

Table A.1. All species sampled by baited underwater stereo-videos (stereo-BRUVs), diver operated stereo-video (stereo-DOV), underwater visual census (UVC), and a remote operated vehicle with a stereo-video attachment (stereo-ROV) in the Jurien Bay Marine Park. Values in cells indicate percentage occurrence of each species in each replicate followed by mean number of individuals of each species observed across all replicates ± 1 .

Family	Species	Stereo-BRUVs	UVC	Stereo-ROV	Stereo-DOV
Apogonidae	<i>Ostorhinchus doederleini</i>		2.27, 0.091 \pm 0.091		
	<i>Ostorhinchus victoriae</i>	24.3, 0.405 \pm 0.157	9.09, 0.568 \pm 0.332	2.70, 0.027 \pm 0.019	11.1, 0.537 \pm 0.267
	<i>Siphamia cephalotes</i>			2.70, 0.054 \pm 0.038	
Aracnidae	<i>Anoplocapros lenticularis</i>			2.70, 0.054 \pm 0.038	
Arripidae	<i>Arripis georgianus</i>	10.8, 0.757 \pm 0.575			
Blenniidae	<i>Aspidontus dussumieri</i>			1.35, 0.014 \pm 0.014	
	<i>Cirripectes hutchinsi</i>		4.55, 0.045 \pm 0.032		
	<i>Plagiotremus rhinorhynchos</i>		4.55, 0.045 \pm 0.032		
Carangidae	<i>Pseudocaranx species</i>	73.0, 6.43 \pm 1.65	4.55, 0.477 \pm 0.455		
	<i>Seriola hippos</i>	27.0, 0.297 \pm 0.085			
Chaetodontidae	<i>Chaetodon assarius</i>	24.3, 0.730 \pm 0.289	27.3, 0.750 \pm 0.287	17.6, 0.284 \pm 0.085	24.07, 1.04 \pm 0.333
	<i>Chelmonops curiosus</i>	21.6, 0.243 \pm 0.081	20.5, 0.273 \pm 0.094	8.11, 0.095 \pm 0.039	9.26, 0.315 \pm 0.181
Cheilodactylidae	<i>Cheilodactylus gibbosus</i>				3.70, 0.037 \pm 0.026
Dasyatiidae	<i>Bathytoshia brevicaudata</i>	10.8, 0.108 \pm 0.052			
Enoplosidae	<i>Enoplosus armatus</i>		9.09, 0.159 \pm 0.079	5.41, 0.203 \pm 0.163	14.8, 0.296 \pm 0.117
Glaucosomatidae	<i>Glaucosoma hebraicum</i>				3.70, 0.037 \pm 0.026
Haemulidae	<i>Plectorhinchus flavomaculatus</i>	45.9, 0.514 \pm 0.100	29.5, 0.568 \pm 0.17	12.2, 0.23 \pm 0.087	20.4, 1.06 \pm 0.486
Kyphosidae	<i>Kyphosus cornelii</i>	48.6, 2.30 \pm 0.628	61.4, 22.9 \pm 9.70	36.5, 8.92 \pm 2.82	33.3, 5.07 \pm 2.73
	<i>Kyphosus sydneyanus</i>	16.2, 0.784 \pm 0.571	20.5, 1.23 \pm 0.546	6.76, 1.01 \pm 0.74	25.9, 3.72 \pm 1.92
	<i>Kyphosus vaigiensis</i>	2.70, 0.027 \pm 0.027			
Labridae	<i>Achoerodus gouldii</i>	2.70, 0.027 \pm 0.027			
	<i>Anampses geographicus</i>	8.11, 0.243 \pm 0.152	13.6, 0.182 \pm 0.081	5.41, 0.095 \pm 0.048	1.85, 0.019 \pm 0.019
	<i>Austrolabrus maculatus</i>	10.8, 0.108 \pm 0.052	27.3, 0.409 \pm 0.119	28.4, 0.635 \pm 0.158	24.1, 0.537 \pm 0.185
	<i>Bodianus frenchii</i>	5.41, 0.081 \pm 0.060	11.4, 0.136 \pm 0.062	2.70, 0.041 \pm 0.03	5.56, 0.074 \pm 0.045
	<i>Chlorurus sordidus</i>		4.55, 0.068 \pm 0.05	2.70, 0.027 \pm 0.019	1.85, 0.019 \pm 0.019
	<i>Choerodon rubescens</i>	67.6, 0.946 \pm 0.160	59.1, 1.36 \pm 0.256	25.7, 0.473 \pm 0.134	42.6, 1.20 \pm 0.3
	<i>Coris auricularis</i>	97.3, 57.9 \pm 6.41	100, 60.3 \pm 6.48	87.8, 30.5 \pm 6.21	79.6, 41.3 \pm 5.95
	<i>Dotalabrus alleni</i>			1.35, 0.014 \pm 0.014	
	<i>Halichoeres brownfieldi</i>	16.2, 0.378 \pm 0.170	13.6, 0.250 \pm 0.142	20.3, 0.649 \pm 0.229	14.8, 0.241 \pm 0.087
	<i>Labroides dimidiatus</i>			1.35, 0.014 \pm 0.014	3.70, 0.037 \pm 0.026
	<i>Leptoscarus vaigiensis</i>		2.27, 0.045 \pm 0.045		
	<i>Notolabrus parilus</i>	97.3, 2.05 \pm 0.208	72.7, 1.75 \pm 0.230	68.9, 2.30 \pm 0.389	63.0, 2.20 \pm 0.381
	<i>Ophthalmolepis lineolatus</i>	16.2, 0.243 \pm 0.119	6.82, 0.136 \pm 0.095	2.70, 0.027 \pm 0.019	16.7, 0.241 \pm 0.083
	<i>Pictilabrus laticlavus</i>	18.9, 0.297 \pm 0.109		1.35, 0.014 \pm 0.014	1.85, 0.019 \pm 0.019
	<i>Pictilabrus viridis</i>	8.11, 0.108 \pm 0.065	4.55, 0.045 \pm 0.032	1.35, 0.014 \pm 0.014	
<i>Pseudolabrus biserialis</i>	21.6, 0.297 \pm 0.109	25.0, 0.409 \pm 0.139	21.6, 0.649 \pm 0.207	20.4, 0.370 \pm 0.119	
<i>Stethojulis bandanensis</i>		4.55, 0.045 \pm 0.032	2.70, 0.027 \pm 0.019	1.85, 0.037 \pm 0.037	
<i>Thalassoma lunare</i>	24.3, 0.838 \pm 0.337	22.7, 0.477 \pm 0.154	4.05, 0.149 \pm 0.112	1.85, 0.019 \pm 0.019	

	<i>Thalassoma lutescens</i>	43.2, 1.05 ± 0.254	45.5, 1.00 ± 0.198	12.2, 0.338 ± 0.136	16.7, 0.407 ± 0.184
	<i>Thalassoma septemfasciatum</i>	13.5, 0.189 ± 0.085	18.2, 0.205 ± 0.07	4.05, 0.041 ± 0.023	1.85, 0.019 ± 0.019
Latidae	<i>Psammoperca datnioides</i>	10.8, 0.135 ± 0.069	6.82, 0.114 ± 0.074		5.56, 0.13 ± 0.084
Latridae	<i>Cheilodactylus rubrolabiatus</i>		2.27, 0.023 ± 0.023		
	<i>Dactylophora nigricans</i>	2.70, 0.027 ± 0.027			
Lethrinidae	<i>Lethrinus genivittatus</i>	2.70, 0.027 ± 0.027			
	<i>Lethrinus nebulosus</i>	5.41, 0.054 ± 0.038			
	<i>Lethrinus punctulatus</i>			1.35, 0.014 ± 0.014	
Monacanthidae	<i>Eubalichthys bucephalus</i>	2.70, 0.027 ± 0.027			
	<i>Eubalichthys mosaicus</i>			1.35, 0.014 ± 0.014	
	<i>Meuschenia flavolineata</i>			2.70, 0.027 ± 0.019	1.85, 0.019 ± 0.019
	<i>Meuschenia galii</i>			5.41, 0.054 ± 0.026	1.85, 0.019 ± 0.019
	<i>Monacanthus chinensis</i>			1.35, 0.014 ± 0.014	
Monodactylidae	<i>Schuettea woodwardi</i>		4.55, 2.84 ± 2.33		9.26, 6.28 ± 3.02
Mullidae	<i>Parupeneus chrysopleuron</i>	16.2, 0.568 ± 0.264		6.76, 0.216 ± 0.124	3.70, 0.167 ± 0.117
	<i>Parupeneus spilurus</i>	62.2, 2.40 ± 0.717	31.8, 1.02 ± 0.342	17.6, 0.541 ± 0.212	13.0, 0.222 ± 0.086
	<i>Upeneichthys vlamingii</i>	5.41, 0.054 ± 0.038	2.27, 0.023 ± 0.023	1.35, 0.014 ± 0.014	
Muraenidae	<i>Gymnothorax species</i>	35.1, 0.351 ± 0.08			
Myliobatidae	<i>Myliobatis tenuicaudatus</i>	5.41, 0.054 ± 0.038			
Nemipteridae	<i>Pentapodus vitta</i>	73.0, 1.92 ± 0.291	11.4, 0.114 ± 0.048	4.05, 0.108 ± 0.073	1.85, 0.037 ± 0.037
Odacidae	<i>Heteroscarus acroptilus</i>	2.70, 0.027 ± 0.027			
	<i>Olisthops cyanomelas</i>	18.9, 0.243 ± 0.09	13.6, 0.477 ± 0.265	29.7, 0.622 ± 0.151	14.8, 1.07 ± 0.782
Pempheridae	<i>Pempheris klunzingeri</i>	10.8, 2.43 ± 1.53	20.5, 4.68 ± 2.48	8.11, 0.554 ± 0.3	16.7, 2.80 ± 1.08
Pinguipedidae	<i>Parapercis haackei</i>		4.55, 0.068 ± 0.050	1.35, 0.014 ± 0.014	1.85, 0.019 ± 0.019
	<i>Parapercis ramsayi</i>				1.85, 0.019 ± 0.019
Pomacanthidae	<i>Pomacanthus semicirculatus</i>			1.35, 0.014 ± 0.014	
Pomacentridae	<i>Abudefduf bengalensis</i>	2.70, 0.027 ± 0.027	2.27, 0.045 ± 0.045	2.70, 0.027 ± 0.019	
	<i>Chromis westaustralis</i>		2.27, 0.023 ± 0.023	5.41, 5.85 ± 3.95	3.70, 1.06 ± 0.858
	<i>Dascyllus trimaculatus</i>		2.27, 0.023 ± 0.023		
	<i>Parma mccullochi</i>	40.5, 0.703 ± 0.189	50.0, 1.52 ± 0.376	50.0, 1.49 ± 0.236	57.4, 1.50 ± 0.252
	<i>Parma occidentalis</i>	21.6, 0.324 ± 0.11	29.5, 0.432 ± 0.123	21.6, 0.365 ± 0.097	18.5, 0.222 ± 0.068
	<i>Parma victoriae</i>		2.27, 0.023 ± 0.023	1.35, 0.014 ± 0.014	
	<i>Pomacentrus milleri</i>	29.7, 0.892 ± 0.402	56.8, 17.1 ± 5.90	36.5, 6.20 ± 3.56	46.3, 11.5 ± 4.36
	<i>Stegastes obreptus</i>		18.2, 0.227 ± 0.085		
Pomatomidae	<i>Pomatomus saltatrix</i>	2.70, 0.027 ± 0.027		1.35, 0.014 ± 0.014	
Pseudochromidae	<i>Labracinus lineatus</i>	54.1, 0.892 ± 0.168	15.9, 0.182 ± 0.067	5.40, 0.054 ± 0.026	7.41, 0.111 ± 0.063
Scaridae	<i>Scarus ghobban</i>	2.70, 0.081 ± 0.081	6.82, 0.114 ± 0.074		3.70, 0.222 ± 0.204
	<i>Scarus rivulatus</i>				3.70, 0.037 ± 0.026
	<i>Scarus sp3</i>			2.70, 0.027 ± 0.019	9.26, 0.241 ± 0.118
Scombridae	<i>Scombridae species</i>	8.11, 0.081 ± 0.045			
Scorpididae	<i>Microcanthus strigatus</i>	5.41, 0.216 ± 0.156	2.27, 0.045 ± 0.045	1.35, 0.081 ± 0.081	
	<i>Neatypus obliquus</i>	35.1, 5.87 ± 1.84	22.7, 0.545 ± 0.217	13.5, 0.757 ± 0.369	27.8, 6.32 ± 2.31
	<i>Scorpis georgiana</i>	16.2, 0.162 ± 0.061	13.6, 0.295 ± 0.154	8.11, 0.081 ± 0.032	7.41, 0.093 ± 0.048
Serranidae	<i>Caesiocorpius theagenes</i>	2.70, 0.676 ± 0.676	2.27, 3.41 ± 3.41		
	<i>Epinephelides armatus</i>	43.2, 0.595 ± 0.131	11.4, 0.136 ± 0.062	1.35, 0.014 ± 0.014	5.56, 0.056 ± 0.031
	<i>Epinephelus coioides</i>				1.85, 0.019 ± 0.019

	<i>Epinephelus rivulatus</i>	10.8, 0.108 ± 0.052		3.70, 0.037 ± 0.026
	<i>Hypoplectrodes nigroruber</i>			1.85, 0.019 ± 0.019
Siganidae	<i>Siganus fuscescens</i>	13.5, 4.76 ± 2.78	2.70, 0.568 ± 0.516	1.85, 0.019 ± 0.019
Sparidae	<i>Chrysophrys auratus</i>	45.9, 1.32 ± 0.412		
	<i>Rhabdosargus sarba</i>	13.5, 0.865 ± 0.409	2.70, 0.054 ± 0.038	
Sphyraenidae	<i>Sphyraena novaehollandiae</i>	5.40, 0.054 ± 0.038		
	<i>Sphyraena obtusata</i>	13.5, 0.649 ± 0.409	4.05, 0.378 ± 0.277	
Terapontidae	<i>Pelates octolineatus</i>	2.70, 0.324 ± 0.324		
	<i>Pelsartia humeralis</i>	2.70, 0.054 ± 0.054		
Tetraodontidae	<i>Lagocephalus sceleratus</i>	2.70, 0.027 ± 0.027		
	<i>Torquigener pleurogramma</i>	10.8, 1.78 ± 1.16	1.35, 0.014 ± 0.014	
Tripterygiidae	<i>Helcogramma decurrens</i>		2.27, 0.023 ± 0.023	
Urolophidae	<i>Trygonoptera ovalis</i>	13.5, 0.135 ± 0.057	4.55, 0.045 ± 0.032	

Table A.2. Indicator species referred to in this study as defined by the state government agency responsible for managing and monitoring marine parks in W.A.

Family	Species	Indicator Type
Carangidae	<i>Seriola hippos</i>	Fishing
Chaetodontidae	<i>Chaetodon assarius</i>	Climate Change
Glaucosomatidae	<i>Glaucosoma hebraicum</i>	Fishing
Labridae	<i>Pictilabrus viridis</i>	Climate Change
Labridae	<i>Pictilabrus laticlavus</i>	Climate Change
Labridae	<i>Austrolabrus maculatus</i>	Climate Change
Labridae	<i>Ophthalmolepis lineolatus</i>	Climate Change
Labridae	<i>Stethojulis bandanensis</i>	Climate Change
Labridae	<i>Choerodon rubescens</i>	Fishing
Pomacentridae	<i>Pomacentrus milleri</i>	Climate Change
Serranidae	<i>Epinephelides armatus</i>	Fishing
Siganidae	<i>Siganus fuscescens</i>	Climate Change
Sparidae	<i>Chrysophrys auratus</i>	Fishing

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