

Towards standardised reef fish
monitoring;
An assessment of stereo-video
techniques to sample shallow and
deep reef fish assemblages

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Abstract

Baited remote underwater stereo-video systems (stereo-BRUVs) were developed to determine fish abundance and size structure in a more unbiased, and relatively non-invasive manner across a broader range of depths and habitats than conventional sampling methods achieve. These characteristics make stereo-BRUVs particularly useful for research inside marine protected areas (MPAs) where researchers are required to minimize disturbance to the protected environment.

Although stereo-BRUVs have been widely used around the world, they are novel to South Africa and standardised protocols for their application are required. As such, this study aimed to address i) the effect of different bait types, namely pilchard (*Sardinops sagax*), squid (*Loligo reynaudi*) and bivalves (*Crassostrea gigas* and *Perna perna*) on the observed reef fish assemblage structure and ii) the effect of artificial illumination (lighting) on the observed reef fish assemblage structure sampled with stereo-BRUVs.

One key component of the stereo-BRUVs methodology is the application of bait to attract fish into the field of view. Globally, pilchard is the most often used bait type for stereo-BRUVs, however, its suitability has never been tested comparatively against other bait types for South African conditions. Significant differences in fish abundance and assemblage structure were recorded between stereo-BRUVs deployments when applying the different bait types. The bivalve baits, oyster and mussel, were typically poor when sampling the reef fish assemblages of the region. Pilchard and squid were sampled in similar communities and were able to attract the majority of the reef fish from the region, suggesting they are suitable bait types for stereo-BRUVs in the Agulhas ecoregion. However, a power analysis indicated that roughly twice the amount of samples are required to effectively sample the same abundances of *Chrysoblephus laticeps* (roman) and *Cheilodactylidae* (fingerfins)

species when sampling with squid as opposed to pilchard. Both bait types can be locally sourced, however, squid is considerably more expensive than pilchard. Considering the difference in cost, together with the fact that twice as much squid bait is required, squid is not a cost-effective option relative to pilchard. As such this study recommends that pilchard is the most appropriate bait for the Agulhas ecoregion of South Africa.

Due to low light levels, artificial lighting is required when conducting remote video research in the sub-photic zone. Blue light has a shorter wavelength than other colour lights and attenuates slower through water. In addition, blue lighting has no recorded effect on fish behaviour and has proven to be a successful light colour for underwater sampling. The use of lighting is unavoidable for sub-photic stereo-BRUVs sampling and is recommended for all stereo-BRUVs sampling for comparability of data from environments characterised by different light levels. A study was conducted to test the necessity of artificial lighting when sampling from environments characterised by ambient light levels adequate for stereo-BRUVs sampling, a comparative to measure the effect of light on the fish assemblage structure. Of the more commonly detected fish species (those observed in >50% of the samples), most were seen at similar abundances in samples using lighting and in those without lighting. No significant differences were observed in fish length data between samples collected with and samples collected without lighting. This suggests that standardised stereo-BRUVs sampling across photic zones on the continental shelf of South Africa can be collected without blue lighting when ambient light levels are sufficient to see the survey area.

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Knowing trees, I understand the meaning of patience.

Knowing grass, I can appreciate persistence.

Hal Borland

Chapter **1**

Introduction

1.1 Importance of monitoring

Global marine fisheries are in a demonstrated decline, with predictions of total stock collapse as early as 2048 (Jackson *et al.* 2001, Worm *et al.* 2006, Hardinge *et al.* 2013). Stock collapse can be a result of a host of anthropogenic factors that include overexploitation of fish stocks and habitat alteration (Granek *et al.* 2008). South Africa's own fisheries have seen a steady decline in catch rates in all recreational and commercial sectors since the 1950s (Bennett 1991, Attwood *et al.* 2010). Heavy fishing pressure has resulted in the collapse of many of our fish stocks, in particular those of the vulnerable slow growing, reef associated species targeted by the line fishery (fishing conducted with either a hand-line or rod and reel and no more than ten hooks per line) (Buxton 1993, Griffiths 2000, Sauer *et al.* 2003). For example, South Africa's formerly most important linefish reef species, *Polysteganus undulosus* (Seventy-four) and the *Petrus rupestris* (Red steenbras) have been declared commercially extinct and severely overexploited, respectively, resulting in subsequent moratoriums (Mann 2013). Regardless, the line fishery remains South Africa's third most important fishery in terms of total tonnage landed and total economic value (FAO 2012). In addition, the line fishery employs the highest number of fishers in South Africa (Sauer *et al.* 2003).

The term 'overexploited' defines a fishery that is "exploited above a level which is believed to be sustainable in the long-term, with no potential room for further expansion and a high risk of stock depletion/collapse" (FAO 2012). Overexploitation has not only ecological, but socioeconomic repercussions. Species under pressure are often important cultural, recreational or commercial resources (Bryant *et al.* 1998, Costanza *et al.* 1998, Marra 2005, Barbier 2007, Hardinge *et al.* 2013). In order to combat these negative impacts effective and strict law enforcement and monitoring as well as adaptive management plans are crucial (FAO 2012, Hardinge *et al.* 2013). Over the last few decades, however, it has become evident that both, the monitoring and management of fish resources have been either inadequate or inappropriate to ensure sustainable fishing of target species (Pauly 1995, Floros 2010).

The single- or multi-species approach of traditional fisheries management is now considered out-dated, particularly for reef fish (Morishita 2008, Floros 2010). Not only are such approaches not suitable due to the longevity, slow growth rate, late maturity and high residency of reef fishes (Buxton 1993, Brouwer and Griffiths 2005), they also are ineffective owing to poor enforcement and low levels of compliance (Hauck and Kroese 2006). As a result, there has been a drive to implement holistic ecosystem based fisheries (EBF) management,

which falls under the ecosystem approach to fisheries management (EAF). Primary management tools within the EAF and EBF are marine protected areas (MPAs), by-catch mitigation and integrated ecosystem approaches (Fletcher *et al.* 2005, Fletcher 2006, Morishita 2008, Fletcher 2010).

1.2 Marine protected areas

Marine protected areas can be defined as “any area of intertidal or subtidal terrain, together with its overlying waters and associated fauna, flora and historical and cultural features, which has been reserved by legislation to protect part or the entire enclosed environment” (Kelleher and Kenchington 1992). Marine protected areas are globally recognized as the most integral part of EBF management (McClanahan 1999, Roberts and Hawkins 2000, Lubchenco *et al.* 2003), and play an explicit role in the conservation of marine resources in South Africa (Attwood *et al.* 2010). The application of MPAs in both fisheries management and the conservation of overexploited stocks and biodiversity are becoming increasingly relevant (Agardy 1994, Roberts 1997, Allison *et al.* 1998, Pauly *et al.* 1998, Willis and Babcock 2000). As a result, there has been a shift to MPAs on global fisheries and conservation agendas (Mallet and Pelletier 2014). The use of MPAs is endorsed by many international agreements to which South Africa is party, such as the Jakarta Mandate, World Summit on Sustainable Development Johannesburg Accord, the Convention on Biological Diversity and the FAO Code of Conduct for Responsible Fisheries (Lemm and Attwood 2003, Floros 2010). South Africa has invested considerable effort into establishing an effective MPA network upon recognition that the stocks of many local linefish species have collapsed and that there are increased demands on marine resources by a growing population (Tunley 2009, Floros 2010, WWF 2012). At present the South African network of MPAs consists of 21 nationally legislated MPAs under the Marine Living Resources Act No. 18 of 1998 (De Vos *et al.* 2014). South Africa's updated draft policies on Marine Fisheries and Biodiversity clearly acknowledge the role of MPAs in the national management plan of the coastal zone (Attwood *et al.* 1997, Attwood *et al.* 2010).

However, implementation of such policies is often problematic and there is extensive literature highlighting a lack of success in the majority of MPA management objectives worldwide (Kelleher *et al.* 1995, Alder 1996, McClanahan 1999, Mora *et al.* 2006). It has been suggested that MPA management in the Western and Eastern Cape of South Africa lacks the capacity to effectively manage MPAs or draft management plans (Tunley 2009). Low priorities are given to marine protection with the focus of the limited staff and funds directed to the

terrestrial components of coastal reserves (Tunley 2009). South Africa's extensive coastal MPA network lacks prioritized baseline assessments and biodiversity monitoring, both within and adjacent to the MPAs and there is an increasing need for research and information (Sink *et al.* 2012). In those MPAs where monitoring has been conducted, efforts have typically been restricted in time and space and there is little standardization between different monitoring programs (Willis *et al.* 2003, Bennett *et al.* 2009). As an example, underwater visual census (UVC) is a popular survey technique. However, how the exact nature of the UVC approach employed (e.g. transects, point-counts or roving diver) varies between the different survey programmes, making data comparisons difficult (Samoilys and Carlos 2000, Edgar *et al.* 2004, Bennett *et al.* 2009, Floros 2010, Kulbicki *et al.* 2010).

More knowledge gaps are prominent in monitoring research, particularly with regard to deeper reefs. It is notoriously difficult to study deep water fish species and their habitats. As such the scientific knowledge often trails behind the effects of fisheries exploitation (Haedrich *et al.* 2001, Sackett *et al.* 2014). Knowledge of the structure and functioning of deep reef ecosystems is essential if appropriate management and conservation systems are to be implemented. It has been found that abiotic factors, such as depth, can directly correlate with, and predict, biotic factors such as fish assemblage structure (Stewart and Jones 2001, Mumby *et al.* 2006, Kellner *et al.* 2010, Edwards *et al.* 2014, Soler *et al.* 2015); yet research indicates that assemblage patterns are sometimes simply driven by abundances of common species that occur across the spectrum of habitats and depths (Sala *et al.* 1998, Edwards *et al.* 2014, Soler *et al.* 2015). Understanding the factors that influence reef fish assemblage structure is ambiguous, emphasizing the need for greater knowledge on deep reef fish populations. This knowledge should include an improved understanding of fish assemblage distribution patterns (Kellner *et al.* 2010, Ban *et al.* 2012, Soler *et al.* 2015) and the role of abiotic factors and their influence on reef habitats, so that they may be appropriately incorporated into analyses (Soler *et al.* 2015).

To develop effective conservation planning and management there is a need for research on a scale that is applicable to MPA management (Jennings and Polunin 1997, Sala *et al.* 1998, Kellner *et al.* 2010, Soler *et al.* 2015). Essential to the effectiveness of MPAs is a holistic approach with the protection of multiple components (e.g. species, habitats, ecosystems and their ecosystem processes) across a spectrum of environments (Rice and Rochet 2005, Mallet and Pelletier 2014, Soler *et al.* 2015). However, in order to manage holistically, one must also monitor holistically.

1.3 Monitoring methods

Monitoring is an integral part of effective MPA management (Sink *et al.* 2012). Monitoring allows for detection of change within the ecosystem and provides the scientific basis for appropriate management plans to be formulated and implemented, whilst ensuring that conservation goals are met (Sumaila *et al.* 2000, Floros 2010, De Vos 2012, Sink *et al.* 2012). In addition, the data can be used to estimate population status and understand the life history of commercially and ecologically important species (Unsworth *et al.* 2014). Such information allows for more effective allocation of management resources, meaning areas of concern can be identified, appropriately mitigated and managed (Risk 1999, Floros 2010). Unfortunately, MPA planning and management are often hindered by inadequate or incomplete species inventories and a poor understanding of species distribution and abundance patterns (Walter and Hollings 1990, Jones 2002, Ban *et al.* 2012, Edgar *et al.* 2014, Soler *et al.* 2015).

Biodiversity as a whole is rarely adequately monitored (Mallet and Pelletier 2014); therefore, a monitoring method that addresses multiple components of the ecosystem and provides unbiased biodiversity monitoring is favourable (Table 1.1; Rice and Rochet 2005, Mallet and Pelletier 2014, Soler *et al.* 2015).

Table 1.1 Favourable qualities of a monitoring method, particularly for use in MPAs.

Effective	The effectiveness of a method will depend on the objectives of the study and whether or not the method can collect data to answer these objectives. Data needs to be precise (yields consistent results) and accurate (yields true results) (Elphick 2008, Mallet and Pelletier 2014).
Easily standardised	The method should allow for repetitive sampling that in turn is comparable over time and space (Bernard <i>et al.</i> 2014, Mallet and Pelletier 2014).
Economically viable	The method needs to be cost-effective enough to make repetitive sampling feasible (Elphick 2008, Mallet and Pelletier 2014).
Institutionally practical	The method must be applicable and within the capabilities of the research institute, both financially and in terms of required skills (Elphick 2008, Mallet and Pelletier 2014)
Comprehensive	The method should collect data across the board, including a wide range of species, depth ranges and habitats so as to be applicable to the Ecosystems Approach to Fisheries Management whilst also being relevant to the objectives of the monitoring program (Bernard <i>et al.</i> 2014, Mallet and Pelletier 2014).
Non-invasive and non-destructive	The method must be environmentally friendly and cause as little impact as possible so as not to change the state to be measured or negate the use of the method within MPAs (Bernard <i>et al.</i> 2014, Mallet and Pelletier 2014).

1.4 Evolving monitoring methods

Marine monitoring methods have evolved over time and numerous methods have been used, some more successfully than others. Below is a summary of monitoring methods that have been applied in MPAs.

1.4.1 Controlled angling

Controlled angling is a commonly employed method that involves the use of hook, line and bait to capture fish (e.g. Bennett and Attwood 1993, Millar and Willis 1999, Götz *et al.* 2008).

This method can be easily standardized by appropriate gear selection, is inexpensive, uses simple equipment and provides fairly precise length measurements (Bennett *et al.* 2009).

However, it is inherently more selective to aggressive species and larger size classes and as a result does not represent the natural fish community or size structure accurately (Hardinge *et al.* 2013). Furthermore, post-capture mortality of fish due to barotrauma is common and

particularly problematic when sampling in MPAs or at depths greater than 20m (Kerwath *et al.* 2013).

1.4.2 Trawl surveys

Trawling involves the use of large vessels and towed nets to capture fish. Fishery independent research trawls are widely used as stock assessment and monitoring tools (Harms *et al.* 2010).

This method has minimal biases and allows for large numbers of fish to be captured with little selectivity. As specimens are collected the fish can be accurately identified and precise measurements can be collected from the samples (Cappo *et al.* 2004).

However, the extractive nature of this method means it cannot be conducted without killing fish and damaging habitats (Cappo *et al.* 2004, Murphy and Jenkins 2010). It is invasive, destructive and generally inappropriate for reef habitat monitoring, particularly in MPAs (Murphy and Jenkins 2010, Harvey *et al.* 2012, Langlois *et al.* 2012b, Bernard 2013).

1.4.3 Fish traps

Fish trapping is an extractive method that involves setting baited traps on the ocean floor for extended 'soak' periods. Data may be collected diurnally and nocturnally over a variety of depths (Harvey *et al.* 2012). This method is commonly used on shallow reefs and has been considered to produce relatively unselective fish assemblage data (Rechsiek *et al.* 1991, Hawkins *et al.* 2007, Locham *et al.* 2010).

However, mortality, injury and barotrauma rates associated with the method remain high (Bernard 2013). There are also inconsistencies in the data collected as more aggressive and carnivorous fish species are likely to be attracted to the traps and once inside preclude the capture of other components of the fish community (Hardinge *et al.* 2013). This suggests that fish traps may be more selective than previously assumed.

1.4.4 Underwater visual census (UVC)

Underwater visual census is an *in situ* method (fish are not extracted, but rather observed in their natural habitat) that is typically conducted by swim transects surveys or stationary point counts via SCUBA (Brock 1954, Russ and Alcala 1996, Zeller and Russ 2000, Bennett *et al.* 2009).

This method is non-destructive and can provide qualitative and quantitative data on the fish

community (Buxton 1987, De Girolamo and Mazzoldi 2001, Bennett *et al.* 2009).

However, the method is invasive as the diver's presence can cause attraction of and disturbances to fish, depending on the species (Chapman *et al.* 1974, Harmelin-Vivien *et al.* 1985, Kulbicki 1998, Dearden *et al.* 2010, Mallet and Pelletier 2014). In addition, UVC is restricted to safe diving depths (typically < 30m) and observation time (Watson *et al.* 2007), and is liable for considerable observer bias in both fish identification and length estimates (Thompson and Mapstone 2002, Bennett *et al.* 2009).

1.4.5 Diver operated video (DOV)

Diver operated video is a method employing video systems operated and manoeuvred by SCUBA divers.

Such methods significantly reduce the biases associated with human error through the opportunity of reanalysis and possibility to archive videos. As with UVC the method is non-destructive and can provide qualitative and quantitative data (Buxton 1987, De Girolamo and Mazzoldi 2001, Bennett *et al.* 2009).

However, DOV like UVC, is limited by depth and may create disturbance to natural fish behaviour by the presence of a diver (Watson *et al.* 2007).

1.4.6 Remotely operated vehicles (ROVs)

Remotely operated vehicles are an *in situ* method that employ highly mechanised underwater vehicles operated remotely from a larger vessel.

This method is non-destructive and not restricted by depth (Shortis *et al.* 2007). Remotely operated vehicles can provide a large amount of data, can cover large areas and can operate for extended survey times (Bernard 2012).

However, the method is not cost-effective as the equipment is expensive, requires highly skilled personnel and a comparatively larger vessel for operations (Bernard 2012).

1.4.7 Remote underwater video systems (RUVs)

Remote underwater video systems are *in situ* boat deployed camera rigs that allow for fish assemblages to be recorded.

This method enables non-destructive sampling without depth limitations that can provide comprehensive data on fish assemblages.

The method is not biased by the presence of bait, however, data is highly variable and as a result large sample sizes are required to achieve appropriate confidence around abundance estimates (Watson *et al.* 2005, Bernard and Götz 2012). This reduces the cost-efficiency of the method. Data obtained through RUVs do not allow for length estimates to be conducted.

1.4.8 Baited remote underwater video systems (BRUVs)

Baited remote underwater video systems are similar to RUVs, however, BRUVs are baited so as to attract fish into the field of view of the cameras. As a result, they are very effective at sampling carnivorous fish. The use of bait does introduce bias, as does the use of light when sampling deeper – such bias may underrepresent herbivorous and zooplanktivores fish species in abundance relative to carnivores and benthic invertivores. In saying that, fewer samples are needed as data variability is reduced when fish are attracted with bait (Watson *et al.* 2005, Bernard and Götz 2012). As with RUVs, BRUVs data do not provide the possibility to extract length estimates.

1.4.9 Stereo-video systems

Stereo-video systems consist of two cameras that allow for a stereo-image to be created with a subsequent possibility to measure objects in the overlapping field of view. Stereo configurations can be applied to any method that uses cameras such as DOV, BRUVs, RUVs, and ROV. As such, stereo-video systems possess all the benefits (and biases) of the original method, with the advantage of obtaining accurate and precise length and distance measurements. This facilitates the measurement of population size structures and the survey area.

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

Developed in 1995, the stereo-video technique was initially aimed to improve fish size estimates from diving surveys (Harvey and Shortis 1995). Since then, the stereo configuration has been applied to many underwater video methods. At the forefront of these advances stand stereo-BRUVs (Watson *et al.* 2007, 2009, Chatfield *et al.* 2010, Mclean *et al.* 2010, Goetze *et al.* 2011, Mclean *et al.* 2011, Merritt *et al.* 2011, Birt *et al.* 2012, Dorman *et al.* 2012, Fitzpatrick *et al.* 2012, Harvey *et al.* 2012a,b,c, Langlois *et al.* 2012a,b) which have enabled

researchers to conduct standardized, statistically rigorous and non-destructive monitoring of shallow and deep-water fish assemblages (Langlois *et al.* 2012).

Baited remote underwater stereo–video systems consist of a pair of high definition digital cameras in underwater housings mounted on a frame to provide an overlapping field of view centred on a baited container that attracts fish. The cameras simultaneously film the same scene and the videos are later precisely synchronized using video processing software. The positions of the cameras are considered during a calibration process using specialised software, which enables the accurate measurement of lengths and distances from the videos post-sampling. This software (CAL) employs calibration files that take into account the exact fine-scale orientation of each pair of cameras (Harvey and Shortis 1996). Samples are then processed in specialized software, *EventMeasure*TM (SeaGIS 2014), which facilitates species identification and the counting and measurement of individuals. This enables the researcher to precisely measure objects independent of their orientation in space between one and ten meters from the cameras, visibility dependent (Mallet and Pelletier 2014).

There have been considerable improvements in stereo-BRUVs over the past 20 years that have raised cost-efficiency consistently. Systems are now smaller, more robust and cheaper. Furthermore, digital cameras possess higher resolution sensors and improved image quality (high definition), batteries are more compact and longer lasting and secure digital (SD) cards can store up to 120 gigabytes of video data (approximately ten hours of footage).

Baited remote underwater stereo–video systems enable researchers to determine fish abundance and population size structure in a relatively unbiased and precise manner across a broader range of depths and habitats than any conventional method (Harvey *et al.* 2001a,b, 2002a, 2004). Fish identification is also improved as analysis can be conducted within the constraints of the observer, for example, analysis may take place within a comfortable time frame (Harvey *et al.* 2003, 2007). This also means quality control by re-analysis of archived videos is possible (Harvey *et al.* 2003, 2007). As a result, the application of stereo-video techniques has increased worldwide, in particular in the last decade (Mallet and Pelletier 2014).

Such systems have only recently been used in South Africa. Previous studies employing stereo-BRUVs have been successfully conducted in New Zealand, Australia, Fiji, Hawaii and the Caribbean, amongst others (Moore *et al.* 2010, Goetze *et al.* 2011, Mallet and Petellier 2014). The amount of research conducted worldwide has resulted in the need for international

standards to be set with regards to the methodology including, but not limited to, bait type, bait quantity, the use of artificial lighting, lighting colour, video duration and camera set up (Priede and Merrett 1998, Bailey and Priede 2002, Harvey *et al.* 2007, Bernard and Götz 2012, Birt *et al.* 2012, Fitzpatrick *et al.* 2013, Hardinge *et al.* 2013, Klages *et al.* 2013).

1.6 Rationale for the examination of methodology

Comprehensive ecological research on continental shelves has been hindered by high costs, a paucity of suitable methods, and a lack of standardisation across geographic and depth gradients (Murphy and Jenkins 2010, Johnson *et al.* 2012). As a result, there is a poor understanding of the effectiveness of South Africa's MPA network; in particular concerning the conservation needs of fish assemblages on deeper reefs (Sink *et al.* 2012). Furthermore, fishing pressure imposed on possible spawning aggregations, and the life history characteristics of slow growing reef dependent fishes make them particularly vulnerable to overexploitation (Buxton 1993, Brouwer and Griffiths 2005, Norse *et al.* 2012). This need for comprehensive ecological research across the continental shelf beckons for a multifaceted method. Baited remote underwater stereo-video systems are a suitable method to fulfil this need, however, potential biases of the method are yet to be determined within the unique South African temperate ecoregion.

Marine ecoregions are defined as "Areas of relatively homogeneous species composition, clearly distinct from adjacent systems. The species composition is likely to be determined by the predominance of a small number of ecosystems and/or a distinct suite of oceanographic or topographic features. The dominant biogeographic forcing agents defining the ecoregions vary between localities but may include isolation, upwelling, nutrient inputs, freshwater influx, temperature regimes, ice regimes, exposure, sediments, currents, and bathymetric or coastal complexity" (Spalding *et al.* 2007). Globally, there are 232 marine ecoregions including the study site in question, the Agulhas ecoregion (Spalding *et al.* 2007). The Agulhas ecoregion falls within the warm-temperate southern African marine province.

The geographical characteristics of the Agulhas ecoregion make for a region that is fairly unique. It is the partial mixing of two currents, one warm (Agulhas Current) and one cool (Benguela Current), which create an area where currents overlap (Hutchings *et al.* 2009, Griffiths *et al.* 2010). Where the mixing of nutrient rich cold waters and hospitable warmer waters occurs, many endemic species including sparid reef fishes can be found (Griffiths *et al.* 2010). The region is also host to important spawning and nursery grounds and supports pelagic

and line-fishing, hake and sole trawl fisheries and a trap fishery for indigenous rock lobster as well as abalone and squid fisheries (Griffiths *et al.* 2010). There are many line fishery important reef dependent fishes present in the ecoregion such as the *Chrysoblephus laticeps*, *Petrus rupestris*, *Polysteganus undulosus*, *Pachymetopon aeneum* (Blue hottentot), *Seriola lalandi* (Yellowtail), *Chrysoblephus cristiceps* (Dageraad), *Chrysoblephus gibbiceps* (Red stumpnose), *Argyrozona argyrozona* (Carpenter), *Cheimerius nufar* (Santer) and *Rhabdosargus globiceps* (White stumpnose) (Bernard 2012, Chalmers 2012, Mann 2013). The Agulhas ecoregion is unique and the monitoring methods employed must be tailored towards this.

Methods research has become a fundamental aspect of science (Elphick 2008). This is due to an increasing emphasis on comparing and contrasting methods and techniques to eliminate or reduce associated biases and limitations. Simultaneously, there is also a need for data to be comparable on a large geographic and temporal scale in order to answer ‘bigger’ ecological questions. This is achieved through the use of standardized methods, or at least the ability to compare results through conversion of population abundance and size structures (Watson *et al.* 2010). While standardisation is important, the method first must be able to effectively collect data to address relevant research questions (Table 1.1).

The present study was deemed necessary to understand whether stereo-BRUVs, as they have been standardized elsewhere, are effective at detecting reef fish species typical to the Agulhas ecoregion. Whilst undoubtedly effective, particulars of the stereo-BRUVs methodology are yet to be investigated. Taking into account that international standards have already been established, it is important to remember that fish communities in different regions of the world may respond differently to stimuli, such as bait (See Chapter 3) and artificial lighting (See Chapter 4). Furthermore, ‘fine-tuning’ the stereo-BRUVs method to South Africa’s Agulhas ecoregion will improve cost-efficiency and the usefulness of the data. A cost-efficient monitoring method is crucial to ensure sustainable monitoring, particularly when considering the current socio-economic environment, both nationally and internationally.

1.7 Bait

Unbaited remote underwater video is not a cost-effective alternative to BRUVs. Natural variability and distributional patchiness in fish populations means unbaited sampling requires large numbers of samples to obtain data with sufficient statistical power (Bernard and Götz 2012). As such, a key component of the stereo-BRUVs methodology is that it requires bait to

attract fish into the field of view. Whilst it is an accepted standard to use pilchard, its suitability has never been tested comparatively to other bait types for local (South African) conditions. Recent research with stereo-BRUVs has demonstrated that the effect of pilchard can be inconsistent between different species and functional groups (Bernard and Götz 2012). One part of this study aimed to investigate the effect of different bait types on the observed fish assemblage structure. The study aimed to determine which bait type provided the most comprehensive and cost-effective assessment of reef fish abundance and diversity when sampling with stereo-BRUVs. The bait types investigated include pilchard, squid and bivalves, represented by brown mussel and oyster.

1.8 Artificial lighting

Knowledge on depth related patterns in fish assemblage composition, abundance and size structure are required to understand the ecology of reef fishes, and to effectively manage resources. As such, a standardized method is needed that can be applied throughout typical distributional ranges of reef fish populations from the shallow subtidal down to the edge of the continental shelf. However, collecting data from the sub-photoc zone requires artificial lighting (referred to from here on as 'lighting') to enable processing of the video footage when using low-cost, off-the-shelf camcorders without low light capabilities. It is therefore necessary to understand how lighting influences the observed fish assemblages. Different colour lighting can have varied effects on fish during deep-water sampling (Widder *et al.* 2005). Bright, white lighting is known to cause damage to eyes and change normal behaviour of fish. In terrestrial studies infrared lighting has been employed as a successful non-invasive light source, however, its low wavelength results in attenuation of the light and a reduced field of view in water (Widder *et al.* 2005). Recently, blue lighting has been suggested as an effective alternative when sampling with underwater video for sampling greater abundances of species (Fitzpatrick *et al.* 2013) with a reduced effect on the behaviour of fishes when compared to white lighting. The second component of this study aimed to investigate the effect of blue lighting on the structure of reef fish assemblages sampled with stereo-BRUVs.

Chapter 2

General Methodology

2.1 Study Areas

South Africa's coastline stretches 3,113km in length (Sink *et al.* 2012). The extensive coastline is characterised by a few large embayments and notoriously strong wave action (Griffiths *et al.* 2010). The Agulhas ecoregion encompasses the southern sector (approximately 800km) of the coastline (Figure 2.1; Hutchings 1994, Heyns 2015). The ecoregion has components of both the Atlantic and Indian Ocean and is subsequently influenced by the cold Benguela (in the west) and warm Agulhas (in the east) currents (Chalmers 2012). Seasonal wind driven upwelling events that drive cold nutrient rich bottom water to the surface, are frequent and result in high variation of nearshore water temperatures, in particular during the summer months (Lutjeharms *et al.* 2000, Lutjeharms 2007, Chalmers 2012). As a result, the Agulhas ecoregion is characterized by high productivity and is home to many endemic reef fish (Bennett 2007). Research trawls have recorded some 160 teleost and chondrichthyan species (Smale *et al.* 1993, Japp *et al.* 1994, Chalmers 2012). In addition, the Agulhas ecoregion is recognized as an important spawning and nursery ground for many of South Africa's economically important fish species (Griffiths *et al.* 2010). Within the Agulhas ecoregion, rocky reefs can be found from the shallow subtidal down to the shelf edge (200m depth), providing essential habitats for fishes, invertebrates and algae that contribute to the biodiversity of the region.

The study areas (Figure 2.1), all located within the Agulhas ecoregion, include the Tsitsikamma National Park (TNP) MPA, the Bird Island (BI) MPA and Riy Banks, the latter two located in the shallow, lunate Algoa Bay (Griffiths *et al.* 2010). The study areas are biologically representative of the ecoregion, as they are spatially distinct and exposed to different environmental conditions (sheltered bay and exposed shore) and levels of anthropogenic disturbance (protected and exploited).

A more detailed analysis of the study areas is provided in each respective data chapter (Tsitsikamma MPA: Chapter 3; Algoa Bay: Chapter 4).

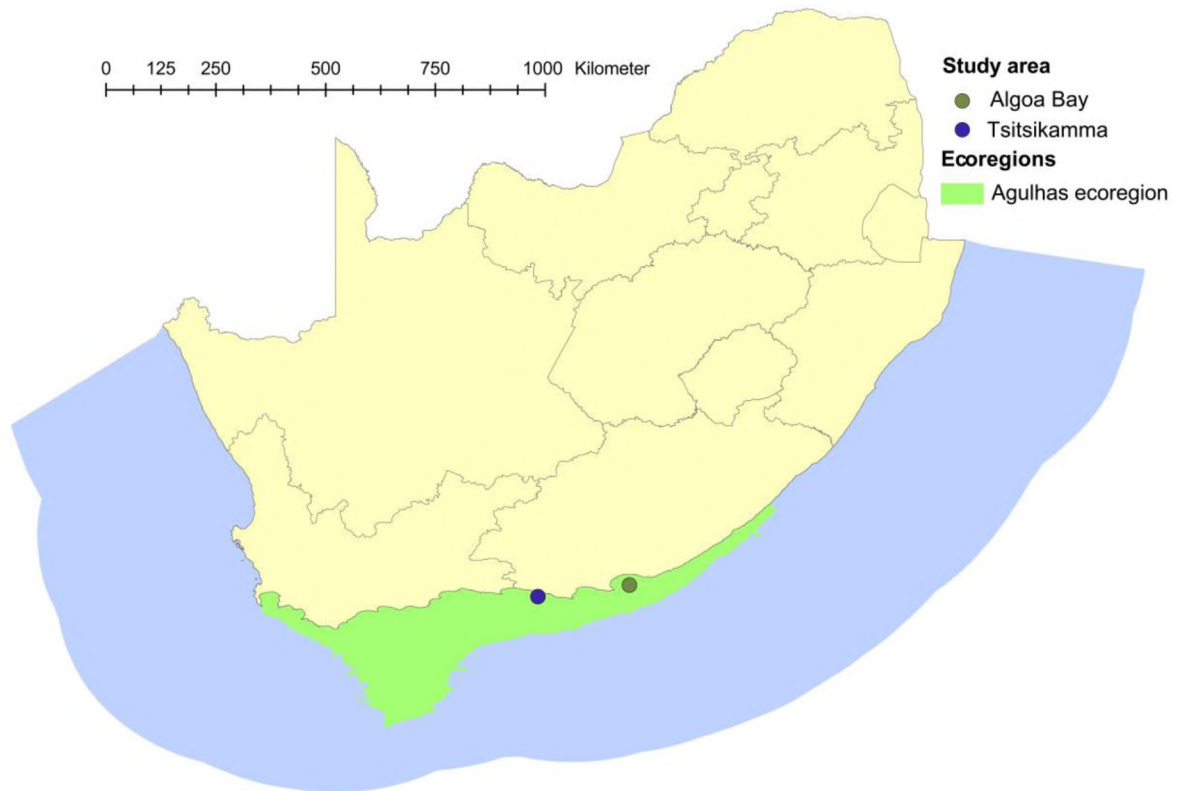


Figure 2.1 Map of South Africa showing the position of the Agulhas ecoregion and the location of the study areas, Algoa Bay and Tsitsikamma National Park MPA.

2.2 Experimental design

Within each of the study areas, one suitable sampling site (containing mostly rocky reef within the depth range of 8-30m) was identified from available bathymetric maps.

To facilitate stratified random sampling within each sampling site a grid of potential sampling points, evenly distributed with 300m separating each point, was superimposed on the existing bathymetric maps. Additional bathymetric mapping was then conducted using a GPS-linked echo sounder, with each mapping transect line passing over a line of potential sampling points. Each potential sampling point was then classified according to its depth and profile in GIS (ArcMap 10). Depth for the sampling point was taken as the depth at the point, while profile was determined by measuring the change in depth within 25m either side of the point. Where the change in depth was less than two meters, the point was assumed to be on sand, less than five meters the point was assumed to be on low profile reef and greater than five meters the point was assumed to be on high profile reef. Sandy habitats were omitted, as they were not the focus of this study. Sampling points classified as being on

sand were discarded from the potential sampling point list. The remaining points were grouped by profile (two fixed levels: high profile and low profile) and depth (two fixed levels: shallow [10-17m] and deep [18-30m]).

Baited remote underwater stereo-video system deployments at each sampling site were based on stratified random principles using a random sequence generator in Excel with an even allocation of effort between each strata combination (shallow-high; shallow-low; deep-high; deep-low). Within the sampling design 16 replicate samples were planned for each strata combination. These combinations included 16 samples of each different bait type (pilchard, squid, oyster and brown mussel; see Chapter 3) and 16 samples each of lighting treatment (with blue lighting and without; see Chapter 4). Replicate sites within sampling sites were separated by 300m (preselected grid) to avoid overlap of the bait plumes and resulting risk of pseudo-replication caused by recounting of fish moving between neighbouring sites (Langlois *et al.* 2010).

2.3 Baited remote underwater stereo-video systems

Four stereo-BRUVs rigs were employed to collect the required data. The stereo-BRUVs were based on the design used by researchers in Australia (Harvey *et al.* 2002, Watson *et al.* 2005, Cappo *et al.* 2004). A stereo-BRUVs consisted of two Canon HF M56 HD video cameras with 0.6 wide angle lens in an underwater housing. The cameras were mounted 0.7m apart on a base and converged inwardly at 8° allowing for a field of view of up to 10m overlapping from 0.5m in front of the cameras (Figure 2.2). A bar holding the synchronising diode (0.6m from the cameras) and the bait canister (1.5m from the cameras) extended perpendicularly from the mid-point between the two cameras (Figure 2.2). The synchronising diode enabled the synchronisation of the video footages from the two cameras which is required when processing the footage for size measurements of fish. The bait canister contained 0.8-1 kilogram of crushed bait, refreshed for every deployment.

The research was conducted off a nine-meter semi-rigid ski-boat set up for stereo-BRUVs surveys with a battery powered capstan-winch and davit-arm for rig deployment and retrieval. Previous research from the Agulhas ecoregion suggested 50-minute deployments were adequate to detect 95% of species at MaxN, however recommended 60-minute deployments to conform to international standards (Bernard and Götz 2012, De Vos *et al.* 2014). Work by Watson *et al.* (2006) indicates that filming must take place for a time period

of at least 36 minutes, ensuring the majority of fish found within the reef are measured. Nevertheless, 60-minute periods were advised so as to include numerous measures of common and rare species (Watson 2006, Watson *et al.* 2010). In this study stereo-BRUVs were left on the ocean floor to film for 60 minutes to conform to local (Bernard and Götz 2012, De Vos *et al.* 2014) and international standards (Watson *et al.* 2010, Goetze *et al.* 2011).

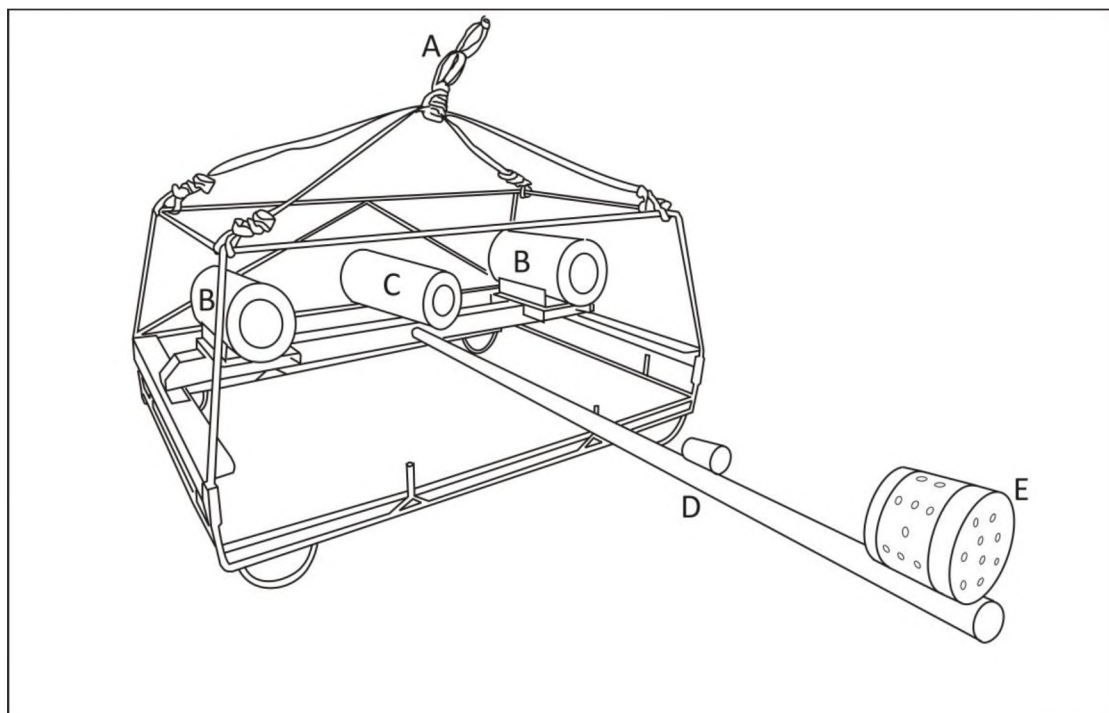


Figure 2.2 Schematic drawing of the baited remote underwater stereo-video system (stereo-BRUVs) employed during this research, showing rope attachment for deployment and retrieval (A), underwater housings for HD video cameras (B), underwater housing for blue LED lighting (C), synchronising diode (D) and bait canister (E). Diagram courtesy of Dr Elodie Heyns.

2.3.1 Calibration of stereo-BRUVs

To enable precise length measurements each pair of stereo-cameras was calibrated before and after each field trip. Two calibration files were required to reduce any possible changes to camera orientations due to dislocation of cameras during travel or deployment.

Calibration is necessary so as to define the interior orientation of the cameras (Harvey and Shortis 1996). This allows for an understanding of internal geometric characteristics including the focal length at particular focus settings, lens distortions, bias between pixel spacing and the principal point (Harvey and Shortis 1996). Secondly, calibration allows for a relative orientation between the two mounted cameras of a pair to be determined (Harvey and Shortis 1996). This takes into account the perspective centres of the lenses, the pointing angles and rotations of the sensors (Harvey and Shortis 1996). Calibration involves filming a test object, in this case a purpose built metre squared open aluminium cuboid (Harvey and Shortis 1996). The cuboid is black in colour with 56 white points or circular targets of known distance apart on the surface (Harvey and Shortis 1996). Calibration took place in a swimming pool to ensure stability and good visibility. Using SCUBA gear, the cube was moved by a diver according to a predefined procedure, including a normal horizontal position and subsequent circuits in other orientations and this was filmed by each camera pair (Harvey and Shortis 1996). Software known as *Cal*[®] (SeaGis 2014) was used to analyse calibration videos. The software creates a three dimensional image of the filmed points, each point is of a known distance apart allowing for standardised information about the particulars of each camera system to be established. The same calibration cube was used for both pre- and post-calibration. A least squares estimation procedure modelled the statistical behaviour of the observed image coordinates which was then used to detect and eliminate observation errors and to compute a calibration file for each system (see Harvey and Shortis 1996). Should differences between pre- and post-calibration values be detected, post-calibration was repeated. If the post-calibration values remained different, the pre-calibration values were discarded and the post-calibration values were applied for subsequent video analysis.

2.4 Environmental variables

Previous studies have shown that environmental conditions, such as habitat structure and water temperature, and biological characteristics, such as biogeography, can influence the type of species detected in a sample and increase the overall variability in datasets (Willis *et al.* 2000, Harvey *et al.* 2007, Colton and Swearer 2010, Pelletier *et al.* 2011, Bernard 2012). For this reason it was important to collect information on environmental variables during the surveys as well.

2.4.1 Water temperature

Water temperature can alter feeding behaviour and potentially alter attraction to the bait canister (Bernard and Götz 2012). Therefore, it was deemed to be important to account for temperature, particularly in the Agulhas ecoregion where drastic temperature changes caused by upwelling events are frequent (Goschen *et al.* 2012).

Temperature loggers (Hobo, Onset Systems) were attached to the frame of each stereo-BRUVs. The loggers recorded water temperature every five minutes and a mean temperature reading from a 30-minute period taken from the middle section of each deployment was used as the sample's temperature.

2.4.2 Bottom type

Habitat can influence the diversity and assemblage structure of the fish community (Moore *et al.* 2010). Bottom type (representing habitat and referring to the nature of the seafloor) was recorded and employed as a covariate in the analyses.

Bottom type was defined from video footage as reef, patch-reef, sand inundated reef or sand. The reef profile was categorized as high or low if the height of the reef in the camera's field of view changed by more or less than one meter, respectively.

2.4.3 Stereo-BRUVs orientation

Depending on the way the stereo-BRUVs settled on the seafloor more or less water column was visible in the video footage at a site. This has the potential to influence the detectability of species that are predominantly found close to the reef, or in the water column. For example, *Cheilodactylidae* are predominantly reef dwelling fish and a video with less water column in view and more reef would be expected to yield more *Cheilodactylidae* (Bernard and Götz 2012). To account for this, the percentage water column (the percentage of the analysed video habitat that was open water i.e. not bottom substrate) was determined for each sample. Percentage obstruction was also determined using the same method, where the field of view was not obstructed by, for example, reef or rope. This was achieved using Vidana[®], a software package for quantifying percentage cover within a quadrat from video footage or still digital images (Marine Spatial Ecology Lab 2016). The percentage of water column and obstruction provides a quantitative description of the habitat in view for analysis. These values were employed as covariates in statistical analysis.

2.4.4 Underwater horizontal visibility

A visibility measure was taken during video analysis for each sample. This was done by creating 3D points in *EventMeasure*TM (SeaGis 2014) at the extent of the visibility where species could still be identified. Visibility was also used to calculate the area (incorporating visibility, camera's horizontal angle of view and obstruction) in which fish were counted. Area was used to standardise MaxN data to account for the variation in visibility between samples and allow unbiased comparisons of samples.

2.5 Video analysis procedure

Videos were analysed by means of specialised stereo-photo comparator software, *EventMeasure*TM (SeaGis 2014). *EventMeasure*TM was developed to analyse stereo-BRUVs data providing species composition, abundance data and the length estimates of objects from underwater stereo-videography (Harvey and Shortis 1996). It allows the analyst to play, rewind, fast forward and step footage during the analysis of two videos simultaneously. Individual fish were identified to species level, recorded and counted. *EventMeasure*TM uses species files collated for southern Africa containing information on family, genus, species and applicable codes for fish identification. The largest number of individuals from a species recorded in one frame (MaxN) was logged for each 60-minute video period and used as a measure of relative abundance (Priede *et al.* 1994). The MaxN procedure avoids repeated counts of the same individuals, subsequently removing any possible pseudo-replication associated with fish re-entering the field of view. Thus MaxN is considered a good, but conservative estimate of relative abundance (Watson *et al.* 2005).

Fish were identified using reference guides, namely Smiths' Sea Fishes (Smith and Heemstra 1986), Two Oceans: a guide to the marine life of southern Africa (Branch *et al.* 2010), Coastal Fishes of Southern Africa (Heemstra and Heemstra 2004) and Southern African Marine Linefish Profiles (Mann 2013). Fish were classified according to trophic groups: generalist carnivores; omnivore; zoo-planktivore; invertebrate carnivores; micro-invertebrate carnivores; piscivores, and herbivores (Heyns pers. com., Smith and Heemstra 1986, Heemstra and Heemstra 2004, Branch *et al.* 2010, Mann 2013).

Once a MaxN frame was identified for each species, these frames were revisited to measure the fork length of all individuals. In each video of the synchronised stereo-video footage pair

two points were made on an individual fish, one on the top lip and one on the fork of the tail (Harvey and Shortis 1996). A three-dimensional distance between these points was then computed automatically according to camera calibrations and specifications (Harvey and Shortis 1996). A detailed description of the measurement process is available in Harvey and Shortis (1996). 'Range', 'RMS' and 'Precision to length' rules were set to alert when size measurements were outside the scope of the rule. Due to generally low visibility in the study areas, no measurements of fish were conducted beyond the five-metre visibility mark, the 'Range' rule was used to reject measurements. When the RMS error was greater than 20, which generally means there is a problem with the calibration files or a fish is too far away for its size, the measurement was rejected at the discretion of the analyzer and the fish was re-measured. Replicate measurements were not made on all fish.

2.6 Statistical analysis

Differences in abundance, length and biomass of the fish assemblages between the different experimental treatments (bait type and lighting) were determined by multivariate community analysis and univariate species-level analysis.

All multivariate and univariate analyses were conducted in the PRIMER v6 package (Clarke and Gorley 2006) with the PERMANOVA+ add-on package (Anderson *et al.* 2008). The generalized linear models, power analysis and kernel density estimations were performed with the statistical program R (R Development Core Team 2015) and the random number generators available in the package MASS (Venables and Ripley 2002).

A more detailed description of the statistical models employed is presented in the research chapters.

Chapter 3

The effect of bait type on reef fish assemblage structure observed with baited remote underwater stereo-video systems

3.1 Introduction

Reef fish monitoring is a fundamental tool in conservation planning and management of marine ecosystems. Contributing to this is an accurate understanding of the distribution and structure of fish assemblages. Fish assemblage data is a useful and sensitive indicator of habitat degradation, ecosystem productivity and overall environmental change (Babcock *et al.* 1999, Willis *et al.* 2003, Cappo *et al.* 2007, Malcolm *et al.* 2007, Watson *et al.* 2007, 2009, Brooks *et al.* 2011, Goetze *et al.* 2011, Langlois *et al.* 2011, 2012, McLean *et al.* 2011, Moore *et al.* 2011, Birt *et al.* 2012, Cheung *et al.* 2012, Dorman *et al.* 2012). Conventional monitoring methods used to collect fish assemblage data such as trawls, angling, fish traps or UVC can be limited by depth range or habitat type and do not allow for comprehensive and precise data to be collected (See Table 1.1; Willis *et al.* 2000, Cappo *et al.* 2004, Watson *et al.* 2005, Colton and Swearer 2010, Pelletier *et al.* 2011, Bernard 2012, Bernard and Götz 2012, Harvey *et al.* 2012). Studies conducted outside of South Africa have shown that BRUVs and stereo-BRUVs outperform these traditional, and often destructive, monitoring methods (Watson *et al.* 2005, Harvey *et al.* 2012, Langlois *et al.* 2012). Recent trials in South Africa have confirmed this for local conditions (Bernard and Götz 2012, De Vos *et al.* 2014).

As the use of stereo-BRUVs grows, so does the range of habitats and ecoregions in which it is applied. In order to standardise the sampling approach it is necessary to understand the method to ensure it sufficiently samples the local fish communities.

3.1.1 Why use bait?

Instrumental to the success of stereo-BRUVs is the application of bait as an attractant. The advantageous effect of bait on the efficiency of marine monitoring is well documented (Wolf and Chislett 1974, Munro 1983, Whitelaw *et al.* 1991, Wraithe *et al.* 2013). Bait allows for a more complete picture of the fish community to be obtained. It also increases the effectiveness of the method by increasing the abundance and diversity of fish observed and can subsequently reduce cost as fewer samples are required. Different baits have different characteristics, including persistence (longevity within the water column) and moisture content (Dorman *et al.* 2012). These influence soak times, dispersal area and bait plume persistence (Dorman *et al.* 2012). Standardizing bait type, the quantity used and the method of delivery, removes any potential bias or selectivity when using bait as an attractant (Dorman *et al.* 2012).

3.1.2 Representation of the true fish assemblage

Studies show that RUVs accurately capture the natural variability found in the distribution of fish populations and fish assemblage data (Bernard and Götz 2012). However, when sampling with RUVs compared to BRUVs, significantly more samples are needed to effectively sample fish populations. In comparison, baited systems allow for more individuals and species to be sampled at an overall lower sampling effort (Harvey 2007, Bernard and Götz 2012). The ability to sample more individuals from a species within different habitats subsequently decreases the data variability (Willis and Babcock 2000, Harvey *et al.* 2007). This results in greater statistical power and more effective sampling (Willis and Babcock 2000, Harvey *et al.* 2007). This is particularly relevant when monitoring carnivorous species, which tend to be highly mobile, occurring at low densities with patchy distributions (Harvey *et al.* 2007).

Furthermore, by attracting more fish closer to the stereo-BRUVs there are more opportunities to acquire precise length measurements (Harvey and Shortis 1996, Harvey *et al.* 2002a,b, Harvey *et al.* 2007). This is important because relative abundance from stereo-BRUVs (in this study MaxN) provides only a conservative estimate of abundance with little supporting information on the structure of the fish assemblage (Willis and Babcock 2000, Harvey *et al.* 2007). Length measurements are essential for a more comprehensive understanding of fish assemblages, as it relates to age structure, reproductive potential and biomass (Willis and Babcock 2000, Harvey *et al.* 2007). In addition, fish length data is highly sensitive to fisheries' impacts as larger individuals are selectively targeted in most fisheries (Murphy and Jenkins 2010, Bernard *et al.* 2014).

3.1.3 Cost-efficiency

Previous work indicates that less variable fish abundance data are collected with BRUVs compared to RUVs (Harvey *et al.* 2007, Bernard and Götz 2012). Given this, fewer samples are needed to effectively sample the fish assemblage when BRUVs are employed, as a representative portion of the fish assemblage can be sampled in less time. The cost per sample is an accumulation of fuel costs, salaries of skippers and deck hands, boat maintenance, equipment longevity and maintenance costs (See Table 3.2 and 3.3, Appendices 3.5 and 3.6). These costs generally dwarf the cost of bait and as a result preference can be given to the baited method where fewer samples are required (Bernard 2012). Economically, this makes baited sampling more efficient.

3.1.4 Bait type

While there are numerous benefits to using bait, it does introduce other biases that must be considered. For example, it would not be expected that herbivorous fish would be attracted to a fish or an invertebrate bait type such as pilchard or squid. Similarly, it would not be expected that piscivorous fishes would be attracted to invertebrate bait types. As such, these groups would be inconsistently sampled or under-represented in the data. If the aim is to measure patterns in fish diversity and community structure, an optimum bait type would permit sampling of the entire fish assemblage inclusive of all trophic guilds (Wraith *et al.* 2013).

Baits release chemical stimuli into the water column, which are then dispersed by currents (Dorman *et al.* 2012). Bait type is known to affect the range of action (the area where the bait is effective as an attractant) of the bait within the water column (Harvey *et al.* 2007, Stobart *et al.* 2007, Wraith 2007, Wraith *et al.* 2013, Mallet and Pelletier 2014). Bait type also influences the type of species attracted as well as the abundance and biomass of the fish observed (Harvey *et al.* 2007, Stobart *et al.* 2007, Wraith 2007, Wraith *et al.* 2013, Mallet and Pelletier 2014). An optimum bait type must be economically efficient, environmentally sustainable and must not introduce pests or disease (Dorman *et al.* 2012). Furthermore, an optimum bait type must be an effective attractant to a wide variety of species, comprising a range of trophic levels and feeding guilds including cryptic species (Dorman *et al.* 2012).

In an effort to achieve this, a myriad of alternative baits and bait combinations have been tested in previous studies. Ellis and Demartini (1995) attached a squid to the outside of a container filled with *Scombridae* sp. (Mackerel), Lowry *et al.* (2012) used vegetable meal and tuna oil, Stobart *et al.* (2007) used a combination of pilchards and effervescent bait pellets (Wraith *et al.* 2013) and a combination of ground anchovy and squid was used to mimic methods employed by traditional fishers when sampling snapper in Hawai'i (Misa *et al.* 2013). Foregoing oily baits entirely, High (1980) investigated the suitability of *Octopus dofleini* (Octopus) as a bait type. Similarly, Wraith *et al.* (2013) tested *Haliotis midae* (Abalone) and *Centrostephanus rodgersii* (Sea urchins), while Dorman *et al.* (2012) went as far as to test cat food and falafel mix (a chickpea, flour combination).

When selecting a suitable bait type it is important to take into consideration the trophic structure of the fish community within the region of interest. Generalist carnivores and

invertebrate carnivores dominate rocky reef fish assemblages in the Agulhas ecoregion (Bernard and Götz 2012, Heyns 2015, Heyns *et al.* 2016). It can therefore be assumed that to monitor the majority of this warm-temperate reef fish community, a fish or invertebrate bait would be most effective. Studies conducted within the Agulhas ecoregion have indicated that larger reef fish are attracted to squid and pilchard baits even when these do not fall into their natural diet (van der Elst 1993, Buxton 1994, Götz 2007).

Baited fish-trap studies indicate that oily baitfish are more effective at catching a wide range of species compared to white-fleshed bait (Whitelaw *et al.* 1991, Wraithe *et al.* 2013). Oily fishes used in previous studies include mackerel, bonito tuna (*Katsuwonus pelamis*) (Brooks *et al.* 2011) and pilchard (Babcock *et al.* 1999, Cappo *et al.* 2004, Watson *et al.* 2005, Watson *et al.* 2007, Watson *et al.* 2010, Westera *et al.* 2003, Bond *et al.* 2012, Dorman *et al.* 2012, Wraithe *et al.* 2013). The alternative oily baits appear to produce similar results to that of the pilchard (Wraithe *et al.* 2013). This suggests pilchard is an effective bait type, with a strong ability to sample the carnivorous guilds (macroinvertebrate carnivores and generalist carnivores) (Wraithe *et al.* 2013). Resultantly, pilchard has become the accepted international standardized bait for BRUVs and stereo-BRUVs research (Watson *et al.* 2010, Mclean *et al.* 2010, 2011, Goetze *et al.* 2011, Bassett and Montgomery 2011, Bernard 2012, Bernard and Götz 2012 Harvey *et al.* 2012a, Langlois *et al.* 2012a,b, Mallet and Pelletier 2014, De Vos *et al.* 2014).

However, recent studies with BRUVs have demonstrated that the effect of pilchard can be inconsistent between different species and functional groups (Bernard and Götz 2012). Whilst pilchard has proven to be an effective bait type in the Agulhas ecoregion through BRUVs and angling research (Götz *et al.* 2007, Götz and Bernard 2012), the use of alternative baits could yield more representative and consistent data.

3.2 Aim

This study aimed to examine the effect of different bait types on the community and size structure of fish assemblage data collected with stereo-BRUVs. The research was conducted in the Agulhas ecoregion of South Africa.

Accepting pilchard as an effective representative of fish baits and in particular, oily fish baits, three additional invertebrate baits were chosen to address this research aim. Oyster and brown mussel represented sessile bivalve invertebrate baits, and squid, a mobile invertebrate bait.

3.3 Methods

3.3.1 Stereo-BRUVs methodology

Stereo-BRUVs were deployed according to the standardized method described in Chapter 2. Four different bait types were applied; pilchard, squid, oyster and brown mussel. To minimise contamination and confounding the results, separate bait canisters were used for the different bait types.

Pilchard

All bait was purchased from local suppliers and the pilchard were caught from within South Africa's pelagic fishing grounds. Pilchard were defrosted and crushed with a wooden pestle to paste-like consistency. Each sample weighed approximately 0.8-1 kilogram. As previously mentioned, pilchard is the accepted international standardized bait for BRUVs and stereo-BRUVs research (Watson *et al.* 2010, Mclean *et al.* 2010, 2011, Goetze *et al.* 2011, Bassett and Montgomery 2011, Harvey *et al.* 2012a, Langlois *et al.* 2012a,b, Mallet and Pelletier 2014). As such, pilchard was selected as the standard bait type. Pilchard is incorporated in a variety of different food webs within the ecoregion and is an important food source for *Cheimarius nufar* (Santer), *Seriola lalandi* (Yellowtail), *Atractoscion aequidens* (Geelbek), *Argyrozona argyrozona* (Carpenter) and *Polysteganus undulosus*, amongst other species (Mann 2013).

Squid

Locally caught squid were purchased as whole animals from local retailers. Animals were cut into roughly 2cm by 2cm cubes and beaten to a similar consistency to that of the standardized pilchard bait. Each sample weighed approximately 0.8-1 kilogram. Due to its popularity with anglers and it being an important food source for a variety of reef and reef associated fish species in the ecoregion, squid was selected as an alternate bait type to pilchard. This highly developed mollusc is recognised as an important food source for a variety of fish present in the Agulhas ecoregion including the *Seriola lalandi*, *Chirodactylus grandis* (Bank steenbras), *Carcharias taurus* (Ragged-tooth shark), *Argyrosomus inodorus* (Kob), *Epinephelus andersoni* (Cat-face rockcod), *Epinephelus marginatus* (Yellowbelly rockcod), *Argyrozona argyrozona*, *Cheimerius nufar*, *Chrysolephus laticeps* (Roman) and the *Petrus rupestris* (Mann 2013).

Oyster

The oysters used were discards from a commercial aquaculture facility in the Port Elizabeth harbour. The oysters were discarded for aesthetic reasons such as doubled shells and damaged shells. The whole animals were crushed up to a similar consistency to that of the standardized pilchard bait. Any large shells with no meat attached were discarded during crushing. Each sample weighed approximately 0.8-1 kilogram. Oysters were selected because they were considered easily accessible through the aquaculture industry, and low cost, as discarded individuals were sourced at no cost. Although never tested as a bait alternative, oysters are naturally occurring bivalves in the Agulhas ecoregion that would be expected to make up some of the diet of generalist carnivores, benthic invertebrate carnivores and omnivores. Molluscs contribute to the dietary needs of *Galeichthys* species (Sea catfish species), *Pomadasys* species (Grunter species), *Umbrina robinsoni* (Baardman), *Boopsoidea inornata* (Fransmadam), *Cymatoceps nasutus* (Black musselcracker), *Lithognathus mormyrus* (Sand steenbras), *Pagellus bellotti natalensis* (Red tjor-tjor), *Rhaphdosargus* species (Stumpnose species) and *Sarpa salpa* (Strepie) (Mann 2013). Bivalves in particular are an important food sources for *Diplodus capensis* (Blacktail) and *Lithognathus lithognathus* (White steenbras) (Mann 2013).

Mussel

The mussel were collected from the Port Elizabeth harbour where they were cleaned from buoy lines and jetty walls. These mussel were freely available, not harvested for human consumption and the harvesting did not affect any natural environment. As a result their harvesting and use as a bait type was considered environmentally and economically sustainable. The bait was crushed up to a similar consistency to that of the standardized pilchard sample. Any large shells with no meat attached were discarded during crushing. Each sample weighed approximately 0.8-1 kilogram. Both oyster and mussel fall under the category of bivalve molluscs. Mussel were selected to replace oyster as bivalve bait following the first survey. This decision was made as oyster proved ineffective in the initial survey, and because no oysters could be sourced at the time of the second survey. The unreliable availability of oysters made them unsuitable as a bait type for standardised monitoring and research. Mussel make up the dietary requirements of a number of fish species and feeding guilds including *Dichistius capensis* (Galjeon), *Cymatoceps nasutus*, *Sparodon durbanensis* (White musselcracker), *Rhadosargus globiceps* (White stumpnose) and *Lithognathus lithognathus* (Mann 2013).

3.3.2 Study site

The Tsitsikamma National Park (TNP) MPA is situated in the centre of the warm-temperate Agulhas ecoregion (Figure 3.1a). The TNP MPA is the oldest (proclaimed in 1964) and one of the largest (323km²) no-take MPAs in southern Africa. The subtidal communities provide one of the best examples of pre-exploitation inshore ecosystems available in southern Africa (Bernard 2012). Using this baseline ecosystem allows for a more thorough assessment of the remote camera method's ability to survey subtidal rocky reef fish communities in the Agulhas ecoregion. All samples were collected on Rheeders Reef, a shallow rocky reef in the centre of the MPA (Figure 3.1b and 3.2) (Bernard 2012). This large reef is part of an on-going long-term monitoring program (Bernard 2012). The reef is diverse with an abundance of high and low profile rocky reefs and intermittent sand patches (Figure 3.2) (Bernard and Götz 2012).

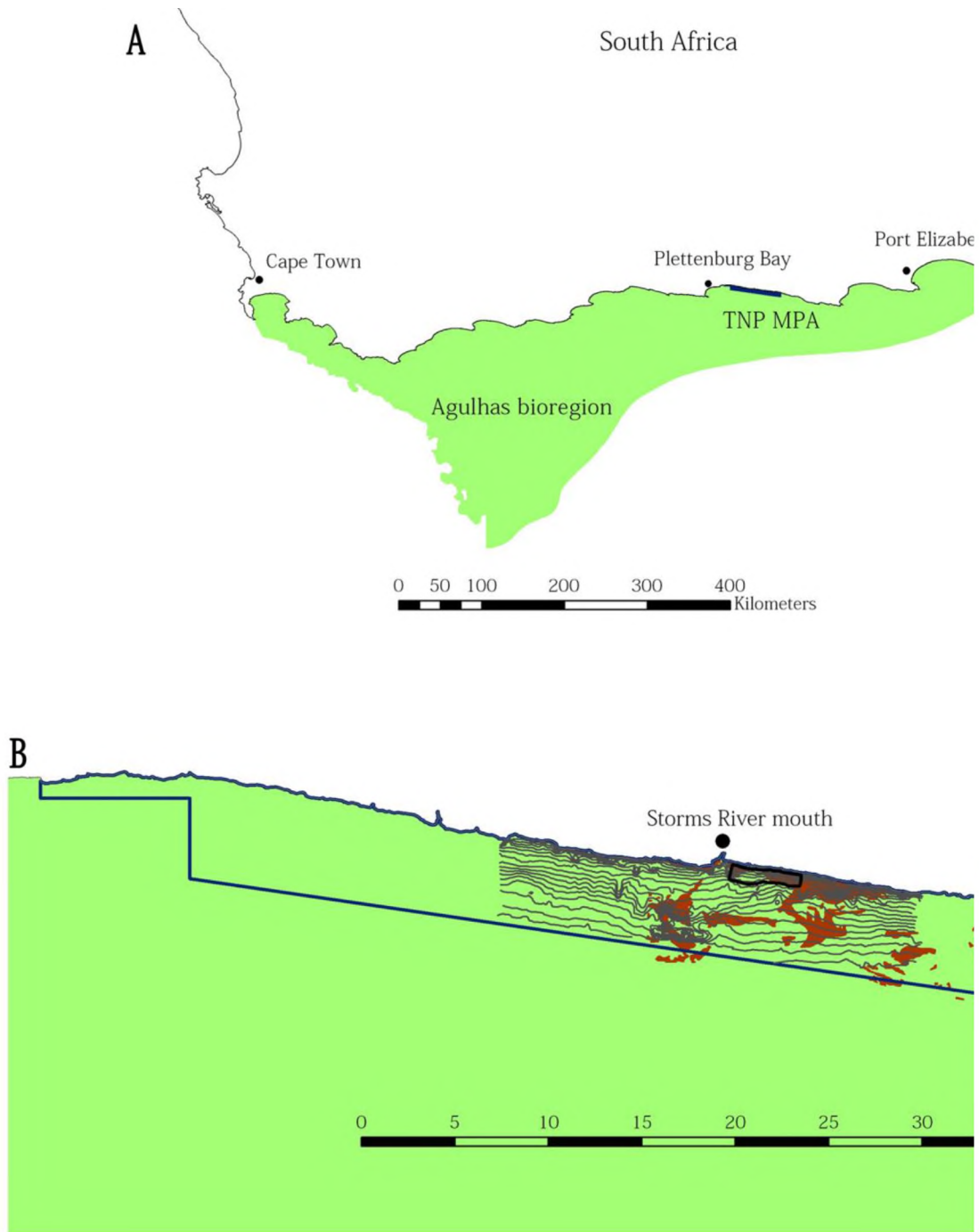


Figure 3.1 The location of the Tsitsikamma National Park Marine Protected Area (TNP MPA) in South Africa (A), with the position of the Rheeders Reef complex within the TNP MPA (B).

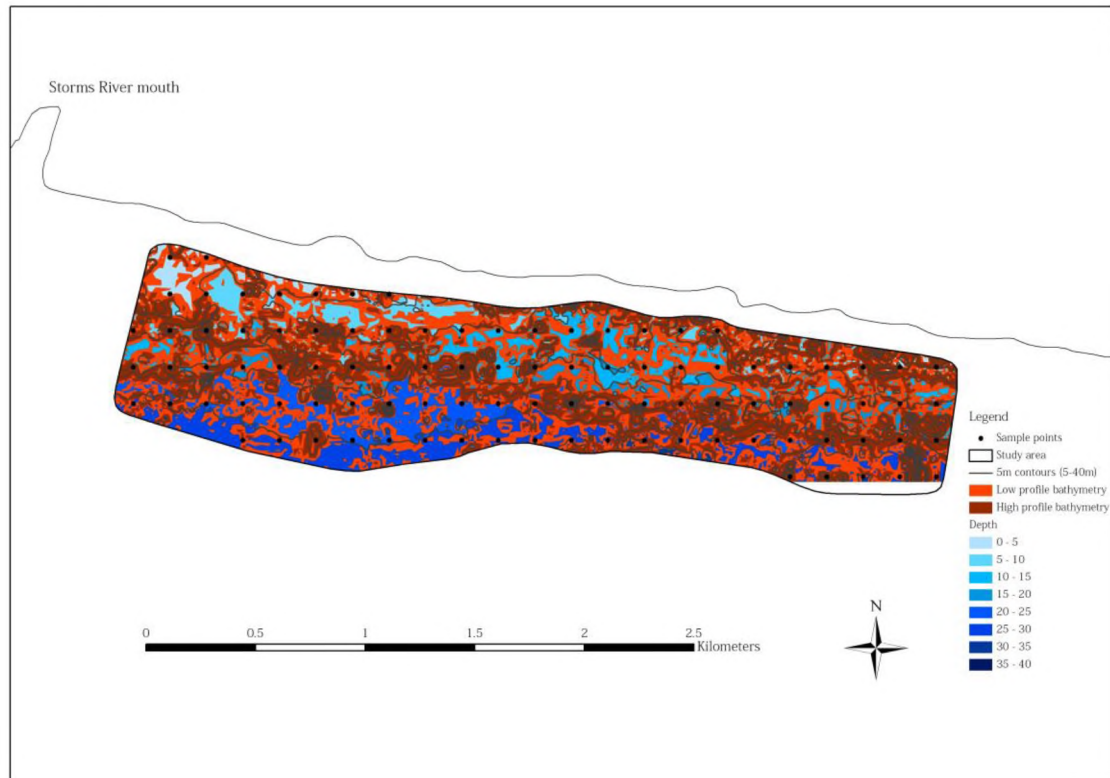


Figure 3.2 The detailed bathymetry of the Rheeders Reef complex, Tsitsikamma National Park Marine Protected Area.

3.3.3 Experimental design

The experimental design consisted of a balanced sampling protocol with 16 samples collected for each bait type. The different bait types were used concurrently during any given sampling day. Sampling sites were selected based on the stratified random sampling approach described in Chapter 2 with an even allocation of samples between high and low profile reef between depths of 8-32m.

Samples employing the pilchard and squid bait types were collected over two sampling trips, February and August 2013. Oysters were used during the first sampling trip, however they were replaced with a second bivalve bait, mussel (see above). Although unavoidable, this complicated the comparisons of the bait types and the analysis was adjusted to accommodate this.

3.3.4 Video analysis

Videos were analysed following the procedure outlined in Chapter 2.

3.3.5 Statistical analysis

The power analysis, generalized linear models (GLMs) and kernel density estimations (KDEs) were performed using the statistical program R (R Development Core Team 2015). All other analyses were conducted using the PRIMER v6 package (Clarke and Gorley 2006) with the PERMANOVA+ add-on package (Anderson *et al.* 2008).

For all analyses of MaxN (relative abundance), apart from that conducted in R, standardised data were used. Area was used to standardise MaxN data to account for variation in underwater visibility between samples and allow unbiased comparisons of treatments. Data were standardised such that each sample accounted for a 5m² area of view. For analyses conducted in R, visibility was treated as a covariate.

3.3.5.1 Testing the effect of Bait type on fish assemblage structure

The response data that was tested included MaxN by species, MaxN by trophic group (a sum of MaxN for each species in the group) and species average length.

Permutational ANOVA

To investigate general effects of bait type on the fish community a one-way permutational ANOVA was conducted to test the effect of bait on species richness, total MaxN and average MaxN. The permutational ANOVA was conducted using the PERMANOVA routine in PRIMER V6 on square-root transformed data using a Euclidean distance resemblance matrix and 9999 unrestricted permutations.

Permutational MANOVA

A one-way non-parametric multivariate analysis of variance (PERMANOVA) tested for differences in the abundance of species in the fish assemblage between different bait treatments. Permutational approaches were used for analyses because the relative abundances of some fish species contained zero counts (Fitzpatrick *et al.* 2013). The PERMANOVA was performed using Modified Gower Logbase 10 resemblance matrix so as to reduce the influence of highly abundant species. The PERMANOVA employed 9999

unrestricted permutations of raw data. Monte Carlo values were acquired for factors with less than 100 unique permutations (Anderson *et al.* 2008, Fitzpatrick 2010).

3.3.5.2 Constrained canonical analysis of principal coordinates

Constrained canonical analysis of principal coordinates (CAP) plots were used to further establish if bait type was an effective predictor of fish species observed when sampling with stereo-BRUVs. The CAP analysis conducts a canonical discriminant analysis on principal coordinate (PCO) axes (Gower 1966, Willis *et al.* 2006). The analysis predicts how data will cluster according to bait type, either data will form discriminated *a priori* groups or groups that have the strongest correlation according to another set of variables, in this instance bait (Anderson and Robinson 2003, Anderson and Willis 2003). This allows for a restricted classification of ecological data (Anderson and Willis 2003). In addition the CAP analysis tests to see what percentage of samples can be correctly assigned to the appropriate group (leave-one-out allocation success) (Dorman *et al.* 2012). High percentage allocation success is indicative of distinction between groups and the potential to correctly predict how samples allocate themselves into similar groups (Heyns 2015). Misclassification error indicates the proportion of misclassified point, allocation success indicates the proportion of correct allocations (calculated as 1 minus the misclassification error).

Constrained canonical analysis of principal coordinates plots were built using Modified Gower (log-base 10) resemblance matrices for abundance (MaxN), length estimates and trophic groups.

3.3.5.3 SIMPER

Similarity percentage (SIMPER) analyses were employed to determine which fish species were most frequently recorded with the bait types. Similarity percentage analyses use the Bray-Curtis measure of similarity, comparing the abundances between groups (i.e. different bait types) and calculates the contribution of individual species to the observed similarity or dissimilarity in assemblage composition. The SIMPER analyses were performed on log (x+1) transformed abundance and trophic group data.

3.3.5.4 Generalized Linear Models

The GLMs were fitted with the MASS package (Venables and Ripley 2002) in the R environment (R Development Core Team 2015). The GLMs were used to investigate the

effect of different bait types on the MaxN of selected fish species (Table 3.1). A GLM is an extension of linear models allowing for variables to be modelled and transformed to linearity (Venables and Ripley 2002). As such, they allow ecological data to be analysed via ‘non-normal’ error structures (Guisan *et al.* 2002).

Table 3.1 Description of the focus fish species and groups of species used as response variables in the generalised linear models.

Focus species/groups	Inclusive of	Motivation	Anthropogenic value
<i>Chysoblephus laticeps</i> (roman)	/	Large generalist carnivore, dominant within the study area, reef associated.	Important in both recreational and commercial fisheries.
<i>Cheilodactylidae</i> family (fingerfin species)	Red fingerfin <i>Cheilodactylus fasciatus</i> , barred fingerfin <i>Cheilodactylus pixi</i> and two-tone fingerfin <i>Chirodactylus</i> <i>brachydactylus</i>	Small and medium sized, omnivorous, representative of non-carnivorous species, common, reef associated.	Conservation importance. Not targeted in fisheries.
Small generalist carnivores	Steentjie <i>Spondyliosoma emarginatum</i> and fransmadam <i>Boopsoidea inornata</i>	Small and medium size generalist carnivores, highly reef abundant, reef associated.	Not targeted in fisheries but captured as by-catch.
Catsharks	Puffadder shyshark <i>Haploblepharus edwardsii</i> , brown shyshark <i>H. fuscus</i> , dark shyshark <i>H. pictus</i> , pyjama catshark <i>Paroderma africanum</i> and leopard catshark <i>P. pantherinum</i>	Small generalist carnivore, common, dominant reef associated shark species.	Not targeted in fisheries but captured as by-catch.
Medium invertebrate carnivores	Blacktail <i>Diplodus capensis</i> and Zebra <i>D. hottentotus</i>	Small and medium sized, omnivores, common, reef and patch-reef associated.	Important in recreational fisheries.
Fisheries important species	Dageraad <i>Chrysoblephus cristiceps</i> , red stumpnose <i>Chrysoblephus gibbiceps</i> , blue hottentot <i>Pachymetopon aeneum</i> and red steenbras <i>Petrus rupestris</i>	More obscure (in comparison to roman), large, generalist carnivores, reef associated.	Important in both recreational and commercial fisheries.

Exploratory analyses were conducted prior to the running of GLMs so as to understand the nature of the data following the protocol set out by Zuur *et al.* (2010). Following this, a standardised full model was run for all analyses. The model was defined as:

$$E(Y) = \beta_0 + (\text{offset}(\log(\text{Area}))) + \beta_1 (\text{Bait type}) + \beta_2 (\text{Temperature}) + \beta_3 (\text{Depth}) + \beta_4 (\text{Bottom type}) + \beta_5 (\text{Percentage water column}) + \varepsilon$$

(Equation 3.1)

where $E(Y)$ is the expected response variable, which is assumed to follow the Poisson distribution, β_0 is the intercept, $\beta_{\#}$ is the estimated coefficient associated with the respective covariates and ε is the error term. Area was treated as an offset to ensure the fitted values and their respective confidence intervals are positive (Zuur *et al.* 2009). See Chapter 2 for definition of covariates (Environmental Variables).

The Poisson distribution which was used as the assumption of homogeneity, required for linear regression, was not met in any of the analyses (Zuur *et al.* 2007). However, where over dispersion occurred the new model was fitted for the negative binomial distribution. The over dispersion in Poisson GLMs was estimated through Pearson residuals (Zuur *et al.* 2007). Where the calculated dispersion parameter was considerably greater than one, the model was recognised as over dispersed and the negative binomial model was applied.

In addition to testing for over dispersion, further model validation investigated if there were patterns in the residuals when plotted against all covariates included in the model, as well as those that were not. If there were no patterns then the model was considered valid. As the GLM technique does not require normal distribution (Zuur *et al.* 2007), histograms and QQ-plots were interpreted according to model fit as opposed to normality of the residuals (Zuur *et al.* 2007).

Likelihood ratio tests (using the 'drop1' function) were employed to see which covariates significantly affected the models. The 'drop1' function is a likelihood ratio test that sequentially removes each parameter from the full model and measures the change in the Akaike Information Criterion (AIC) and the deviance explained by the resultant model (Zuur *et al.* 2013). This determines if the parameter that was dropped significantly contributed to the model fit (Zuur *et al.* 2013). Parameters that were found not to influence the model fit were dropped from the model. The AIC is a measure of goodness of fit whereby the lower the AIC the better the model fits the data (Zuur *et al.* 2009). The model with the lowest AIC

was used in this case, including the covariate to be tested (bait type), representing the most parsimonious model, balancing model fit and simplicity (Bernard 2012).

3.3.5.5 Kernel density estimation

The KDE analysis was employed to statistically compare the length-frequency distributions of *Chrysoblephus laticeps* sampled with the different bait types. *Chrysoblephus laticeps* is a dominant and fisheries important species in the Agulhas ecoregion. It was observed with all bait types and thus was selected as the species for examination.

The KDE is a non-parametric, data-driven, more sensitive method for density estimations. The KDE analysis was realized here by means of R-code provided by Langlois *et al.* (2012). The KDE analysis tested for differences in the shape (of the distribution, indicated by changes in mode or median relative to mean) (Langlois *et al.* 2012) and locality (mean length) of the length-frequency distributions of *Chrysoblephus laticeps* sampled by the different bait types. Biases towards smaller or larger fish are illustrated by dissimilarities in locality, whilst dissimilarities in shape illustrate bias towards a particular length class. The 'sm.density.compare' function was employed to produce a plot with a grey standard error band representative of the null model of no difference between bait types (see Langlois *et al.* 2012). Significance tests were conducted as permutation tests of the area between the probability density functions (Langlois *et al.* 2012). Where the KDE curves were outside of the standard error band (deviated from the null model) a significant difference was assumed. Raw length data was standardised by median and variance to provide a test of shape only ($y = x - \text{median} / \text{stdev}$) (Bowman and Azzalini 1997, Langlois *et al.* 2012).

3.3.5.6 Cost effectiveness

Power analysis

The power analysis calculated the number of stereo-BRUVs samples needed per bait type to detect an annual 10% population increase (r) over the five year set period, with 80% power, at a significance level of $\alpha < 0.05$. The five-year time frame was selected to represent a theoretical MPA evaluation cycle. The analysis focussed on two contrasting, but important, reef fish species, *Chrysoblephus laticeps* and *Cheilodactylidae*. *Chrysoblephus laticeps* were selected as they are a primary target species within recreational and commercial fisheries as a large generalist carnivore. Alternatively, *Cheilodactylidae* are not a targeted species, but a

reef fish of conservation importance and represent generalist invertebrate carnivores abundant on reefs in the Agulhas ecoregion.

The power analysis employed the Monte-Carlo simulation approach following that described by De Vos *et al.* (2014) and other authors (Porch *et al.* 2004, Bolker 2007, Blanchard *et al.* 2007).

A deterministic trend in average MaxN was based on the following equation:

$$\text{MaxN}_{z, y+1} = \text{MaxN}_{z, y} \times e^r$$

(Equation 3.2)

where $\text{MaxN}_{z,t}$ is the predicted relative abundance value for bait type (z) and year (y) and r is the rate of increase per year (i.e. 10% growth). For each bait type, 1000 simulations were generated to determine the power associated with the sample size (n), where n is the number of samples conducted per year.

A Poisson GLM was fitted to the MaxN values under the assumption that counts followed a Poisson distribution where the variance is equal to the mean (Zuur *et al.* 2009). A test of fit established the dispersion parameter, where the dispersion was greater than 1 (i.e. the data were over dispersed). Where dispersion was evident, a negative binomial GLM was used in place of the Poisson GLM. The power analysis was run on the data for each bait type separately with no additional environmental covariates (i.e. the Null model). This was done to avoid over fitting the data, which would result in underestimating the number of samples required to achieve a power of 80%. The structure of the GLM fitted for each simulation assumed the following equation:

$$\ln(\mu_i) = \alpha + \beta_r \times \text{year}_i$$

(Equation 3.3)

where α is the intercept and β_r is the estimated year trend (1 - 5). Where factor β_r was significant ($p < 0.05$) the number of simulations was taken into account. Where μ_i is the mean assumes MaxN follow a Poisson distribution.

The statistical power for sample size n was calculated as the proportion of simulations that detected a significant trend (De Vos *et al.* 2014, Blanchard *et al.* 2008). The sample size n

was increased stepwise by 4, starting at 4.

An asymptotic growth function was fitted to simulated power curve results:

$$power = 1 - e^{-bn}$$

(Equation 3.4)

where b is the rate of increase as power approaches one. Sample sizes required to achieve 80% power for each bait type were calculated as:

$$np80 = b^{-1} \ln(1 - 0.8)$$

(Equation 3.5)

(Adapted from Bernard 2012 and De Vos 2014)

Power analyses were conducted by means of the MASS package (Venables and Ripley 2002) in the R environment (R Development Core Team 2015).

Cost benefit analysis

A cost benefit analysis is a systematic procedure that determines economic feasibility and sustainability of projects. Here the cost benefit analysis was conducted to determine which bait provided the most cost-efficient means of sampling the populations of *Chrysoblephus laticeps* and *Cheilodactylidae*. The costing used was updated from data provided by Bernard (2012).

Cost per sample

For each bait type, a cost per sample was calculated (Table 3.2). This included the costs of consumable materials required in the field and processing time, post sample collection. Bait costs were standardized to 900g per sample. The data processing times of 3.0 hours were allocated for each sample, at a cost of R100 per hour (Table 3.2 Appendices 9).

Table 3.2 Breakdown of costs per field day to accommodate four field workers and boat use.

The costs are adapted from a previous cost benefit analysis by Bernard (2012).

Per field day					
#	Item	Quantity	Unit	Cost	Total
1	Subsistence for research team	4,00	people	R 75,00	R 300,00
2	Boat running (btw sample sites = 1.43km)	69,60	km	R 15,00	R 1 044,00
3	Boat running (to&from study area)	8,00	km	R 15,00	R 120,00
4	Time for research team	3,00	people	R 553,60	R 1 660,80
5	Skipper	1,00	people	R 553,60	R 553,60
				Total/day	R 3 678,40
				Total	R 14 713,60
				Total (bad weather days)	R 10 057,60

Cost per day

The cost per day is a consolidation of subsistence, size of the monitoring team, number of days spent in the field, running cost of the vessel, average number of samples collected per day, average distance travelled between sample sites based on the stratified random sample site allocation, and the daily distance travelled to and from the study area.

Table 3.3 The cost of bait for a stereo-BRUVs sample (correct at time of sampling).

Bait cost per sample	
Pilchard	R33.50
Squid	R85.00
Oyster	n/a
Mussel	n/a

The size of the monitoring team varied between three and four people (one skipper and two or three deckhands). Staff time while in the field was calculated to R 69.2 per hour or R 553.6 per day (Bernard 2012).

The average distance travelled between sample sites is dependent upon the size of the study area. Using a standard 6 x 3km study area, divided into 300 x 300m blocks classified according to depth and reef profile, the average (\pm SD) distance between 100 randomly

selected sampling points from all strata was estimated to be 2.9 (± 2.0 km) (Bernard 2012). Sites were visited twice, for deployment and retrieval, at 1.43 km traveling distance between sites, which equates to an average distance of 5.8km per sample (Bernard 2012).

The number of hours spent at sea was standardised to six hours per day. For stereo-BRUVs surveys it was estimated that with four systems, 12 samples could be collected per day, each deployed for 60 minutes with 20 minutes between successive deployments to allow for transit and preparation of equipment.

The number of field days required was then calculated as the necessary number of samples, determined from the power analysis described above, divided by the estimated number of samples collectable per day. In addition to the cost per day of fieldwork, loss of days due to bad weather was considered. From past seagoing experience with smaller vessels in the Agulhas ecoregion 50% of the days are too poor to head out to sea, so the allotted bad weather days was equal to the calculated number of sea-going days required. The cost of bad weather days was taken as the employee's daily rate and subsistence for the monitoring team with no additional costs.

See Appendices 3.5 and 3.6 for detailed cost breakdown.

3.4 Results

A total of 81 samples were collected on Rheeders Reef in the TNP MPA with the four bait types (pilchard, squid, oyster, mussel). Of the 81 samples, 31 had pilchard as the bait type, 24 squid, and 26 bivalves (16 oyster and 10 mussel). Fewer mussel samples were collected due to poor weather conditions at the time of the field trip. Pilchard and squid were used in both summer and winter, and as a result more samples could be collected. Oysters were only used in summer, as they were not available for sampling in winter.

A total of 1,088 MaxN counts were conducted of 4,766 individual fish from 58 different species. From these 2,545 length measurements were extracted (see Appendices 3.7, 3.8, 3.9 and 3.10 for full species' lists).

Of the 58 species, sampling with pilchard provided 43 species, squid 43 species, oyster 31 species and mussel 25 species (combined total of 37 species for bivalve baits, oyster and mussel). A total of six species were unique to samples with squid bait and five to pilchard bait. Only three species were unique to oyster and one to mussel bait.

3.4.1 The effect of bait type on MaxN

Univariate ANOVA

Squid and pilchard produced similar average species richness, total MaxN and MaxN per species (Table 3.4). In addition, pilchard and squid samples recorded considerably more species and at higher abundances in comparison to oyster and mussel samples (Table 3.4).

Table 3.4 Total number of species (species richness), average species richness, average total MaxN, average MaxN per species and average length for different bait types tested with stereo-BRUVs.

Bait types	Species Richness	Average Species Richness	Average Total MaxN	Average MaxN per species	Average Length (mm)
Pilchard	43	10 (± 6)	92.4 (± 94.8)	9.2 (± 8.6)	249.7 (± 151.7)
Squid	43	10 (± 5)	107.3 (± 126.3)	11.2 (± 12.8)	277.1 (± 146.3)
Oyster	31	6 (± 3)	22.4 (± 22.0)	4.8 (± 6.5)	201.9 (± 102.0)
Mussel	25	9 (± 5)	49.5 (± 33.8)	6.6 (± 6.0)	209.9 (± 73.9)

A univariate ANOVA tested the effect of bait type on species richness, total MaxN and average of MaxN (Table 3.5).

A significant difference in species richness between samples collected with different bait types was found ($P < 0.05$). The pairwise test indicated that the species richness observed with oyster bait was significantly lower than that recorded from samples collected with both squid and pilchard bait types (Table 3.5).

Bait type did not significantly affect average MaxN ($P > 0.05$). However, a significant difference was found in total MaxN between species ($P < 0.01$). Oyster samples recorded significantly less fish (Total MaxN) compared to all other bait types (pilchard, squid and mussel) (Table 3.5).

Table 3.5 Results from the pair-wise tests from the Permutational ANOVA testing the effect of bait type on univariate measures of the fish assemblage. *Indicates the level of significance P with: * <0.05 , ** <0.01 , * ** <0.001 .

	Species Richness	Total MaxN
Pilchard, Oyster	0.0168*	0.0015**
Pilchard, Squid	0.8838	0.7216
Pilchard, Mussel	0.7538	0.2427
Oyster, Squid	0.0074**	0.002**
Oyster, Mussel	0.0845	0.0223*
Squid, Mussel	0.6502	0.2016

Permutational MANOVA (PERMANOVA)

A one-way PERMANOVA tested the effect of bait type on MaxN for species and trophic groups and the average length for species (Table 3.6). Significant differences in MaxN between samples with different bait treatments were found (Pseudo-F = 2.2171, $P = 0.0004$). The fish community sampled with oyster bait was significantly different from that sampled with all other bait types (Table 3.6). A significant difference was also found between the fish communities sampled with pilchard and mussel bait types. However, there was no significant difference between the fish communities sampled with squid and mussel baits and pilchard and squid baits (Table 3.6). The PERMANOVA showed no significant difference

in average fish length between samples of different bait treatments (Pseudo-F = 1.0222, P = 0.4137). The PERMANOVA of fish length data indicated no significant differences between all pairwise analyses of bait types (Table 3.6). The PERMANOVA investigating the effect of bait type on the abundance of fish within the different trophic groups indicated a significant difference (Pseudo-F = 2.9478, P = 0.0021). Significant differences between oyster and all other bait types were observed ($p < 0.05$, Table 3.6). No other significant differences were found between bait types.

Table 3.6 Results from the pair-wise tests from the PERMANOVA testing the differences between fish assemblages sampled with different bait types. *Indicates the level of significance P with: * < 0.05, ** < 0.01, *** < 0.001.

	MaxN	Length estimates	Trophic groups
Pilchard, Oyster	0.0008 ***	0.1126	0.0016 **
Pilchard, Squid	0.3264	0.6215	0.7752
Pilchard, Mussel	0.0180 *	0.9193	0.2376
Oyster, Squid	0.0048 **	0.1256	0.0020 **
Oyster, Mussel	0.0186 *	0.4206	0.0032 **
Squid, Mussel	0.0700	0.7842	0.3496

3.4.2 CAP analysis

The CAP analysis showed both an overlap of MaxN and length data for most samples, particularly for those using pilchard and squid as attractants (Figure 3.3 and 3.4). Pilchard and squid samples showed a definitive overlap in MaxN and length estimate data for observed fish, suggesting that the fish communities sampled were similar. There was some distinction in the fish assemblage MaxN data between samples employing the mussel and oyster bait compared to pilchard and squid bait (Figure 3.3). Mussel and oyster samples also showed little data overlap between samples (Figure 3.3). This distinction was less so when observing length data, with oyster only showing some deviation from the other bait types (Figure 3.4).

For the MaxN data (Figure 3.3) the first eight axes explained 63.0% of the variance ($m = 8$, proportion (prop) = 63.0%) and there was an overall leave-one-out allocation success of 62.5%, indicating that community composition was a moderate (60%) proxy for bait type. A

similar leave-one-out allocation success was achieved for length measurements (Figure 3.4) at 64.9%, with the first 19 axes explaining 91.0% of the variance ($m = 19$, prop = 91.0%).

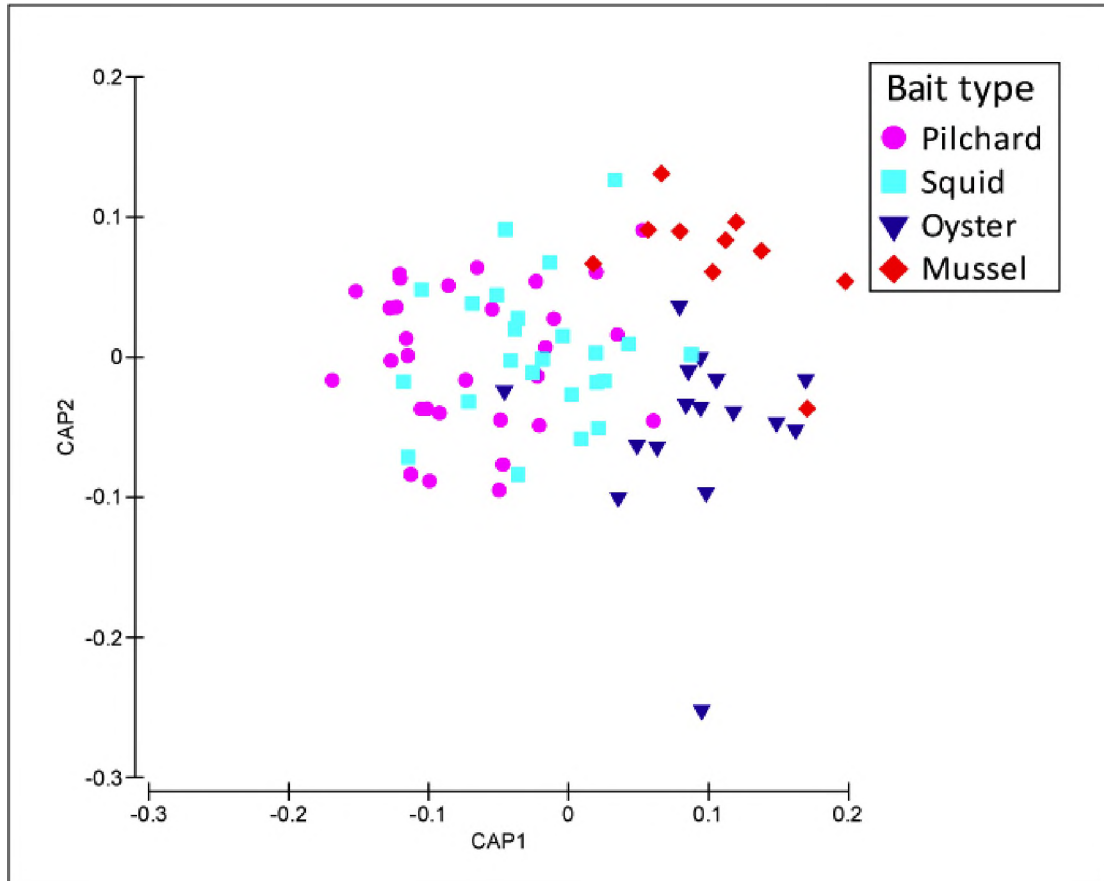


Figure 3.3 Canonical analysis of principle coordinates (CAP) ordination plot of MaxN data of four different bait treatments from stereo-BRUVs sampling in the Agulhas ecoregion.

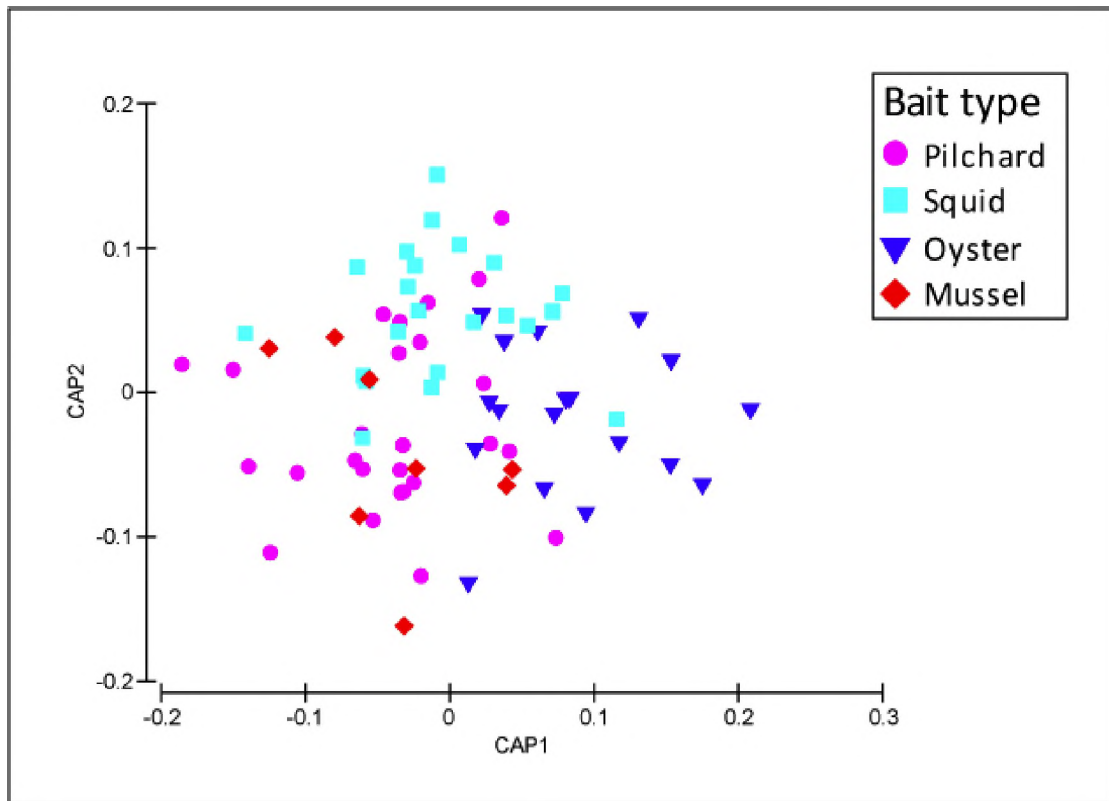


Figure 3.4 Canonical analysis of principle coordinates (CAP) ordination plot of length estimates of four different bait treatments from stereo-BRUVs sampling in the Agulhas ecoregion.

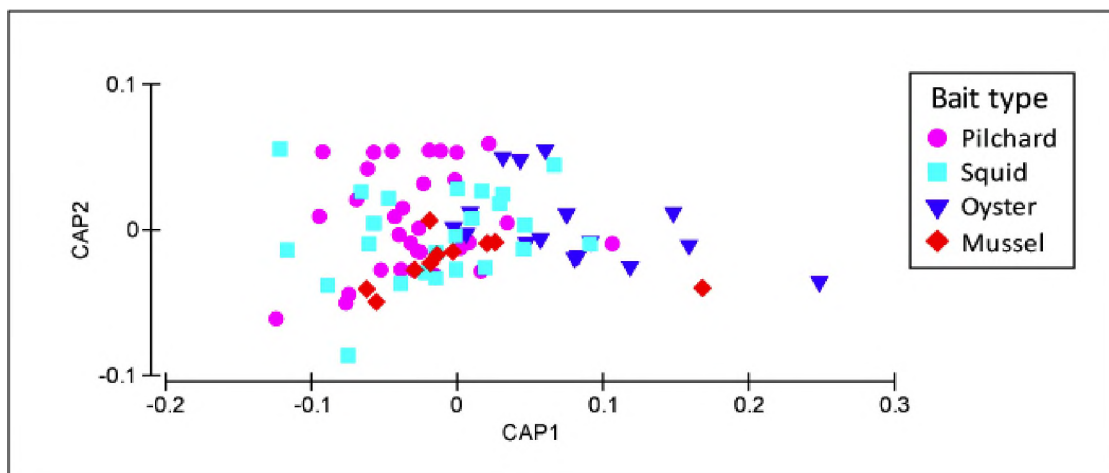


Figure 3.5 Canonical analysis of principle coordinates (CAP) ordination plot of trophic group data of four different bait treatments from stereo-BRUVs sampling in the Agulhas ecoregion.

The CAP analysis showed an overlap of samples collected with the different bait types when examining trophic groups (Figure 3.5). Samples collected with mussel and oyster bait were more distinct from the rest. However, the majority of samples overlapped one another within a similar distribution area for all four bait types. For the data collected, the first four axes explained 83.9% of the variance ($m = 8$, prop = 83.9.0%) and there was a poor leave-one-out allocation success of 43.2%, suggesting that the trophic composition of the attracted fish community was not strongly determined by bait type.

3.4.3 SIMPER

The SIMPER analysis on log (x+1) transformed abundance data, was employed to identify those species that contributed most to the within-sample similarity for each bait treatment (Table 3.7 and 3.8). *Chrysolephus laticeps* and *Boopsoidea inornata*, both fisheries important generalist carnivores, contributed high relative abundances in all samples, particularly for those collected with pilchard, squid and oyster baits (Table 3.7). *Pagellus bellottii natalensis* (Red tjob-tjob) contributed highly when sampling with oyster (Table 3.7). *Gymnocratophus curvidens* (Janbruin) and *Oplegnathus conwayi* (Cape knife-jaw) contributed highly when sampling with mussel (Table 3.7).

Table 3.7 SIMPER (similarity percentage) results comparing the dominant species by abundance for the different bait types. Analysis was conducted on transformed ($\text{Log}(x+1)$) MaxN data and indicates the percentage contribution of the species to the within-group similarity. The colours and sub-script numbers show ranking of species according to highest percentage contribution within each bait type.

Species	Contribution (%)			
	Pilchard	Squid	Oyster	Mussel
<i>Amblyrhynchotes honckenii</i>	3.22	5.73 ₅		
<i>Boopsoidea inornata</i>	13.84 ₃	13.47 ₃	5.17 ₅	10.06 ₃
<i>Cheilodactylus fasciatus</i>			2.15	
<i>Cheilodactylus pixi</i>			2.02	3.94
<i>Chirodactylus brachydactylus</i>	3.9	2.68	17.91 ₃	9.52 ₄
<i>Chrysolephus cristiceps</i>	3.1			
<i>Chrysolephus laticeps</i>	21.01 ₁	24.79 ₁	29.31 ₁	15.12 ₁
<i>Diplodus capensis</i>	4.67	2.51		2.29
<i>Galeichthys feliceps</i>	2.2			
<i>Gymnocrotaphus curvidens</i>				10.06 ₂
<i>Haploblepharus edwardsii</i>	3.69	3.06		
<i>Mustelus mustelus</i>	5	5.19		
<i>Myliobatis aqulia</i>				2.68
<i>Oplegnathus conwayi</i>				6.71
<i>Pachymetopon aeneum</i>	4.94 ₅	4.72		7.16 ₅
<i>Pagellus bellottii natalensis</i>			15.57 ₄	
<i>Poroderma africanum</i>	5.41 ₄	6.3 ₄		
<i>Pterogymnus laniarius</i>	2.64			
<i>Sarpa salpa</i>	2.56	2.36		
<i>Spondyliosoma emarginatum</i>	15.81 ₂	20.53 ₂	18.16 ₂	

Samples collected with squid and pilchard bait types showed similar trophic composition, with samples dominated by generalist carnivores, invertebrate carnivores and benthic invertebrate carnivores (Table 3.8). As expected for the ecoregion, omnivores were poorly represented in the data (Mann 2013). Oyster showed similar ranking of trophic groups to pilchard and squid. However, using mussel ranked invertebrate carnivores as its most abundant trophic group and omnivores as the third, markedly different from the results of pilchard, squid and oyster. Importantly, across all bait types the same fish species were often ranked highest, however, the order of ranking was markedly dissimilar for oyster and mussel compared to squid and pilchard, which were nearly identical.

Table 3.8 SIMPER (similarity percentage) results comparing bait types. Analysis was conducted on transformed (Log(x+1)) MaxN data and indicates the contribution percentages of trophic groups. The colours and sub-script numbers show ranking of species according to highest percentage contribution within each bait type.

Trophic group	Contribution (%)			
	Pilchard	Squid	Oyster	Mussel
Generalist carnivores	61.94 ₁	51.03 ₁	42.22 ₁	36.76 ₂
Invertebrate carnivores	21.66 ₂	25.78 ₂	24.31 ₃	39.86 ₁
Benthic invertebrate carnivores	8.26 ₃	10.29 ₃	31.65 ₂	11.05
Omnivores		8.98		12.33 ₃

3.4.4 Generalized linear models

To accommodate the issues encountered in standardising the bivalve bait type, four different sets of analyses were conducted on the MaxN data. The relevant results are presented per species/group and the detailed results can be found in the appendices at the end of this chapter.

For the results of the most parsimonious models and model selection process for each analysis please refer to Appendices 3.1, 3.2, 3.3 and 3.4.

Table 3.9 Results from the likelihood ratio test (drop1) demonstrating the effect of bait type on MaxN for the dominant species. P-value reported. Analysis 1: all data, all seasons; Analysis 2: oyster, pilchard and squid sampled only in "summer"; Analysis 3: mussel, pilchard and squid sampled only in "winter"; Analysis 4: pilchard and squid bait types sampled both seasons.

Species	Analysis			
	1	2	3	4
<i>Chrysoblephus laticeps</i>	0.000 ***	0.000 ***	0.052	0.155
<i>Cheilodactylidae</i>	0.063	0.489	0.157	0.476
<i>Spondyliosoma emarginatum</i> & <i>Boopsoidea inornata</i>	0.000 ***	0.058	0.005 **	0.120
Catsharks	0.000 ***	0.000 ***	0.003 **	0.807
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	0.001 ***	0.000 ***	0.293	0.142
Fisheries important Species	0.004 **	0.018 *	0.986	0.317

Significance level: "****"<0.001, "***"<0.01, "**"<0.05

Table 3.10 Summary of the generalized linear model (GLM) output showing the effect of bait type on the model fit for the focus species. The values reported include the estimate of MaxN, the Z value (P statistic). Negative estimates and Z-values imply a negative effect of that bait type on the intercept and the P statistic indicates whether or not the effect is significantly different from zero and does not compare bait types. Analysis 1; all data (Model intercept is mussel), all seasons, Analysis 2: oyster, pilchard and squid sampled only in "winter" (Model intercept is oyster), Analysis 3; mussel, pilchard and squid sampled only in "winter" (Model intercept is mussel), Analysis 4; pilchard and squid bait types sampled both seasons (Model intercept is pilchard).

Species	Analysis	Bait types							
		Pilchard		Squid		Oyster		Mussel	
<i>Chrysolephus laticeps</i>	1	0.792	1.86 (0.064)	1.329	3.03 (0.002) **	-0.808	-1.63 (0.104)	-2.363	-2.46 (0.014) *
	2	1.805	3.80 (0.000) ***	2.088	4.49 (0.000) ***	-4.048	-3.28 (0.001) **		
	3	0.577	1.65 (0.100)	0.992	2.39 (0.017) *			-0.247	-0.64 (0.523)
	4	-1.499	-1.49 (0.135)	0.456	1.51 (0.132)				
<i>Cheilodactylidae</i>	1	-1.099	-2.57 (0.010) *	-1.140	-2.40 (0.017) *	-0.957	-2.03 (0.042) *	-1.737	-1.55 (0.122)
	2	-0.263	-0.51 (0.613)	-0.668	-1.23 (0.218)	-3.453	-2.40 (0.016) *		
	3	-0.918	-1.93 (0.054)	-0.626	-1.04 (0.299)			0.521	1.00 (0.317)
	4	-2.134	-2.06 (0.040) *	-0.285	-0.72 (0.470)				
<i>Spondyliosoma emarginatum & Boopsoidea inornata</i>	1	1.014	2.08 (0.038) *	0.715	1.43 (0.153)	-0.897	-1.63 (0.103)	-2.128	-2.27 (0.023) *
	2	1.891	2.27 (0.023) *	1.598	2.01 (0.045) *	-3.014	-2.05 (0.040) *		
	3	1.241	3.52 (0.000) ***	0.723	2.33 (0.020) *			-3.503	2.02 (0.044) *
	4	-2.591	-2.88 (0.004) **	-0.572	-1.55 (0.121)				
Catsharks	1	1.815	3.07 (0.002) **	1.883	3.14 (0.002) **	-0.269	-0.39 (0.700)	-0.334	-0.38 (0.705)
	2	2.477	5.09 (0.000) ***	1.995	4.05 (0.000) ***	-1.815	-3.21 (0.001) **		
	3	1.593	2.54 (0.011) *	2.087	3.15 (0.002) **			-2.116	-3.78 (0.000) ***
	4	1.433	2.90 (0.036) *	0.075	0.26 (0.793)				
<i>Diplodus capensis & Diplodus hottentotus</i>	1	0.291	0.46 (0.644)	-0.385	-0.57 (0.569)	-2.791	-3.18 (0.002) **	-3.948	-2.88 (0.004) **
	2	4.077	5.91 (0.000) ***	1.498	2.08 (0.038) *	-5.074	-4.23 (0.000) ***		
	3	0.869	1.72 (0.089)	0.683	1.09 (0.272)			-0.581	-1.12 (0.262)
	4	-2.749	-2.30 (0.022) *	-0.703	-1.58 (0.114)				
Fisheries important species	1	0.311	0.50 (0.619)	-0.529	-0.72 (0.471)	-2.587	-2.77 (0.006) **	-10.685	-5.06 (0.000) ***
	2	2.849	2.48 (0.013) *	0.884	0.73 (0.463)	-15.487	-4.21 (0.000) ***		
	3	0.014	0.03 (0.977)	-0.949	-0.13 (0.900)			-2.790	-3.18 (0.002) **
	4	-10.749	-4.66 (0.000) ***	-0.615	-1.08 (0.279)				

Significance level: "****"<0.001, "***"<0.01, "**"<0.05

Chrysolephus laticeps

The model selection process identified depth, temperature, percentage water column and bottom type to significantly influence the MaxN or observed abundance of *Chrysolephus laticeps* (see Appendices 3.5, 3.6, 3.7 and 3.8 for a summary of model output). *Chrysolephus laticeps* were observed with all bait types and, depending on bait type, significant differences in the abundance of this species were observed (Table 3.9; Analysis 1, 2 and 3). There were no significant differences in observed abundance of *Chrysolephus laticeps* when comparing samples using pilchard and squid bait types ($p>0.1$) (Table 3.9). Significantly higher abundances of *Chrysolephus laticeps* were observed when samples were collected using pilchard and squid bait types (Table 3.10) suggesting they are relatively good bait types to attract this fisheries important sparid. On the other hand, bivalve baits (oyster and mussel) had less of an effect on the observed abundance of *Chrysolephus laticeps* (Table 3.10).

Cheilodactylidae

In the model selection process bottom type, percentage water column and depth had a significant effect on the abundance of *Cheilodactylidae* (see Appendices 3.5, 3.6, 3.7 and 3.8 for summary of model output). The *Cheilodactylidae* family are benthic invertebrate carnivores, however, species were observed with all bait types. *Cheilodactylidae* showed no significantly different abundances between bait types (Table 3.9). This suggests bait is a poor indicator of abundance for *Cheilodactylidae* species.

Small generalist carnivores

The model selection process identified bottom type, temperature and percentage water column as a significant influence on the observed abundances of small generalist carnivores (i.e. *Spondylisoma emarginatum* (Steentjie) and *Boopsoidea inornata*; see Appendices 3.5, 3.6, 3.7 and 3.8 for summary of model output). The abundance of small generalist carnivores illustrated significant differences between different bait types for all analyses apart from squid versus pilchard bait, suggesting there is no difference in abundance between the two bait types when sampling these species. In the analysis of all bait types (Analysis 1), pilchard sampled a higher abundance of these species whilst mussel sampled fewer and had less of an effect on the observed abundance of small generalist carnivore species (Table 3.10). In

the analysis of summer samples, a higher abundance was sampled with both pilchard and squid bait types, but a significantly smaller abundance was sampled with oyster, indicating it is a poor bait type to sample these ecologically important sparids (Table 3.10).

Catshark species

The model selection process for the catshark data identified bottom type, percentage water column and temperature as significantly influencing their abundance (see Appendices 3.5, 3.6, 3.7 and 3.8 for summary of model output). The abundance of the reef associated catsharks and shysharks (*Haploblepharus edwardsii*, *H. fuscus*, *H. pictus*, *Poroderma africanum* and *P. pantherinum*) was significantly affected by bait type (Table 3.9; Analysis 1, 2 and 3). In the analysis of between pilchard and squid as bait types (Table 3.9; Analysis 4) no significant effect on MaxN for the catshark species was observed. Samples collected with squid and pilchard recorded higher abundances of the species, for all analyses (Table 3.10). Samples collected with oyster and mussel observed lower abundances suggesting they are an ineffective bait type for sampling catshark species (Table 3.10).

Medium invertebrate carnivores

Temperature and bottom type were identified in the model selection process as significantly influencing the observed abundance of medium invertebrate carnivores (*Diplodus capensis* and *Diplodus hottentotus*) (see Appendices 3.5, 3.6, 3.7 and 3.8 for summary of model output). Bait type had a significant effect on the abundance of medium invertebrate carnivores samples (Table 3.9). When pilchard was employed as a bait type a larger abundance of *Diplodus* species was seen (Table 3.10). A significantly smaller abundance was observed when using oyster and mussel as bait types (Table 3.10; Analysis 1). A smaller abundance of these species were sampled in summer when oyster was used as a bait type (Table 3.10). No significant differences in abundance of *Diplodus* species were observed when using mussel bait (Table 3.10), suggesting oyster is a poor bait type to sample medium invertebrate carnivorous fish.

Fisheries important species

The model selection process recognised bottom type, depth, temperature and percentage water column to significantly influence the abundances of the fisheries important species group (see Appendices 3.5, 3.6, 3.7 and 3.8 for summary of model output). Species of

fisheries importance (*Chrysoblephus cristiceps*, *Chrysoblephus gibbiceps*, *Pachymetopon aeneum* and *Petrus rupestris*) showed significant differences in abundance for the differing bait types (Table 3.10; Analyses 1 and 2). When samples were collected using pilchard as a bait type, a significant positive effect on the abundance of these species was observed, whilst fewer fisheries important species were recorded in the samples collected with squid, oyster and mussel (Table 3.10). This suggests oyster, mussel and squid are less effective bait types in comparison to pilchard when aiming to detect species of fisheries importance.

3.4.5 KDEs

The KDE analysis tests for significant differences for both location (difference in mean length) and shape (difference in mode or median in relation to mean) (Langlois *et al.* 2012).

Table 3.11 Kernel density estimation significance tests of raw and standardised length estimates for *Chrysoblephus laticeps*. *Indicates the level of significance (p(permutation)) with: * < 0.05, ** < 0.01, *** < 0.001.

Groups	Raw length data	Standardised length data
Pilchard, Squid	0.106	0.492
Oyster, Pilchard	0.056	0.750
Mussel, Pilchard	0.584	0.240
Oyster, Squid	0.006 **	0.838
Mussel, Squid	0.186	0.266
Mussel, Oyster	0.156	0.071

A significant effect (Table 3.11) of bait type on the location and shape of length frequency data was found between squid and oyster (Figure 3.6), where squid sampled larger *Chrysoblephus laticeps*.

Once data were standardised, and the statistics were sensitive to shape alone, no significant differences were found (Table 3.11). Pilchard and squid as bait types showed no significant differences. This suggests bait type does not influence the shape of length-frequency distributions of *Chrysoblephus laticeps* observed in stereo-BRUVs sampling (Figure 3.6).

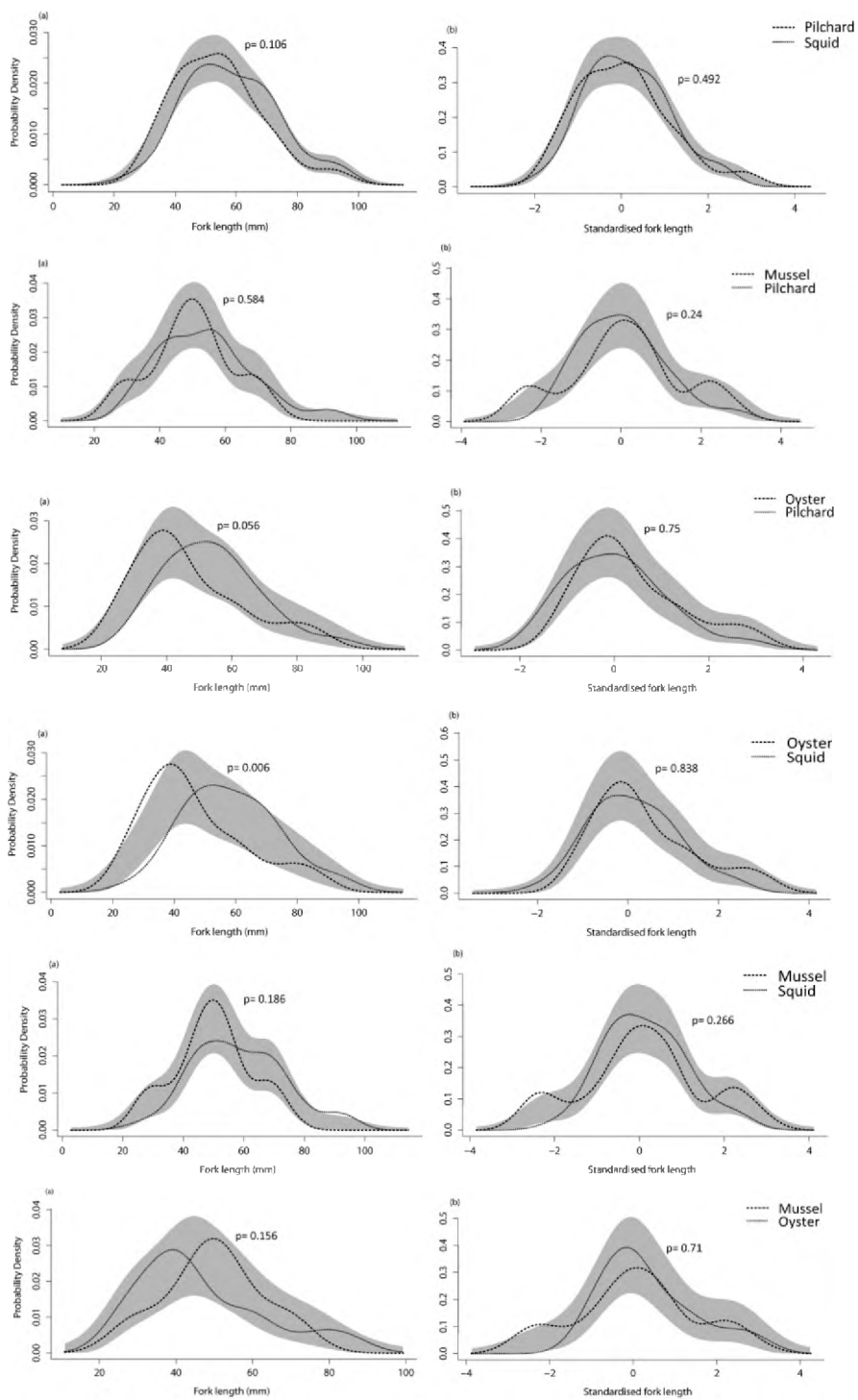


Figure 3. 6 Kernel density estimation (KDE) probability density functions using fork length estimates of *Chrysolephus laticeps* observed with comparable baittypes, for a) raw and b) standardised data. The dashed and dotted lines represent KDE functions. Grey bands represent one standard error either side of the null model of no difference between the KDEs of each bait type (Langlois *et al.* 2012).

3.4.6 Cost-efficiency analysis

Power analysis

For the pilchard bait type the power analysis indicated that 20 samples would be needed to detect an annual 10% growth in abundance of *Chrysoblephus laticeps* over a period of five years with a statistical power of 80% at a significance level of 5%. In contrast, 44 samples were required for squid, and both the oyster and mussel bait types required 36 samples (Table 3.12, Figure 3.12). The power analysis indicated that for effective sampling of the *Cheilodactylidae* population 68 samples would be required when sampling with pilchard as a bait type. Sampling *Cheilodactylidae* with squid as a bait type would require 108 samples. On the other hand, the power analysis estimated that with the oyster bait type 44 samples would be required and with the mussel bait type 58 samples would be required to sample the *Cheilodactylidae* population (Table 3.12, Figure 3.13). This suggests that in terms of number of samples needed, pilchard is the most efficient bait type to adequately sample *Chrysoblephus laticeps*, and oyster is the most efficient bait type to adequately sample *Cheilodactylidae*.

Cost-benefit analysis

Costs were calculated under the assumption of 12 stereo-BRUVs samples per day, at a cost per sample of R318, cost per field day of R4,492 and cost per non-field day (i.e. bad weather day) of R2,514.40 (Appendix 3.5, 3.6). Using the results from the power analysis, the number of sampling days to achieve the required sampling effort for the different bait types was calculated and converted to an overall cost per bait type.

Squid is a very expensive bait type to use in comparison to pilchard, mussel and oyster (Table 3.3). The power analysis indicated that sampling with squid requires the most sampling days (Figure 3.7, 3.8). Results indicate that squid is the least cost-effective bait type (Table 3.12).

Oyster is both the most time- effective and cost-efficient bait type when sampling *Cheilodactylidae*, with pilchard and mussel coming in second (Table 3.12).

When sampling *Chrysoblephus laticeps*, pilchard is both the most effective and cost-efficient bait type (Table 3.12).

Table 3.12 Cost-benefit analysis for different bait types according to the number of samples needed predicted by a power analysis to sample (a) *Chrysolephus laticeps* and (b) *Cheilodactylidae sp.*

(a)	Samples needed	Field days	Non-field days	Bait cost per sample	Total cost
Pilchard	20	2	2	R33.50	R21 042.80
Squid	44	4	4	R85.00	R45 757.60
Oyster	36	3	3	n/a	R32 467.20
Mussel	36	3	3	n/a	R32 467.20

(b)	Samples needed	Field days	Non-field days	Bait cost per sample	Total cost
Pilchard	68	7	7	R33.50	R72 946.80
Squid	108	11	11	R85.00	R120 594.40
Oyster	44	4	4	n/a	R42 017.60
Mussel	68	7	7	n/a	R70 668.80

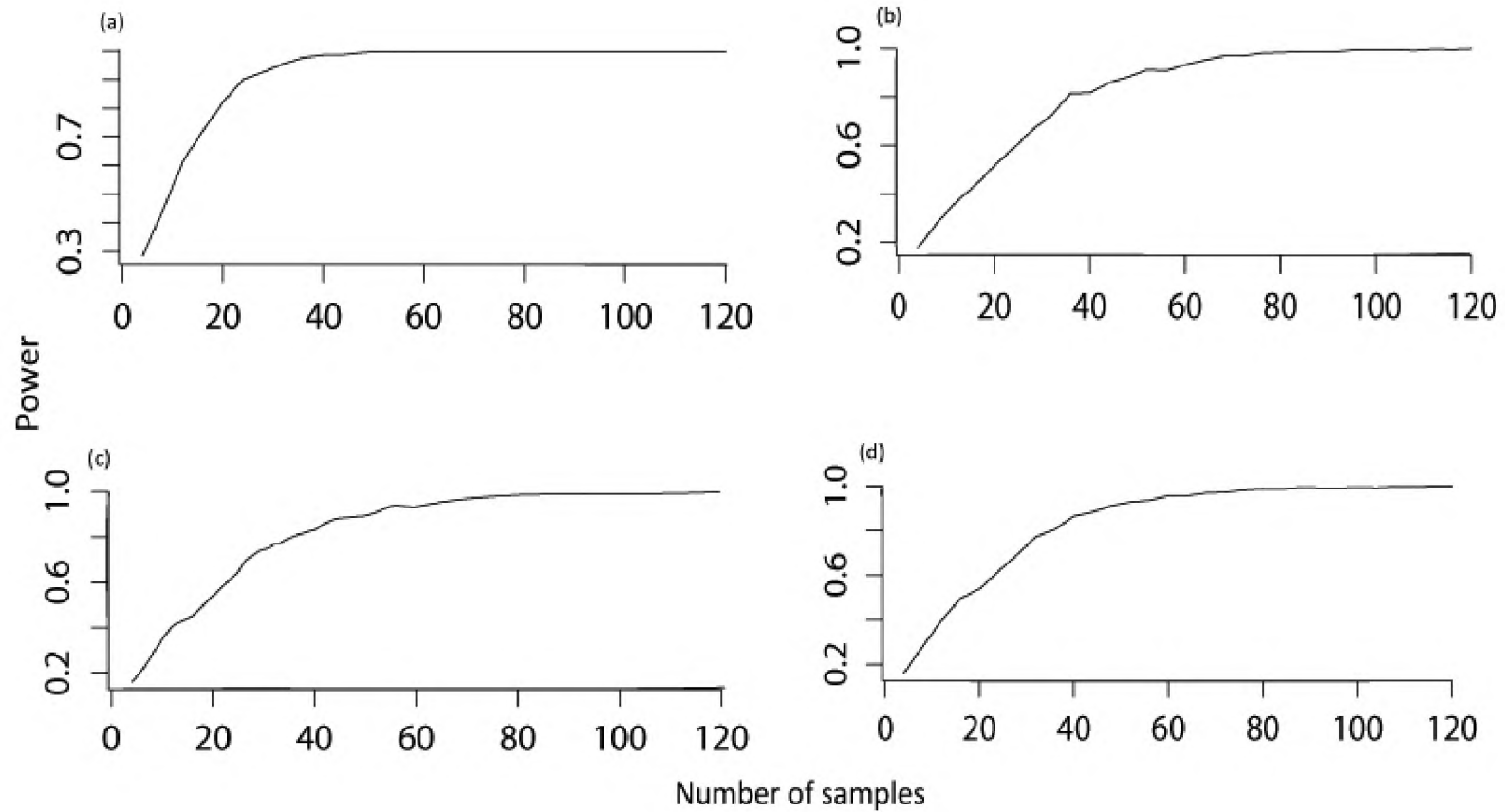


Figure 3.7 Relationship between sample size and statistical power to detect an annual 10% growth in *Chrysolephus laticeps* relative abundance (MaxN) over a period of five years for different bait types, pilchard (a), squid (b), oyster (c), and mussel (d).

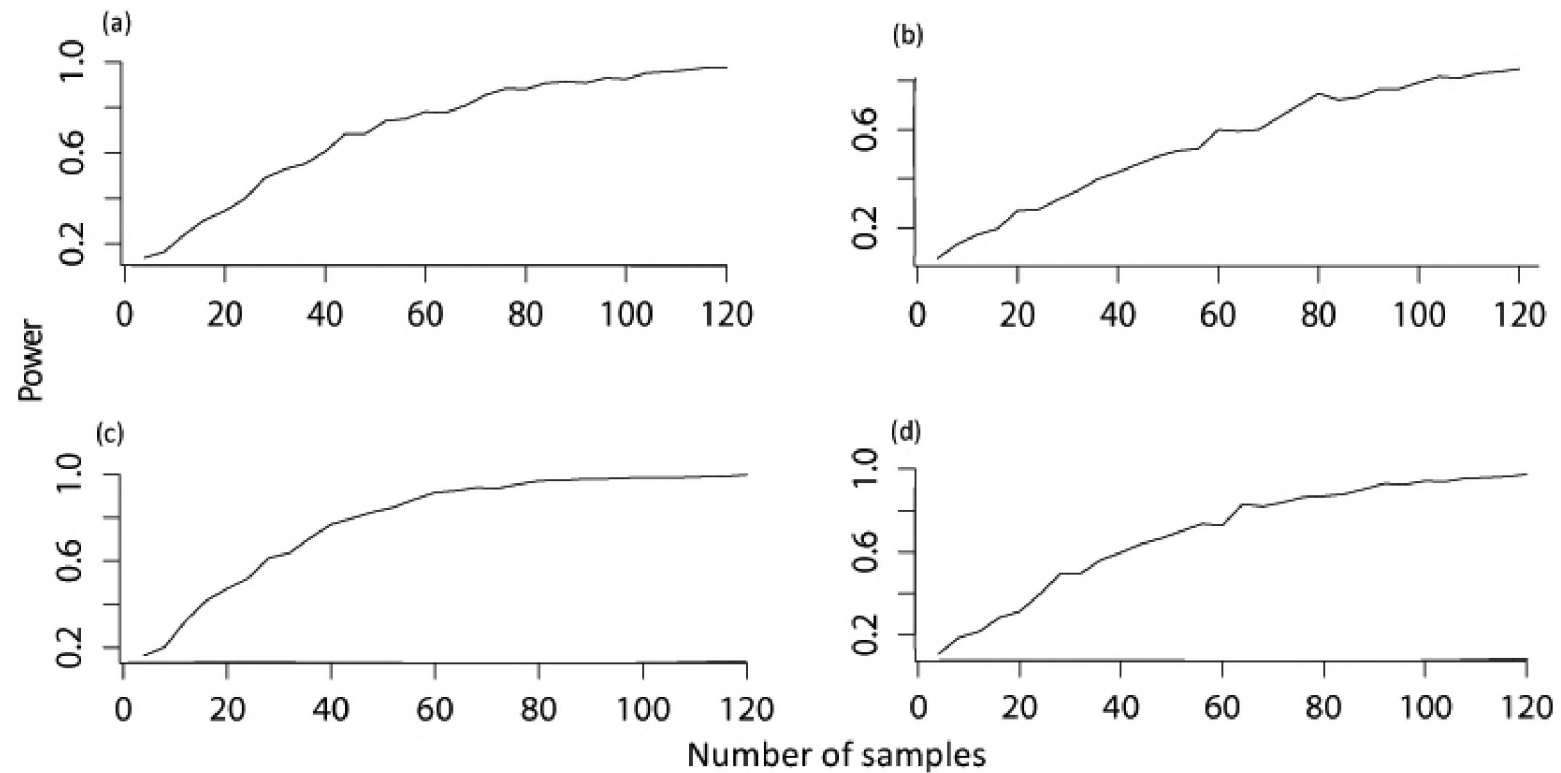


Figure 3.8 Relationship between sample size and statistical power to detect an annual 10% growth in *Cheilodactylidae* relative abundance (MaxN) over a period of five years for different bait types, pilchard (a), squid (b), oyster (c), and mussel (d).

3.5 Discussion

For effective long-term monitoring there is a need for a robust sampling method with an emphasis on data quality and field costs (Cappo *et al.* 2003). Stereo-video is a well-documented and effective sampling method for fish assemblage studies (Watson *et al.* 2005, 2010, Langlois *et al.* 2010, Bernard and Götz 2012, Harvey *et al.* 2012). The method has been developed to use bait attached to the video systems to attract fish into the field of view (Ellis and DeMartini 1995, Priede and Merret 1996, Willis and Babcock 2000, Willis *et al.* 2000, Westera *et al.* 2003, Capo *et al.* 2004, Denny and Babcock 2004, Denny *et al.* 2004, Watson *et al.* 2005, Harvey *et al.* 2007). Sampling collected without bait is inefficient as it requires large sample sizes to reduce high variation in fish assemblage data (Watson *et al.* 2005, Harvey *et al.* 2007, Bernard and Götz 2012). Previous studies demonstrated that the presence of bait is essential for effective sampling (Watson *et al.* 2005, Harvey *et al.* 2007, Bernard and Götz 2012).

Fundamental to the application of bait in remote video sampling is the type of bait employed, as the choice of bait may create bias within the data (Wraith *et al.* 2013). Previous studies in Australia indicated that observed fish assemblages and species richness are strongly influenced by bait type (Wraith *et al.* 2013). This was noted too in this study within the Agulhas ecoregion, with significant differences in the fish assemblages observed with different bait types.

An effective bait type will attract a representative range of species and size classes. In order to quantify this, the performance of a bait type was determined by the number of species and total abundance recorded as well as the ability to consistently detect different species. Pilchard is the internationally accepted standardized bait type (Harvey *et al.* 2007, Watson *et al.* 2010, Mclean *et al.* 2010, 2011, Bassett and Montgomery 2011, Goetze *et al.* 2011, Harvey *et al.* 2012a, Langlois *et al.* 2012a,b, Mallet and Pelletier 2014). It is not only the preferred bait for BRUVs research, but has also historically been preferred for controlled angling in South Africa (Götz *et al.* 2007) and elsewhere (Wolf 1992). Previous work using BRUVs confirmed that oily baits, such as pilchard, were the most effective at attracting fishes (Wraith *et al.* 2013). As was to be expected, pilchard was an effective stereo-BRUVs bait type in the Agulhas ecoregion, with a high species richness and total MaxN recorded. Pilchard showed a significantly positive effect on the presence of species important to local fisheries. Similarly, a controlled angling survey conducted by Götz *et al.* (2007) in the Agulhas ecoregion indicated that fisheries species such as *Cheimereus nufar* and *Pachymetopon aeneum* were more often caught with pilchard or squid as bait compared to an alternative invertebrate bait type.

That being said, squid too proved to be an effective bait type in this study as no significant differences were found between pilchard and squid, both for species richness and length estimates recorded. This suggests that pilchard and squid are potentially effective bait types for stereo-BRUVs surveys in the Agulhas ecoregion. Alternatively, the bivalve baits (mussels and oysters) were significantly less effective, recording fewer species at typically lower abundances.

Oysters naturally occur in the Agulhas ecoregion (Branch *et al.* 2010); as such it was hypothesized that this bait type would attract the species frequently observed, but also those that normally shy away from pilchard bait, such as benthic invertebrate carnivores. The oysters used in this study were farm grown and because of slight aesthetic issues discarded and available at no cost. This made oyster a particularly attractive bait type as they came at no cost and did not have to be harvested from the wild. However, the data analysis indicated oyster was particularly ineffective as a 'generalist' bait type in comparison to pilchard, squid or mussel. This was verified in the CAP analysis where the community sampled with this bait type was markedly different, and the univariate analysis where oyster produced low species richness, total MaxN and average MaxN values. Furthermore, the difficulty in sourcing oysters for the follow-up survey meant that their availability was unreliable, making them a poor option for any research that relies on consistency (i.e. long-term monitoring).

As a result, mussels were used as alternate bivalve bait to oysters. Mussels also occur naturally in the Agulhas ecoregion and form part of the diet of many sparid species (Mann 2013), particularly those that prey upon benthic invertebrates. However, like oyster, mussel proved to be an ineffective bait type in comparison to pilchard and squid. Whilst there were no significant differences, univariate analysis showed mussel produced lower average MaxN values and species richness compared to pilchard and squid. The tendency of mussel to produce low MaxN values in comparison to other bait types was also recorded in the PERMANOVA results. The PERMANOVA illustrated a significant difference between the standardised MaxN of pilchard versus mussel as well as oyster versus mussel. Using mussel resulted in low MaxN for catsharks, small carnivores and fisheries important species (Table 3.10). Although mussel outperformed oyster as a bait type, relative to pilchard, it attracted fewer species and typically at lower abundances.

3.5.1 The influence of environmental covariates

Whilst bait type was identified as a contributing factor to the observed fish assemblage structure, as expected, the GLM results indicated that bottom type, temperature, depth and percentage water column were influential. These are known parameters influential on fish diversity and abundance (Anderson and Millar 2004, Bennett *et al.* 2009, Bernard 2012).

Temperatures during sampling ranged from 9.8°C to 25.1°C. The Agulhas ecoregion is known for localised, wind-driven upwelling events that cause prominent drops in water temperatures (Bernard 2012), as was the case in this study. Previous studies in the TNP MPA also found that water temperature can explain variability in fish abundance (Bernard 2012). Lower abundances of fish associated with colder water may be a result of fish sheltering in caves and crevices (Kerwath *et al.* 2007, Bernard 2012). This could be accounted for as a detectability bias, as fish are present in the survey area, but not available for detection (Elphick 2008, Bernard 2012).

Previous studies have associated fish assemblage structure to bottom and topographic complexity (Choat and Ayling 1987, McCormick 1994, Jenkins and Sutherland 1997, Ault and Johnson 1998, Jenkins and Wheatley 1998, Hyndes *et al.* 1999, O'Hara 2001, Gust *et al.* 2001, Curley *et al.* 2002, Giannoulaki *et al.* 2003, Harman *et al.* 2003, Hyndes *et al.* 2003, Willis and Anderson 2003, Almany 2004, Anderson and Millar 2004, Watson *et al.* 2005, Cappo *et al.* 2007, Moore 2010). Bottom type was found to significantly affect the MaxN for the majority of the focus species. Many of these species are reef dependent (Mann 2013) and therefore it was expected that their abundance would be influenced by the type of reef and profile of the reef as well as the amount of reef cover. Significant differences in MaxN as a result of bottom type were observed for catsharks, small generalist carnivores, medium invertebrate carnivores and fisheries important species. It was expected that many of these species would be observed on reef habitats as reefs provide feeding grounds and protection (Trow 1982, Buxton and Smale 1984, van der Elst 1988, Burger 1990, van der Elst and Adkin 1991, Smith 2005, Mann 2013).

3.5.2 Examining the detectability of trophic groups with different bait types

The use of bait in remote video techniques is said to bias the sampled community towards predatory or scavenging species while at the same time exclude herbivorous and, to some extent, omnivorous species (Harvey *et al.* 2007, Stobart *et al.* 2007, Colton and Swearer 2010, Wraith *et al.* 2013). This has also been observed in studies with baited and unbaited fish traps (Newman 1990, Harvey *et al.*

2007). However, a study conducted by Harvey *et al.* (2007) showed that baiting RUVs was not a deterrent to herbivorous species and attracted more species than the unbaited systems, possibly an effect of fish activity and curiosity. As a result of these contradictory opinions, it was unknown whether different bait types caused differences observed between trophic levels.

The bivalve bait types were tested in this study as it was assumed that they may sample alternate feeding guilds. The use of oyster and mussel as a bait type contributed high abundance percentages of fish species (such as *Pagellus bellottii natalensis*, *Oplegnathus conwayi*, *Gymnocratophus curvidens* and the *Cheilodactylidae* species (Table 3.8)) belonging to previously under sampled trophic groups. This suggests oyster and mussel had the potential to efficiently sample these groups. Mussel sampled significantly different trophic groups compared to oyster (Table 3.6). However, this difference was a result of the oyster bait type failing to effectively sample the fish community producing fewer species and lower abundances. Conclusively, oyster is an ineffective bait type, both as a generalist species bait and as an attractant for alternative species that may not be drawn to oily fish baits.

The mussel bait type recorded higher frequencies and abundances of *Gymnocratophus curvidens* and *Oplegnathus conwayi*. These species are common reef fishes that were under-sampled when using pilchard and squid as bait types. Mussel too recorded high frequencies and abundances of omnivores compared to other bait types. Significant differences and positive effects on small generalist carnivores and fisheries important species were also observed when sampling with mussel bait. However, mussel did not sample significantly different trophic guilds compared to pilchard and squid (Table 3.6). Pilchard and squid showed no significant differences between different focus species and species groups; these groups were developed according to trophic characteristics. This indicates that pilchard and squid attract similar feeding guilds. All bait types contributed highly to the abundance of generalist carnivore and invertebrate carnivore species sampled. This was an expected result as Harvey *et al.* (2007) also identified a significant positive effect of bait on MaxN of piscivores, generalist carnivores, micro-invertebrate carnivores and invertebrate carnivores. Similar results were found in past research at TNP MPA, indicating that from 2012 to present, generalist carnivores and invertebrate carnivores dominated rocky reef fish assemblages (Bernard and Götz 2012). Piscivorous, zooplanktivorous, omnivorous and herbivorous species are not as abundant on reefs in the Agulhas ecoregion. Recording smaller contributions of these species was anticipated. In summation, both squid and pilchard are effective bait types, representative of the trophic guild

structure of the Agulhas ecoregion. While it is possible that the use of mussel bait type may provide data on normally under sampled species, the results were not conclusive.

3.5.3 Effect of bait type on size distributions

Previous BRUVs studies have shown that bait types can sometimes affect the abundance (Dorman *et al.* 2012, Wraith *et al.* 2014) and diversity of fish species observed (Cappo *et al.* 2004, Harvey *et al.* 2007, Watson *et al.* 2010, Wraith *et al.* 2014). However, studies also show that bait type causes little difference in the biomass or size of fish sampled (Dorman *et al.* 2012, Götz *et al.* 2007). However, Götz *et al.* (2007) conducted a controlled angling survey in the Agulhas ecoregion which showed that *Chrysolephus laticeps* caught with red bait were significantly smaller than those caught with pilchard or squid. The study also indicated that pilchard and squid were the preferred bait types for larger reef fish, even for species that were not normally piscivorous (Götz *et al.* 2007). Similar effects of bait on reef fish within South Africa have been reported (Buxton 1984, van der Elst 1993). Kernel density estimation results indicated squid sample larger *Chrysolephus laticeps* than oyster. However, no other significant differences in fish length estimates were found between different bait types in this study. Bait type did not affect overall fish lengths for stereo-BRUVs sampling collected in the Agulhas ecoregion (Table 3.4, 3.6).

Pilchard and squid are seemingly better bait types to effectively sample a more representative selection of the fish assemblage as well as attracting more individuals into the field of view. More fish are observed and more length estimates could be made meaning improved statistical power of the data and higher confidence around results.

3.5.4 A cost-benefit analysis for different bait types

The implementation of MPAs must be accompanied by effective research and monitoring (Cappo *et al.* 2003). Research, whether it is a rapid assessment or long-term monitoring, is often constrained by the need for a balance of field costs and data quality (Cappo *et al.* 2003). Therefore, the most effective and cost-efficient bait type is preferable for monitoring, particularly in the long-term where sustainability is key.

The project management triple is a concept derived from engineering and is used as a measure of project success (Cueller 2010). Globally, economic constraints force a compromise or trade-off between data quality/quantity and costs, known as the project management triple constraint

(Schwalbe 2007, Cueller 2010). The concept assumes that for project success a balance is needed between the three often competing goals of scope (data quality), time (data quantity) and costs (Schwalbe 2007, Cueller 2010). Relating this to sampling with stereo-BRUVs we assume that the more samples are needed, the more time is required to complete the sampling, in turn increasing the costs. Linked to this, the data 'quality' (i.e. its ability to address a projects' research aims) will be determined by the methods ability to sample the target species or community and the consistency at which the species are detected. Using this assumption, one may eliminate bait types according to their inability to sample the target community. This is of primary importance; if data acquired does not meet the objectives even the most cost-effective method is economically inefficient and futile. The number of samples is dependent on the efficacy of the bait, the more samples needed to sample a population effectively the more expensive the operation becomes. Thus, a good bait type must be effective enough such that fewer samples are needed to sample the population efficiently.

The results from this study indicate that both the bivalve bait types tested were not suitable for stereo-BRUVs research and are not cost-effective as a result of being poor amassers of relevant data. On the other hand, pilchard and squid appear to be suitable bait types. Squid is caught primarily off the Eastern Cape coast (Schon *et al.* 2000, Cochrane *et al.* 2014) making it a geographically relevant bait for the Agulhas ecoregion. The same can be said for pilchard with an annual catch of 4,000 tons in the Eastern Cape (WWF SASSI 2015). Furthermore, the 'green' status on the South African Sustainable Seafood Initiative (SASSI) list suggests that both bait types are environmentally sustainable (WWF SASSI 2015). Both bait types can be locally sourced, however, in recent years the South African squid fishery has seen numerous fluctuations in landings as a result of not only natural dips in wild populations, but was also unhinged by changes in the rights of squid fishermen and stock management (Cochrane *et al.* 2014). These fluctuations have caused unstable and often high prices of squid. On average, it is more expensive than most fish on a per-weight basis (Götz *et al.* 2007). Pilchard on the other hand has a much more stable market and is a cheaper bait type. Cost-efficiency is the trade-off between the number of samples needed and the days at sea required. The power analysis showed that in the instance of both *Chrysoblephus laticeps* and *Cheilodactylidae*, more samples are needed when sampling with squid. This makes squid, whilst effective at sampling a wide range of species, an expensive bait type and economically unsustainable. When sampling for *Cheilodactylidae*, pilchard was not the most cost-efficient, due to higher variability in the data, with oyster treatments needing the least number of samples and being the most cost-efficient. Unbaited

RUV sampling by Bernard and Götz (2012) showed similar results to those of oyster when observing *Cheilodactylidae*, needing approximately half the number of samples in comparison to baited surveying. The high data variability caused by pilchard and squid was observed in the CAP analysis and was a determining factor of the power analysis. This is likely to be a result of these bait types attracting more individuals and a larger variety of species, reducing the ability to predict the population. As a result of the small values for MaxN and species richness observed when sampling with oyster relative to other bait types, the amount of samples needed to effectively collect data is smaller than for other bait types. Power analysis was used to determine the optimal sample size to detect an effect, a predefined population change. Power analysis does not determine the effectiveness of attracting a representative fish population compared to other bait types. The inability of oyster to sample the majority of the species present in the area limits its usefulness as a standardised bait type.

3.6 Critical analysis

A shortcoming of this study was the discontinuation of sampling using oyster. Oyster was replaced with mussel in this bait type study as oyster was no longer available for use. Oyster were only sampled within the summer months and mussel, as an alternate sessile bivalve bait type, were only sampled within the winter months. Whilst a preliminary analysis of oyster showed that it was a poor bait type, ideally both oyster and mussel should have been sampled during both the summer and winter trips. Fewer samples and the occurrence of chance events such as 66 *Sarda orientalis* recorded during a single deployment (Appendix 3.9) further distort the results obtained.

Another shortcoming of the bait study was the small sample size with mussel bait type. This was a result of poor sea conditions experienced during winter. Ideally, more samples should have been collected as this may have strengthened observed differences, particularly when attracting species previously under sampled by other methods. However, it is felt that the results still address the research question that was put forward and provide a valuable assessment of the different bait types for sampling reef fish in the Agulhas ecoregion of South Africa.

3.7 Further work

The present study successfully examined the effectiveness of different bait types in the application with stereo-BRUVs in the Agulhas ecoregion; however there is scope for more research. An understanding of the full effect of the bait plume, its dispersion and full impact on fish assemblages

for each bait type was difficult to address. Mussel and oyster are relatively ineffective bait types for stereo-BRUVs research in the Agulhas ecoregion. This poor performance of bivalve baits could be attributed to their different tissue composition in comparison to a significantly oilier bait type such as pilchard. Differences in physical properties, such as composition of the bait type may reduce the size and intensity of the bait plume emitted (Dorman *et al.* 2012). Poor performance of these bait types could also be explained simply in that oyster and mussel are not a popular prey item for the majority of the fishes. This remains a challenge, which is also recognized across the research spectrum (see Priede *et al.* 1991 and Wraith *et al.* 2013). There is a need for experimentation with more alternative bait types or rather bait types in different 'states'. As an example, a prospective bait type in an alternate 'state' is fish oil. Fish oils would be expected to achieve a similar efficiency to pilchard, perhaps more efficient as the plume will be more easily dispersed. Fish oils may be more cost-effective and more feasible, as fish oils are more readily available than the acquisition of fresh or even frozen bait types, particularly in remote locations of Africa. Many fish species undertake ontogenetic migrations into deeper waters as they mature (Mann 2000, Heyns 2015), and the diet of many fish species shifts with age (van der Elst 1993). As an example, adult *Chrysolephus laticeps* favour high profile reefs up to 100m in depth, whilst juveniles favour shallow reefs up to 30m in depth (Mann 2013). This would suggest the need for a similar study of bait types across a broader depth spectrum in order to provide generally applicable results. Furthermore, a study inside and outside of protected areas may be useful as accessibility and abundance of prey are thought to be important determinants in diet and feeding strategies of fishes (Sanchez-Hernandez *et al.* 2011). Some studies have used mixed baits (Ellis and Demartini 1995, Stobart *et al.* 2007, Lowry *et al.* 2012, Misa *et al.* 2013, Wraith *et al.* 2013). As an example, for the bait types examined in this study it is possible that mussel and pilchard would be an effective combination, as mussel as a bait type sampled species that were under-sampled by pilchard and squid. This provides scope for further work using bait combinations to further improve sampling efficiency.

3.8 Conclusion

Long-term monitoring and the application of stereo-BRUVs internationally has sparked the need for a standardized methodology. Here we examined standardization of the bait component of the method. Most existing studies have used pilchards as bait and this study encourages the continuation of this practice. Wraith *et al.* (2013) suggested that the limitations and biases of BRUVs

be assessed in multiple locations so as to improve the utility of the method.

The results suggest that pilchard is the most appropriate bait, in terms of effectiveness as a 'generalist' bait type, cost-efficiency, time-efficiency and availability for the Agulhas ecoregion. It is recommended that pilchard be continued as a primary bait type for stereo-BRUVs research in the Agulhas ecoregion. Dorman *et al.* (2012) illustrate that the use of bait, in the instance of their pilchard study, can discriminate the fish assemblages observed between tropical and temperate regions. As such, it is recommended that preliminary studies be conducted in the sub-tropical areas of South Africa before pilchard can confidently be used across South Africa. Other bait types may be used for specific research questions involving specialist groups, for example *Cheilodactylidae*, *Gymnocratophus curvidens* and *Oplegnathus conwayi* may be better sampled with an alternative bait type such as mussel or oyster.

Overall, pilchard being the most effective bait type for this ecoregion is a favourable result, as it will allow future projects to be comparable to global datasets that employ the same bait type. Fisheries management can be volatile in that local research is often collected without prior efforts to link it within a global context. By increasing the functionality of research conducted within South Africa there is scope to bridge not only the geographic gap, but also the technological gap. Furthermore, this result highlights that caution should be taken when looking for ecological processes using data collected with different bait types.

Appendix

Appendix 3.1a Generalised linear model summary, Analysis one.

Focus species/groups	AIC		Deviance explained	Best fit model
	Best fit model	Full model		
<i>Chrysolephus laticeps</i>	407.87	598.3	43.60%	glm.nb(formula = MaxN ~ Bait type + Depth + Bottom type + % Water column + offset(log(Area))) + Temp
<i>Cheilodactylidae</i>	270.64	296.77	50.75%	glm.nb(formula = MaxN ~ Bottom type + Depth + Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Spondyliosoma emarginatum</i> & <i>Boopsidea inornata</i>	558.83	1542.5	35.32%	glm.nb(formula = MaxN ~ Bait type + Bottom type + % Water column + offset(log(Area))) + Temp
Catsharks	308.51	344.96	37.14%	glm.nb(formula = MaxN ~ Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	242.04	296.18	46.75%	glm.nb(formula = MaxN ~ Bottom type + Temp + % Water column + 1 + Bait type + offset(log(Area)))
Fisheries important species	173.2	193.5	50.14%	glm.nb(formula = MaxN ~ Bottom type + Depth + Temp + 1 + Bait type + offset(log(Area)))

Appendix 3.1b Generalised linear model summary, Analysis one.

Focus species/groups	Results of Drop1 likelihood ratio tests					GLM summary for Best fit model							
	Df	Deviance	AIC	LRT	Pr(>Chi)	Estimate	Std. Error	z-value	Pr(> z)				
<i>Chrysolephus laticeps</i>	Bait type	3	116.131	431.03	31.1642	7.85e-07	***	(Intercept)	-2.363127	0.959092	-2.464	0.01374	*
	Depth	1	100.012	418.91	15.0456	0.0001049	***	Bait typeOyster	-0.807739	0.496249	-1.628	0.10359	
	Bottom type	3	96.803	411.70	11.8360	0.0079667	**	Bait typePilchard	0.791537	0.426774	1.855	0.06364	.
	% Water column	1	89.214	408.11	4.2472	0.0393140	*	Bait typeSquid	1.329494	0.438449	3.032	0.00243	**
	Temp	1	88.673	407.57	3.7066	0.0541979	.	Depth	0.062525	0.015911	3.930	8.5e-05	***
								Bottom typeReef high	0.182439	0.382570	0.477	0.63345	
								Bottom typeReef low	-0.278868	0.370813	-0.752	0.45202	
								Bottom typeSand	-1.219632	0.462483	-2.637	0.00836	**
								% Water column	-0.010101	0.004476	-2.257	0.02401	*
								Temp	0.078186	0.040058	1.952	0.05096	.
<i>Cheilodactylidae</i>	Bottom type	3	96.165	282.03	19.3907	0.000227	***	(Intercept)	-1.736711	1.122563	-1.547	0.12184	
	Depth	1	82.310	272.17	5.5361	0.018628	*	Bottom typeReef high	0.423861	0.426848	0.993	0.32071	
	Temp	1	79.190	269.05	2.4158	0.120118	.	Bottom typeReef low	0.513397	0.413127	1.243	0.21397	
	% Water column	1	84.962	274.83	8.1883	0.004216	**	Bottom typeSand	-1.936002	0.714503	-2.710	0.00674	**
	Bait type	3	84.062	269.93	7.2883	0.063255	.	Depth	0.044104	0.018029	2.446	0.01443	*
								Temp	0.079220	0.048086	1.647	0.09946	.
								% Water column	-0.015916	0.005197	-3.063	0.00219	**
								Bait typeOyster	-0.956729	0.471147	-2.031	0.04229	*
								Bait typePilchard	-1.099109	0.427616	-2.570	0.01016	*
								Bait typeSquid	-1.140016	0.475542	-2.397	0.01652	*
<i>Spondyllosoma emarginatum</i> & <i>Boopsoidea inornata</i>	Bait type	3	111.533	569.64	18.8081	0.0002995	***	(Intercept)	-2.128175	0.938007	-2.269	0.0233	*
	Bottom type	3	101.773	559.88	9.0478	0.0286616	*	Bait typeOyster	-0.896546	0.550517	-1.629	0.1034	
	% Water column	1	95.696	557.80	2.9712	0.0847609	.	Bait typePilchard	1.014213	0.487454	2.081	0.0375	*
	Temp	1	106.584	568.69	13.8597	0.0001970	***	Bait typeSquid	0.715229	0.500466	1.429	0.1530	
								Bottom typeReef high	0.133921	0.467891	0.286	0.7747	
								Bottom typeReef low	0.238904	0.454829	0.525	0.5994	
								Bottom typeSand	-1.011248	0.522191	-1.937	0.0528	.
								% Water column	-0.009391	0.005398	-1.740	0.0819	.
								Temp	0.193416	0.046473	4.162	3.16e-05	***
Catsharks	Temp	1	84.279	307.62	3.110	0.07783	.	(Intercept)	-0.334267	0.882146	-0.379	0.70474	
	% Water column	1	84.789	308.13	3.620	0.05710	.	Temp	-0.072570	0.036588	-1.983	0.04732	*
	Bait type	3	113.794	333.14	32.624	3.865e-07	***	% Water column	-0.009623	0.004840	-1.988	0.04680	*
								Bait typeOyster	-0.269648	0.699242	-0.386	0.69977	
								Bait typePilchard	1.814611	0.590729	3.072	0.00213	**
								Bait typeSquid	1.882906	0.600255	3.137	0.00171	**
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	Bottom type	3	81.748	253.75	19.7059	0.0001953	***	(Intercept)	-3.948207	1.369945	-2.882	0.003951	**
	Temp	1	69.361	245.36	7.3187	0.0068242	**	Bottom typeReef high	-1.784692	0.627860	-2.842	0.004476	**
	% Water column	1	64.226	240.22	2.1830	0.1395455	.	Bottom typeReef low	-0.407625	0.541110	-0.753	0.451262	
	Bait type	3	78.547	250.55	16.5047	0.0008934	***	Bottom typeSand	-3.401067	1.168702	-2.910	0.003613	**
								Temp	0.258351	0.074140	3.485	0.000493	***
								% Water column	-0.010550	0.007305	-1.444	0.148690	
								Bait typeOyster	-2.791388	0.877940	-3.179	0.001475	**
								Bait typePilchard	0.291260	0.631073	0.462	0.644418	
							Bait typeSquid	-0.385081	0.675766	-0.570	0.568784		
Fisheries important species	Bottom type	3	64.592	178.84	13.644	0.003431	**	(Intercept)	-10.68464	2.11265	-5.057	4.25e-07	***
	Depth	1	61.398	179.65	10.451	0.001226	**	Bottom typeReef high	0.76080	0.68113	1.117	0.264006	
	Temp	1	70.133	188.39	19.186	1.186e-05	***	Bottom typeReef low	-0.44728	0.69558	-0.643	0.520206	
	Bait type	3	64.163	178.42	13.216	0.004192	**	Bottom typeSand	-2.01902	1.08496	-1.861	0.062756	.
								Depth	0.10945	0.03183	3.439	0.000583	***
								Temp	0.39691	0.09997	3.970	7.18e-05	***
								Bait typeOyster	-2.58708	0.93544	-2.766	0.005681	**
								Bait typePilchard	0.31054	0.62491	0.497	0.619237	
							Bait typeSquid	-0.52934	0.73338	-0.722	0.470427		

Appendix 3.2a Generalised linear model summary, Analysis two.

Focus species/groups	AIC		Deviance explained	Best fit model
	Best fit model	Full model		
<i>Chrysoblephus laticeps</i>	246.76	409.17	54.14%	glm.nb(formula = MaxN ~ Bottom type + Depth + Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Cheilodactylidae</i>	157.05	167.77	30.70%	glm.nb(formula = MaxN ~ Bottom type + Depth + Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Spondylisoma emarginatum</i> & <i>Boopsoidea inornata</i>	500.28	1476.2	34.05%	glm.nb(formula = MaxN ~ Bait type + Bottom type + % Water column + offset(log(Area)) + Temp)
Catsharks	196.19	210.71	51.32%	glm.nb(formula = MaxN ~ Bottom type + % Water column + 1 + Bait type + offset(log(Area)))
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	152.46	189.1	64.60%	glm.nb(formula = MaxN ~ Bottom type + Temp + 1 + Bait type + offset(log(Area)))
Fisheries important species	90.187	106.32	67.77%	glm.nb(formula = MaxN ~ Bottom type + Depth + Temp + % Water column + 1 + Bait type + offset(log(Area)))

Appendix 3.2b Generalised linear model summary, Analysis two.

Focus species/groups		Results of Drop1 likelihood ratio tests				GLM summary for Best fit model							
		Df	Deviance	AIC	LRT	Pr(>Chi)	Estimate	Std.Error	z-value	Pr(> z)			
<i>Chrysolephus laticeps</i>	Bottom type	3	58.756	248.66	9.9074	0.01937	*	(Intercept)	-4.048119	1.232633	-3.284	0.001023	**
	Depth	1	67.127	261.03	18.2791	1.908e-05	***	Bottom typeReef high	0.152189	0.517317	0.294	0.768613	
	Temp	1	54.775	248.68	5.9261	0.01492	*	Bottom typeReef low	-0.634740	0.522196	-1.216	0.224167	
	% Water column	1	53.975	247.88	5.1271	0.02356	*	Bottom typeSand	-1.470268	0.626292	-2.348	0.018896	*
	Bait type	2	69.080	260.99	20.2319	4.043e-05	***	Depth	0.095759	0.023577	4.061	4.88e-05	***
								Temp	0.115644	0.047866	2.416	0.015693	*
								% Water column	-0.018037	0.006884	-2.620	0.008791	**
								Bait typePilchard	1.804982	0.475299	3.798	0.000146	***
								Bait typeSquid	2.088132	0.465211	4.489	7.17e-06	***
<i>Cheilodactylidae</i>	Bottom type	3	55.102	158.42	9.3662	0.024798	*	(Intercept)	-3.452591	1.436776	-2.403	0.01626	*
	Depth	1	50.734	158.05	4.9976	0.025382	*	Bottom typeReef high	0.783855	0.633900	1.237	0.21625	
	Temp	1	49.494	156.81	3.7584	0.052542	.	Bottom typeReef low	0.474430	0.604159	0.785	0.43229	
	% Water column	1	52.412	159.73	6.6760	0.009772	**	Bottom typeSand	-1.458429	0.846066	-1.724	0.08475	.
	Bait type	2	47.168	152.49	1.4317	0.488788		Depth	0.059403	0.025862	2.297	0.02162	*
								Temp	0.111953	0.056668	1.976	0.04820	**
								% Water column	-0.022040	0.008309	-2.653	0.00799	**
								Bait typePilchard	-0.262772	0.519933	-0.505	0.61328	
								Bait typeSquid	-0.667727	0.541992	-1.232	0.21795	
<i>Spondyllosoma emarginatum</i> & <i>Boopsidea inornata</i>	Bait type	2	96.614	510.02	15.7455	0.0003810	***	(Intercept)	-3.014348	1.000075	-3.014	0.002577	**
	Bottom type	3	88.547	499.96	7.6785	0.0531457	.	Bait typePilchard	1.890963	0.451578	4.187	2.82e-05	***
	% Water column	1	83.716	499.13	2.8479	0.0914905	.	Bait typeSquid	1.597665	0.453152	3.526	0.000422	***
	Temp	1	93.348	508.76	12.4801	0.0004113	***	Bottom typeReef high	0.249068	0.539956	0.461	0.644602	
								Bottom typeReef low	0.360546	0.505649	0.713	0.475824	
								Bottom typeSand	-0.902829	0.592059	-1.525	0.127285	
								% Water column	-0.010405	0.006092	-1.708	0.087655	.
								Temp	0.190442	0.048387	3.936	8.29e-05	***
Catsharks	Bottom type	3	58.567	198.54	10.345	0.01585	*	(Intercept)	-1.814950	0.565198	-3.211	0.00132	**
	% Water column	1	51.063	195.04	2.841	0.09189	.	Bottom typeReef high	0.357722	0.439596	0.814	0.41579	
	Bait type	2	78.374	220.35	30.152	2.835e-07	***	Bottom typeReef low	-0.898746	0.455907	-1.971	0.04869	*
								Bottom typeSand	0.333037	0.521951	0.638	0.52343	
								% Water column	-0.011521	0.006638	-1.735	0.08266	.
								Bait typePilchard	2.477011	0.486451	5.092	3.54e-07	***
								Bait typeSquid	1.995398	0.492150	4.054	5.03e-05	***
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	Bottom type	3	48.980	156.89	12.432	0.006040	**	(Intercept)	-5.07353	1.20051	-4.226	2.38e-05	***
	Temp	1	45.894	157.81	9.346	0.002235	**	Bottom typeReef high	-1.81583	0.62920	-2.886	0.00390	**
	Bait type	2	95.701	205.61	59.153	1.429e-13	***	Bottom typeReef low	-1.55499	0.60365	-2.576	0.01000	**
								Bottom typeSand	-1.83765	0.75903	-2.421	0.01548	*
								Temp	0.16801	0.05895	2.850	0.00437	**
								Bait typePilchard	4.07739	0.68816	5.925	3.12e-09	***
							Bait typeSquid	1.49762	0.71972	2.081	0.03745	*	
Fisheries important species	Bottom type	3	33.056	93.126	10.9397	0.01206	*	(Intercept)	-15.48716	3.68056	-4.208	2.58e-05	***
	Depth	1	25.756	89.827	3.6402	0.05640	.	Bottom typeReef high	1.38680	1.37047	1.012	0.311578	
	Temp	1	40.803	104.874	18.6869	1.54e-05	***	Bottom typeReef low	-1.22065	1.40875	-0.866	0.386230	
	% Water column	1	27.174	91.244	5.0578	0.02452	*	Bottom typeSand	-1.84699	1.79333	-1.030	0.303048	
	Bait type	2	30.153	92.224	8.0372	0.01798	*	Depth	0.13977	0.06691	2.089	0.036724	*
								Temp	0.55150	0.15001	3.676	0.000237	***
								% Water column	-0.03566	0.01547	-2.305	0.021169	*
								Bait typePilchard	2.84989	1.14833	2.482	0.013073	*
								Bait typeSquid	0.88404	1.20369	0.734	0.462679	

Appendix 3.3a Generalised linear model summary, Analysis three.

Focus species/groups	AIC		Deviance explained	Best fit model
	Best fit model	Full model		
<i>Chrysoblephus laticeps</i>	148.65	151.4	38.50%	glm.nb(formula = MaxN ~ Bottom type + 1 + Bait type + offset(log(Area)))
<i>Cheilodactylidae</i>	118.87	123.62	50.02%	glm.nb(formula = MaxN ~ Bottom type + % Water column + 1 + Bait type + offset(log(Area)))
<i>Spondylisoma emarginatum</i> & <i>Boopsoidea inornata</i>	475.84	1476.2	24.30%	glm.nb(formula = MaxN ~ Bait type + Temp + % Water column + 1 + offset(log(Area)))
Catsharks	113.31	123.23	26.69%	glm.nb(formula = MaxN ~ 1 + Bait type + offset(log(Area)))
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	125.92	128.6	46.29%	glm.nb(formula = MaxN ~ Bottom type + 1 + Bait type + offset(log(Area)))
Fisheries important species	81.426	83.163	43.57%	glm.nb(formula = MaxN ~ Bottom type + Depth + 1 + Bait type + offset(log(Area)))

Appendix 3.3b Generalised linear model summary, Analysis three.

Focus species/groups	Results of Drop1 likelihood ratio tests					GLM summary for Best fit model							
	Df	Deviance	AIC	LRT	Pr(>Chi)	Estimate	Std.Error	z-value	Pr(> z)				
<i>Chrysolephus laticeps</i>	Bottom type	3	57.759	160.30	19.6519	0.0002004	***	{Intercept}	-0.2465	0.3862	-0.638	0.52332	
	Bait type	2	44.030	148.57	5.9227	0.0517481	.	Bottom typeReef high	0.3592	0.4172	0.861	0.38924	
								Bottom typeReef low	-0.1605	0.3819	-0.420	0.67427	
								Bottom typeSand	-2.1068	0.6822	-3.088	0.00201	**
								Bait typePilchard	0.5767	0.3500	1.648	0.09945	.
								Bait typeSquid	0.9924	0.4159	2.386	0.01704	*
<i>Cheilodactylidae</i>	Bottom type	3	43.844	126.81	15.9483	0.001162	**	{Intercept}	5.205e-01	5.206e-01	1.000	0.3174	
	% Water column	1	31.185	118.16	3.2892	0.069739	.	Bottom typeReef high	3.079e-01	5.375e-01	0.573	0.5668	
	Bait type	2	31.602	116.57	3.7068	0.156707	.	Bottom typeReef low	3.123e-01	5.516e-01	0.566	0.5712	
								Bottom typeSand	-3.703e+01	2.349e+07	0.000	1.0000	
								% Water column	-1.196e-02	5.904e-03	-2.026	0.0428	*
								Bait typePilchard	-9.182e-01	4.760e-01	-1.929	0.0537	.
							Bait typeSquid	-6.260e-01	6.029e-01	-1.038	0.2991	.	
<i>Spondylisoma emarginatum & Boopsoides inornata</i>	Bait type	2	82.365	476.51	6.6762	0.0355049	*	{Intercept}	-3.502730	1.018876	-3.438	0.000586	***
	Temp	1	90.136	486.28	14.4473	0.0001441	***	Bait typePilchard	1.241197	0.469525	2.644	0.008205	**
	% Water column	1	77.953	474.10	2.2637	0.1324365	.	Bait typeSquid	0.723322	0.481284	1.503	0.132865	.
								Temp	0.261482	0.050251	5.204	1.96e-07	***
								% Water column	-0.007831	0.005559	-1.409	0.158944	.
Catsharks	Bait type	2	43.600	118.95	11.636	0.002974	**	{Intercept}	-2.1163	0.5597	-3.781	0.000156	***
								Bait typePilchard	1.5925	0.6255	2.546	0.010891	*
								Bait typeSquid	2.0871	0.6622	3.152	0.001622	**
<i>Diplodus capensis & Diplodus hottentotus</i>	Bottom type	3	53.412	137.84	19.9214	0.0001762	***	{Intercept}	-5.812e-01	5.176e-01	-1.123	0.2615	
	Bait type	2	35.945	122.38	2.4542	0.2931448	.	Bottom typeReef high	-5.419e-01	6.049e-01	-0.896	0.3703	
								Bottom typeReef low	-7.037e-03	4.972e-01	-0.014	0.9887	
								Bottom typeSand	-3.760e+01	2.364e+07	0.000	1.0000	
								Bait typePilchard	8.697e-01	5.062e-01	1.718	0.0858	.
								Bait typeSquid	6.831e-01	6.218e-01	1.099	0.2719	.
Fisheries important species	Bottom type	3	36.579	83.815	10.3892	0.01553	*	{Intercept}	-2.790e+00	8.774e-01	-3.179	0.00148	**
	Depth	1	31.094	82.329	4.9040	0.02679	*	Bottom typeReef high	7.240e-01	5.840e-01	1.240	0.21507	
	Bait type	2	26.218	75.454	0.0282	0.98601	.	Bottom typeReef low	5.418e-02	5.876e-01	0.092	0.92654	
								Bottom typeSand	-3.604e+01	2.469e+07	0.000	1.00000	
								Depth	6.368e-02	2.825e-02	2.254	0.02418	*
								Bait typePilchard	1.436e-02	5.030e-01	0.029	0.97722	
							Bait typeSquid	-9.496e-02	7.590e-01	-0.125	0.90042	.	

Appendix 3.4a Generalised linear model summary, Analysis four.

Focus species/groups	AIC		Deviance explained	Best fit model
	Best fit mod.	Full model		
<i>Chrysoblephus laticeps</i>	314.16	495.13	25.03%	glm.nb(formula = MaxN ~ Depth + Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Cheilodactylidae</i>	176.1	200.65	18.29%	glm.nb(formula = MaxN ~ Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Spondylisoma emarginatum</i> & <i>Boopsoidea inornata</i>	416.4	1246.9	18.24%	glm.nb(formula = MaxN ~ Bait type + Temp + 1 + offset(log(Area)))
Catsharks	255.01	289.36	10.16%	glm.nb(formula = MaxN ~ Temp + % Water column + 1 + Bait type + offset(log(Area)))
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	197.01	239.46	35.13%	glm.nb(formula = MaxN ~ Bottom type + Temp + % Water column + 1 + Bait type + offset(log(Area)))
Fisheries important species	136.23	141.78	35.13%	glm.nb(formula = MaxN ~ Depth + Temp + 1 + Bait type + offset(log(Area)))

Appendix 3.4b Generalised linear model summary, Analysis four.

Focus species/groups		Results of Drop1 likelihood ratio tests					GLM summary for Best fit model					
		Df	Deviance	AIC	LRT	Pr(>Chi)		Estimate	Std.Error	z-value	Pr(> z)	
<i>Chrysoblephus laticeps</i>	Depth	1	68.597	314.71	4.5415	0.033083	*	(Intercept)	-1.499797	1.004408	-1.493	0.13538
	Temp	1	68.939	315.05	4.8834	0.027116	*	Depth	0.041372	0.020241	2.044	0.04096
	% Water column	1	73.372	319.48	9.3161	0.002271	**	Temp	0.101935	0.045134	2.258	0.02392
	Bait type	1	66.082	312.19	2.0267	0.154556		% Water column	-0.017331	0.005593	-3.099	0.00194
								Bait typeSquid	0.456030	0.302886	1.506	0.13217
<i>Cheilodactylidae</i>	Temp	1	57.353	175.72	3.6150	0.057260	.	(Intercept)	-2.133609	1.036420	-2.059	0.03953
	% Water column	1	61.040	179.40	7.3026	0.006886	**	Temp	0.115947	0.056881	2.038	0.04151
	Bait type	1	54.247	172.61	0.5089	0.475623		% Water column	-0.019922	0.007407	-2.690	0.00715
								Bait typeSquid	-0.285087	0.394208	-0.723	0.46956
<i>Spondyliosoma emarginatum & Boopsoidea inornata</i>	Bait type	1	66.566	414.82	2.4154	0.1201476		(Intercept)	-2.59105	0.90124	-2.875	0.00404
	Temp	1	77.727	425.98	13.5761	0.0002291	***	Bait typeSquid	-0.57190	0.36838	-1.552	0.12055
								Temp	0.26456	0.05278	5.013	5.36e-07
Catsharks	Temp	1	58.878	253.44	2.4318	0.11890		(Intercept)	1.43283	0.68279	2.098	0.0359
	% Water column	1	60.379	254.94	3.9326	0.04736	*	Temp	-0.06747	0.03825	-1.764	0.0777
	Bait type	1	56.506	251.07	0.0600	0.80645		% Water column	-0.01085	0.00524	-2.070	0.0385
								Bait typeSquid	0.07531	0.28707	0.262	0.7931
<i>Diplodus capensis & Diplodus hottentotus</i>	Bottom type	3	63.595	202.78	13.7623	0.003247	**	(Intercept)	-2.749642	1.198040	-2.295	0.0217
	Temp	1	52.864	196.05	3.0311	0.081682	.	Bottom typeReef high	-1.435403	0.790371	-1.816	0.0694
	% Water column	1	51.963	195.15	2.1298	0.144459		Bottom typeReef low	0.287657	0.647718	0.444	0.6570
	Bait type	1	51.994	195.18	2.1609	0.141563		Bottom typeSand	-2.230094	1.259292	-1.771	0.0766
								Temp	0.177452	0.076559	2.318	0.0205
								% Water column	-0.011433	0.008511	-1.343	0.1792
								Bait typeSquid	-0.703486	0.445214	-1.580	0.1141
Fisheries important species	Depth	1	47.969	139.87	7.6351	0.005724	**	(Intercept)	-10.74856	2.30559	-4.662	3.13e-06
	Temp	1	56.881	148.78	16.5476	4.744e-05	***	Depth	0.11422	0.03768	3.031	0.00244
	Bait type	1	41.334	133.23	0.9998	0.317369		Temp	0.40812	0.10284	3.969	7.23e-05
								Bait typeSquid	-0.61497	0.56769	-1.083	0.27868

Appendix 3.5 Breakdown of costs per sample i.e. costs needed for each sample undertaken with stereo-BRUVs, adapted from previous cost analysis by Bernard (2012).

	Species Richness	Total MaxN	Average of MaxN
Pilchard, Oyster	0.0168*	0.0008***	0.03*
Pilchard, Squid	0.8838	0.3264	0.7084
Pilchard, Mussel	0.7538	0.018*	0.3546
Oyster, Squid	0.0074**	0.0048**	0.0476*
Oyster, Mussel	0.0845	0.0186*	0.3636
Squid, Mussel	0.6502	0.07	0.3276

Appendix 3.6 Breakdown of costs once off i.e. once off purchases needed to develop stereo-BRUVs ready for use, adapted from previous cost analysis by Bernard (2012).

Once off					
#	Item	Quantity	Unit	Cost	Total
1	Cameras	8	#	R8 000,00	R64 000,00
2	Wide-angle lenses	8	#	R1 000,00	R8 000,00
3	Housings	8	#	R6 500,00	R52 000,00
4	Synchronizing diodes	4	#	R500,00	R2 000,00
5	Underwater lights				R0,00
6	Frame	4	#	R8 000,00	R32 000,00
7	Calibration cude	1	#	R26 350,00	R26 350,00
8	CAL software	1	licence	R29 750,00	R29 750,00
9	EventMeasure	1	licence	R29 750,00	R29 750,00
10	Rope	600	m	R4,00	R2 400,00
11	Big buoys	4	#	R500,00	R2 000,00
12	Small buoys	4	#	R150,00	R600,00
13	SD cards (8GB)	80	#	R50,00	R4 000,00
14	Micilaneous extras	1	lump	R5 000,00	R5 000,00
				Total	R257 850,00

Appendix 3.7 MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group and species recorded with pilchard bait type from 31 samples.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Acanthistius sebastoides</i>	1	19	9	5	150-243	Small & medium sized generalist carnivore
<i>Amblyrhynchotes honckenii</i>	4	45	34	19	115-211	Small & medium sized generalist carnivore
<i>Argyrozona argyrozona</i>	1	16	6	4	145-307	Dominant large generalist carnivore
<i>Boopsoides inornata</i>	30	68	454	225	108-264	Small & medium sized generalist carnivore
<i>Carcharhinus brachyurus</i>	1	6	4	1	1328	Sharks, skates & rays
<i>Chaetodon marleyi</i>	1	3	1	1	162	Benthic invertebrate carnivore
<i>Cheilodactylus fasciatus</i>	1	23	11	4	156-203	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	3	16	12	5	89-172	Benthic invertebrate carnivore
<i>Cheimerus nufar</i>	8	29	29	18	112-277	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	9	55	54	24	174-351	Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	2	52	34	12	133-463	Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	1	3	1	1	205	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	14	81	202	128	48-487	Dominant large generalist carnivore
<i>Clinus superciliosus</i>	2	6	4	3	131-222	Benthic invertebrate carnivore
<i>Cymatoceps nasutus</i>	1	3	1			Benthic invertebrate carnivore
<i>Dasyatis brevicaudata</i>	1	10	5	2	896-1279	Sharks, skates & rays
<i>Diplodus capensis</i>	12	48	129	63	110-324	Herbivore & omnivore
<i>Diplodus hottentotus</i>	2	32	21	9	114-233	Herbivore & omnivore
<i>Epinephelus marginatus</i>	1	3	1	1	417	Scarce large generalist carnivore
<i>Galeichthys feliceps</i>	3	35	22	18	240-359	Small & medium sized generalist carnivore
<i>Gymnocrotaphus curvidens</i>	2	29	16	7	238-373	Herbivore & omnivore
<i>Gymnura natalensis</i>	1	3	1	1	947	Sharks, skates & rays
<i>Haploblepharus edwardsii</i>	2	45	26	15	252-539	Cryptic reef sharks
<i>Lithognathus mormyrus</i>	2	13	6	3	180-227	Benthic invertebrate carnivore
<i>Mustelus mustelus</i>	3	48	32	19	462-1399	Sharks, skates & rays
<i>Myliobatis aquila</i>	1	10	3	2	520-810	Sharks, skates & rays
<i>Notorynchus cepedianus</i>	1	3	1	1	1699	Sharks, skates & rays
<i>Oplegnathus conwayi</i>	1	23	12	5	215-416	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	25	55	152	76	101-430	Small & medium sized generalist carnivore
<i>Pagellus bellottii natalensis</i>	50	16	91	49	105-222	Small & medium sized generalist carnivore
<i>Parascorpius typus</i>	1	3	1			Benthic invertebrate carnivore
<i>Petrus rupestris</i>	2	19	11	6	254-378	Scarce large generalist carnivore
<i>Porcostoma dentata</i>	2	3	4	2	118-123	Small & medium sized generalist carnivore
<i>Poroderma africanum</i>	5	55	54	36	454-958	Cryptic reef sharks
<i>Poroderma pantherinum</i>	1	6	3	2	549-561	Cryptic reef sharks
<i>Pterogymnus lineatus</i>	5	35	29	20	58-321	Dominant large generalist carnivore
<i>Rhabdosargus holubi</i>	3	23	15	10	138-263	Small & medium sized generalist carnivore
<i>Rhinobatos annulatus</i>	3	3	3	3	630-983	Sharks, skates & rays
<i>Rostroraja alba</i>	1	3	1	2	1666	Sharks, skates & rays
<i>Sorpa salpa</i>	64	35	384	115	111-247	Herbivore & omnivore
<i>Serranus knysnaensis</i>	2	3	2	2	171-188	Small & medium sized generalist carnivore
<i>Sporodon durbanensis</i>	1	3	2			Benthic invertebrate carnivore
<i>Spondyllosoma emarginatum</i>	48	65	739	383	32-294	Small & medium sized generalist carnivore

Appendix 3.8 MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group) and species recorded with squid bait type from 24 samples.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Amblyrhynchotes honckenii</i>	6	42	37	17	109-238	Small & medium sized generalist carnivore
<i>Argyrozona argyrozona</i>	1	4	1			Dominant large generalist carnivore
<i>Atractoscion oequidens</i>	1	4	1	1	1265	Scarce large generalist carnivore
<i>Boopsoida inornata</i>	28	67	135	118	100-337	Small & medium sized generalist carnivore
<i>Carcharhinus brachyurus</i>	1	13	3	2	1440-1709	Sharks, skates & rays
<i>Choetodon marleyi</i>	2	8	3	1	114	Benthic invertebrate carnivore
<i>Cheilodactylus fasciatus</i>	1	29	9	4	119-204	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	1	8	3	1	158	Benthic invertebrate carnivore
<i>Cheimerius nufar</i>	3	21	10	10	159-224	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	3	42	25	17	128-302	Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	5	17	11	8	176-560	Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	1	4	2	2	139	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	26	79	142	120	109-489	Dominant large generalist carnivore
<i>Clinus superciliosus</i>	2	8	5			Benthic invertebrate carnivore
<i>Dasyatis brevicaudata</i>	1	17	7	3	853-992	Sharks, skates & rays
<i>Diplodus capensis</i>	13	38	40	32	105-244	Herbivore & omnivore
<i>Diplodus hottentotus</i>	2	13	7	2	167	Herbivore & omnivore
<i>Epinephelus marginatus</i>	1	4	1	1	194	Scarce large generalist carnivore
<i>Galeichthys feliceps</i>	3	21	9	7	251-337	Small & medium sized generalist carnivore
<i>Gymnocrotaphus curvidens</i>	2	29	12	7	200-315	Herbivore & omnivore
<i>Gymnura natalensis</i>	1	4	1	1	1213	Sharks, skates & rays
<i>Haploblepharus edwardsii</i>	1	42	15	11	235-1426	Cryptic reef sharks
<i>Haploblepharus fuscus</i>	1	8	2	1	607	Cryptic reef sharks
<i>Haploblepharus pictus</i>	1	4	2	1	500	Cryptic reef sharks
<i>Lithognathus mormyrus</i>	1	8	3	1	224	Benthic invertebrate carnivore
<i>Mustelus mustelus</i>	2	46	18	12	761-1568	Sharks, skates & rays
<i>Myliobatis aquila</i>	1	21	7	4	328-1196	Sharks, skates & rays
<i>Oplegnathus conwayi</i>	2	25	7	5	321-604	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	10	46	52	34	117-349	Small & medium sized generalist carnivore
<i>Pachymetopon blochii</i>	1	4	1			Small & medium sized generalist carnivore
<i>Pogellus bellottii natalensis</i>	29	21	58	36	95-270	Small & medium sized generalist carnivore
<i>Petrus rupestris</i>	1	13	4	4	321-463	Scarce large generalist carnivore
<i>Pomodasys olivaceum</i>	125	4	125	42	103-360	Small & medium sized generalist carnivore
<i>Paroderma africanum</i>	4	54	25	18	521-1171	Cryptic reef sharks
<i>Paroderma pantherinum</i>	1	8	3			Cryptic reef sharks
<i>Pterogymnus laniarius</i>	1	8	2	6	154-248	Dominant large generalist carnivore
<i>Rhabdosargus holubi</i>	1	8	3	2	157-162	Small & medium sized generalist carnivore
<i>Sarda orientalis</i>	24	4	24	5	811-935	Piscivores
<i>Sorpa salpa</i>	159	29	264	107	104-283	Herbivore & omnivore
<i>Seriola lalandi</i>	4	8	6	5	848-1114	Scarce large generalist carnivore
<i>Sparodon durbanensis</i>	1	8	2	3	531-1030	Benthic invertebrate carnivore
<i>Spandyllosoma emarginatum</i>	37	75	297	209	38-280	Small & medium sized generalist carnivore
<i>spp.</i>	1	4	2	1	255	
<i>Triakis megalopterus</i>	1	4	1			Sharks, skates & rays

Appendix 3.9 MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group and species recorded with oyster bait type from 16 samples.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Amblyrhynchotes honckenii</i>	1	13	2	2	48	Small & medium sized generalist carnivore
<i>Boopsoidea inornata</i>	8	31	21	14	153-263	Small & medium sized generalist carnivore
<i>Cheilodactylus fasciatus</i>	1	25	4	3	184-191	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	2	25	5	1	133	Benthic invertebrate carnivore
<i>Cheimerius nufar</i>	3	25	7	7	146-224	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	6	63	23	20	126-224	Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	1	6	1			Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	2	6	2	2	127-195	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	6	69	32	19	131-432	Dominant large generalist carnivore
<i>Clinus superciliosus</i>	1	6	1			Benthic invertebrate carnivore
<i>Dasyatis chrysonata</i>	1	6	1			Sharks, skates & rays
<i>Diplodus capensis</i>	7	13	9	3	195-203	Herbivore & omnivore
<i>Galeichthys feliceps</i>	7	6	7	3	309-370	Small & medium sized generalist carnivore
<i>Gymnacrotaphus curvidens</i>	1	6	1	1	301	Herbivore & omnivore
<i>Mustelus mustelus</i>	1	13	2	1	1147	Sharks, skates & rays
<i>Myliobatis aquila</i>	1	6	1			Sharks, skates & rays
<i>Oplegnathus conwayi</i>	2	13	3	3	97-328	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	2	25	5	2	113-136	Small & medium sized generalist carnivore
<i>Pachymetopon blochii</i>	1	6	1	1	75	Small & medium sized generalist carnivore
<i>Pagellus bellottii natalensis</i>	8	44	20	14	121-218	Small & medium sized generalist carnivore
<i>Porcostoma dentata</i>	1	6	1	1	51	Small & medium sized generalist carnivore
<i>Poroderma africanum</i>	2	19	4	1	522	Cryptic reef sharks
<i>Pterogymnus laniarius</i>	2	19	4	3	140-205	Dominant large generalist carnivore
<i>Rhabdosargus holubi</i>	3	19	5	2	108-173	Small & medium sized generalist carnivore
<i>Rostroraja alba</i>	1	6	1	1	733	Sharks, skates & rays
<i>Sarda orientalis</i>	31	13	66	31	195-623	Piscivores
<i>Sarpa salpa</i>	8	13	17	16	139-164	Herbivore & omnivore
<i>Serranus knysnaensis</i>	1	6	1			Small & medium sized generalist carnivore
<i>Spondylisoma emarginatum</i>	33	56	102	54	139-164	Small & medium sized generalist carnivore
<i>Trachurus trachurus</i>	1	6	1			Small & medium sized generalist carnivore
<i>Umbrina robinsoni</i>	1	6	1	1	86	Scarce large generalist carnivore

Appendix 3.10 MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group) and species recorded with mussel bait type from 10 samples.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Amblyrhynchotes honckenii</i>	1	20	4	2	30-202	Small & medium sized generalist carnivore
<i>Boopsoidea inornata</i>	6	60	44	18	152-240	Small & medium sized generalist carnivore
<i>Chaetodon marleyi</i>	1	20	4	1	102	Benthic invertebrate carnivore
<i>Cheilodactylus fasciatus</i>	1	30	6	2	202-246	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	2	50	12	1	150	Benthic invertebrate carnivore
<i>Cheimerius nufar</i>	7	20	16	7	178-233	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	6	60	38	21	75-361	Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	3	30	12	6	189-570	Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	1	30	6	3	160-185	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	7	70	48	22	138-374	Dominant large generalist carnivore
<i>Clinus superciliosus</i>	1	10	2			Benthic invertebrate carnivore
<i>Dasyatis brevicaudata</i>	1	10	2	1	974	Sharks, skates & rays
<i>Diplodus capensis</i>	3	40	12	5	195-974	Herbivore & omnivore
<i>Diplodus hottentotus</i>	2	30	8	4	176-412	Herbivore & omnivore
<i>Gymnacrotaphus curvidens</i>	2	70	20	9	197-319	Herbivore & omnivore
<i>Myliobatis aquila</i>	3	20	6			Sharks, skates & rays
<i>Oplegnathus conwayi</i>	6	60	26	11	113-455	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	8	60	40	18	91-293	Small & medium sized generalist carnivore
<i>Petrus rupestris</i>	1	10	2	1	387	Scarce large generalist carnivore
<i>Poroderma africanum</i>	1	30	6	2	659-849	Cryptic reef sharks
<i>Poroderma pantherinum</i>	1	10	2	1	573	Cryptic reef sharks
<i>Pterogymnus lineatus</i>	1	10	2	1	189	Dominant large generalist carnivore
<i>Sarpa salpa</i>	1	10	2			Herbivore & omnivore
<i>Seriola lalandi</i>	1	10	2	1	1225	Scarce large generalist carnivore
<i>Spondylisoma emarginatum</i>	12	80	82	34	96-213	Small & medium sized generalist carnivore
<i>spp</i>	1	10	2			

Chapter 4

The effect of lighting on the reef-fish
assemblage structure observed with
baited remote underwater stereo-video

4.1 Introduction

To effectively manage resources, knowledge on the depth related patterns in fish assemblage composition, abundance and size structure and their vulnerabilities are required (Johnson *et al.* 2012, Heyns 2015). Comprehensive ecological research on continental shelves has been hindered by high costs, a paucity of suitable methods, and a lack of standardization across geographic and depth gradients (Murphy and Jenkins 2010, Johnson *et al.* 2012).

To date, only limited fisheries independent research on reef ecosystems in South Africa, particularly within MPAs, has been conducted at depths greater than 30m. In part, this is because established sampling techniques, such as SCUBA based UVC are restricted to shallow water depths for safety reasons (Heyns 2015). Methods used for deep-water sampling such as controlled angling and trawl surveys often result in mortality of fish and destruction of habitats (Bernard 2013, Murphy and Jenkins 2010, Harvey *et al.* 2012, Langlois *et al.* 2012b, Kerwath *et al.* 2013). The destructive impacts on the fish assemblage and fish habitats make these methods unsuitable for standardized sampling across MPAs and fishing areas. Some deep reef research has been conducted with ROVs or manned submersibles (Heyns 2015). Typically, however, such studies sample reefs deeper than 150m (Heyns 2015, Stein *et al.* 2005, Sink *et al.* 2006, Colaço *et al.* 2013). As such, there remain significant knowledge gaps of subtidal reef communities, in particular between 30 and 150m of water depth (Heyns 2015, Sink *et al.* 2006, Karpov *et al.* 2006, Love & Schroeder 2007).

Many reef fish species, including species of commercial importance, undertake ontogenetic migrations, and occupy broad depth ranges during the course of their lives (Collins and McBride 2010). In South Africa, this is true for most of the important reef dwelling species targeted by the line fishery, with some species occurring throughout wide depth ranges of up to 200m (Mann 2000). It has been suggested that depth can play a role as a natural refuge for fishes because some fisheries tend to target shallower hard substrates (Goetze *et al.* 2011). However, as inshore resources become scarce, increased fishing pressure is being imposed on fish stocks in deeper waters further offshore (Heyns 2015, Morato *et al.* 2006, Watson & Morato 2013). Notably, the life history characteristics of the targeted slow growing deep-sea fishes make them particularly vulnerable to overexploitation (Norse *et al.* 2012).

There is a need to implement a suitable comprehensive and cost-effective method for monitoring

(See Chapter 1 for detailed analysis of suitable monitoring method characteristics). Importantly, more research is needed on deep-water habitats so as to obtain an understanding of these habitats and their current threats. In addition, to effectively sample a population, the research and monitoring need to cover the full distribution of the target fish species in a standardised fashion, such that data are comparable.

Baited remote underwater stereo-video systems are gaining recognition as a highly suitable, and easily standardised method for monitoring reef fish assemblages throughout their depth distribution and across geographic boundaries (Harvey *et al.* 2004, 2012a, 2012b, Watson *et al.* 2005, 2010, Langlois *et al.* 2010, 2012b). Although stereo-BRUVs have been recognised as an effective fish assemblage sampling tool, deep-water sampling is not simple. Apart from logistical difficulties to collect suitable data at depth, low ambient light levels further compromise the usability and comparability of the collected data. This is particularly true for the Agulhas ecoregion of South Africa, where high water turbidity results in a shallow photic zone, with little to no light penetrating below 50 - 80m of water depth (Heyns 2015). To effectively collect video samples from the sub-photic zone the sampling site needs to be illuminated with artificial lighting. This facilitates the processing of the video footage when using low-cost, off-the-shelf camcorders without low light capabilities. To ensure a true understanding of fish communities and populations, sampling must, as best as possible, be conducted without altering fish behaviour with lighting (Widder *et al.* 2005). It is therefore necessary to understand how lighting influences the observed fish assemblages.

4.1.1 The effect of lighting on fish

Different colour lighting, characterised by differing wavelengths, are known to have varied effects on fish during deep-water sampling (Widder *et al.* 2005, Raymond and Widder 2007). Fish are known to exhibit phototactic behaviour, i.e. movement as a response to lighting (Fitzpatrick 2010). Consequently, fish assemblage composition can be affected by artificial lighting (Herring *et al.* 1999, Widder *et al.* 2005). Some fish are attracted by artificial lighting and some fish are repelled or shy away (Herheijen 1958, Forward 1988, Widder *et al.* 2005, Fitzpatrick *et al.* 2013). Phototactic behaviour is attributed to the presence or absence of lighting as well as the specific wavelengths (colours) of the light (Widder *et al.* 2005, Raymond and Widder 2007). These behavioural responses are thought to be a result of species-specific characteristics and adaptations to the environments where the fish are found (Bowmaker 1990, Downing and Litvak 2001, Fitzpatrick 2010).

The sensitivity of a fish to a specific light colour is determined by the structure of their eyes and their ability to process particular wavelengths via photoreceptors (Bowmaker 1995, Warrant 2004, Fitzpatrick *et al.* 2013). Reactions to light and light colour may be species-dependent. For example, deep-water fish species and nocturnal fish species have developed structural and physiological adaptations that allow for increased detection of prey in low light habitats (Warrant 2004, Fitzpatrick *et al.* 2013). These species have increased proportions of rods to cones, with rods being the photoreceptors that are active in low light levels as they are sensitive to all wavelengths (Von der Emde *et al.* 2004, Fitzpatrick *et al.* 2013). Cones are the photoreceptors active in bright light levels and allow for colour vision and, in turn diurnal fish species have more cones than rods (Von der Emde *et al.* 2004, Fitzpatrick *et al.* 2013). One may assume that the effect lighting has on fish species may be reduced if the colour is out of the spectral sensitivity range (Fitzpatrick *et al.* 2013) as many fish species rely on vision as a primary source of sensory information (Guthrie and Muntz 1993, Utne-Palm 2000).

4.1.2 White lighting

In the past, nocturnal and deep-water fish observations have primarily been conducted with bright incandescent white lighting (Widder *et al.* 2005). However, bright white lighting (i.e. light covering the full colour spectrum) is known to damage eyes and change the normal behaviour of fish (Widder *et al.* 2005). This may influence the detectability and abundance patterns of fish (Widder *et al.* 2005). For example, mobile, swimming animals have exhibited varied behaviours when exposed to white lighting, with some swarming around the lights (Fitzpatrick *et al.* 2013) while others stayed away (Verheijen 1958, Forward 1988, Widder *et al.* 2005). Sedentary animals were seen to 'shrink back' or to 'cease their normal activities' in the presence of white lighting (Herring *et al.* 1999, Widder *et al.* 2005). The change of normal behaviour, whether it be related to attraction and/or intimidation of fish, makes white an unsuitable lighting colour dependent on intensity for monitoring fish populations as it will impart a consistent bias on the data (Edgar *et al.* 2004, Fitzpatrick *et al.* 2013).

4.1.3 Red lighting

Fish, particularly non-target species, are less likely to be deterred when sampled with red lighting than with white lighting (Fitzpatrick 2013). Infrared lighting has been successfully applied in

terrestrial ecosystems to investigate the nocturnal behaviour of animals (Widder *et al.* 2005). Under terrestrial conditions, infrared lighting has proved to sufficiently illuminate an area, whilst at the same time being non-disruptive, with no noticeable effects on animal behaviour (Widder *et al.* 2005). However, infrared lighting is less effective in water as it attenuates rapidly and at 700nm, infrared light can only penetrate 1.5m into the water column (Matsuoka *et al.* 1997, Widder *et al.* 2005). For effective sampling with stereo-BRUVs the ideal visible distance would be 5m and further. Red lighting works similarly, but attenuates less rapidly than infrared light (Fitzpatrick *et al.* 2013). As expected, red lighting, in comparison to blue or white lighting, is the least disruptive, and is recommended for behavioural studies or stock assessments of deep-water fishes that experience low levels or no natural light (Widder *et al.* 2005, Raymond and Widder 2007). This is because the longer wavelengths associated with red lighting are thought to be outside the visual sensitivity of many fish species (Fitzpatrick *et al.* 2013). However, the longer wavelength means that red light dissipates rapidly, resulting in attenuation of the lighting and a smaller area of illumination (Widder *et al.* 2005). Cameras are available that can operate under low light conditions which could allow for fish to be counted and measured under red light, but they are considerably more expensive than off-the-shelf alternatives (Widder *et al.* 2005). Therefore, whilst red lighting is less disruptive, it is not effective enough for standard stereo-BRUVs and sampling is not economically feasible should specialty cameras be used. Recently, blue lighting has been suggested as an effective alternative when sampling with underwater video (Fitzpatrick *et al.* 2013).

It is important to note that while red lighting may be less disruptive to the normal behaviour of fish, in comparison to white and blue lighting, it may be associated with its own set of biases (Widder *et al.* 2005). For example, Widder *et al.* (2005) noted that when presented with bait illuminated with red lighting, fish were seemingly attracted to the bait in a reactionary response to feed, which overrode the aversion to lighting. This resulted in fish swimming into the illuminated area, due to the bait attraction, but subsequently fleeing in an apparent negative response to the red lighting (Widder *et al.* 2005).

4.1.4 Blue lighting

The available evidence suggests that rods and cones in the eyes of deep-water fishes, restricted to the sub-photic zones, are more sensitive to the shorter wavelengths associated with blue lighting (Partridge *et al.* 1988, Lythgoe *et al.* 1994). However, nocturnal fish studies in shallower sub-photic environments have indicated that blue lighting has little influence on the observed abundance of the dominant fisheries species (Fitzpatrick *et al.* 2013). Research has shown that many coastal fish

species possess a visual spectrum sensitive to wavelengths of around 500nm (Fitzpatrick 2013), although some species do differ and have shown to possess UV vision (10-400nm) (Zamzow *et al.* 2008). Blue lighting falls between the 420-470nm spectrum and attenuates slowly in water, in comparison to red or infrared lighting. As the blue light can travel further, a larger area can be illuminated. This is beneficial to detection, correct identification, counting and measuring of fish from stereo-BRUVs samples (Fitzpatrick *et al.* 2013, Harvey *et al.* 2012a, 2012b). It is apparent that for effective stereo-BRUVs sampling in sub-photic environments a compromise is needed to ensure adequate illumination of the reef, while at the same time limiting the sampling bias for fish species deterred by the selected lighting colour. From the available information, red lighting would be the best choice due to its short wavelength, which allows for the least obtrusive form of observation (Widder *et al.* 2005). However, red lighting does not provide an adequate area of illumination to make for feasible cost-effective sampling with off-the-shelf video cameras (Widder *et al.* 2005). On the other hand, the disruptive nature of white lighting on fish behaviour makes it an undesirable choice too. Although previous research suggests that blue lighting does disrupt the normal behaviour of fish, its short wavelength reduces the light attenuation, thereby illuminating a sufficiently large enough area for cost-effective stereo-BRUVs sampling (Widder *et al.* 2005). This is because the larger illuminated area increases the probability of detecting fish, thereby reducing the potential variability between the samples and increasing the effectiveness of the sampling method. It is possible that this makes blue lighting an economically feasible choice whilst limiting the disturbances to fish behaviour during sampling.

This study was designed to analyse the effect of blue lighting on fish assemblage structure recorded from stereo-BRUVs. Lighting is particularly important when sampling deeper sites as the absence of natural light make it a prerequisite. Whilst sampling without lighting is possible in shallow waters the use of lighting in deep-water is unavoidable. This creates a potential inconsistent sampling bias between data collected from photic (where lighting is not used) and sub-photic (where lighting is needed) habitats which may confound comparisons.

It is therefore necessary to determine how artificial lighting influences the assemblage structure of reef fish. This can be done via a field experiment that directly compares the composition, abundance and size structure of the fish assemblages sampled by stereo-BRUVs collected with and without lighting.

As the use of lighting is unavoidable in sub-photic environments it can be argued that a standardised stereo-BRUVs sampling method must always include lighting, irrespective of the ambient light levels. Following this, the comparative field experiment can only be conducted in the photic zone. Under this design the null hypothesis would be that lighting has no effect on fish assemblage structure. Acceptance of the null hypothesis would indicate that there is no need for lighting when sampling in the photic zone, while failure to accept the null hypothesis would indicate that lighting is necessary regardless of ambient light levels and water depth.

4.2 Aim

It is thus the aim of the research to:

Identify the effect of lighting employed with stereo-BRUVs on the population structure of observed reef fish typical to photic reefs on the southeast coast of South Africa.

Specific objectives

1. Establish the optimal lighting colour for effective low light condition sampling with stereo-BRUVs from a desktop study of previously published and unpublished works.
2. Conduct a comparative field experiment to measure the effect of lighting on the fish community structure (composition, abundance and size structure) detected on photic zone reefs, by comparing stereo-BRUVs samples illuminated by the optimal lighting colour (from 1.) against samples under ambient light conditions.

4.3 Methods

4.3.1 General stereo-BRUVs methodology

Baited remote underwater stereo-video systems were deployed according to standardised methods described in Chapter 2. Following the results from Chapter 3, standardised bait consisting of 0.8-1 kilogram crushed pilchard was used and refreshed for each sample.

4.3.2 Lighting

Lighting systems were made available from the University of Western Australia. Each system consisted of a light emitting diode (LED) panel with seven Cree XLamps XP-E LEDs that each delivered a radiant flux of 350-425 mW. Royal Blue LEDs emitting a wavelength ranging from 450-465 nm were used. The LED panels were powered by 12 V batteries and were contained within an aluminium housing. The housing was mounted in between the two video cameras of the stereo-BRUVs and positioned to illuminate the overlapping field of view (Figure 2.1).

4.3.3 Study site

Sampling took place to the east of Tsitsikamma in Algoa Bay (Figure 4.1). Data was collected over two sampling trips within Algoa Bay (Figure 1), one at Riy Banks reef (Figure 4.2) and the other at Bird Island MPA (Figure 4.3).

Algoa Bay is characterised by the large metropolitan area of Port Elizabeth with over 1.2 million inhabitants and two commercial ports. In contrast to TNP MPA (See Chapter 3), Algoa Bay contains multiple management zones with two small MPAs, namely Bird Island (BI) and Sardinia Bay, leaving the remaining reef habitat open to fisheries exploitation (Chalmers 2012, Griffiths *et al.* 2010, Thompson 1913).

The BI MPA (Figure 4.3) consists of a group of islands (Bird Island, Seal Island, Stag Island and Black Rocks) and the surrounding waters (Chalmers 2012). The MPA encompasses 70.1km² with Bird Island sitting in the centre. Located 53km due east of Port Elizabeth and 10km offshore from the Woody Cape headland, the MPA is a seaward extension of the Greater Addo Elephant National Park (Chalmers 2012). Declared for biodiversity conservation in 2004, the BI MPA is one of South Africa's youngest no-take MPAs (DEAT 2004, Chalmers 2012).

Riy Banks reef complex (Figure 4.2) is a rocky bank at the southwestern entrance of Algoa Bay, approximately 22km offshore from Port Elizabeth (Figure 4.1). The reef starts at a depth of 12m and extends to a water depth of 80m. As opposed to the protected BI MPA, Riy Banks is heavily exploited by the commercial and recreational line fisheries (Chalmers 2012).

Both reef systems support similar fish assemblages (Chalmers 2012). They provide a continuum of reef habitats representing both a protected and an exploited reef. This allows for a comprehensive study on the effect of lighting on the fish assemblages occurring on the Algoa Bay photic reef systems.

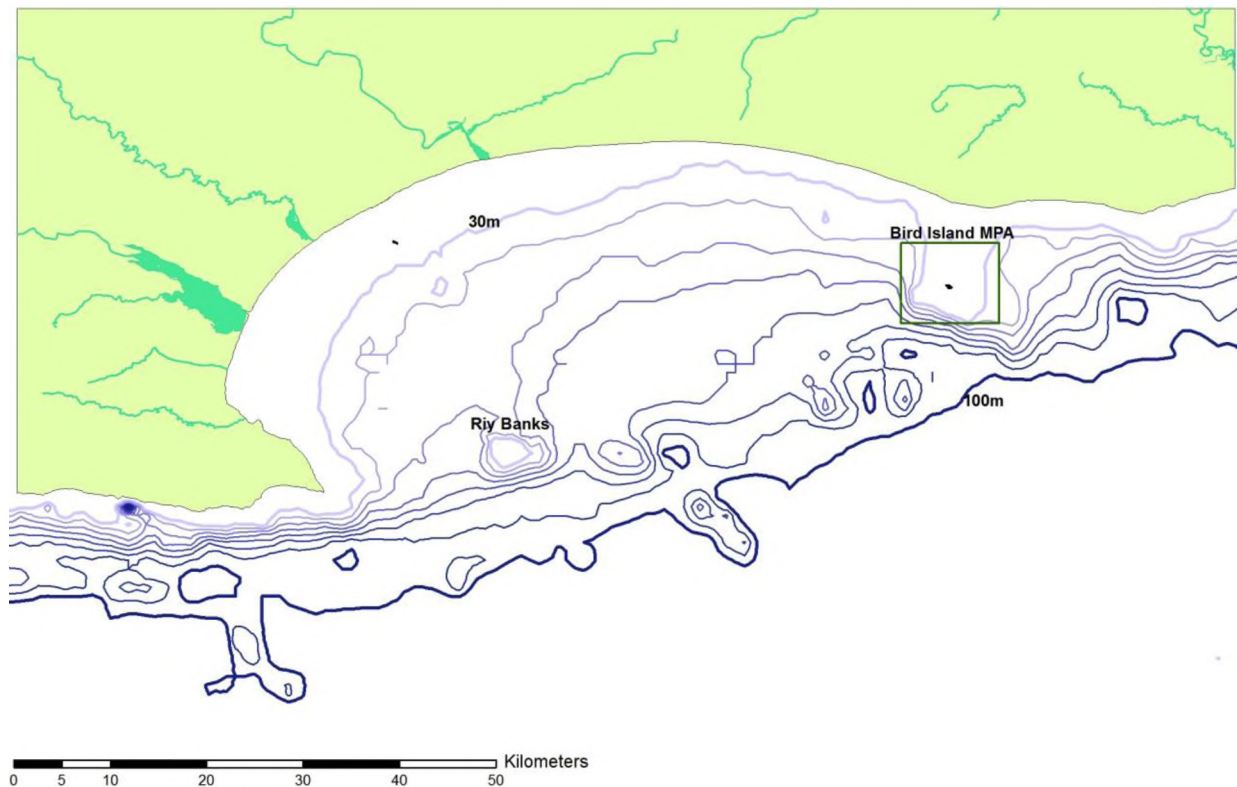


Figure 4.1 Bathymetric map of Algoa Bay, which includes the sampling site of Riy Banks and Bird Island marine protected area.

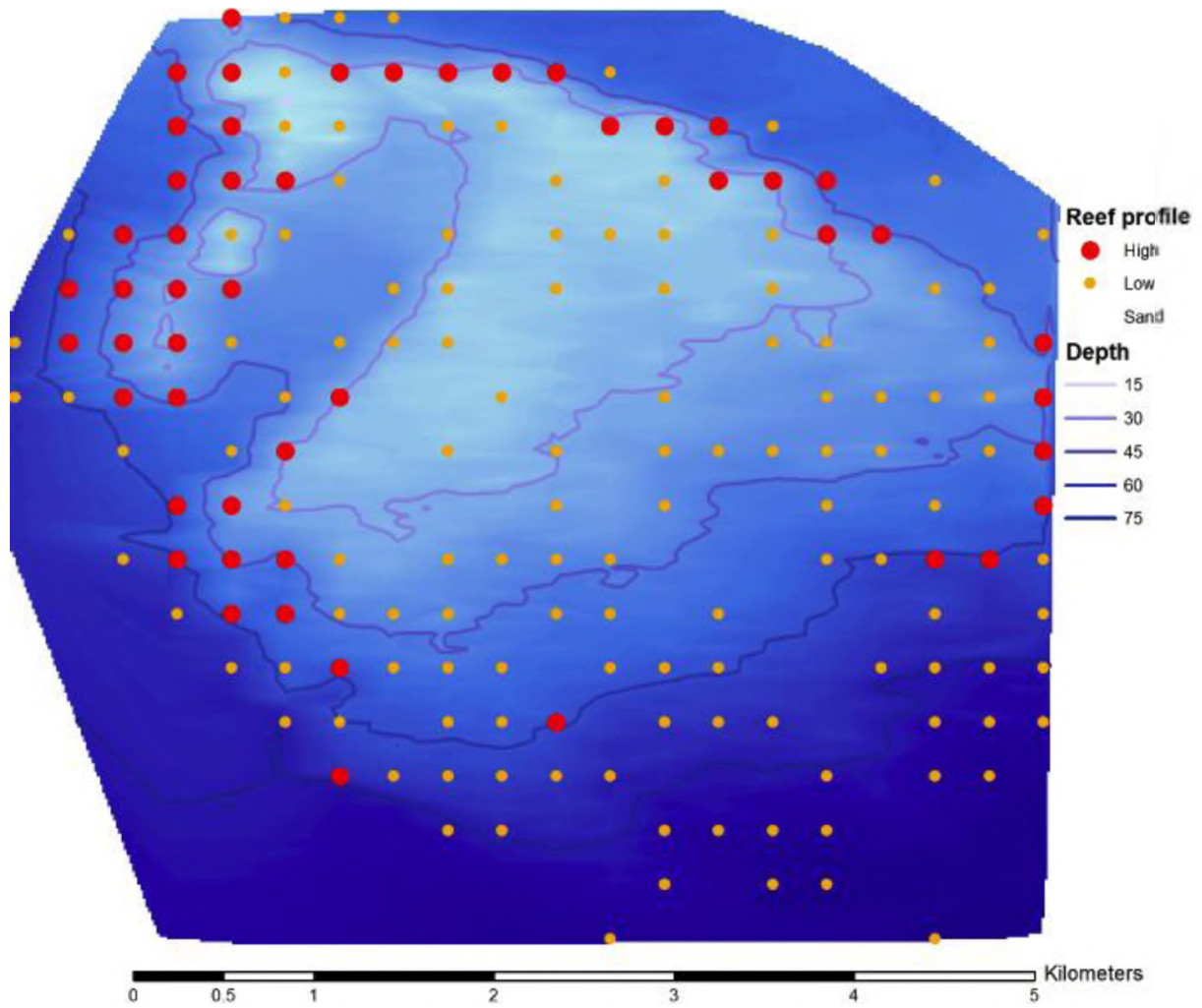


Figure 4.2 Bathymetric map of Riy Banks sampling site, including depth gradients and reef profile.

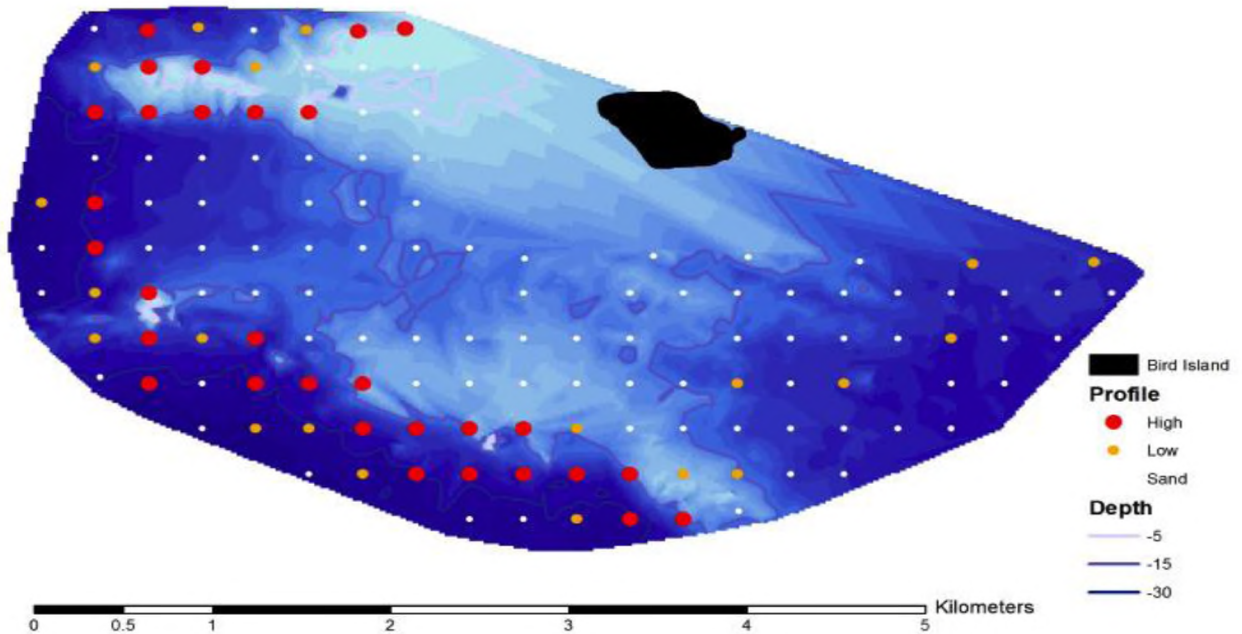


Figure 4.3 Bathymetric map of the Bird Island marine protected area sampling site, including depth gradients and reef profile.

4.3.4 Experimental Design

The study consisted of a balanced design with two sampling sites (BI MPA and Riy Banks; Figure 4.1). At each sampling site it was planned to collect 20 stereo-BRUVs samples, ten with blue lighting and ten without. Sampling stations were selected based on the stratified random sampling approach described in Chapter 2, with an even allocation of samples between high and low profile reef within a depth range of 15-30m. Sampling took place over a two month period; Riy Banks (Figure 4.3) was sampled in March 2014 and BI MPA (Figure 4.3) was sampled in April 2014. Different lighting treatments were used concurrently throughout one sampling day. Replicate sites within sampling locations were separated by 300m (preselected grid) to avoid overlap of the bait plumes and resulting risk of pseudo-replication caused by recounting of fish moving between neighbouring sites (Langlois *et al.* 2010).

4.3.5 Video analysis

Videos were analysed following the procedure outlined in Chapter 2.

4.3.6 Statistical analysis

All multivariate and univariate analyses were conducted in PRIMER with the PERMANOVA+ add-on package (Anderson *et al.* 2008). Generalized linear models were performed using the statistical program R (R Development Core Team 2015).

For all analyses of MaxN (relative abundance), apart from that conducted in R, standardised data were used. Area was used to standardise MaxN data to account for variation in underwater visibility between samples and allow unbiased comparisons of treatments. Data were standardised such that each sample accounted for a 5m² area of view. For analyses conducted in R, visibility (area) was treated as an offset.

Environmental variables were measured to establish their effect as predictor variables. These variables included depth, temperature, percentage water column, with or without lighting, bottom type and visibility (area). Detailed descriptions are provided in Chapter 2.

4.3.6.1 The affect of lighting of fish assemblage structure

The response data that was tested included MaxN by species, MaxN by trophic group and species average length. See Chapter 3 for further details on statistical analysis.

Permutational ANOVA

Differences in abundance, size and length estimates of the fish assemblages between the different experimental treatments (with and without lighting) were determined by univariate species-level analysis. The permutational ANOVA was conducted using the PERMANOVA routine in PRIMER V6 on square-root transformed data using a Euclidean distance resemblance matrix and 9999 unrestricted permutations. See Chapter 3, Section 3.4.1 for a detailed description of the statistical approach.

Permutational MANOVA

A one-way non-parametric multivariate analysis of variance (PERMANOVA) tested for differences in fish assemblages between samples collected with lighting and those collected without (relative

abundance and average length). A PERMANOVA was conducted between samples with lighting and without lighting. The analyses were conducted using standardised MaxN data for species, MaxN data for different trophic groups and average length for species. The PERMANOVA employed 9999 unrestricted permutations of raw data. Monte Carlo values were acquired for factors with less than 100 unique permutations (Anderson *et al.* 2008, Fitzpatrick 2010). See Chapter 3, Section 3.3.5.1.

4.3.6.2 CAP analysis

Constrained canonical analysis of principal coordinates (CAP) plots were employed to investigate if lighting was an effective predictor of fish assemblage composition. The CAP plots were useful to visually compare fish assemblages observed with and without lighting. Constrained canonical analysis of principal coordinates plots were built using Modified Gower (log-base 10) resemblance matrices for abundance (MaxN), length estimates and trophic groups. Additionally the CAP analysis tests the percentage of samples that correctly assigned to the appropriate group (leave-one-out allocation success) (Dorman *et al.* 2012). See Chapter 3, Section 3.3.5.2.

4.3.6.4 Generalized linear models

Generalized linear models (GLMs) were fitted using the MASS package (Venables and Ripley 2002) in the R environment (version 2.13.0, R Development Core Team 2015). The GLMs were used to investigate the effect of lighting on the MaxN of selected fish species (Table 3.1). See Chapter 3, Section 3.3.5.4.

Exploratory analyses were conducted prior to the running of GLMs so as to understand the nature of the data following the protocol set out by Zuur *et al.* (2010). Following this, a standardised full model was run for all analyses. The model was defined as:

$$E(Y) = \beta_0 + (\text{offset}(\log(\text{Area}))) + \beta_1 (\text{Lighting}) + \beta_2 (\text{Temperature}) + \beta_3 (\text{Depth}) + \beta_4 (\text{Bottom type}) + \beta_5 (\text{Percentage water column}) + \varepsilon$$

(Equation 4.1)

where $E(Y)$ is the expected response variable, which is assumed to follow the Poisson distribution, β_0 is the intercept, $\beta_{\#}$ is the estimated coefficient associated with the respective covariates and ε is the error term. Area was treated as an offset to ensure the fitted values and their respective confidence

intervals are positive (Zuur *et al.* 2009). See Chapter 2 for definition of covariates (Environmental Variables). See Chapter 3 for model selection and model validation.

For parsimonious models and model selection for each analysis please refer to appendices (See Appendix 4.2).

4.4 Results

Although it was planned to collect 40 samples under a balanced sampling design, low visibility and poor weather limited sampling to 34 stereo-BRUVs stations. Of these, 19 samples were collected with lighting and 15 were collected without lighting. Thirteen samples were collected from BI MPA (with lighting = 8, without lighting = 5) and 21 from Riy Banks (with lighting = 11, without lighting = 10). The analysis of the video footage provided 466 observations of 40 different species with a total of 2,211 fish counted. Of these, 1,667 length measurements were made. All samples were taken within the same season, between March and April 2014 and within 20 days of each other.

Samples collected with lighting recorded all 40 species whilst those collected without lighting only recorded 33 species. Piscivorous (with lighting = 1.8%, without lighting = 1.8%) and omnivorous fish species (with lighting = 3.5%, without lighting = 2.9%) were scarce in all samples. However, samples collected with lighting recorded nearly 5% more invertebrate carnivores, nearly twice as many micro-invertebrate carnivores and 7% more herbivores. On the other hand, generalist carnivores were more prevalent (15%) in the samples collected without lighting.

Spondyllosoma emarginatum and *Boopsoidea inornata* produced higher MaxNs in samples collected with lighting (MaxN Total with lighting: 640, without lighting: 319) (Figure 4.4c). However, the MaxN data from all other species detected by both lighting treatments was very similar (Figure 4.4). Similarly, little difference was observed in length estimates between treatments (Figure 4.5).

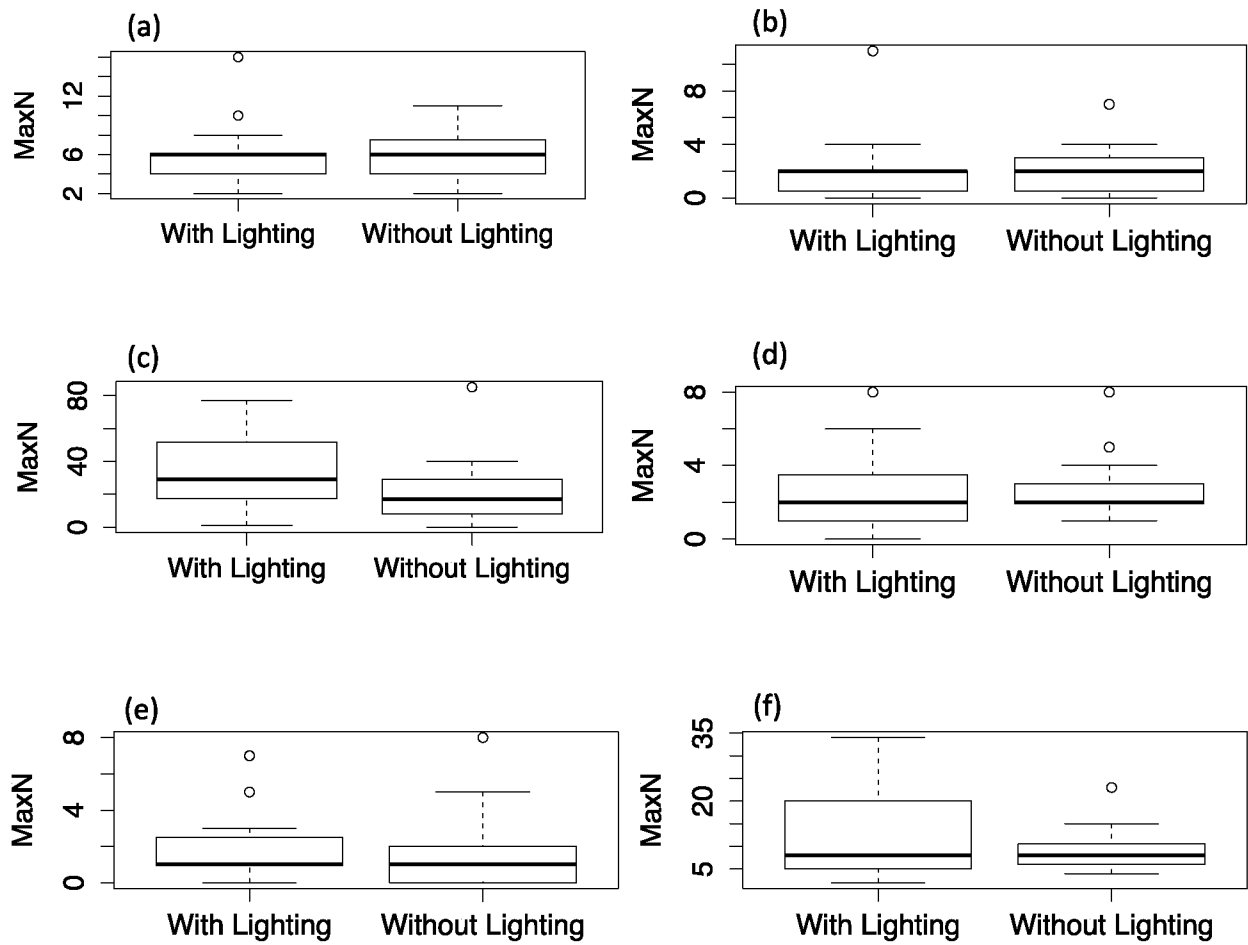


Figure 4.4 Boxplots comparing the abundance (MaxN) range under different lighting treatments for (a) *Chrysolephus laticeps* (b) *Cheilodactylidae* species (c) small generalist carnivores (d) catsharks (e) medium invertebrate carnivores (f) other fisheries important species. Whisker plots represent minimum and maximum values. See Table 3.1 for detailed description of the species. See Table 4.7 for significant differences in lighting treatments.

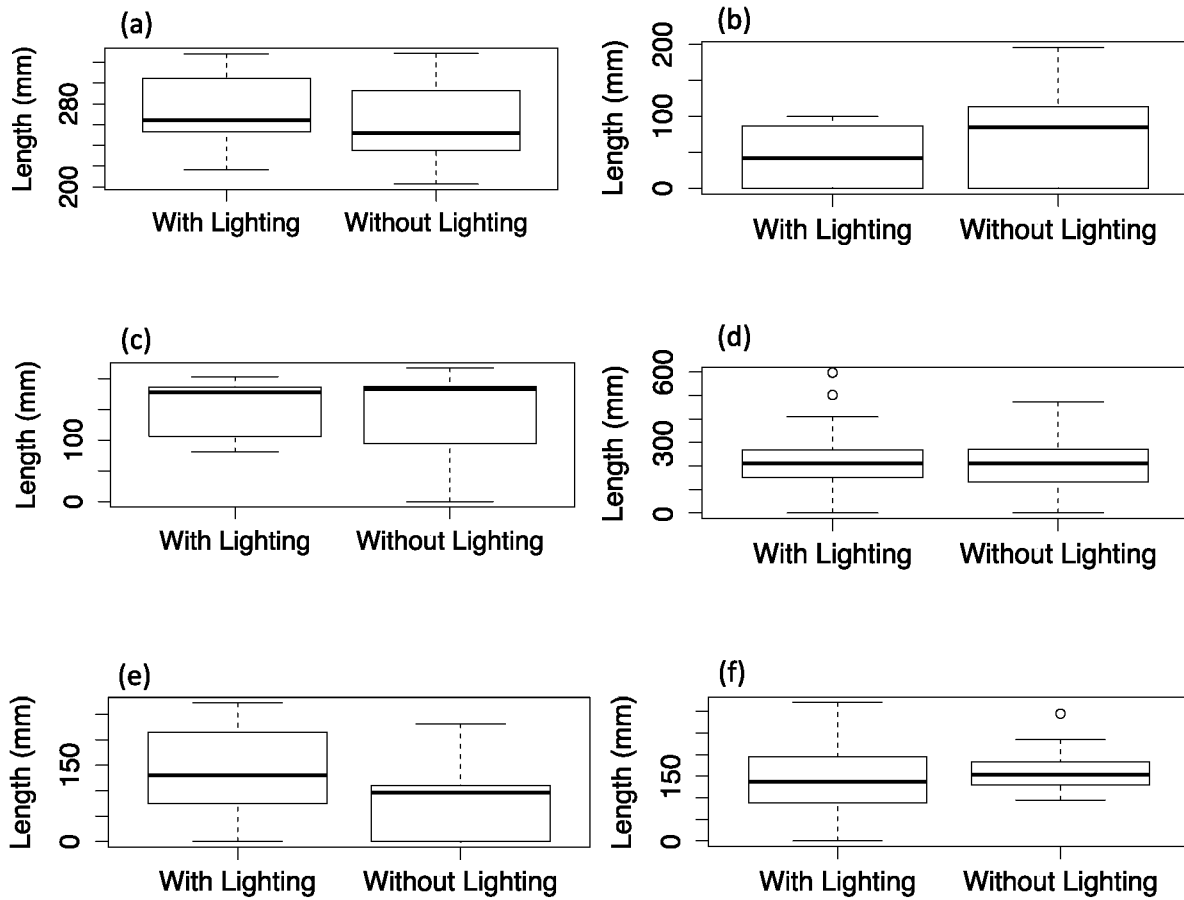


Figure 4.5 Boxplots comparing the size (Length estimates) range under different lighting treatments for (a) *Chrysolephus laticeps* (b) *Cheilodactylidae* species (c) small generalist carnivores (d) catsharks (e) medium invertebrate carnivores (f) other fisheries important species. Whisker plots represent minimum and maximum values. See Table 3.1 for detailed description of the species.

4.4.1 The affect of lighting of fish assemblage structure

Permutational ANOVA

PERMANOVA pair-wise tests of fish assemblage data (MaxN data) sampled with stereo-BRUVs comparing samples collected with and without lighting showed no significant effect of lighting on

species richness ($P > 0.05$), total MaxN ($P > 0.05$), average MaxN ($P > 0.05$) or average fish lengths per sample ($P > 0.05$). Although not significantly different (Table 4.1) samples with lighting did, on average, record seven more species compared to samples without lighting. However, these additional species were rare and recorded at low abundances (Appendix 4.1). Importantly, the sample size was larger for samples conducted with lighting (19) compared to without (15); species accumulation may account for this difference in species richness.

Table 4.1 Total number of species (species richness), average species richness, Average Total MaxN, Average MaxN and Average Length for different light treatments employed with stereo-BRUVs.

Treatment	Species Richness	Average Species Richness	Average Total MaxN	Average MaxN	Average Length (mm)
With Lighting	40	14 (± 4)	110.7 (± 108.5)	7.8 (± 7.0)	365.3 (± 273.4)
Without Lighting	33	13 (± 3)	103.8 (± 102.2)	8.3 (± 8.6)	373.1 (± 302.4)

Permutational MANOVA

A one-way permutational multivariate analysis of variance tested the effect of lighting on MaxN and on average length per species. The PERMANOVA showed no significant effect of lighting on the multivariate abundance data (Pseudo-F = 0.88053, $P = 0.5677$). Equally, the PERMANOVA indicated that lighting had no significant effect on the multivariate fish length data (Pseudo-F = 0.93642, $P = 0.522$).

As with the species assemblage data, the multivariate analysis of different trophic groups showed that lighting did not significantly affect the abundance of fish within the different trophic groups (Pseudo-F = 0.66977, $P = 0.6147$).

4.4.2 The canonical ordination of principles (CAP) analysis

Canonical ordination of principles graphs showed considerable overlap and minimal differences between samples collected with lighting and those collected without lighting (Figure 4.6 and 4.7). The CAP analysis on average length data also showed a small degree of separation between samples collected with and without lighting, however, this was not significant (Figure 4.7 and 4.8). Both CAP analyses for length estimates and MaxN displayed sample number 33 as an outlier; further examination of the data revealed that the sample had very poor visibility.

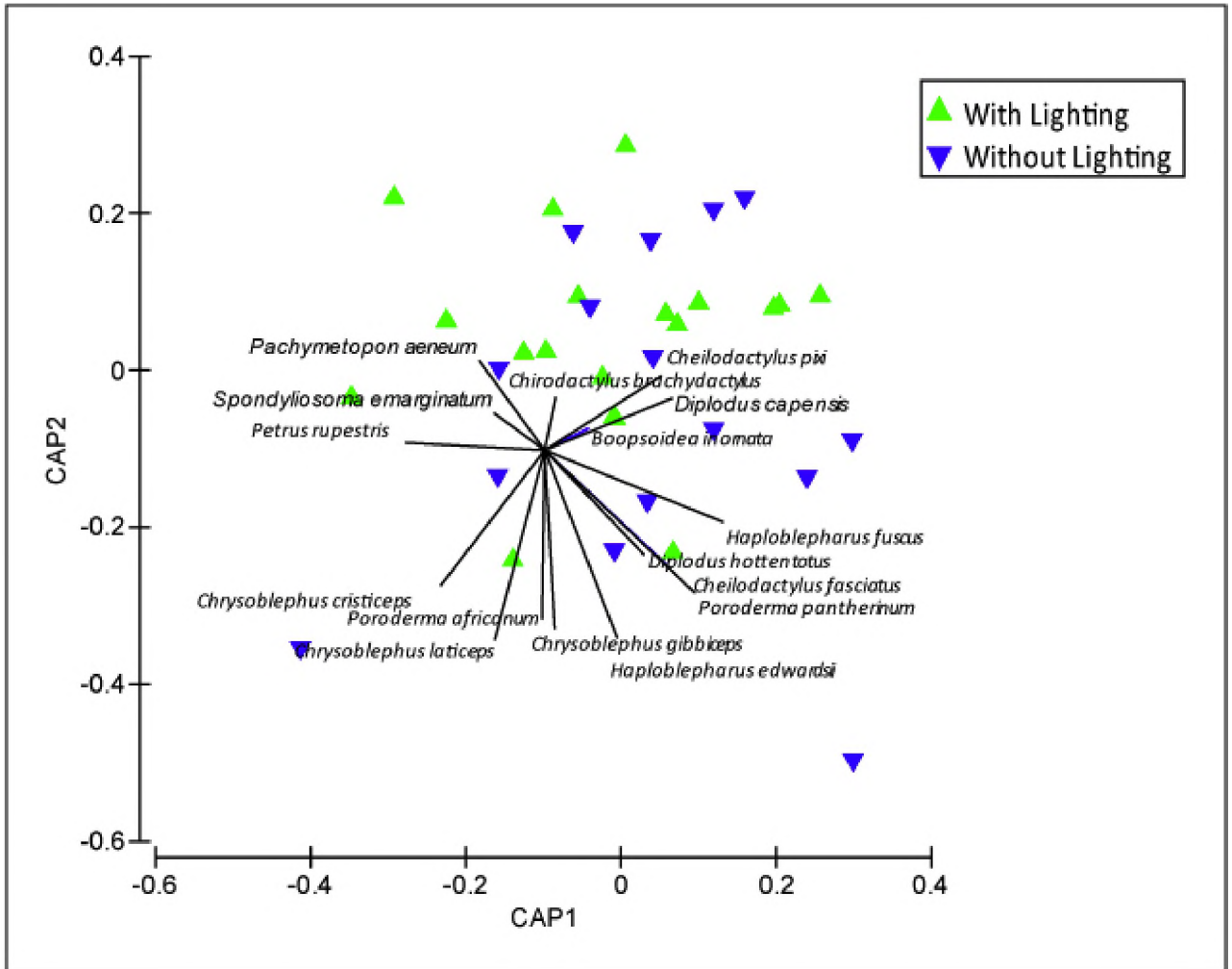


Figure 4.6 Canonical analysis of principles coordinates (CAP) ordination showing the effect of lighting on MaxN of reef fish sampled with stereo-BRUVs, with an overlay of fisheries or biodiversity important focus species (see Table 3.1 for detail of focus species).

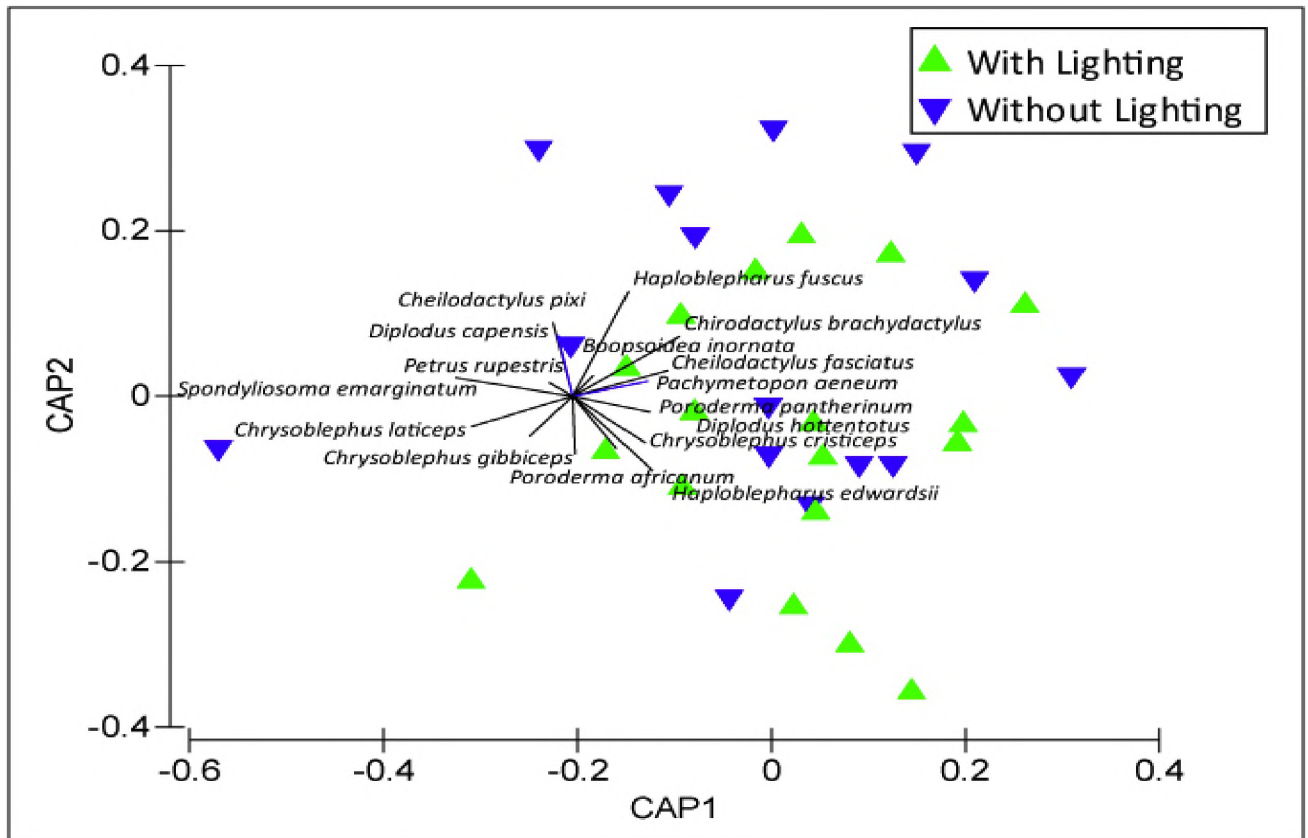


Figure 4.7 Canonical analysis of principles coordinates (CAP) ordination for samples with and without lighting during stereo-BRUVs deployments for average length estimates, with an overlay of fisheries or biodiversity important focus species (see Table 3.1 for detail of focus species).

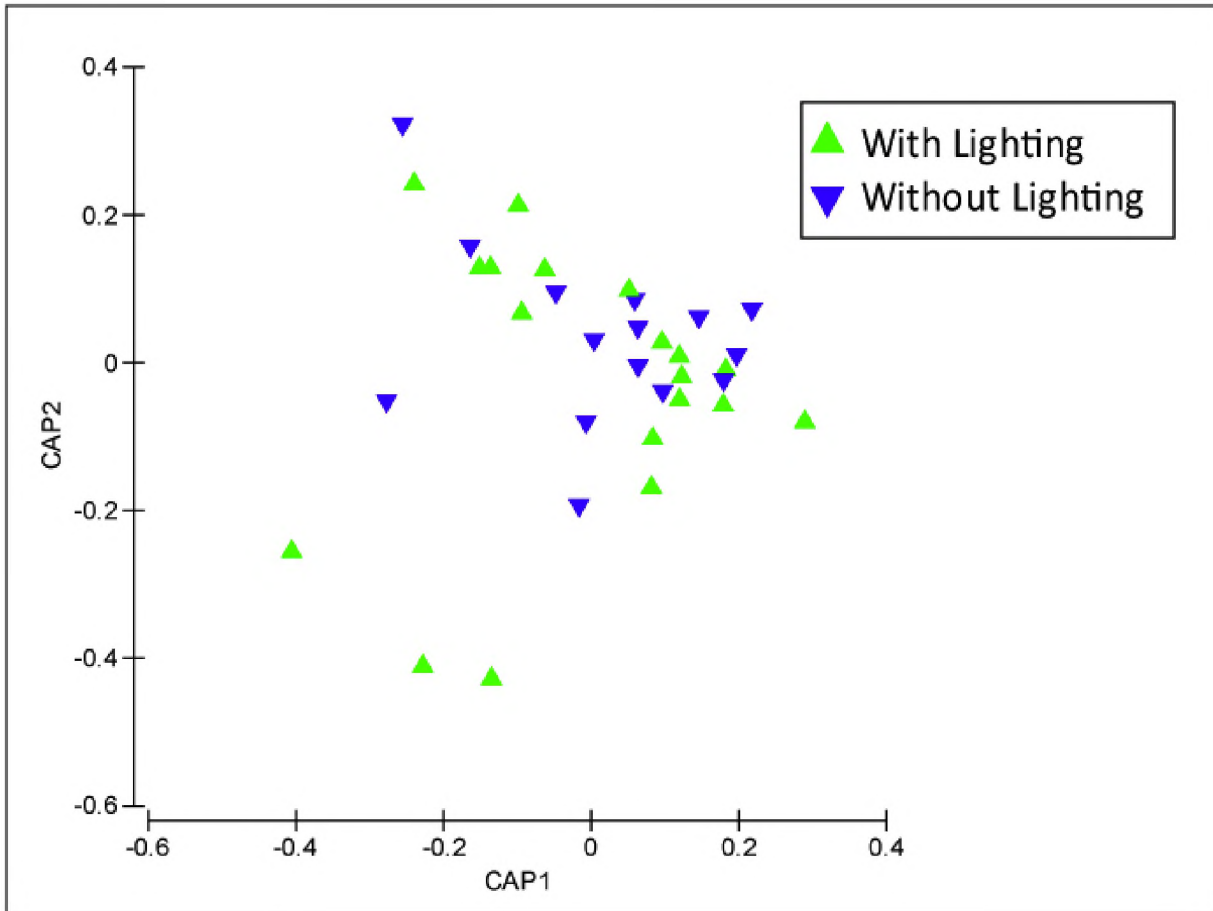


Figure 4.8 Canonical analysis of principles coordinates (CAP) ordination plots of trophic groups comparing samples collected with and without lighting during stereo-BRUVs deployments.

The 'leave-one-out' procedure provided an estimation of the misclassification error. For MaxN the CAP on the factor Lighting gives a misclassification error of 35%, with 68% successful allocation. Length estimate data produced a misclassification error of 33%, with a successful allocation of 67%.

The CAP analysis of trophic groups showed an overlap of samples conducted with lighting and those conducted without. The 'leave-one-out' procedure gave an estimation of the misclassification error of 52%, with successful allocation of 47%.

4.4.3 Generalized linear models

Generalized linear models addressed differences in MaxN data of focus species and groups (Table 3.1) for samples collected with lighting and those collected without. Only the relevant outputs of the GLMs are presented here. Full outputs can be reviewed in Appendix 4.2.

The results revealed that lighting had no significant effect on abundance for any of the species (or groups of species) included in the analysis.

Table 4.2 Summary of the generalized linear model output showing the effect of lighting on the model fit for the focus species. The values reported in the table include the estimate, the Z value (P statistic). Negative estimates and Z-values imply a negative effect of that lighting treatment on the intercept and P-statistic indicates whether or not the effect is significantly different from zero and does not compare lighting treatments (Model intercept is Without Lighting).

Focus species/groups	Treatment					
	With Lighting			Without Lighting		
<i>Chrysolephus laticeps</i>	3.819	3.56	(0.000) ***	0.062	0.39	{0.696}
<i>Cheilodactylidae</i>	2.706	4.41	(0.000) ***	0.227	0.87	{0.386}
<i>Spondylisoma emarginatum</i> & <i>Boopsoidea inornata</i>	3.929	13.76	(0.000) ***	-0.433	-1.33	{0.183}
Catsharks	-3.633	-1.82	(0.069) .	-0.121	-0.56	{0.575}
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	-9.486	-3.42	{0.001} ***	-0.173	-0.54	{0.588}
Fisheries important species	7.483	5.88	(0.000) ***	-0.008	-0.40	{0.968}

Significance level: "****"<0.001, "***"<0.01, "**"<0.05, "."<0.1

Chrysolephus laticeps

The model selection process identified temperature as having a significant effect on the abundance of *Chrysolephus laticeps* (see Appendix 4.2 for summary of model output). The best-fit model explained 8.6% of the variability observed in the MaxN data. No significant difference in abundance of *Chrysolephus laticeps* was found in the likelihood ratio test, with the model summary suggesting that *Chrysolephus laticeps* were slightly more abundant in the samples collected with lighting compared to the samples collected without lighting (Table 4.2).

Cheilodactylidae

Depth and percentage water column were found to have a significant effect on the abundance of *Cheilodactylidae* (see Appendix 4.2 for summary of model output). Of the variability observed in the MaxN data, 49.4% was explained by the best-fit model. No significant differences were found between treatments however the model summary suggests *Cheilodactylidae* were more abundant in samples collected without Lighting (Table 4.2).

Small generalist carnivores

Bottom type had a significant effect on the abundance of *Spondylisoma emarginatum* and *Boopsoidea inornata* (see Appendices 4.2 for summary of model output). Of the variability observed in the MaxN data, 17.2% was explained by the best-fit model. No significant differences were observed between the two lighting treatments, however, as suggested by the model summary a slightly larger abundance of small generalist carnivores was observed in samples collected with lighting (Table 4.2).

Catshark species

The model selection process identified that temperature, area, bottom type and depth significantly influenced the abundance of catsharks (see Appendix 4.2 for summary of model output). The best-fit model explained 36.9% of the variability in the MaxN data. Lighting had no effect on the abundance of catshark species with little observed difference between the average MaxN values however the model summary suggests catsharks were slightly more abundant in samples collected with lighting (Table 4.2).

Medium invertebrate carnivores

Temperature had a significant negative influence on the abundance of medium sized invertebrate carnivores (*Diplodus capensis* and *Diplodus hottentotus* Zebra) (see Appendix 4.2 for summary of model output). The best-fit model explained 31.7% of the variability observed in the MaxN data. The effect of lighting on the abundance of medium invertebrate carnivores was not significant, however, the model summary suggests their abundances was larger in the samples where lighting was present (Table 4.2).

Fisheries important species

The model selection process found that area and temperature significantly influenced the abundance of fisheries important species (see Appendix 4.2 for summary of model output). Of the variability observed in the MaxN data the best-fit model explained 40.2%. No significant difference was observed between lighting treatments for fisheries important species however the model summary suggests a slightly higher abundance in samples collected with lighting (Table 4.2).

4.5 Discussion

Knowledge of depth related patterns in fish assemblage composition are required to identify distribution ranges, critical habitats and effectively manage fisheries resources (Heyns 2015). Many South African fish species exhibit broad depth distributions from the shallow photic zone (5m) to the edge of the continental shelf (200m) (Mann 2013). Importantly, the data collected across this broad depth range must be comparable and it is recommended that a standardised sampling method be employed.

However, data collected from deep-water benthic habitats is compromised by low ambient light conditions and as such lighting is required to observe the fish community. It would be expected that fish assemblage data from these samples might exhibit some bias, attributed to the presence of lighting, in comparison to samples collected from shallow waters that rely on natural light. Lighting is not a requirement for sampling in the photic depth zone, however, it must be used when sampling in the sub-photoc depth zone. Whilst the use of lighting presents biases this is unavoidable when sampling in the sub-photoc depth zone. Furthermore, testing the bias caused by lighting at sub-photoc depths is complicated, as some form of lighting is needed to observe the fish, i.e. a control without lighting is impossible. For this reason, sampling with and without lighting was conducted in the photic zone to understand any biases that may alter the fish community. Little deep-water work has been conducted in the Agulhas ecoregion and little is known about fish abundances beyond the shallow zones (Heyns 2015). Wraith *et al.* (2013) states “to improve the generality of BRUVs as a methodology, an understanding of its biases is essential across localities”. As such, the research presented in this chapter will contribute towards understanding how the inherent biases of stereo-BRUVs in warm-temperate areas can influence the data.

4.5.1 The influence of environmental covariates on fish assemblage structure as a whole

The GLM results indicated that bottom type, temperature and percentage water column were influential on the observed fish assemblages. These are parameters that were known to influence fish diversity and abundance (Anderson and Millar 2004, Bennett *et al.* 2009, Bernard 2012).

Considerable research indicates associations between fish assemblage structure and the nature of the benthos and/or topographic complexity (Choat and Ayling 1987, McCormick 1994, Jenkins and

Sutherland 1997, Ault and Johnson 1998, Jenkins and Wheatley 1998, Hyndes *et al.* 1999, Gust *et al.* 2001, O'Hara 2001, Curley *et al.* 2002, Giannoulaki *et al.* 2003, Harman *et al.* 2003, Hyndes *et al.* 2003, Willis and Anderson 2003, Almany 2004, Anderson and Millar 2004, Watson *et al.* 2005, Cappo *et al.* 2007, Moore 2010). Due to the reef dependent nature of many of the fish species observed in this study, fluctuations in abundance influenced by bottom type was to be expected (See Chapter 3, Section 3.5 which details the influence of bottom type on fish assemblages).

Water temperatures recorded during sampling ranged from 14.4°C to 21.3°C. The Agulhas ecoregion is characterised by localised, wind-driven upwelling events causing rapid drops in water temperature (Goschen *et al.* 2012), as was the case in this study. Previous studies in the Tsitsikamma MPA by Bernard (2012) and in this study have also found water temperature is a consistent explanatory factor of variability in fish abundance (See Chapter 3, Section 3.5 which illustrates the contributing effect of temperature on observed fish assemblages).

The percentage water column in each sample contributed to differences in abundance data for *Cheilodactylidae* species. These fishes are closely associated with the reef where they forage and rest on the bottom, supported by their extended pelvic fins. Sampled videos with a higher percentage water column observe less reef habitat, it can then be assumed that reef associated species would be observed less often.

4.5.2 The effect of Lighting on fish assemblage structure

The results from the data analysis demonstrate that the presence of lighting with stereo-BRUVs did not significantly alter the observed fish assemblage structures, relative to samples that relied on the ambient levels of natural light. The analysis showed no effect of Lighting on species richness. Previous studies have observed differences in species richness between different coloured lights (Fitzpatrick *et al.* 2013) There were seven additional species recorded in samples collected with Lighting, relative to samples collected without lighting, previous studies have too observed this difference between different coloured lights (Fitzpartick *et al.* 2013). On average though, species richness recorded by the two treatments were similar. This implies that the effect of blue lighting relative to ambient lighting was nominal. Suggesting that blue lighting is either not within the visual spectrum of the species present or that it is non-invasive. Previous studies have established that UV light is within the visual spectrum of some fish species, and that UV light may be visible at depths as deep as 200m (Zamzow *et al.* 2008). Blue light falls within the same spectrum, the species-specific

behaviour of some species possessing UV or blue light vision (Losey *et al.* 1999) may account for differences in species richness.

The univariate analysis indicated that whilst the average lengths of some fishes were larger when sampling with lighting, the size estimates of the fish assemblage was not significantly different. Equally, the analysis showed that the presence of lighting had little effect on the total (per sample) and average (per species per sample) abundance of fish recorded by the stereo-BRUVs. In summary, this suggests the use of blue lighting in the photic zone did not change the fish assemblages observed with stereo-BRUVs in Algoa Bay.

Similarly, no significant differences were observed for abundance, length estimate or trophic data in the PERMANOVA. The CAP analysis demonstrated overlapping of data for samples collected with lighting and for samples collected without. This suggests little difference in the fish assemblages when sampling with lighting or without. However, sample number 33 was an outlier. Reviewing this sample showed it was collected during poor visibility conditions, illustrating the effect visibility plays in effective stereo-BRUVs sampling. Assuming a successful allocation value of 50% (half of the samples correctly allocated) represents no ability to predict which treatment a sample belongs to, then the CAP analysis is inconclusive with regards to the effect of lighting on fish abundance, size and trophic diversity.

4.5.3 Species-specific effects This study indicated some species-specific responses to the presence of lighting on stereo-BRUVs (Figure 4.4c). These species-specific responses must be considered biases of the method. For example, lighting resulted in considerably higher abundances of *B. inornata* (Appendices 4.1). *Boopsoidea inornata* may exhibit a level of attraction to blue lighting. Past studies have shown that such behaviour in fishes may be a result of an increased number of marine plankton (Thorson 1964, Crisp and Ritz 1973, Forward 1988, Fitzpatrick *et al.* 2013). Many marine plankton species are known to show attraction to light, particularly within the blue-green light spectrum (Thorson, 1964; Crisp and Ritz, 1973; Forward, 1988, Fitzpatrick *et al.* 2013). It is possible that this predator to prey attraction is the reason for the increased abundance in *Boopsoidea inornata*. However, marine plankton were not noticeably observed in this study. One must then take the precautionary assumption that the increased abundance of *Boopsoidea inornata* was a result of natural variation. *Boopsoidea inornata* are known to school, therefore, as no significant difference

was observed one may assume samples collected with lighting may have encountered more schools by chance.

Nocturnal studies conducted by Fitzpatrick *et al.* (2013) yielded similar results with swarming bait fish being present in blue and white lighting and subsequent inflated abundances of predatory species; no evidence for this was found in this study.

4.5.4 The effect of lighting on different trophic groups

Studies suggest species-specific adaptations to light (Warrant 2004, Fitzpatrick *et al.* 2013), however, this study was conducted in habitats with ambient light and therefore the species present would already be adapted to tolerate lighting. As such, it was expected that no difference in the trophic group structure would be found when using lighting. Multivariate analysis of trophic groups showed no significant differences in abundance data categorised by trophic groups. The use of lighting had no significant effect on the trophic groups of fish assemblage structure observed in the Agulhas ecoregion.

4.6 Further work

Critical analysis

The experimental design of this study was compromised by issues with the lighting supplier. The experiment was designed initially to compare a variety of light colours, however, a compromise had to be reached when the lighting could not be delivered within the time frame of the project. The use of low-light camera systems too would have made for a more robust study from an ecological perspective, however, due to high costs was not a suitable option for long term monitoring. As a result, the findings of this study were less comprehensive but nevertheless addressed relevant questions.

Different wavelengths

The use of red lighting at the time of this study was not an option due to supplier constraints. Consequently, some preliminary work conducted on the sub-photoc reefs in Algoa Bay using stereo-BRUVs illuminated with red lighting suggested that red light may be an effective light source (R. Juby pers. comm.). However, due to the rapid attenuation of red light in water, together with the typically turbid water conditions experienced in the Agulhas ecoregion, the area of illumination by red light is

very small. However, it would be useful to compare the effect of blue and red lighting, even with a reduced field of view, on fish species assemblage composition in the Agulhas ecoregion. Previous studies substantiate the suitability of blue lighting (Fitzpatrick *et al.* 2012) for low light sampling. However, to ascertain any species-specific discrepancies caused by lighting colour when used in sub-photic environments it is recommended a study of different colour lights be conducted. Studies suggest some species of fish possess blue light or UV light vision (Losey *et al.* 1999). This may affect the ethology of the system in particular social interactions and predation (Losey *et al.* 1999). Should there be species-specific differences between light colours, an appropriate protocol can be established. This will allow future studies to be conducted with objectives structured according to the appropriate light colour relevant to the species under investigation. Similarly, Fitzpatrick *et al.* (2013) established that whilst blue light did not influence the abundance of the dominant fisheries species, it did influence the presence of non-target species. Compared to samples with red lighting, non-target species were recorded less frequently with blue lighting (Fitzpatrick *et al.* 2013). An in-depth study with lighting of different wavelengths (colours) is required.

Turbidity measures

Under ideal conditions, the euphotic zone extends 200m deep into the ocean where light is believed to penetrate (Sosik and Johnsen 2004). Light penetration, however, is determined by the presence of plankton and sediment, which absorb and scatter light (Sosik and Johnsen 2004). Coastal waters tend to be murkier allowing less light penetration and subsequently a shallow penetration depth (Sosik and Johnsen 2004). In the Agulhas ecoregion this is dependent on season, upwelling events and turbidity. A limitation of many deep-water sampling attempts undertaken so far with stereo-BRUVs in the Agulhas ecoregion is high concentrations of suspended particles. This high turbidity is particularly evident in winter when upwelling events are infrequent. High turbidity reduces visibility and even with the use of lighting can make analysis difficult if not impossible. Visibility was a contributing factor to differences in fish assemblage data as indicated in this study. Evidence suggests highly turbid conditions can also result in behavioural stress responses in fishes (Bruton 1985, Utne-Palm 2001). I would suggest water turbidity be tested as an environmental covariate in future studies. Water turbidity can then be added as a factor when examining fish assemblage data. It is important to establish when low light conditions are a result of depth or of highly turbid water that restrict natural light. Subsequently, differences may be accounted for accordingly.

A cost effective method to determine turbidity could be secchi disk measurements conducted from the surface, however, this may be biased particularly for deeper samples and where thermoclines are present. An alternative cost effective method would be to develop a scaling system (e.g. 1- water clear to 5- water high particle abundance, water highly turbid) to be used during video analysis at the discretion of the individual analysing the video.

Diurnal vs. Nocturnal

Sampling with lighting allows species to be recorded beyond the diurnal time constraints currently experienced with stereo-BRUVs. It would be useful to establish a review of fish species with regard to whether they are either diurnally or nocturnally active. Subsequently, it would be beneficial to examine the effect of different colour lighting on these species groups, diurnal or nocturnal. As previously mentioned many marine plankton species are attracted to blue-green light (Thorson, 1964; Crisp and Ritz, 1973; Forward, 1988, Fitzpatrick *et al.* 2013). Many planktivorous fishes partake in vertical migrations, using depth as a diurnal refuge and ascend nocturnally to feed on marine plankton (Neilson and Perry 1990, Ryer *et al.* 2002). It would be expected that larger numbers of these planktivorous species would be observed in blue-light night sampling and less so in different wavelengths (colours). However, not many planktivorous fishes are found in the ecoregion, suggesting blue lighting may still be appropriate for both diurnal and nocturnal studies.

4.7 Conclusion

This chapter aimed to determine if lighting was required in the photic zone to ensure standardisation, and consistent bias, with data obtained from sub-photic depths. The multivariate analysis showed no effect of blue lighting on the reef fish assemblage from photic depths. As a result, it is concluded that blue lighting for stereo-BRUVs sampling in the photic depths is not necessary for standardisation purposes. The data collected from samples with lighting should be seen as comparable to samples collected without lighting. Further study of the suitability of blue lighting for non-discriminate deep-water sampling is required as species-specific phototactic behaviour can be expected with deep-water sampling where species have not been previously exposed to light.

This is a favourable result. Locally, stereo-BRUVs sampling can address and compare fish assemblages that occupy both the photic (10-40/50m depth) and sub-photic zones (>50depths). A suggested sampling protocol would be to sample the shallower (10-50m depth) photic zone without

the application of lights and to apply lighting at depths beyond 50m. The depth cut-off of 50m is based on observations from preliminary deep-water work. Understanding of light systems and their biases in the ecoregion mean a more effective application of the stereo-BRUVs sampling method so that it may be standardised (Wraith *et al.* 2013). Following on this understanding, sampling efforts across depth ranges utilising lighting are comparable regionally and internationally. This is encouraging for the application of stereo-BRUVs in South Africa. A standardised method will allow projects of a similar nature to be comparable, making the method that much more universally applicable and relevant.

Appendix

Appendix 4.1a MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group) and species seen for baited samples with Lighting.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Acanthistius sebastoides</i>	1	21	5	2	203-329	Small & medium sized generalist carnivore
<i>Amblyrhynchotes honckenii</i>	1	11	2	2	192-25	Small & medium sized generalist carnivore
<i>Argyrozona argyrozona</i>	2	5	2	2	186-193	Dominant large generalist carnivore
<i>Boopsoides inornata</i>	76	89	550	416	139-284	Small & medium sized generalist carnivore
<i>Carcharhinus brachyurus</i>	1	11	2	1	894	Sharks, skates & rays
<i>Carcharhinus obscurus</i>	1	5	1			
<i>Chaetodon marleyi</i>	1	5	1			Benthic invertebrate carnivore
<i>Cheilodactylus fasciatus</i>	1	11	2	1	144	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	3	21	7	4	62-114	Benthic invertebrate carnivore
<i>Cheimenus nufar</i>	5	95	51	45	202-543	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	11	63	29	24	175-424	Benthic invertebrate carnivore
<i>Chirodactylus grandis</i>	3	16	6	4	247-369	Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	10	47	28	26	154-500	Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	3	89	29	24	244-540	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	16	100	114	106	157-421	Dominant large generalist carnivore
<i>Cymatoceps nasutus</i>	1	5	1	1	708	Benthic invertebrate carnivore
<i>Dasyatis brevicaudata</i>	2	26	6	5	817-1477	Sharks, skates & rays
<i>Diplodus capensis</i>	6	47	20	19	137-302	Herbivore & omnivore
<i>Diplodus hottentotus</i>	5	68	18	13	230-332	Herbivore & omnivore
<i>Galeichthys feliceps</i>	6	47	17	13	201-274	Small & medium sized generalist carnivore
<i>Gymnacrotaphus curvidens</i>	1	11	2	2	222-263	Herbivore & omnivore
<i>Haploblepharus edwardsii</i>	3	21	6	6	299-493	Cryptic reef sharks
<i>Haploblepharus fuscus</i>	1	21	4	3	445-657	Cryptic reef sharks
<i>Mustelus mustelus</i>	1	21	4	4	956-1189	Sharks, skates & rays
<i>Notothenchus cepedianus</i>	1	5	1			Sharks, skates & rays
<i>Oplegnathus conwayi</i>	2	37	10	8	253-331	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	22	68	118	62	123-300	Small & medium sized generalist carnivore
<i>Pagellus bellottii natalensis</i>	1	5	1	1	192	Small & medium sized generalist carnivore
<i>Petrus rupestris</i>	2	16	5	5	278-329	Scarce large generalist carnivore
<i>Pomadasys olivaceum</i>	19	5	19			Small & medium sized generalist carnivore
<i>Porcostoma dentata</i>	3	37	13	11	108-173	Small & medium sized generalist carnivore
<i>Poroderma africanum</i>	4	63	24	19	437-931	Cryptic reef sharks
<i>Poroderma pantherinum</i>	3	47	15	11	410-723	Cryptic reef sharks
<i>Pterogymnus lanarius</i>	3	16	7	4	200-227	Dominant large generalist carnivore
<i>Rhabdosargus giobiceps</i>	1	16	3	3	242-326	Scarce large generalist carnivore
<i>Rhabdosargus holubi</i>	1	16	3	3	194-263	Small & medium sized generalist carnivore
<i>Sarpa salpa</i>	58	16	95	38	137-236	Herbivore & omnivore
<i>Seriola lalandi</i>	8	42	29	20	537-1611	Scarce large generalist carnivore
<i>Serranus knysnaensis</i>	1	5	1	1	118	Small & medium sized generalist carnivore
<i>Spondyliosoma emarginatum</i>	23	79	90	63	100-250	Small & medium sized generalist carnivore

Appendix 4.1b MaxN (Maximum, % Abundance of MaxN counts in all samples, total MaxN), number of length measurements, size range of length measurements, trophic group) and species seen for baited samples without Lighting.

	Max.	% Abundance	Total	Number of length measurements	Size range (mm)	Trophic group
<i>Acanthistius sebastoides</i>	2	47	8	5	145-222	Small & medium sized generalist carnivore
<i>Amblyrhynchotes hænckenii</i>	1	7	1	1	203	Small & medium sized generalist carnivore
<i>Boopsoides inornata</i>	85	67	256	198	143-292	Small & medium sized generalist carnivore
<i>Carcharhinus brachyurus</i>	1	7	1	1	1341	Sharks, skates & rays
<i>Cheilodactylus fasciatus</i>	1	7	1	1	196	Benthic invertebrate carnivore
<i>Cheilodactylus pixi</i>	5	27	11	9	93-153	Benthic invertebrate carnivore
<i>Cheimarius nufar</i>	8	100	36	33	178-463	Scarce large generalist carnivore
<i>Chirodactylus brachydactylus</i>	3	73	18	15	205-415	Benthic invertebrate carnivore
<i>Chirodactylus grandis</i>	1	7	1	1		Benthic invertebrate carnivore
<i>Chrysoblephus cristiceps</i>	5	60	18	16	154-391	Scarce large generalist carnivore
<i>Chrysoblephus gibbiceps</i>	3	100	23	22	249-487	Scarce large generalist carnivore
<i>Chrysoblephus laticeps</i>	11	100	92	83	167-429	Dominant large generalist carnivore
<i>Dasyatis brevicaudata</i>	2	27	7	7	568-1457	Sharks, skates & rays
<i>Diplodus capensis</i>	4	60	19	16	163-254	Herbivore & omnivore
<i>Diplodus hottentotus</i>	6	20	8	2	235-258	Herbivore & omnivore
<i>Galeichthys feliceps</i>	2	33	7	5	193-286	Small & medium sized generalist carnivore
<i>Gymnocrotaphus curvidens</i>	1	13	2	2	248-283	Herbivore & omnivore
<i>Haploblepharus edwardsii</i>	2	20	4	3	235-368	Cryptic reef sharks
<i>Haploblepharus fuscus</i>	1	33	5	5	432-561	Cryptic reef sharks
<i>Mustelus mustelus</i>	1	20	3	3	1037-1347	Sharks, skates & rays
<i>Oplegnathus conwayi</i>	1	20	3	2	229-376	Herbivore & omnivore
<i>Pachymetopon aeneum</i>	14	87	62	55	118-244	Small & medium sized generalist carnivore
<i>Petrus rupestris</i>	1	13	2	1	428	Scarce large generalist carnivore
<i>Porcostoma dentata</i>	3	60	13	9	129-244	Small & medium sized generalist carnivore
<i>Poroderma africanum</i>	3	67	17	12	446-866	Cryptic reef sharks
<i>Poroderma pantherinum</i>	3	73	16	12	321-645	Cryptic reef sharks
<i>Pterogymnus lanarius</i>	2	13	3	3	181-307	Dominant large generalist carnivore
<i>Rhabdosargus globiceps</i>	3	13	4	4	186-239	Scarce large generalist carnivore
<i>Rhabdosargus holubi</i>	2	27	5	3	188-209	Small & medium sized generalist carnivore
<i>Sarpa salpa</i>	1	13	2	2	199-277	Herbivore & omnivore
<i>Seriola lalandi</i>	4	33	11	5	595-1171	Scarce large generalist carnivore
<i>Serranus knysnaensis</i>	1	13	2	2	144-201	Small & medium sized generalist carnivore
<i>Spondylisoma emarginatum</i>	18	80	63	50	118-237	Small & medium sized generalist carnivore

Appendix 4.2a Parsimonious models and Akaike information criterion (AIC) scores used for generalized linear models (GLM) in Rgui.

Focus species/groups	AIC		Deviance explained	Best fit model
	Best fit model	Full model		
<i>Chrysolephus laticeps</i>	167.84	172.05	8.6%	glm.nb(formula = MaxN ~ Temp + 1 + Lighting)
<i>Cheilodactylidae</i>	115.11	119.89	49.4%	glm.nb(formula = MaxN ~ Depth + % Water column + 1 + Lighting)
<i>Spondyliosoma emarginatum</i> & <i>Boopsoidea inornata</i>	296.79	301.29	17.2%	glm.nb(formula = MaxN ~ Bottom type + 1 + Lighting)
Catsharks	132.58	135.32	36.9%	glm.nb(formula = MaxN ~ Area + Bottom type + Temp + 1 + Lighting)
<i>Diplodus capensis</i> & <i>Diplodus hottentotus</i>	120.93	128.33	31.7%	glm.nb(formula = MaxN ~ Temp + 1 + Lighting)
Fisheries important species	214.27	244.38	40.2%	glm.nb(formula = MaxN ~ Area + Temp + 1 + Lighting)

Appendix 4.2b Parsimonious models and Akaike information criterion (AIC) scores used for generalized linear models (GLM) in Rgui.

Focus species/groups	Results of Drop1 likelihood ratio tests					GLM summary for Best fit model							
	Df	Deviance	AIC	LRT	Pr(>Chi)	Estimate	Std.Error	z-value	Pr(> z)				
<i>Chrysoblephus laticeps</i>	Temp	1	34.902	167.17	3.3333	0.06789	(Intercept)	3.81861	1.07278	3.560	0.000372	***	
	Lighting	1	31.723	163.99	0.1533	0.69536	Temp	-0.11368	0.06021	-1.888	0.059012	.	
							Without Lighting	0.06181	0.15807	0.391	0.695772		
<i>Cheilodactylidae</i>	Depth	1	37.866	113.54	2.4355	0.1186	(Intercept)	2.70603	0.61325	4.413	1.02e-05	***	
	% Water col.	1	59.413	135.09	23.9832	9.718e-07	***	Depth	-0.04497	0.02924	-1.538	0.124	
	Lighting	1	36.177	111.85	0.7466	0.3876	% Water column	-0.02182	0.00464	-4.703	2.56e-06	***	
							Without Lighting	0.22723	0.26224	0.866	0.386		
<i>Spondylisoma emarginatum & Boopsoides inornata</i>	Bottom type	1	46.553	299.16	6.3743	0.01158	*	(Intercept)	3.9286	0.2856	13.755	<2e-16	***
	Lighting	1	41.924	294.53	1.7453	0.18647		Bottom type	-0.8179	0.3308	-2.473	0.0134	*
								Without Lighting	-0.4333	0.3256	-1.331	0.1833	
Catsharks	Area	1	39.860	133.22	4.6407	0.03122	*	(Intercept)	-3.63292	1.99597	-1.820	0.068739	.
	Bottom type	1	50.410	143.77	15.1913	9.715e-05	***	Area	-0.02925	0.01405	-2.082	0.037330	*
	Temp	1	40.721	134.08	5.5018	0.01900	*	Bottom type	0.96618	0.26303	3.673	0.000239	***
	Light	1	35.534	128.89	0.3148	0.57475		Temp	0.23958	0.10712	2.237	0.025312	*
							Without Lighting	-0.12098	0.21585	-0.560	0.575151		
<i>Diplodus capensis & Diplodus hottentotus</i>	Temp	1	48.928	132.37	15.4426	8.505e-05	***	(Intercept)	-9.4858	2.7727	-3.421	0.000624	***
	Lighting	1	33.780	117.22	0.2951	0.587		Temp	0.5541	0.1489	3.720	0.000199	***
								Without Lighting	-0.1726	0.3184	-0.542	0.587812	
Fisheries important species	Area	1	38.937	215.91	5.6379	0.01758	*	(Intercept)	7.483045	1.272330	5.881	4.07e-09	***
	Temp	1	50.152	227.12	16.8526	4.04e-05	***	Area	0.023308	0.010000	2.331	0.0198	*
	Lighting	1	33.301	210.27	0.0016	0.96849		Temp	-0.302743	0.072065	-4.201	2.66e-05	***
								Without Lighting	-0.007594	0.187756	-0.040	0.9677	

Chapter 5

Synthesis and Recommendations

5.1 Thesis rationale

This thesis addressed two research questions with the aim to optimize and standardize the application of stereo-BRUVs for monitoring reef fish in South Africa, with a focus on the Agulhas ecoregion. South Africa's Agulhas ecoregion represents an important component of South African marine life. Evidence suggests the Agulhas Current is warming (Rounualt *et al.* 2009, Von der Heyden 2011). There is much uncertainty surrounding the effect that climate change poses (Rijnsdopr *et al.* 2009, Sorte *et al.* 2010, Von der Heyden 2011). Regardless of these uncertainties distinct shifts to fish assemblage structures are anticipated (Rijnsdopr *et al.* 2009, Sorte *et al.* 2010, Von der Heyden 2011). It is hypothesized that the warmer Agulhas waters and associated changes to the Benguela waters will mean warm water fish species will extend their ranges from tropical areas into the Agulhas ecoregion (Von der Heyden 2011, Potts *et al.* 2015). This amplifies an increasing need for long-term monitoring (Goodwin 2007, Bennett 2007). Long-term monitoring is vital to marine conservation and biodiversity planning and its implementation (McKenna and Allen 2005, Bennett 2007). Long-term monitoring accounts for changes in indicators of species depletion and allows for timely remedial action (Underwood 1991, Bennett 2007). Importantly too, monitoring can gauge the effectiveness of management measures (Vos *et al.* 2000) and as such play a crucial role in MPA management (Attwood *et al.* 1997, Bennett 2007). Key to this is monitoring and research that is both optimized and standardized. A standardized method makes research relevant and comparable between monitoring sites and with other studies, both locally and internationally (Sutherland 1996, ICES 2006, Bennett 2007).

Baited remote underwater stereo-video systems prove a long-term monitoring tool that improves considerably on method requirements (Langlois *et al.* 2012). The method is a comprehensive, cost-effective and easily standardized approach to identify trends in South Africa's marine ecology (Bernard 2012). The research potential of stereo-BRUVs in South Africa, as tools for long-term monitoring, is yet to be fully realized.

The first research question attempted to determine what bait type was most effective at surveying the reef fish community. To do this, four different bait types were compared to investigate the differences in fish assemblage structure recorded from stereo-BRUVs samples. Bait types were selected for their relevance to the Agulhas ecoregion. The second research question attempted to

determine the effect of artificial illumination (lighting) on the sampled reef fish. To address this, stereo-BRUVs samples were collected with and without the presence of lighting from shallow photic reefs. The optimal approach (bait type or with/without lighting) was determined by which method:

- a) Detected the majority of reef fish species typical to the region and,
- b) Was most cost-effective (applicable to bait type study only).

Methods research plays an integral role in applied ecology (Elphick 2008). The ecosystem approach to fisheries management (EAF) recommends that for the longevity of monitoring systems they must be applicable to policy development (Garcia *et al.* 2003). However, there are current limitations in the understanding of the role of methodology and its effect on policy (Elphick 2008). A sampling method that accurately represents the system in question can influence policy adequately; likewise, poor research has the potential to power poor policy decision-making. Institutional support, particularly governmental, is often hinged on cost-efficiency and the ability to replicate research for comparisons in time and space, particularly regarding long-term monitoring. Fundamental to this is an adept understanding of the selected sampling methodology.

5.2 Key findings

As a novel method in South Africa, this thesis provides a critical understanding of the usefulness and effectiveness of the stereo-BRUVs for surveying the reef fish community typical to the area.

While similar research has been conducted elsewhere around the world, this is the first time such research has been conducted in South Africa and as such the findings advance our understanding of the stereo-BRUVs sampling method in relation to the South African environment.

The first research chapter of this thesis tested different bait types to determine which was most suited to sample the reef fish community. The stereo-BRUVs method was developed so as to survey fisheries target species previously under-sampled by methods that did not employ bait for attraction (Bernard 2014, Babcock *et al.* 1999, Willis and Babcock 2000).

It was anticipated that the sessile bivalve bait types of oyster and mussel would either prove to be an effective bait type to survey the entire community or that they successfully sampled alternate species previously missed by more traditional baits such as pilchard. This, however, was not the

case. Sessile bivalve baits, whilst available at no cost, proved to be poor bait types and, with respect to oyster, an unreliable bait source.

Squid and pilchard were demonstrated to be suitable bait types. No significant difference for both abundance and size estimates were found between the bait types. Favourably, however, pilchard was found to be the most effective and most cost-efficient bait type. This is consistent with other BRUVs studies where pilchard is the accepted international norm. It is recommended pilchard be used for BRUVs and stereo-BRUVs research in the Agulhas ecoregion. This is an important result as it allows one to compare South African datasets with international counterparts.

Despite squid being outperformed by pilchard in the cost benefit analysis, knowing it is an effective bait type is still favourable. Knowledge of substitute bait types, such as squid, broadens the application of stereo-BRUVs in the event pilchard is not available.

Another important application of stereo-BRUVs is sampling at depth, to do so requires lighting. This thesis examined the effect of lighting on the fish assemblage structures observed with stereo-BRUVs. The chapter examined whether using lights when sampling in shallow habitats was a necessity for deep water sampling. Sampling was conducted in the photic zone to be able to compare samples collected with and without lighting. The potential biases associated when sampling with lighting, such as the phototactic behaviour of some fish species, may require special consideration when comparing samples collected with lighting and without lighting. No significant differences were found in fish assemblage data (MaxN, length estimates, trophic groups) of samples collected with lighting and those collected without. This result suggests that samples, whether collected with or without lighting from the photic zone, are comparable. In turn, data collected with stereo-BRUVs without lighting from shallow photic environments can be compared to data collected with stereo-BRUVs employing blue lighting from sub-photoc environments, as biases caused by not using lighting in the photic zone are negligible.

The study identified some species-specific patterns that may need to be considered. Phototactic behaviour was anticipated, and some species did exhibit either attraction or aversions to lighting. Small generalist carnivores increased in abundance and larger Pyjama catsharks were observed more frequently when sampled with lighting. However, the statistical analysis revealed that none of these trends were significant, suggesting that the phototactic behaviour was negligible. Current stereo-

BRUVs research in South Africa has focused on sampling the whole fish assemblage. However, some BRUVs research has focused on targeting one species or one trophic guild in particular. For example, Willis & Babcock (2000) focus singularly on sampling snapper (*Pagrus auratus*). Should this be the case for the Agulhas ecoregion, I would suggest that the use of lighting be viewed as a potential bias for *Boopsoidea inornata*.

South Africa's deep near-shore reef communities are under-researched (Heyns 2015). One confounding factor is the difficulty to sample such habitats (Heyns 2015). Deep-water sampling requires a compromise between the desire to sample with the least impact to reduce bias and the unavoidable necessity to employ lighting in low ambient light conditions.

5.3 Critical evaluation

Riy Banks, BI MPA and TNP MPA are characteristic of reef habitats found in the Agulhas ecoregion. Ideally, however, data from a wider variety of locations would have strengthened that analysis and enabled broader generalization of the results across a larger area. This is particularly true for the bait type study where only one study area was sampled. Targeting multiple areas was simply not feasible given time constraints of this MSc project, however generality can also be drawn from the results of international studies. It is important to take into account that this study was the first application of stereo-BRUVs in the country and as was to be expected, some logistical setbacks did occur that hindered initial progress.

Many fish species undertake ontogenetic migrations into deeper waters as they mature (Mann 2000). It is important to consider that:

- a) The diet of many fish species shifts with age, and
- b) Older adult fish species that have occupied deep-water habitats for numerous years may have heightened sensitivity to light and may avoid areas illuminated with lighting.

This suggests that the fish assemblages observed in deeper waters, and their behaviour, might be different to those observed in shallower waters. Considering the broad depth range inhabited by many of the reef fish species occurring in the Agulhas ecoregion of South Africa it would have been useful to have had assessed a wider depth range. Whilst this is a short coming in the bait study it was unavoidable in the lighting study. It was not possible to test the effect of omitting lighting in the

sub-photic (deeper) zone due to the low light conditions and not being able to see the fish. Previous studies have been conducted comparing red and blue lighting, which could be useful in testing fish assemblages observed in the sub-photic zone. Initial planning of the lighting study included an experiment to test the effect of a variety of light colours. However, to develop the necessary lighting systems required materials that were not available in South Africa. Consequently, pre-made lighting systems were supplied from Australia and only blue lighting systems were available at the time.

5.4 Application of the research

Biological monitoring plays an important role in EAF (Garcia *et al.* 2003). In southern Africa there is a compelling and urgent need for effective monitoring of marine ecosystem trends (Hutchings *et al.* 2009). Importantly, monitoring is necessary so as to provide objective and sound scientific advice for decision makers in government and industry (Hutchings *et al.* 2009). The knowledge obtained from this research is instrumental to improve the effectiveness of stereo-BRUVs as a monitoring tool in South Africa.

The EAF requires comprehensive data collection systems, relative to conventional management, to monitor fisheries (Garcia *et al.* 2003) and stereo-BRUVs present an exciting platform for development. This methodology study on stereo-BRUVs in South Africa has identified potential biases that are similar to, or can be expected of, ecology studies (Elphick 2008). In applying the results of this study one will be able to mitigate biases. This will ensure stereo-BRUVs in South Africa are more effective and more efficient, and provide more comprehensive data sets of fish assemblages. An important component to the effectiveness of the method is cost-efficiency and achieving economies of scale. A cost-efficient method is a sustainable method. This is of increasing relevance with much incentive being placed on sustainability of data collection and analysis in southern African fisheries management and conservation (Hutchings 2009). The use of pilchard has shown to require the least number of samples to effectively survey the fish assemblage present, reducing sampling costs whilst providing an important measure of ecosystem integrity. Similarly, by foregoing the need for lighting in photic sampling the cost of comparative stereo-BRUVs across a range of depths is reduced.

The research within this thesis successfully provided insights into the methodology for monitoring reef fish with stereo-BRUVs in the Agulhas ecoregion of South Africa. The results are relevant and comparable with international studies addressing similar questions in other locations, adding to the growing global dataset from stereo-BRUVs research.

5.5 Future work

This thesis formed part of a larger project and recommendations for future work are in many instances being addressed in other studies or are planned to be addressed. It is, however, useful to reiterate Wraithe *et al.* (2013) and highlight the importance of ensuring standardization in the use of stereo-BRUVs not only in South Africa, but internationally. Spatial variation, the differences between geographical areas, can influence fish diversity and abundances of fish observed. Wraithe *et al.* (2013) highlight the importance of addressing stereo-BRUVs associated limitations and biases across a variety of locations to improve the utility of underwater video techniques. It is suggested too that this thinking be applied to a variety of depths. Recently in South Africa, moratoriums on important slow growing sparid species and no-take MPAs have been legally challenged. It is of increasing importance that research is conducted not only to be scientifically credible, but also to withstand challenges induced by increasing population pressures.

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