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# **Study of fish assemblages in <em>Ecklonia radiata</em> dominated rock reefs in subtropical eastern Australia and dietary analyses of three species of generalist predators of the family Labridae**

Sanchez Peregrin, Laura

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radiata* dominated rocky reefs in  
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predators of the family Labridae**

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**Masters by Research**

**7<sup>th</sup> of December 2017**

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I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. I certify that I have complied with the rules, requirements, procedures and policy of the University (as they may be from time to time).

A handwritten signature in blue ink, appearing to be a stylized 'J' or a similar character.

Signed ..... Date: 7<sup>th</sup> of December, 2017

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## Abstract

Fish are one of the most conspicuous components of reef ecosystems and have been found to be good surrogates in the study of rocky reefs communities. I examined fish assemblages in three *Ecklonia radiata* dominated rocky reefs of the Coffs Coast, in subtropical eastern Australia (a recognised hotspot for climate change). The results determined that adult kelp is strongly correlated with fish community composition, Labridae (wrasses) is the most common fish family in these reefs, and labrids *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* are the most abundant species. These species were chosen to study the role of predators in threatened kelp habitats. Dietary analyses were undertaken using a cutting edge technique, DNA metabarcoding, in order to test the effectiveness of this technique in the study of food webs in these ecosystems. In total, 70 different Operational Taxonomic Units were identified across the gut content samples of 14 fish. Previous reports of wrasse as generalist, opportunistic feeders are supported by the findings of this study. The results provided novel information on prey diversity of wrasses and indicated that these species may occupy a high trophic level in the food web, given they feed on carnivorous fish. Small sample sizes limited the ability to draw conclusions on competition or food partitioning among these species, or on how kelp loss is going to affect their trophic ecology. However, this study enabled the identification of a wide range of prey from highly digested gut contents, indicating that DNA metabarcoding is a powerful technique for dietary analysis of generalist predatory fish. Future studies on the fish community structure in these ecosystems should implement a multi-method approach to study fish assemblages. Future dietary studies of these wrasse species should include more replicates and should target individuals in other habitats in the region. Furthermore, these studies should assess the present residency of these wrasses in kelp forests in order to investigate their habitat alternatives after the loss of *Ecklonia radiata* canopies. Further research should also focus on the invertebrate community structure in these habitats and in the surrounding areas, with depth as a factor, to predict changes in prey assemblages in the context of climate change.

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## Abbreviations

Av – Average	MW – Maori wrasse
BOLD – Barcode Of Life Database	N – Nitrogen
BLAST – Basic Local Alignment Search Tool	NGS – Next Generation Sequencing
Bp – Base pairs	<i>Ngym</i> – <i>Notolabrus gymnogenis</i>
BRUV – Baited Remote Underwater Video	nMDS – Non-Metric Multidimensional Scaling
BT – Belt Transect	NR – North Rock
C – Carbon	NSW – New South Wales
CBW – Crimson-banded wrasse	Olin – <i>Ophthalmolepis lineolata</i>
CPCe – Coral Point Count with Excel extensions	OTU – Operational Taxonomic Unit
CO <sub>2</sub> – Carbon dioxide	PCR – Polymerase Chain Reaction
COI – Cytochrome C Oxidase subunit I	PERMANOVA – Permutational Analysis of Variance
CBOL – Consortium for the Barcode of Life	Pgue – <i>Pseudolabrus guentheri</i>
DistLM – Distance based Linear Modelling	POM – Particulate Organic Matter
DNA – Deoxyribonucleic Acid	S1 – Kelp Stage 1
DPI – Department of Primary Industries	S2 – Kelp Stage 2
EAC – Eastern Australian Current	S3 – Kelp Stage 3
ENSO – El Niño Southern Oscillation	SCUBA – Self-Contained Underwater Breathing Apparatus
GSR – Great Southern Reef	SIA – Stable Isotope Analysis
GW – Gunthers wrasse	SIMP – Solitary Islands Marine Park
HD – High Definition	SIMPER – Similarity Percentages Analysis
HPZ – Habitat Protection Zone	SPC – Stationary Point Counts
iBOL – International Barcode of Life	SSTs – Sea Surface Temperatures
KelpCam – GoPro Kelp Camera	St Err – Standard Error
Lo – Location	RUV – Remote Underwater Video
MBI – Muttonbird Island	UVC – Underwater Visual Census
Me – Method	UV-Vis – Ultraviolet-visible spectroscopy
Mo – Month	WBCs – Western Boundary Currents
MPA – Marine Protected Area	WR – Woolgoolga Reef

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# Chapter 1 – Literature Review

## 1.1 Kelp forests

### 1.1.1 General introduction to kelp forests

Kelps primarily comprise brown algae from the Order Laminariales and are conspicuous features in rocky reefs in intertidal and subtidal ecosystems (Steneck et al. 2002) along temperate, boreal and subantarctic regions (Lüning et al. 1990, Schiel 1994, Christie et al. 2009), and they are present on all continents except for the Antarctica (Moe and Silva 1977). Temperature, nutrients and light generally limit kelp to mid-latitudes (40°-60°) in the Northern and Southern hemispheres (Steneck et al. 2002), although northern range limits of the kelp *Ecklonia radiata* in Australia extend to ~27° (Marzinelli et al. 2015b). In general, kelp distribution is considered to be restricted towards the tropics by the 20°C summer isotherm of surface temperature (Mann 1973). However, growth of gametophytes and sporophytes of *E. radiata* is optimal between 15°C and 22°C (Mabin et al. 2013). The environmental factors limiting growth and mortality of kelp are light, wave height and nutrient levels (Graham et al. 2007b). Depending on how much light these forests are exposed to, wave action and the activity of grazers, these ecosystems can be found from the shoreline to depths of 30-40 m (Marzinelli et al. 2015b), but have been found at depths below 60 m in some areas (Graham et al. 2007a).

As primary producers, these macrophytes are among the most productive on the planet (Mann 1973b, Schiel and Foster 1986, Lüning et al. 1990, Barrón et al. 2003, Abdullah and Fredriksen 2004) and represent a key food source to some of the associated fauna (Graham 2004). Furthermore, they are also promoters of secondary productivity (Teagle et al. 2017), as they provide three-dimensional habitat structure, functioning as ecological engineering-foundation species (Dayton 1975, Barry and Dayton 1991). Similarly to other benthic foundation species like seagrasses, massive sponges and hard corals, kelp increase the volume and heterogeneity of the habitat (Dayton 1985, Bruno and Bertness 2001). They also provide refuge and nursery habitats for invertebrates and fish (Willis and Anderson

2003, Graham 2004, Coleman et al. 2007). All these features result in the support of high biodiversity. As large macrophytes, they modify the environment and the resources for other organisms (Dayton 1985, Foster and Schiel 1985, Kendrick et al. 1999, Wernberg et al. 2005, Wernberg et al. 2010, Wernberg et al. 2016), influencing light within the borders of the kelp forest (Kennelly 1989, Connell 2003b, Clark et al. 2004). They also alter the substratum for other organisms to live (Christie et al. 2007) and modify other environmental factors such as sedimentation (Connell 2003a) and water flow dynamics (Eckman et al. 1989). Furthermore, the three-dimensional structure of kelp forests provides a physical barrier that alters water current velocity (Jackson 2008), thus reducing the water flow and increasing the retention of sediment particles and organic material that will be consequently deposited under the canopy (Smith 2000).

These macrophytes form beds and forests that are energy-rich habitats of great diversity (Mann 1973a) and ecological and economic importance (Foster and Schiel 1985, Leet 2001, Graham et al. 2007b, Beaumont et al. 2008, Christie et al. 2009, Almanza and Buschmann 2013, Costanza et al. 2014, Bennett et al. 2015). They support large faunal density (Smith et al. 1996b, Wernberg et al. 2005, Graham et al. 2008, Tuya et al. 2008, Christie et al. 2009), especially in shallow habitats (Coleman et al. 2007), and provide protection to an extensive amount of marine organisms, including commercially (Bologna and Steneck 1993) and conservationally important species (Steneck et al. 2002, Smale et al. 2013). Kelp habitats are ecologically significant for their high productivity, and their support of complex food webs and high diversity in coastal ecosystems (Steneck et al. 2002, Graham 2004, Coleman et al. 2007, Christie et al. 2009, Mann 2009, Almanza and Buschmann 2013). Furthermore, they play a major role in energy exportation to other systems (Harrold et al. 1998, Graham 2004, Branch 2008, Bishop et al. 2010, Krumhansl and Scheibling 2012), providing organic matter and enhancing secondary production in communities located from a few meters to hundreds of kilometres from the production source (kelp forests).

The most complex microhabitat provided by these macrophytes is the holdfast structure, which anchors the thallus of the macroalgae to the substratum (Arnold et al. 2016) and comprises intertwined haptera that forms a structurally complex lattice, supporting diverse assemblages of invertebrates and fish (Smith 2000, O'hara 2001, Vega et al. 2005, Coleman et al. 2007, Goodsell and Connell 2008,

Villegas et al. 2008). The habitat provided by the holdfast is extensive and stable for most species of kelp and the gaps between the substratum they latch on and the haptera make favourable, protective spaces for colonising animals trying to avoid predators and hostile environmental factors (Teagle et al. 2017). Furthermore, because these haptera increase the area and the volume of the substrata available, organic matter accumulates in these spaces and sessile and mobile organisms attach to and colonise the haptera and the substrata beneath and in the surrounding area (Ojeda and Santelices 1984), increasing the diversity per square meter. Typically, the species richness supported by a single adult kelp holdfast may be 30-70 macrofaunal species, with some cases reaching 90 species per holdfast (Jones 1972, Smith et al. 1996a, Thiel and Vásquez 2000, Christie et al. 2003). These figures may vary with the species of macrophyte, but generally those species with laminarian type of holdfast (*Ecklonia radiata*, *Laminaria hyperborea*) support the largest biodiversity (Teagle et al. 2017).

Animals from more than 10 phyla can be found in kelp forests: Chordata, Arthropoda, Annelida, Echinodermata, Bryozoa, Cnidaria, Mollusca, Platyhelminthes, Brachiopoda and Porifera (Steneck et al. 2002). Holdfast assemblages are normally dominated by mobile invertebrates that can quickly colonise new available habitat (Teagle et al. 2017). The most conspicuous mobile taxa are copepods, polychaetes, gastropods and amphipods, but also sessile animals like bryozoans, sponges and bivalves can be found amongst and around the haptera (Moore 1973, Ojeda and Santelices 1984, Norderhaug et al. 2002, Christie et al. 2003, Arroyo et al. 2004, Anderson et al. 2005, Ríos et al. 2007, Blight and Thompson 2008, Christie et al. 2009, Schaal et al. 2012). Amphipods and polychaetes normally dominate faunal assemblages in these microhabitats, sometimes representing over 75% of the total abundance of animals in the holdfast (Smith et al. 1996a).

Furthermore, kelp canopies promote conditions for the development of understory macroalgae assemblages, which will be mainly dominated by red algae (Clark et al. 2004, Flukes et al. 2014). This is due to the kelp providing a buffer for benthic macro-algal species from high wave energy (Almanza and Buschmann 2013), also regulating the shading of the habitat (Kennelly 1987b, Arkema et al. 2009) and altering the water flow (Eckman et al. 1989). These understorey macroalgae will function as habitat for both sessile and mobile invertebrate fauna (Flukes et al.

2014, Lavender et al. 2017). Most of the common algal species found under the canopies of kelp are tolerant to low light conditions, growing more rapidly and successfully when irradiances are low (Jones et al. 1971, Norton et al. 1977). Understorey algal assemblages have been shown to have higher diversity in reefs with canopy than those lacking a canopy (Melville and Connell 2001), probably due to these canopies increasing the heterogeneity of the habitat and creating more suitable environmental conditions (Teagle et al. 2017).

### **1.1.2 Trophic ecology of kelp forests**

Food supply in most kelp forest habitats is not considered a limiting factor, as it comes from detrital kelp lamina, other dead macroalgae and phytoplankton that gets deposited amongst the kelp (Schaal et al. 2012). Trophic pathways are primarily detrital based (Smith 2000, Graham et al. 2007b), and previous studies have identified a trophic flow from kelp, via invertebrates, to predatory fish (Norderhaug et al. 2003, Norderhaug et al. 2005). Furthermore, considerable amounts of fixed carbon (drift kelp) (Graham et al. 2003) and particulate organic matter (POM) is lost from the ecosystem by export (Christie et al. 2009).

Thanks to the complexity of the holdfast interstices, pieces of kelp that litter the benthos accumulate within the holdfast, this acting as a trap for sediment and particulate organic matter (POM). Then, these may be consumed by invertebrates and microbes (Moore 1973, Smith 1996, Arroyo et al. 2004). Therefore, kelp communities are often dominated by filter feeders and deposit feeders (Smith et al. 1996a, Schaal et al. 2012). However, grazers, scavengers and predators are also present (McKenzie and Moore 1981, Smith et al. 1996a).

There is also a pathway of herbivores feeding directly on kelp canopies, and in some kelp ecosystems herbivory is an important process (Harley et al. 2012b) because mesograzers keep the kelps free of epiphytic algae and other competitors (Fowler-Walker and Connell 2002, Moksnes et al. 2008). However, sometimes large herbivores may over-graze new kelp recruits, specially after severe physical disturbances (Steneck et al. 2002, Sala and Dayton 2011). This may result in phase shifts from complex and diverse habitats to less diverse and productive ones, such as urchin barrens (Steneck et al. 2002, Ling 2008, Ling et al. 2009a, Johnson et al. 2011b, Ling et al. 2015), simplifying the whole food web (Byrnes et al. 2011).

However, fish predation seems to regulate the densities of mesograzers in healthy kelp habitats (Davenport and Anderson 2007, Moksnes et al. 2008).

I will expand more on food webs in kelp forests and the methods used to study them in section 1.3.1.

### **1.1.3 Threats to kelp forests**

Kelp forests are subjected to a range of anthropogenic stressors. These include climate change, reduced water quality, overgrazing driven by trophic cascade effects caused by overfishing and/or range expansion of herbivores, and in some countries, high kelp extraction pressure (Steneck et al. 2002, Johnson et al. 2005, Ling 2008, Vásquez 2009, Johnson et al. 2011b, Wernberg et al. 2012, Almanza and Buschmann 2013, Smale et al. 2013, Steneck and Johnson 2013, Brodie et al. 2014, Verges et al. 2014, Ling et al. 2015, Byrnes et al. 2016, Vergés et al. 2016). Each of these may result in ecological phase shifts (Steneck et al. 2002, Worm and Lotze 2006, Jackson 2008), that drive the ecosystem to an alternate state (Wright et al. 2005, Ling 2008), such as the transition from kelp forests to urchin barren grounds (Ling 2008, Ling et al. 2009b, Lenanton et al. 2017)

#### **1.1.3.1 Overgrazing caused by overfishing**

Kelp harvesting has become common and is certainly growing, due to the demand for human consumption, the growing alginate extraction industry and its use as a food source for abalone cultivation (Troell et al. 2006, Buschmann et al. 2008, Vásquez 2008). For example, in both Chile and Norway, between 130,000 and 200,000 tonnes of kelp are extracted every year (Vásquez 2008, Vea and Ask 2011). While a limited harvest may be sustainable if properly managed, kelp removal has shown to have negative consequences for kelp communities, the functioning of these ecosystems and the services they provide to other ecosystems (Christie et al. 1998, Anderson et al. 2006, Vásquez 2008, Krumhansl and Scheibling 2012). For example, the removal of *Macrocystis pyrifera* in Chile by abalone farmers, who require biomass all year round (Gutierrez et al. 2006), has led to processes of succession whereby other species invade, occupy and colonise the habitat made available (Almanza and Buschmann 2013), thus affecting other economically important species (e.g. *Cochleopas concholepas*, *Fisurella* sp.).

Thus, while the removal of this habitat-forming species and main primary producer in the ecosystem may be sustainable, the potential for impact to the services kelp provides may be considerable (Smale et al. 2013). The associated assemblages may take longer to recover (Christie et al. 1998): for example, fish abundance may be reduced and so is the habitat preferred by foraging seabirds (Lorentsen et al. 2010). Therefore, kelp harvesting may lead to the collapse of the ecosystem (Christie et al. 2009). When this happens, original energy provision from phytodetritus derived from macroalgae may shift to ephemeral microalgae and phytoplankton (Graham 2004).

#### **1.1.3.2 Overgrazing caused by overfishing**

The most important regulators of kelp grazing organisms are their predators (Estes and Duggins 1995, Sala et al. 1998, Steneck 1998, Johnson et al. 2011a, Ling et al. 2015) and their diseases (Scheibling et al. 1999, Brady and Scheibling 2005). When these predators, such as lobsters (Ling et al. 2009b) or fish (Tegner and Dayton 2000) are intensively harvested, cascading down the food web may occur, for example, via the proliferation of kelp grazers such as sea urchins (Bourque et al. 2001, Steneck et al. 2004, Ling et al. 2009b, Foster and Schiel 2010).

Sea urchins have led to destructive grazing of large areas of kelp forests in different regions of the planet (Sivertsen 1997, Bourque et al. 2001, Steneck et al. 2002, Steneck et al. 2004, Graham et al. 2007a, Ling 2008, Ling et al. 2009b, Norderhaug and Christie 2009), affecting regulatory processes within the ecosystems and impeding efficient kelp recruitment (Henríquez et al. 2011). When kelp bed decimation occurs, in extreme cases, the loss of biogenic habitat causes phase-shifts from complex kelp forests to impoverished barrens (Bourque et al. 2001, Steneck et al. 2002, Johnson et al. 2011a, Filbee-Dexter and Scheibling 2014, Ling et al. 2015). Furthermore, this reduction in kelp causes cuts in the supply of kelp phytodetritus to kelp habitats and to other ecosystems (Krumhansl and Scheibling 2012), thus leading to shifts from primary consumers which feed on kelp and their epiphyte algae, to consumers of phytoplankton and its phytodetritus (Graham 2004).

As the habitat changes, diversity and ecological processes are altered as well: when sea urchins remove kelp from the reef, the system shifts to a new state, and may become a turf algae-dominated habitat (Moy et al. 2008) with lower faunal

densities and species richness, supporting few species of animals and plants (Elner and Vadas 1990, Steneck et al. 2002, Graham 2004). Production in these modified ecosystems can be two orders of magnitude lower than for the original kelp forest (Chapman 1981, Ling et al. 2009b). This state of reduced diversity and species richness may persist until the population of urchins decreases (Norderhaug and Christie 2009). However, returning to the original state requires much lower abundances of urchins than the threshold at which overgrazing occurred in the first place (hysteresis) (Ling et al. 2009b). In the worst-case scenario, sedimentation in urchin barrens may occur, preventing the recruitment of macroalgae, and thus leading to the perpetuation of the barren state, even in the absence of urchins (Valentine and Johnson 2005).

Despite all of the above, overgrazing is a rather unusual event (Christie et al. 2009), as kelp state may resist urchin grazing through stabilising feedbacks. This suggests that self-regulation occurs in healthy kelp forests, for example with these macroalgae providing habitat for the predators of urchin recruits (Elahi and Sebens 2013). Thus, persistence, resilience and vulnerability of kelp habitats are dependent on their structural complexity, the interaction between these macrophytes, epiphytes, grazers and predators (Scheibling et al. 1999, Christie et al. 2009), and on the diversity within different functional groups (Steneck et al. 2002). Similar roles in the community, known as ‘functional redundancy’, such as competition amongst different species of grazers and predators for the same food source, is a key factor in the stability of kelp forests (Duffy et al. 2001, Steneck et al. 2002). This functional redundancy may provide “biological insurance” (Elahi and Sebens 2013) in the context of warming water temperatures (Stocker et al. 2013) and biodiversity loss (Hooper et al. 2012).

### **1.1.3.3 Climate change**

Climate change will involve increases in atmospheric and oceanic temperatures, changes in rainfall pattern, an increase in the frequency of extreme weather events and, in the marine environment, oceanic currents will be altered and pH will keep decreasing (Stocker et al. 2013). These changes may cause direct and indirect effects on species, communities and ecosystems (Bellard et al. 2012, Zarnetske et al. 2012, Poloczanska et al. 2013, Vergés et al. 2016), and for this reason it is crucial to understand and predict the impacts of these changes. It is important to

note that, within the projected increase in water temperatures, there are regional differences, with localised areas of higher rate of warming, commonly known as hotspots for climate change (Sen Gupta et al. 2013). For example, western boundary currents (WBCs) –Japan, eastern USA, eastern Australia, northern Brazil and southeastern Africa– are warming 2-3 times faster than the global mean (Wu et al. 2012) and are considered to be potential hotspots for biological change (Verges et al. 2014). Ocean circulation influences the distribution of marine species (Ling 2008, Coleman et al. 2011, Coleman et al. 2013, Cetina- Heredia et al. 2015, Vergés et al. 2016), affecting larval dispersal and recruitment, and connectivity of macroalgae, fishes and sea urchins, amongst others (Ling et al. 2009a, Verges et al. 2014, Coleman et al. 2017). Currently, there is strong evidence that warmer waters are altering the dispersal and increasing the expansion range of several marine species (Sagarin et al. 1999, Ling 2008, Ling et al. 2009a, Figueira and Booth 2010, Baird et al. 2012, Wernberg et al. 2013, Goatley and Bellwood 2014, Verges et al. 2014, Nimbs et al. 2015, Scott et al. 2015, Nimbs and Smith 2016, Vergés et al. 2016, Lenanton et al. 2017, Nimbs et al. 2017).

Kelps are cool water macroalgae which are stressed by high temperatures (Steneck et al. 2002). Rising temperatures in the ocean negatively affect their productivity, reproduction, recruitment, growth and capacity to compete with other species for space (Wernberg et al. 2010, Merzouk and Johnson 2011, Harley et al. 2012b, Mabin et al. 2013, Provost et al. 2017). Warmer temperatures also restrict the range of kelp forests to colder waters, causing latitudinal shifts in distribution in the direction of the poles (Polunin 2008, Brierley and Kingsford 2009). Due to their high response to environmental conditions, kelps are considered good sentinels of climate change (Wernberg et al. 2013, Bell et al. 2015).

With the frequency and severity of extreme warming events increasing (Smale and Wernberg 2013), as well as the gradual longer-term ocean warming (Wernberg et al. 2011a), kelp populations are facing widespread losses (Raybaud et al. 2013, Voerman et al. 2013). For example, warming is occurring 2-4 times the global average in western and south-eastern Australia, respectively (Pearce and Feng 2007, Ridgway 2007, Hobday and Pecl 2014). In the eastern side, this is due to the strengthening of the Eastern Australia Current (EAC) and the increased frequency and size of its eddies. The EAC is propagating polewards towards Tasmania (Johnson et al. 2011b), increasing the influence of warm, nutrient-poor water, which

is associated with dramatic losses of giant kelp (*Macrocystis pyrifera*) (Johnson et al. 2011b, Smale and Wernberg 2013).

Furthermore, tropical herbivores are also expanding their ranges pole-wards, favoured by warmer temperatures, and subtropical and temperate kelp forests are becoming exposed to a great diversity and density of new herbivores capable of high levels of deforestation (Sivertsen 1997, Steneck et al. 2002, Steneck et al. 2004, Graham et al. 2007a, Ling 2008, Ling et al. 2009b, Norderhaug and Christie 2009, Vergés et al. 2016). Thus, projected levels of global warming are a cause of serious concern, as they may lead to permanent changes in habitats (Poloczanska et al. 2007, Hawkins et al. 2008, Wernberg et al. 2011c, Díez et al. 2012, Beger et al. 2014), which may affect the whole community (Schiel et al. 2004, Wernberg and Goldberg 2008) and cause severe loss of biodiversity (Graham 2004, Blight and Thompson 2008, Ling 2008, Vergés et al. 2014). All of the above may result in changes in interspecific interactions (Kordas et al. 2011), trophic cascades (Graham 2004, Steneck et al. 2004, Schiel 2011) and phase shifts (Sivertsen 1997, Steneck et al. 2002, Steneck et al. 2004, Ling 2008, Ling et al. 2009b, Norderhaug and Christie 2009). All these phenomena may have, not only important ecological consequences, but also socio-economic ones, for example, leading to the complete collapse of fisheries (Serisawa et al. 2004).

Ocean acidification seems to have negative effects on macroalgal communities (Porzio et al. 2011, Wernberg et al. 2011a, Provost et al. 2017). Projected moderate acidification and temperatures may have a synergetic positive effect on kelp competitors, such as non-calcareous algal turfs, which may exacerbate kelp loss (Connell and Russell 2010, Connell et al. 2013, Falkenberg et al. 2013b, Provost et al. 2017). Natural disturbances are part of the dynamics of kelp forests (Steneck et al. 2002, Reed et al. 2011, Wernberg et al. 2012). And despite disturbances being common, kelp communities do not constantly shift from one state to another. Whether environmental change is sudden or gradual, change in the community may be minimal thanks to compensatory mechanisms, such as trophic compensation (Ghedini et al. 2015). Nevertheless, sometimes disturbance is larger than the capacity of compensatory mechanisms.

For example, wave action is important in maintaining diversity within kelp forest, and it promotes turnover of nutrients and species (Smale et al. 2010, Smale and

Vance 2016). However, extreme wave action may cause severe damage to kelp and the associated fauna, and may lead to high mortality rates and severe loss of habitat (Reed et al. 2011, Filbee-Dexter and Scheibling 2012, Krumhansl and Scheibling 2012). Climate change is intensifying the global hydrological cycle, with water being cycled more rapidly between the atmosphere and the oceans (Held and Soden 2006). As a consequence of this, storms are projected to become more intense and more frequent as surface temperatures increase (Meehl et al. 2000, Stocker et al. 2013). Furthermore, wave height and speed are also projected to increase (Young et al. 2011). Storms and increased wave action can remove entire kelp plants, leaving large areas of the reef without canopy cover (Thomsen et al. 2004, Reed et al. 2011). This may negatively affect the entire ecosystem, by altering light, hydrodynamics and the entire three-dimensional structure of the habitat (Graham et al. 2007b) and possibly facilitating the invasion of non-native species (Edgar et al. 2004b). Thus, the ecosystem suffers losses in diversity and complexity, as species go locally extinct, leading to the simplification of food webs (Byrnes et al. 2011). For example, in California, El Niño Southern Oscillation (ENSO) events, which are projected to intensify in the next century (Stocker et al. 2013), affect *Macrocystis pyrifera* in two ways: primarily through the deepening of the thermocline, causing nutrient stress, and by the increase of kelp-destructing storm waves (Graham et al. 2007b). These stressors have led to the decimation of kelp beds in the past and have also favoured kelp overgrazing by urchins and crustaceans (Steneck et al. 2002).

Recovery of kelp after storms may be impeded by the effects of increased temperature on their physiology and reproduction (Wernberg et al. 2010), the presence of new grazers favoured by environmental changes (Ling 2008, Verges et al. 2014), and anthropogenic disturbances (Gutierrez et al. 2006, Russell et al. 2009), some of which may be already causing shifts in the trophic balance (Smith 2000, Graham et al. 2007b). Furthermore, physiological stresses on kelp may make these macroalgae more susceptible to diseases that can cause mass mortality (Cole and Babcock 1996) or have sub-lethal impacts, reducing growth and fecundity (Wahl et al. 2015), ultimately affecting their provision of biogenic habitat.

## 1.2 The future for kelp forests in Australia

### 1.2.1 Climate change in Australia

In Australia, on both the east and the west coasts of the continent, ocean temperatures have already warmed and salinity has increased in the pole-ward flowing currents (Pearce and Feng 2007, Ridgway 2007, Hobday and Pecl 2014). These physical changes are associated with biological changes in abundance, geographic range, phenology and community structure of marine species (Sagarin et al. 1999, Hobday et al. 2007, Poloczanska et al. 2007, Ling 2008, Ling et al. 2009a, Figueira and Booth 2010, Wernberg et al. 2011b, Baird et al. 2012, Wernberg et al. 2013, Verges et al. 2014, Nimbs et al. 2015, Scott et al. 2015, Lenanton et al. 2017, Nimbs et al. 2017). Furthermore, the effects of climate change on Australia's marine organisms are often increased and potentially masked by anthropogenic disturbances (Hobday and Lough 2011). By the 2030s, Sea Surface Temperatures (SSTs) are projected to be ~1°C higher (than those in 1980-1999) around Australia, and by 2070s, SSTs are projected to be 1.5-3.0 °C higher (Hobday and Lough 2011, Collins et al. 2013). South-eastern Australia shows the greatest projected rate of warming to the end of the 21st Century, as a result of both rising atmospheric temperatures and the strengthening of the Eastern Australian Current (EAC) (Ridgeway and Hill 2012), which translates to warm and nitrate-poor waters pushing further south for longer periods of time (Cai et al. 2005, Pearce and Feng 2007, Ridgway 2007, Hobday and Pecl 2014). By 2050, average SSTs in this region are projected to be 2°C higher than the 1990-2000 average. Intensification of the EAC's southward flow and intensification of its anti-cyclonic eddies are expected to continue over the next 100 years (Poloczanska et al. 2007, Ridgway 2007, Ridgeway and Hill 2012, Cetina- Heredia et al. 2015), with the east-coast region of Australia confirmed as a hotspot for climate and biological change (Hobday and Lough 2011, Wu et al. 2012, Verges et al. 2014). These changes may impact dispersal and connectivity within the EAC (Cetina- Heredia et al. 2015), having important implications for genetic diversity (Coleman et al. 2017), ranges of species (Ling 2008) and ecological interactions (Johnson et al. 2011b, Verges et al. 2014).

Along with rises in sea temperature, other effects of climate change are expected to have severe consequences. Rising sea levels will cause inundation of coastal

regions and coastal erosion, which will not be uniform around Australia and will affect estuarine habitats in particular (Gillanders et al. 2011). Furthermore, the chemistry of the ocean is changing, with the pH of surface waters decreasing and projected to be ~0.3 units lower by 2100 (Collins et al. 2013, Stocker et al. 2013, Ciais et al. 2014). This is critically important for marine organisms: it poses a threat to the structure of coral-reef communities (Hoegh-Guldberg et al. 2007), to macroalgal habitats (Porzio et al. 2011, Provost et al. 2017), to coralline algae in temperate environments (Wernberg et al. 2011a), to larvae, to adult benthic and pelagic calcifying organisms (Przeslawski et al. 2008, Brennand et al. 2010), as well as to tropical fish (Pankhurst and Munday 2011).

### **1.2.2 *Ecklonia radiata* forests in a changing climate**

Shallow (<30 m) subtropical and temperate reefs in the Australian continent are characterised by the presence of *Ecklonia radiata* kelp forest. These span more than 8000 km of coastline the south of Queensland (~27°S) (Phillips and Price 1997, Marzinelli et al. 2015b), down the east coast of the continent, around Tasmania, along the southern coastline of Australia and to the Kalbarri region in Western Australia (27.7°S) (Bennett et al. 2015). *E. radiata* was also once present in Lord Howe Island (Lucas 1935) but has since disappeared (Millar and Kraft 1994).

These subtropical and temperate reefs are starting to be considered as an entity, the Great Southern Reef (GSR) (Bennett et al. 2015), made up of thousands of reefs dominated by *E. radiata* forests and connected to each other through oceanographic (Coleman et al. 2011, Wernberg et al. 2013) ecological (Irving and Connell 2006, Connell and Irving 2008, Vanderklift and Wernberg 2010) and evolutionary processes (Phillips 2001). The Great Southern Reef is under threat given the growing pressures from climate change, and the increasing urbanisation and development of coastal areas, with their associated pollution and growing fishing pressure (Bennett et al. 2015). It has become important to study and manage local conditions to alleviate, and hopefully reverse these stressors (Wernberg et al. 2011a, Falkenberg et al. 2013a). A better understanding of how the GSR functions is necessary in order to focus the research towards this new ‘big picture’, with special interest on the linkages and contrasts across this system (Bennett et al. 2015). However, exhaustive local research needs to be carried out in order to understand those linkages and differences between ecosystems.

*E. radiata* is the most abundant and widely-distributed, low, canopy-forming kelp in Australia (Coleman 2013, Mabin et al. 2013) and probably the most ecologically important macroalgae in temperate Australasia (Wernberg et al. 2010). It plays a major role as a foundation species (Dayton 1975) and it contributes to the high biodiversity (Smith 1996, Coleman et al. 2007), and to the ecological functioning, of most coastal rocky reefs in temperate Australasia (Irving and Connell 2006). In southern Australia, kelp forests have the highest rates in the world of regional endemism in both marine algae and invertebrates (Phillips 2001, Kerswell 2006). In Tasmania, kelp is economically important as it supports the southern rock lobster and the abalone industries (Ling et al. 2009b, Mabin et al. 2013).

*Ecklonia radiata* appears to be vulnerable to increasing temperature (Bearham et al. 2013), which can impair photosynthesis, respiration and cellular function of kelp (Staehr and Wernberg 2009, Wernberg et al. 2010). This may cause a reduction in size in adult plants (Mabin et al. 2013) and ultimately lead to recessions or local disappearance (Millar 2007, Connell et al. 2008, Wernberg et al. 2010, Verges et al. 2014). Kelp disappearance may be the result of combined effects of climate change (Wernberg et al. 2012) and local anthropogenic stressors (Russell and Connell 2009, Russell et al. 2009, Foster and Schiel 2010, Wernberg et al. 2011a, Russell and Connell 2012).

However, warmer water temperatures may not affect adult plants directly, having primary effects on young recruits by metabolic adjustments that lead to a reduced capacity to respond to physical perturbations (Wernberg et al. 2010). Warmer water temperatures also seem to affect reproduction directly (Mabin et al. 2013). The ecological performance of kelp recruits and the recovery of the adult canopy of *E. radiata* seem to be affected by a combination of the effects of severe perturbations and warm temperatures (Wernberg et al. 2012). This implies that kelp beds in warm waters have a diminished capacity for recovery following intense physical disturbance (i.e., they have a lower resilience), and the vulnerability of early life-stage processes seem to drive this impact (Wernberg et al. 2010).

*Ecklonia radiata* kelps are hosts for microbial communities. These are different subsets of bacteria that have closely related affinity to host traits or the microenvironment provided by it, or that may be correlated as a result of ecological interactions. These communities are strongly and consistently associated with the

host condition. Bacteria within surface biofilms can provide key nutrients, be integral to the normal development of the hosts and may also contribute to the defenses against natural enemies (Nasrolahi et al. 2012, Egan et al. 2013). A changing environment may lead to microbial communities on healthy hosts to break down when kelps are stressed. This may result in a disruption of the relationship between hosts and their associated microbial communities, consequently having strong detrimental effects on the host (kelps) (Marzinelli et al. 2015a).

In the context of climate change, physiological adjustments may enable the existence of *E. radiata* kelp beds in warm environments. But these may be in a state in which they are less resilient to physical disturbances such as wave action (Wernberg et al. 2010), grazing (Verges et al. 2014, Vergés et al. 2016), or to regional and local stressors, such as pollution (Russell and Connell 2009, Foster and Schiel 2010), disease (Wernberg et al. 2011a, Marzinelli et al. 2015a) and overfishing (Ling et al. 2009a, Ling et al. 2009b, Foster and Schiel 2010).

If compensatory mechanisms, such as trophic compensation (Ghedini et al. 2015) and phenological processes (de Bettignies et al. 2015) are weaker than these disturbances, the stability of the population cannot be maintained. Then the consequences of these stressors and the reduced resilience of *E. radiata* will potentially lead to the loss of kelp beds or increase their fragmentation (Wernberg et al. 2011a, Wernberg et al. 2016, Coleman et al. 2017), causing loss of biodiversity and ecological function (Steneck et al. 2002, Graham 2004). Eventually, survival of kelp forests may be limited by their potential to expand and increase their abundance at higher latitudes (Lima et al. 2007). However, kelps cannot extend their range further south in Australasia. Thus, a southward shift of the distribution will imply a net loss in abundance for this species (Wernberg et al. 2011b).

*E. radiata* plants may not be directly influenced by ocean acidification; however elevated CO<sub>2</sub> levels have a positive effect on species that compete with *E. radiata* for space, such as algal turfs, which are natural components of kelp habitats(Provost et al. 2017). When favoured by the environmental conditions, these competitors may inhibit kelp recruitment (Connell and Russell 2010, Connell et al. 2013, Falkenberg et al. 2013b). The combined effects of ocean warming and acidification

may affect kelp forests in low-latitude regions via consumer pressure and via competitive interactions (Verges et al. 2014, Provost et al. 2017).

Storms are a major source of physical disturbance, and wave exposure is probably the most commonly identified cause of *E. radiata* canopy loss (Kennelly 1987a, Wernberg and Thomsen 2005). As storm events and wave height and speed increase, and the EAC becomes stronger, these will have important implications for kelp forest architecture and on how *E. radiata* engineers the surrounding environment (Wernberg and Thomsen 2005). Furthermore, these disturbances will also have strong impacts on the kelp-associated species and their trophic ecology (Byrnes et al. 2011, Johnson et al. 2011b). After a storm event, the canopies need to regenerate. This process requires either the existing recruits to acclimatise to the new canopy-free conditions or the settlement of new recruits. These processes are crucial for the maintenance of kelp beds and the preservation of their ecological function (Wernberg et al. 2010) if storm intensity increases as predicted (Stocker et al. 2013).

The EAC also influences larval availability (Booth et al. 2007), affecting the distribution of many species across the shelf. The strengthening of the EAC will benefit important macrophyte grazers, such as the barrens-forming urchin *Centrostephanus rodgersii* (Fletcher 1987, Andrew and Underwood 1993, Connell and Irving 2008). Temperatures in Tasmania now exceed the 12°C threshold for successful development of the larvae of these urchins (Ling 2008, Ling et al. 2009a) and, since its arrival to the island, previously nonexistent urchin barrens are now present throughout north-eastern Tasmania and are expected to keep expanding (Johnson et al. 2005). This species is already causing a shift in the structure and the dynamics of eastern-Tasmanian rocky reef communities, forming barrens, and consequently resulting in cascading effects and ecological change in benthic and pelagic systems (Johnson et al. 2011b).

Other taxa close to their minimum thermal tolerances are also being positively affected by the strengthening of the EAC: pole-ward expansion has already occurred for intertidal species in south-eastern Australia (Wernberg et al. 2011a), for invertebrates such as sea slugs (Nimbs et al. 2015, Nimbs et al. 2016, Nimbs and Smith 2016) and for dozens of species of fish (Last et al. 2011), the latter including herbivorous species (e.g. the herring cale *Odax cyanomelas*), fish with

territorial behaviour (e.g. the damselfish *Parma microlepis*) and predatory fish species (e.g. Port Jackson Shark, *Heterodontus portusjacksoni*), with potential of altering macroalgal habitats. Taxonomic and functional diversity of herbivorous fish on temperate systems is generally low (Floeter et al. 2005). Thus, the addition of new species of tropical fish that feed directly upon the macroalgae, may have strong impacts in kelp forests of temperate Australia, causing decreases in the resilience of these ecosystems and preventing the recovery of kelp beds after physical disturbances (Wernberg et al. 2013, Verges et al. 2014).

Last but not least, there is strong evidence that corals are increasing their range pole-wards, influenced by the strengthening of oceanic currents (Verges et al. 2014). Thus, with the EAC becoming warmer and stronger, authors expect more coral-macroalgal interactions in subtropical and temperate regions of eastern Australia (Beger et al. 2014). Tropical herbivores may become important in the mediation of these interactions (McCook et al. 2001), as the ability of the macroalgae to compete depends on their accumulation of sufficient biomass to overgrow corals (Miller and Hay 1998). If kelp forest resilience is low, phase shifts to coral-dominated benthos may occur, as it has happened before in other regions (e.g. Japan -(Mezaki and Kubota 2012)).

## 1.3 Food webs in kelp forests

### 1.3.1 General characteristics

In kelp habitats, thousands of species rely on the foundation species (Byrnes et al. 2011), many directly depending on fixed carbon by these macroalgae, or indirectly through a predatory trophic web (Graham et al. 2008). Either way, food supply in most kelp forest is not considered a limiting factor. Trophic pathways in these ecosystems are primarily detrital based (Smith 2000, Graham et al. 2007b) (Yorke et al. 2013) and generally energy flows from kelp, via invertebrates, to predatory fish (Norderhaug et al. 2003, Norderhaug et al. 2005). Furthermore, considerable amounts of fixed carbon (drift kelp) (Graham et al. 2003) and particulate organic matter (POM) are exported (Christie et al. 2009) to, for example, intertidal habitats (Branch 2008), soft-sediment habitats (Bishop et al. 2010) and deep water systems (Yorke et al. 2013).

Different pathways to higher trophic levels exist within kelp forest ecosystems:

Storms and waves break and rip kelps off (Byrnes et al. 2011), mostly by fragmentation of the old lamina (Leclerc et al. 2013), sending the detritus to either the benthos in that same kelp forest or to other systems (Branch 2008, Bishop et al. 2010, Yorke et al. 2013). Often, pieces of kelp litter the benthos in the forest and detritus accumulates within the holdfast, thanks to its complexity: the interstices between haptera (particularly laminarian holdfasts, e.g. *E. radiata*) act as a trap for sediment and POM. These may then be consumed by invertebrates and microbes (Moore 1973, Smith 1996, Arroyo et al. 2004).

Kelp detritus is a rich source of carbon and nitrogen (Mann 1988, Fielding and Davis 1989, Bustamante and Branch 1996, Krumhansl and Scheibling 2012). However, most fauna living in kelp forests do not feed on fresh kelp detritus directly, as kelps do not seem to be a suitable food source until released as particulate organic matter (POM) and degraded by bacteria (Norderhaug et al. 2003). This is associated with their high C:N ratio (Norderhaug et al. 2003, Schaal et al. 2010) and to kelp species having anti-herbivory products in their tissues (Bustamante and Branch 1996, Duggins and Eckman 1997, Norderhaug et al. 2003). Thus, In kelp forests under stable conditions, herbivores rarely consume more than 10% of the living biomass (Mann 2009). However, herbivores and detritivores feeding directly on kelp are also present in these ecosystems (Vahl 1983, Duggins et al. 1989, McGrath 2001). For example, sometimes if large pieces of detritus stay in kelp forests, they may be directly consumed by crustaceans (amphipods, isopods and decapods), asteroids, molluscs and fishes (Graham et al. 2008). Furthermore, smaller detrital particles (i.e. particulate organic matter, POM) become accessible to many more detritivores, filter feeders and suspension feeders (e.g., bivalves, sponges, polychaetes, ophiuroids, mysids) (Smith 1996, Graham et al. 2007b, Graham et al. 2008). Therefore, the holdfast community is often dominated by filter feeders and deposit feeders (Smith et al. 1996a, Schaal et al. 2012). However, grazers, scavengers and predators are also present (McKenzie and Moore 1981, Smith et al. 1996a). Many taxa recorded amongst the holdfasts are normally found in the surrounding habitat, and beneath other macroalgae in the ecosystem, rather than being obligated only to kelp holdfast habitats (Smith et al. 1996b, Smith and Rule 2001).

Some authors believe that, in these ecosystems, smaller particles of kelp represent the major contributor of POM (Leclerc et al. 2013). However, others suggest that POM derived from kelp detritus is not enough to sustain all suspension feeders, and that phytoplankton contributes to most of the POM pool (Yorke et al. 2013). Regardless the source of organic matter, diversity of filter feeders and herbivores can be extremely high in these ecosystems, and many of these taxa tend to disappear during storms and severe wave events, leading to a simplification of the whole food web (Byrnes et al. 2011).

Secondly, there is a pathway of herbivores feeding directly on kelp canopies (Graham et al. 2008): molluscs, crustaceans, echinoderms and fish that graze on the biomass of these macroalgae having little impact on it (Graham et al. 2007b). In some kelp habitats, grazing is a fundamental process (Harley et al. 2012): mesograzers, such as sea urchins, feed on the surface of these macrophytes and successfully remove epiphytic foliose algae, playing a key role in keeping the kelp free of overgrowth by competitors (Fowler-Walker and Connell 2002, Moksnes et al. 2008). In some cases, reduction in the abundance of small grazers can result in overgrowth by epiphytic algae, which may lead to severe effects on the habitat-forming macrophytes (Moksnes et al. 2008). Generally, these are not the preferred food source for sea urchins, but increases in the numbers of these mesograzers may result in their overgrazing kelp canopies (Steneck et al. 2002, Steneck et al. 2004, Norderhaug and Christie 2009). However, fish predation seems to regulate mesograzer densities in healthy kelp habitats (Davenport and Anderson 2007, Moksnes et al. 2008).

Nonetheless, in some instances, grazing by larger herbivores (e.g. sea urchins) can diminish the available kelp habitat through over-grazing kelp sporophytes. In such situations, this may have a strong negative effect in the numbers of new kelp recruits, preventing the recovery of kelp canopies after physical disruptions occur (Steneck et al. 2002, Sala and Dayton 2011). Sea urchins in particular can have a great impact on kelp forests, in some cases detaching entire kelp plants and keeping the system deforested by grazing on new recruits (Steneck et al. 2002, Ling 2008, Christie et al. 2009, Norderhaug and Christie 2009). This may result in phase shifts from complex and diverse habitats to poor urchin barrens (Steneck et al. 2002, Ling 2008, Ling et al. 2009a, Johnson et al. 2011b, Ling et al. 2015).

There are several mechanisms that inhibit kelp overgrazing by sea urchins. For example, in the northern hemisphere, specifically in southern California predation by sea otters prevent sea urchin overgrazing on kelp *Macrocystis pyrifera* (Graham et al. 2007b). When these mammals are absent, then lobsters, fish and asteroids control sea urchin population by feeding on adult individuals (Steneck et al. 2002, Graham et al. 2008, Daly and Smale 2013). Abiotic factors, such as storms and diseases, also control sea urchin populations, wiping out large aggregations of these grazers (Lafferty 2004). A common feature of kelp forests worldwide is that the more diverse the ecosystem is, especially in high trophic levels, more resilient to the outbreaks of grazers (Graham et al. 2007b).

### 1.3.2 Trophic cascades and control

The latest literature defines trophic cascades as indirect species interactions that originate with predators and spread downward through food webs (Ripple et al. 2016). For example, in a three-level food chain, a high abundance of top predators causes low abundance of the mid-level consumers and a higher abundance of primary producers (Steneck et al. 2002, Estes et al. 2004, Graham et al. 2007b). When the top predator is removed, this may lead to greater abundance in consumers and a decline in the primary producers (Estes et al. 2004). Associated with trophic cascades, there are terms that describe how food webs are regulated: “top-down control” refers to the control of the upper-level predator on the lower food web components. In contrast, “bottom-up control” refers to the regulation of the food web levels by either primary producers or limiting nutrients (Pace et al. 1999). Trophic cascades may be transitory and they commonly result in dramatic shifts in energy provision in the ecosystems (Graham 2004) and the abundances of the food web components (Steneck et al. 2004).

Physical disturbances are the principal factor determining net primary production in kelp forests (Reed et al. 2011). For example, ENSO in California (and the associated warm water, low nutrients and storms) has caused large declines in *Macrocystis pyrifera* in the past (Foster and Schiel 2010). After severe loss of kelp, understory algae may increase their richness and primary consumer diversity may boost, thus resulting in a bottom-up trophic cascade that drives changes in the complexity of food web (Byrne et al. 2011).

Kelp forests are energy rich systems, with complex, non-linear trophic interactions (Pace et al. 1999, Steneck et al. 2002). Diversity and productivity of these ecosystems are primarily driven by oceanographic processes (Graham et al. 2007b), and climate change is expected to affect these parameters differently at different trophic levels (Byrne et al. 2011). For this reason, it has become important to understand the ecological role of consumers and predators in kelp communities, trophic interactions in between them, and the shifts that they mediate on temperate reefs, with the aim of providing adaptive management in the context of climate change, which is predicted to have severe effects on food webs (Steneck et al. 2002, Graham et al. 2007b).

Improving the understanding of marine community assemblages and the functioning of ecosystems may help translate projected climate change into biological impacts, and help determine the vulnerability of species and habitats (Hobday and Lough 2011). Ecological studies should not only focus on top-down effects (Steneck et al. 2002, Steneck et al. 2004) from removal of predators and herbivores, but also should take into account the decreasing resilience of kelp beds through impacts on bottom-up processes (Wernberg et al. 2010).

Furthermore, it is important to develop models that consider the effect of species adaptation to climate change, as well as the combined effects of rising temperatures and anthropogenic causes of disturbance, such as fishing pressure, habitat loss and eutrophication (Wolf et al. 2010). With these models, we should be able to better project future ecological trends and to maybe predict with more accuracy the impacts of climate change on kelp habitats.

## 1.4 Approaches to study food webs in marine ecosystems

In this section, I examine different approaches to study marine food webs and trophic interactions. There is not a best one, but all of them can be used successfully depending on the type of ecosystem and the species studied. As a matter of fact, many ecologists studying marine food webs combine two or three methodologies, resulting in comprehensive examination of trophic relationships, energy flow and function within the ecosystems. Here I describe some of the most commonly used approaches at present, including a cutting-edge technique that has

the potential to become a very valuable tool for marine biodiversity and the assessment of food web studies.

#### **1.4.1 Observational methods**

In the attempt to study trophic interactions within complex food webs, many authors have tried to record feeding behaviour by the use of observational techniques, to characterise the frequency of encounter between food web constituents. Video and direct observational data can provide information on the interaction between prey-predator and here are a few examples: the feeding behaviour of marine mammals has been previously recorded (Davis et al. 1999) and marine mammals themselves have been used to record their own prey by attaching a camera to their body (Fuiman et al. 2002). Grazer activity has also been filmed, for example to study the effects of urchins on kelp (Lauzon-Guay and Scheibling 2007). Underwater remote video has been used to successfully record feeding behaviour of lobsters at continental shelf depths (Hudson and Wigham 2003). For fish feeding behaviour, data has been obtained by SCUBA divers in situ (Wańkowski and Thorpe 1979, Fox and Bellwood 2007) or by the use of remote underwater video (Fox and Bellwood 2008).

Despite all the above, in a comprehensive study of marine food webs, it is not possible to study trophic relationships between the different trophic levels by sending divers to record data or by analysing remote video footage only (Sheppard and Harwood 2005). That is because information is limited to interactions within the field of view or time of the day (Sheppard and Harwood 2005, Lowry et al. 2012, Assis et al. 2013), which normally represents a very small fraction of the ecosystem's predation event. For this reason, trying to study a food web by the use of visual methods, recording prey-predation encounters, not only would be time-cost ineffective (Gillies 2012), but it would be very difficult with small, cryptic or elusive species (Lowry et al. 2012, Assis et al. 2013).

#### **1.4.2 Gut content visual analysis**

The collection of organisms and posterior post-mortem analyses of gut content and/or faecal analysis has been a very important tool for food web studies (Sheppard and Harwood 2005, Preti et al. 2012), and it is normally used in combination with other techniques (Würzberg et al. 2011, Dromard et al. 2014).

The dietary components of sampled fish have been used to determine principal food items, prey preferences, and to place the functional group of these fish within food webs (Morton et al. 2008, French et al. 2013). French et al. (2013) examined the gut contents of 35 abundant predatory fish in south Western Australia to reveal their trophic level, using a statistical approach to construct the food web of a demersal fish community. They discovered that, with an increase in size, some species moved higher on the hierarchy with a change in diet accompanying the size increase, whereas other species remained in the same predator guild despite the size increase. They also carried out analysis to identify prey guilds, also lined in a trophic hierarchy. Both the predator and prey guilds were then plotted against each other to show percentages of diet contribution of each prey guild to the predator guilds.

As mentioned above, in combination with other methodologies, analysis of gut content can be a very useful tool. However, the general disadvantage of this technique is that some preys are too small or sometimes the component of the diet is not resistant to the digestion (Corse et al. 2010). Thus, in some cases there are not solid remains left, which can lead to bias in taxonomic identification of partially digested prey (Morton et al. 2008, Corse et al. 2010). Furthermore, this technique is also considerably time consuming and it is best used in studies that focus in single prey-predator relationships rather than in the studies of a whole trophic structure (Gillies 2012).

#### **1.4.3 Stable Isotope Analysis (SIA)**

To overcome the challenges of studying trophic relationships and the diets of consumers, trophic ecologists designed indirect techniques that are less invasive than gut content analysis. For example, in the study of fish diet, these analyses can be carried out by biopsy of muscular tissue or by taking scale samples.

These analyses are performed by using stable isotopes and amino acid (fatty lipid) markers. However, the latter technique is not as good at providing trophic-level information in higher trophic levels, and also it is more complicated than the stable isotopes method (Gillies 2012). Both techniques rely on the identification of chemical signatures in the tissues of sampled individuals, which are originally derived from their diet. It is based on the “you are what you eat” concept (Gillies 2012).

Stable isotopes analyses (SIA) have been extensively used to describe trophic relationships, migratory movements and residency in marine ecosystems (Vizzini and Mazzola 2009, Hussey et al. 2012, Wyatt et al. 2012, Daly et al. 2013, Jaime-Rivera et al. 2013, Malpica-Cruz et al. 2013, Cresson et al. 2014, Davis et al. 2014a, Hussey et al. 2014, Davis et al. 2015). This technique has provided information about assimilated food and prey preferences, trophic interactions in marine food web, intrinsic tissue signatures for different locations, and migration patterns. Specifically, the fractionation of nitrogen isotopes ( $^{15}\text{N}:^{14}\text{N}$ ) between prey and consumers has been used to examine diet, trophic position and food web structure, while the fractionation of carbon ( $^{13}\text{C}:^{12}\text{C}$ ) has been used to find the relationship between primary consumers and predators, providing a tool to reveal dietary sources and migration patterns.

The science behind this technique is based on the presence of two naturally occurring isotopes for N and C in nature, one more abundant and “lighter” ( $^{14}\text{N}$  and  $^{12}\text{C}$ ) and one less abundant and “heavier” ( $^{15}\text{N}$  and  $^{13}\text{C}$ ). A faster loss of the lighter through metabolic activity and excretion in general leaves the consumers with a higher ratio of  $^{15}\text{N}:^{14}\text{N}$  and  $^{13}\text{C}:^{12}\text{C}$  than that in their diet (primary producer or prey). This process is called trophic “fractionation” or “enrichment”. By convention, isotopic ratios are expressed per mil ( $\text{\textperthousand}$ ), and represent the relative abundance of the two stable isotopes to one another in a particular sample, compared with the same ratio in atmospheric N and C. For example, higher  $^{15}\text{N}$  values mean enrichment of the sample of the heavier isotope  $^{15}\text{N}$ , relative to  $^{14}\text{N}$  (standard) and this is the basis of stable isotopes in food ecology (Page et al. 2013).

For example, Vizzini and Mazzola (2009) studied the feeding and foraging habits and trophic positions of littoral fishes in the Mediterranean Sea using stable isotopes. The differences in isotopic compositions showed variation between islands with benthic feeder fishes compared to planktivorous fishes. This study showed resource partitioning, which allows co-existence of similar species within the same ecosystem. It also revealed the isotopic signature and trophic level variation of fishes according to location.

In another study (Page et al. 2013) stable nitrogen isotopes were used to determine the trophic relationships and diet of consumers in a temperate kelp forest in southern California and in a tropical Pacific coral atoll. Their results showed an

increase in nitrogen isotopes from herbivores, such as invertebrates and fish, to higher level consumers, such as predatory invertebrates and fish. Isotope analysis also showed that some high level consumers on rocky reefs were feeding mainly on invertebrates rather than fish. Trophic levels determined by the nitrogen isotope analysis in this study could also be used to determine short and long term influences of natural and human induced changes within kelp forest and coral reef food webs.

Despite the advantages of this technique, stable isotope analyses do not have the power of providing accurate quantitative estimates of the diet of consumers and they only reflect the diet over a temporal scale, accumulated over weeks or months, and incorporated into the tissues. These techniques do not provide a snapshot of the present moment, but an integrated history of the diet of the organism. Although it is definitely a good tool in delineating trophic relationships, it relies on indirect evidence and it requires a complete knowledge of prey isotopic signatures, which can be difficult to obtain (Corse et al. 2010). For this reason, in many studies with stable isotopes, the results need to be compared with visual observations of the diet (Page et al. 2013).

#### **1.4.4 DNA analyses of gut content**

DNA barcoding was initially proposed fourteen years ago as a fast and effective tool to identify species and to assess biodiversity (Hebert et al. 2003). Initially this technique focused on the description and documentation of biodiversity, but more recently DNA barcoding was promoted as a tool to study trophic interactions and food webs in both terrestrial and aquatic ecosystems (Valentini et al. 2009, Carreon- Martinez and Heath 2010, Pompanon et al. 2012, Joly et al. 2014, Bartley et al. 2015). This is due to the fact it represents an extremely powerful tool to carry out dietary analysis of organisms from their gut contents (Deagle et al. 2005a, Smith et al. 2005, Barnett et al. 2010, Dunn et al. 2010, Braid et al. 2012, Hargrove et al. 2012, Valdez-Moreno et al. 2012, Cote et al. 2013, Jung 2014, Paquin et al. 2014, Moran et al. 2015, Sakaguchi et al. 2017), their faeces (Deagle et al. 2005b, Deagle et al. 2009, Clare et al. 2011, De Barba et al. 2014) or from regurgitates (Alonso et al. 2014). This has become particularly useful when prey cannot be identified by morphological characteristics or because it has been partially or totally digested. This technique is also useful when feeding behavior cannot be directly observed.

Several dietary studies targeting organisms from aquatic ecosystems have used DNA barcoding as a tool for the identification of prey in gut contents (Smith et al. 2005, Barnett et al. 2010, Dunn et al. 2010, Braid et al. 2012, Hargrove et al. 2012, Valdez-Moreno et al. 2012, Cote et al. 2013, Arroyave and Stiassny 2014, Jung 2014, Bartley et al. 2015, Moran et al. 2015, Aguilar et al. 2016). In the process, DNA is extracted from the semi-digested prey. Then, the species-specific standardised DNA region (DNA barcode, e.g. COI) is PCR amplified using universal primers (Folmer et al. 1994, Leray et al. 2013b), and then PCR amplicons are sequenced. Unknown samples are then identified by comparing the sequences against a database of known and curated COI sequences (e.g. Barcode of Life Database - BOLD) derived from reference specimens (Ratnasingham and Hebert 2007). This technique has the power to detect prey-specific DNA fragments from the gut contents of predators, even several hours after digestion (Deagle et al. 2005a, Sheppard and Harwood 2005).

However, there is a limitation to this technique: degraded prey items, or those in a liquid form, present a challenge and cannot be easily isolated for identification. And in many cases, predatory species feed either on soft-bodied metazoans or consume small preys that are digested quickly (Randall 1967, Denny and Schiel 2001, Morton et al. 2008). Therefore, digested items cannot be easily separated, identified, stored and sub-sampled. Thus, the gut content available for analysis is semi-digested, homogenised tissue that may contain DNA traces from a large pool of consumed prey and DNA from the predator together.

The introduction of next generation sequencing (NGS) and its application in DNA barcoding enabled the development of DNA metabarcoding. This is a powerful tool that allows the direct characterisation of dozens of samples containing thousands of sequences per PCR product and it has been applied to samples from the gut of generalist predators or environmental samples containing heavily degraded DNA (soil, water, faeces, etc.) (Leray et al. 2013b, De Barba et al. 2014, Leray et al. 2015, Albaina et al. 2016, Gebremedhin et al. 2016, Granquist et al. 2016, Harms-Tuohy et al. 2016, Kasapidis et al. 2016, Guillerault et al. 2017, Kamenova et al. 2017). DNA metabarcoding has great potential to boost the acquisition of data in biodiversity research (Taberlet et al. 2012) and in the study of food webs (Pompanon et al. 2012), and has recently been applied in a few dietary studies in

marine ecosystems (Leray et al. 2013b, Leray et al. 2015, Harms-Tuohy et al. 2016, Sakaguchi et al. 2017).

Where other techniques fail to detect and identify prey items to species or higher taxonomic levels, DNA metabarcoding has the potential to assess highly digested consumed prey that could otherwise be unidentifiable. Therefore, it is becoming a powerful tool for ecologists in food web studies (Bartley et al. 2015), as the capacity to quantify the complexity of food webs and to understand energy flow can therefore be considerably enhanced by the use of this cutting-edge technique (Yoccoz 2012). Furthermore, it has been estimated that the time required to identify prey from gut contents can be halved through the use of DNA techniques (Dunn et al. 2010).

## **1.5 The present study**

### **1.5.1 The area of the study**

This study took place at three near-shore reefs in the Coffs Coast, situated in the subtropical east coast of Australia, a region confirmed as a globally relevant hotspot for climate-related change (Hobday and Lough 2011, Wu et al. 2012, Verges et al. 2014, Vergés et al. 2016). Specifically, the sites of this study are located within the Solitary Islands Marine Park (SIMP). This includes the farthest offshore shallow (<25 m deep) reef system on the continental shelf of New South Wales and comprises islands off the coast that are surrounded by shallow reefs, as well as shelf waters up to 70 m deep (Malcolm et al. 2011a). Offshore locations in SIMP experience a stronger EAC influence than inshore reefs, thus temperature increases from inshore to offshore consistently. This has a strong influence on the community composition and creates an evident cross-shelf pattern (Malcolm et al. 2011a). The general biotic pattern in the region is for near-shore reefs to be *Ecklonia radiata* dominated (Millar and Kraft 1994, Mau et al. 1998), mid reefs to support both macroalgae and coral, and shallow offshore reefs to support the most diverse communities of coral (Harriott et al. 1994). Patterns for fish communities are similar to these, with the diversity of tropical fishes increasing towards the outer reefs of the SIMP (Malcolm et al. 2010a, Malcolm and Smith 2010, Malcolm et al. 2010b). With the forecasted strengthening of the EAC, inshore reefs may be flooded with warmer waters more frequently and for longer periods, this resulting

in changes in sea-temperature patterns in the region and consequently in the composition of biotic communities (Poloczanska et al. 2007).

Therefore, the near-shore reefs in the Coffs Coast are under threat. This including not only climate change, but a range of anthropogenic factors on adjacent land, such as dredging, beach-nourishing programs, river run-off and marine debris (Smith et al. 2011). Furthermore, these near-shore reefs are subjected to human use, including activities such as boating, fishing and spear-fishing and diving. Thus, it has become important to monitor these inshore reefs in the SIMP, to evaluate and predict the effects of global warming, population growth, increasing fishing pressure and changes in land-use patterns.

### **1.5.2 The aims of the study**

Current predictions with respect to changing climate within the region include a reduction in the cover of kelp *Ecklonia radiata* in shallow reefs (Wernberg et al. 2011a, Marzinelli et al. 2015, Coleman et al. 2017). This study therefore aims to document fish species that are dependent on *Ecklonia radiata* forests and that may be affected by their contraction. This will provide an indication of the potential implications of kelp loss in these ecosystems. The second aim is to explore potential impacts of kelp loss on a key ecological process through the quantification of food webs. I will do this by focusing on the role of selected carnivorous fish in *Ecklonia radiata* dominated rocky reefs. As reviewed, food webs are a key component of ecosystems which summarise species interactions as energy flow through the systems. These trophic interactions can be used to describe, hypothesise, test and compare how species, populations and communities survive natural or anthropogenic perturbations.

Specifically, this study aimed to:

1. Quantify spatial and temporal variation in the fish communities associated with *Ecklonia radiata* forests and identify species that are consistently associated with these forests.

Fish communities in kelp forests were investigated at three locations in the Coffs Coast, at multiple sample times. Three different observational techniques that are commonly used in the study of fish communities were to be assessed with the aim of determining the most cost-effective method for this type of study. A key

outcome would be a list of carnivorous fish common to all three locations that can be targeted for subsequent dietary analysis.

2. Quantify the trophic ecology of selected carnivorous fish through the analysis of gut contents.

The second stage of the study had the objective of exploring the diets of the target carnivorous fish by the use of a novel technique: DNA metabarcoding. This aimed to reveal dietary components for these fish in *Ecklonia radiata* forests, and to provide information on prey diversity. This may help understand the potential impacts loss of kelp habitat may have on these common predatory fish and on prey assemblages in the near-shore reefs of this recognised hotspot for climate change.

# Chapter 2 – Fish community structure in *Ecklonia radiata* dominated rocky reefs

## 2.1 Abstract

Fish are one of the most conspicuous components of reef ecosystems and have been found to be good surrogates in the study of rocky reefs communities. This part of the study examined fish assemblages in *Ecklonia radiata* dominated rocky reefs of the Coffs Coast, in subtropical eastern Australia (a recognised hotspot for climate change). The aims were to: 1) measure spatial and temporal variation in the structure of fish assemblages in three different near-shore reefs by adopting a multi-method approach (25 m Belt-Transects, Underwater Remote Cameras and Stationary Point Counts), 2) explore fish-habitat associations, and 3) provide a list of carnivorous fish commonly occurring in the three locations to further carry out dietary analysis. Multivariate and univariate analyses were conducted by the use of PRIMER-E. In total, 27,834 individuals were recorded, belonging to 121 species, from 47 different families of fish. Labridae (wrasses) was the most represented family, and the labrids *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* were the most abundant species. Significant differences in fish community structure were found between locations and sampling times. Results showed that density of Stage 3 of kelp (adults) was a key variable limiting fish community composition in all locations and times. Significant differences between sampling methods were found, and each one of these observational techniques showed strengths and limitations. I recommend adopting a multi-method approach to study fish assemblages in this type of ecosystems.

## 2.2 Introduction

### 2.2.1 Fish communities

One of the most conspicuous elements of reef communities are fish: a resource that represents an important component of marine biodiversity and that includes species that are both commercially and recreationally important (Henry and Lyle 2003), some of which are severely exploited and require conservation management. Fish

communities also include species that can influence the assemblage structure of other taxa, providing top-down control on reef ecosystems through trophic cascades (Babcock et al. 1999, Shears and Babcock 2003).

Fish have been found to be good surrogates in the study of rocky reefs communities (Malcolm and Smith 2010) and they are generally easier to sample than other groups of organisms. Studying the distribution of reef fish assemblages is an important step towards the better understanding of ecological processes occurring in reef ecosystems (Underwood et al. 2000). Given the threats near-shore reefs of the SIMP are exposed to (section 1.5.1), there is an interest to study the present fish community composition in this region, in order to evaluate and predict changes associated with global warming and other anthropogenic pressures in the area.

### **2.2.2 Methods to study fish communities**

Underwater Visual Censuses (UVC) is an approach frequently taken by marine scientist to estimate species richness and fish abundance. This non-destructive, non-invasive technique has been used to accurately identify species and estimate their abundances, and it is a common method to study fish communities in marine protected areas (Curley et al. 2003, Malcolm and Smith 2010). This method has shown to provide acceptable results for a range of species in past studies in reef habitats of NSW (Kingsford 1998, Curley et al. 2003).

However, even though divers can research the habitat in a way that cameras cannot (Lowry et al. 2012), UVC relies on the ability of the diver to identify fish. Furthermore, regardless of the experience of the diver, cryptic species may be underestimated when using this technique (Colton and Sweare 2010, Dalben and Floeter 2012).

On the other hand, the increasing use of underwater video for management and surveys (Pelletier et al. 2011, Longo and Floeter 2012, Assis et al. 2013, Mallet and Pelletier 2014) has allowed non-invasive, very cost-effective, accurate monitoring of fish assemblages, and has been proven to minimise observer bias in underwater surveys (Cappo et al. 2003) and to remove the diver effect (Assis et al. 2013). It has helped overcome fish sampling limitations imposed by depth, fish behaviour and seafloor rugosity (Cappo et al. 2006) and it has provided an increased coverage of diver-averse species as well as information regarding the behaviour of the species

identified (Lowry et al. 2012). Furthermore, it also represents a useful tool for sampling in marine protected areas, where non-destructive methodologies are essential (Cappo et al. 2003, Willis and Anderson 2003, Cappo et al. 2006, Langlois et al. 2006, Heagney et al. 2007), with the added advantage of providing a permanent record that can be stored for future consideration or secondary analyses (Longo and Floeter 2012).

Remote Underwater Video (RUV) has been used in a large number of behaviour-related studies during the last fifty years (Mallet and Pelletier 2014). It comprises a very useful technique to examine their natural behaviour and interactions (Watson et al. 2005) and it provides a powerful tool for the evaluation of fish communities across several functional groups and trophic categories in highly diverse ecosystems (Longo and Floeter 2012).

Even though Baited Remote Underwater Video (BRUV) is a well-established, effective technique to study fish communities, it was not considered in this study in order to avoid bias (Harvey et al. 2007). In past surveys large predatory or scavenging fish have been proved to influence fish assemblages (Klages et al. 2014) or to dominate significantly the bait stations, resulting in a reduced observation of the small fish (Watson et al. 2005, Cappo et al. 2006) and the underestimation of herbivores and territorial species (Colton and Swearer 2010). Thus, overestimation or underestimation of certain fish species are a likely source of bias and they would not be convenient in the present study. Instead, I used non-baited RUV, which has been proven to be an effective tool to perform censuses in fish communities (Assis et al. 2013). This method still has limitations which are mainly due to the passive nature of the cameras, as sampling efficiency relies on the movement of fish into the field-of-view (Lowry et al. 2012, Assis et al. 2013). Furthermore, visibility and light loss may also be limiting factors when using any type of underwater video (Cooke and Schreer 2002) and also when surveying species with crypto-benthic habits (i.e. Blenniidae and Gobiidae) (Cappo et al. 2006, Colton and Swearer 2010, Longo and Floeter 2012, Lowry et al. 2012).

## 2.3 Aims

1. To measure spatial and temporal variation in the structure of fish assemblages on sub-tidal, *Ecklonia radiata* dominated rocky reefs in three different locations of the Coffs Coast, in subtropical eastern Australia, by adopting a multi-method approach using standardised underwater techniques.

The null hypothesis is:

- ‘There is no difference in fish community structure and species richness between methods, between locations and between survey times’.
2. To explore fish-habitat associations, and to understand how the presence of fish species is influenced by the environment, by including correlations with benthic composition and environmental variables.

The null hypotheses are:

- ‘There are no differences in benthic community structure between locations and survey times’.
  - ‘Benthic composition and environmental variables do not explain patterns of fish community structure’.
3. To provide a list of carnivorous fish commonly occurring in the three locations, to further carry out dietary studies.

## 2.4 Materials and methods

### 2.4.1 Study sites

I carried out underwater censuses in 3 study sites chosen randomly from a range of near-shore, kelp-dominated, shallow reefs on the Coffs Coast (north side of Muttonbird Island (MBI) 30°18'12.88'' S, 153°09'03.41'' E; Woolgoolga Reef (WR) 30°05'33.88'' S 153°12'28.14'' E and North Rock (NR) 29°58'16.05'' S, 153°14'44.63'' E) (Figure 1). The three sites are Habitat Protection Zones (HPZ) in the Solitary Islands Marine Park (SIMP). The site at MBI is 750 m from the mainland, WR is 740 m from the mainland and NR is 1,900 m from the mainland. The sites at MBI and WR are separated by 24km, MBI and NR are separated by

38.4 km, and WR and NR are separated by 14.5 km (Figure 1). These reefs are not connected, as there are big patches of sand between them. I knew there was a high abundance of kelp *Ecklonia radiata* in these sites, which had been observed in previous studies (Smith, pers. comm.).



**Figure 1.** The three locations where this study took place (MBI = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock) of the Coffs Coast of NSW, in subtropical eastern Australia.  
Images Source: Google Earth, 2017.

All sites are remote from the closest fresh water and run-off sources: MBI is 1,300 m from Coffs Creek HPZ; WR is 1,000 m from Woolgoolga Lake and 4,000 m from Arrawarra Creek HPZ; and NR is located 2,700 m from Corindi River Sanctuary Zone and 2,500 m from Station Creek Sanctuary Zone. The predominant swell direction in these sites is S-SE. The site at MBI is exposed to northern and eastern winds and swells, but is protected from southerly swell and wind by Muttonbird Island. The WR and NR sites, however, have limited protection from swells and winds.

I conducted three sets of surveys, in June 2014, August 2014 and June 2015. All surveys were conducted between 0900 and 1400 hours when the water visibility was greater than 5 m. The depth of the replicate transects varied slightly at each site and in each transect, but it was always between 7-12 m.

## **2.4.2 Survey methodologies**

### **2.4.2.1 Stationary Point Count (SPC)**

Using SCUBA, four measuring tapes were placed randomly along the bottom at each site, although separated by 10 metres from each other. The 0m mark was placed on the point where a GoPro camera would be deployed. A single stationary observer undertook a visual fish census for 5 min (Hussey et al. 2012, Lowry et al. 2012, McCauley et al. 2012) within a segment of the water column defined by the angle of view of the camera ( $127^\circ$ ). Divers recorded fish species and abundances. This 5-min point count was followed by a 5 min active search to record cryptic fish species. Juveniles (young-of-the-year) were excluded from the surveys and analysis, as their small size leads to problems and biases in their identification. The area covered by this method is  $27\text{m}^2$ .

### **2.4.2.2 25 m Belt Transect (BT)**

After the SPC, four randomly placed  $25 \times 5\text{m}$  UVC Belt-Transects (Kingsford 1998, Watson et al. 2005, Bassett and Montgomery 2011) were conducted in each site, starting from the point where the SPC had been carried out. All species of fish were recorded, as well as their abundance. Counts were carried out 2.5 m either side of the 25 m tape measure. The time taken to complete the transects varied between 20-25 min, depending on the species richness and abundance of the species found. The area of reef covered by each transect was  $125\text{ m}^2$ . It was not logistically possible for a single diver to perform the 4 transects, therefore several experienced fish experts participated in every sampling day (3-4 divers), although any possible variation due to different observers was not measured. Juveniles (young-of-the-year) were excluded from the surveys and analysis.

### **2.4.2.3 KelpCam**

Once the SPC and BT surveys had been completed, GoPro video cameras were deployed by a single diver at the start of each transect line and were left recording

for one hour. The video system consisted of a horizontal outward-looking GoPro Hero 4 HD camera mounted over a 1.5 kg weight, placed within the kelp forest in an area with both kelp canopy and both sandy and rocky patches. These cameras have an angle of vision of 127° and allow the recognition of fish to species level when these are closer than 5 m. Therefore, the area that the cameras can reliably cover is 27 m<sup>2</sup>. All surveys were conducted in depth-range of 7-12 m as has been done for other studies in nearshore reefs and *Ecklonia radiata* forests studies (Smith et al. 2008, Wernberg et al. 2010, Malcolm et al. 2011b, Mabin et al. 2013). To maintain consistency, the first 2 minutes of each video were discarded to account for the artefacts associated with the camera deployment (e.g. sand stirring, kelp canopy disturbance, diver presence, etc.) (Salter 2010). Only 45 min of video footage was analysed by a single observer. Juveniles (young-of-the-year) were excluded from the surveys and analysis.

The average maximum distance in which we could identify an individual down to species level was 5 m, given the visibility and the kelp cover in all locations.

The data obtained after these fish community surveys was a list of species and their abundances for each location, time and method. There were four replicas for each treatment, so I ended up with 108 samples that were merged and placed in the same worksheet.

### **2.4.3 Other variables**

The structural complexity of the habitat (Rugosity), Kelp Density, Benthic Cover and temperature are factors that can influence fish assemblage structure. Consequently, each of these was assessed during the field work at each site and time.

#### **2.4.3.1 Benthic composition**

The structure of sessile, benthic communities is an important biological feature of reefs, and it is strongly correlated with several biological and physical factors: depth, wave exposure, sediment cover, surrounding habitat type (Toohey 2007). Generally, it influences other type of organisms within the ecosystem by creating suitable conditions (Teagle et al. 2017) and providing refuge, nursery and foraging areas for invertebrates and fish (Krajewski and Floeter 2011, Flukes et al. 2014,

Lavender et al. 2017). For all these reasons, the assessment of benthic structure is an important component for reef studies (Smith et al. 2008, Smith et al. 2011).

Benthic cover was assessed by the use of underwater HD video (GoPro 3 cameras). Video transects were performed by pointing the video camera downwards, 0.5 m above the substratum and offset from the centre of each transect. The diver slowly swam from along each transect line recording the substrate over a period of approximately 5 min.

Four Benthic Cover videos (replicates) were recorded in every location, therefore I ended up with 36 replicates after the three rounds of sampling. I assigned 300 points per Benthic Cover video with the use of CPCe software (Kohler and Gill 2006). Biotic (e.g. algae, coral) and abiotic (e.g. sand, boulders, debris) components were identified in the laboratory by pausing the footage at predetermined intervals of time and determining the taxa lying under 5 points placed on the monitor. These taxa were identified to the lowest taxonomical level and their growth form were described (e.g. coral branching, coral encrusting). These taxa were placed in higher taxonomic groups (e.g. corals, sponges, red algae) and were quantified by calculating the per cent cover of dominant benthic categories along each transect.

#### **2.4.3.2 Habitat complexity (Rugosity)**

Habitat complexity (Rugosity) can have a strong effect on fish assemblages in terms of composition and species richness (Gratwicke and Speight 2005, Carraro and Gladstone 2006, Lingo and Szedlmayer 2006). For this reason, it was important to assess complexity as a covariate in this study. In total, I obtained 36 different Rugosity measurements (one per each transect performed).

The “rope-and-chain” method was used (Luckhurst and Luckhurst 1978), in which a taut tape measure is run-out horizontally and secured to the substrate. A second tape-measure is then contoured to the substrate immediately below the first tape: the ratio of the horizontal and contoured distances represents a simple measure of rugosity (Luckhurst and Luckhurst 1978, McCormick 1994, Toohey 2007).

#### **2.4.3.3 Kelp Density**

Kelp density was estimated using 1 m<sup>2</sup> quadrats (Underwood et al. 1991) along the established transects. I randomly placed 10 quadrats on each transect and counted

the number of kelp plants within them (Scheibling et al. 1999). The density of different kelp life stages (Kirkman 1981) was also recorded: Stage 1 (S1, 6.5-27 cm), the small recruit stage where the thallus is a single blade and the lamina is undifferentiated; Stage 2 (S2, 19-34 cm), the old recruit stage where the lamina starts to develop primary laterals; Stage 3 (S3 > 30 cm) the adult stage where the thallus becomes fully differentiated into complex laterals. The average and standard deviation for kelp plants were then calculated for each life stage based on the 10 observations in each transect. I ended up with 36 different measurements for each kelp Stage.

#### **2.4.4 Statistical methods**

Multivariate and univariate analyses were conducted using procedures in the PRIMER-E software package (Clarke and Warwick 2005), in order to answer the following questions:

##### **2.4.4.1 Question one: Are there differences in fish community structure and species richness between locations and survey times, depending on the survey method used?**

In these analyses, only the abundance of benthos-associated species was accounted for. Schooling species were assigned for presence and absence (1 if they were present, 0 if they were not) to avoid their dominance. Data were pre-treated using square-root transformation to avoid dominance of common species and to allow greater contribution from rare species (Clarke and Warwick 1994). Dissimilarities between pairs of samples were determined using the Bray-Curtis dissimilarity coefficient. Non-metric multidimensional scaling (nMDS) ordination was used as ordination method, to visualise variation in the structure of fish assemblages, across the factors in the design.

I assessed differences in fish assemblages across the full design using Permutational Analysis of Variance (PERMANOVA) (Anderson 2005). The experimental design consisted of the following factors: location (random with 3 levels, Muttonbird Island (MI), Woolgoolga Reef (WR), and North Rock (NR)), method (fixed with 3 levels, 25-m belt transects (25 m BT), Stationary Point Counts (SPC) and Kelp Camera (KelpCam); and time (random with 3 levels, June 2014, August 2014, and June 2015). Significant differences were further explored using

suitable pair-wise tests and the average dissimilarities between groups was also obtained.

The individual species contributing to the observed differences amongst locations and methods were identified using similarity percentage (SIMPER) analysis. This was done using the transformed data from each time (JUN14, AUG14, JUN15). Species identified as driving differences across factors were further explored using univariate PERMANOVA of abundances using Euclidean Distance as the distance measure. Species richness was calculated for each replicate using the DIVERSE function and analysed across the full design using PERMANOVA of Euclidean Distances.

#### **2.4.4.2 Question two: How do the benthic community structure (Benthic Cover) and other environmental variables (Kelp Density and Rugosity) affect fish assemblages? Do they explain differences in fish community structure between locations and times?**

Benthic Cover data in each transect were pre-treated using fourth-root transformation to avoid dominance of common organisms or substrate type and to allow greater contribution from rare categories (Clarke and Warwick 1994). Dissimilarities between pairs of samples were determined using the Bray-Curtis dissimilarity coefficient. Non-metric multidimensional scaling (nMDS) ordination was used, to visualise variation in benthic composition at each location and time.

I assessed differences in benthic assemblages across the full design using Permutational Analysis of Variance (PERMANOVA) (Anderson 2005). The experimental design consisted of the following factors: Location (random with 3 levels, Muttonbird Island (MI), Woolgoolga Reef (WR), and North Rock (NR)), and Month (random with 3 levels, June 2014, August 2014, and June 2015). Significant differences were further explored using appropriate pair-wise tests and the average dissimilarities between groups was also obtained. The next step was to calculate similarity and dissimilarity percentages between groups (SIMPER), to reveal which substrate type or organism contributed the most to the observed differences amongst months and locations. This was done using the un-transformed data. Those benthic organisms or substrata providing higher percentage of dissimilarities were tested separately. First, a resemblance matrix based on Euclidean Distance on the untransformed data was created, and then I ran a

PERMANOVA on each single type or organism, to test significant differences between locations.

The aim of this component was to reveal if the benthic composition affected fish assemblages. For that reason, I used the fish abundance data obtained by the 25 m Belt-Transect method (as the benthic composition data was also obtained along the 25m transect lines) and I pre-treated this data using a square-root transformation. I created a resemblance matrix based on Bray-Curtis similarity measure related both 25 m BT and Benthic Composition matrices using the Spearman correlations in the ‘RELATE’ routine on PRIMER-E. This tests the null hypothesis of no relation between multivariate pattern from two sets of dissimilarity matrices (Clarke and Warwick 2001). This computes a rank correlation coefficient ( $\rho$ ) between two Bray-Curtis similarity or dissimilarity matrices, where values approaching 1 indicate a high level of concordance.

The next step was to determine how two environmental variables (Rugosity and the density of different Kelp Stages) might affect the fish community composition. Kelp Stage density and Rugosity data were firstly normalised. Then the relationship between fish assemblages data (resemblance matrix of the 25 m BT data) and these environmental variables was investigated using a non-parametric multiple regression test, the Distance based Linear Model (DistLM in PRIMER-E) (Legendre and Anderson 1999, McArdle and Anderson 2001), with a ‘step-wise’ procedure and criterion ‘AIC’. First, this test analysed each environmental factor separately (ignoring any other factor) to evaluate the potential relationship between fish community structure and the environmental variables (marginal test). Then, the environmental factors were subjected to a best-selection procedure, in which the amount of variability explained by each environmental factor (%) is conditional upon the factors previously included (sequential test). Both these tests were based on the Bray-Curtis dissimilarity index.

## 2.5 Results

### 2.5.1 General results

In these surveys, I recorded a total of 27,834 individuals of fish, belonging to 121 species, from 47 different families. The numbers recorded by each method and location were the following: 12,926 individuals recorded using 25 m Belt-Transects, from 89 species (61 in MBI, 43 in WR, 56 in NR); 5,790 individuals recorded with Stationary Point Counts, from 42 species (41 in MBI, 34 in WR, 33 in NR); 9,118 individuals recorded with KelpCam, from 84 species (54 in MBI, 36 in WR, 55 in NR).

Appendix 1 shows a comparison of the differences in sampling effort for every method used to assess the fish community composition in this study, and the results obtained (number of species and number of families).

Table 1 shows those species most commonly recorded during my surveys, regardless of the method and sample time. The wrasses *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri*, along with the damselfish *Parma unifasciata* are the most abundant species, but other species of wrasses such as *Austrolabrus maculatus*, *Labroides dimidiatus*, the red morwong *Cheilodactylus fuscus*, and the herring cale *Odax cyanomelas* were recorded in high numbers.

Table 2 shows the numbers of species recorded by family, by method and location. The most represented families in our study were the wrasses (Labridae), with a total of 29 species recorded, and the damselfish (Pomacentridae) with 14 species. These were followed by the butterflyfish (Chaetodontidae) with 6 species, and the leatherjackets (Monacanthidae) with 5 species.

**Table 1.** Most recorded benthic-associated species (no schooling or pelagic species).

Family	Common name	Species	Records	MBI	WR	NR
Cheilodactylidae	Red Morwong	<i>Cheilodactylus fuscus</i>	94	50	32	12
Labridae	Eastern Blue Groper	<i>Achoerodus viridis</i>	38	17	12	9
	Black-spotted Wrasse	<i>Austrolabrus maculatus</i>	119	22	38	59
	Snakeskin Wrasse	<i>Eupetrichthys angustipes</i>	28	1	2	25
	Clouded Wrasse	<i>Halichoeres nebulosus</i>	27	3	3	21
	Blue-streak Cleaner Wrasse	<i>Labroides dimidiatus</i>	87	32	17	38
	Crimson-banded Wrasse	<i>Notolabrus gymnogenis</i>	547	159	200	188
	Maori Wrasse	<i>Ophthalmolepis lineolata</i>	201	66	36	99
	Senator Wrasse	<i>Pictilabrus laticlavius</i>	39	16	10	13

	Gunther's Wrasse	<i>Pseudolabrus guentheri</i>	249	47	101	101
	Moon Wrasse	<i>Thalassoma lunare</i>	33	1	4	28
Monacanthidae	Pygmy Leatherjacket	<i>Brachaluteres jacksonianus</i>	29	10	9	10
Mullidae	Black-saddled Goatfish	<i>Parupeneus spilurus</i>	56	44	7	5
	Blue-lined Goatfish	<i>Upeneichthys lineatus</i>	22	15	2	5
Odacidae	Herring Cale	<i>Odax cyanomelas</i>	77	15	32	30
Pomacentridae		<i>Mechaenichthys immaculatus</i>	54	36	14	4
		<i>Parma oligolepis</i>	46	39	6	1
		<i>Parma unifasciata</i>	569	149	219	201
		<i>Pomacentrus coelestis</i>	39	7	15	17

**Table 2.** Number of species of the most common families and their relative abundance for each Method and Location.

	25m BT MBI	25m BT WR	25m BT NR	SPC MBI	SPC WR	SPC NR	KelpCam MBI	KelpCam WR	KelpCam NR
Chaetodontidae	5 (8.20%)	1 (2.33%)	0 (0.00%)	2 (4.88%)	0 (0.00%)	0 (0.00%)	4 (7.41%)	0 (0.00%)	0 (0.00%)
Labridae	13 (21.31%)	14 (32.56%)	20 (35.71%)	9 (21.95%)	10 (29.41%)	12 (36.36%)	12 (22.22%)	13 (36.11%)	20 (36.36%)
Monacanthidae	1 (1.64%)	2 (4.65%)	2 (3.57%)	1 (2.44%)	1 (2.94%)	1 (3.03%)	4 (7.41%)	3 (8.33%)	4 (7.27%)
Pomacentridae	8 (13.11%)	6 (13.95%)	8 (14.29%)	7 (17.07%)	4 (11.76%)	5 (15.15%)	6 (11.11%)	4 (11.11%)	3 (5.45%)

In the length of these surveys, I detected 22 Families in MBI, 15 in WR, and 18 in NR. Those species that contributed for the most counts (more than 5% contribution) in MBI belonged to the families Labridae, Pomacentridae, Mullidae and Cheilodactylidae. In WR, these were Labridae, Pomacentridae, Plesiopidae, Microcanthidae, Cheilodactylidae and Odacidae. Finally, in NR, the families that contributed the most were Labridae, Pomacentridae and Plesiopidae.

In all the length of my surveys I detected 38 families using 25 m BT, 19 families using SPC and 19 families using KelpCam. The most common families detected by the use of BT (more than 5% contribution) were Labridae, Pomacentridae and Apogonidae. When using SPC, the most common families detected were Labridae, Pomacentridae, Plesiopidae and Cheilodactylidae. Finally, when using KelpCam, the most commonly detected families were Labridae, Pomacentridae, Carangidae, Monacanthidae, Microcanthidae and Odacidae.

Results showed that, in general, the 25 m BT method detects more families and more species per family than SPC. However there are a few exceptions. During this study, SPC detected species from the families Clinidae, Platycephalidae,

Dasyatididae and Ostraciidae when 25 m BT did not detect them. Nonetheless, SPC failed to detect 9 families that 25 m BT did detect. Between 25 m BT and KelpCam, results are more even. In general, the 25 m BT method detects more species per family, but there were many exceptions in which KelpCam was more effective. In the length of this study, KelpCam detected more Carangidae, Monacanthidae, Myliobatidae, Odacidae, Aplodactylidae, Haemulidae, Monodactylidae, Sparidae, Scorpidae, Dinolestidae and Microcanthidae. And this method also detected five families that 25 m BT failed to detect: Diodontidae, Hemiramphidae, Lutjanidae, Narkidae and Ostraciidae. Furthermore, KelpCam detected the same number of species for the families Enoplosidae, Pinguipedidae and Gerreidae than the 25 m BT method. On the other hand, BT detected more species per family than KelpCam for the families Labridae, Pomacentridae, Apogonidae, Serranidae, Mullidae, Acanthuridae, Chaetodontidae, Orectolobidae, Pempheridae, Cheilodactylidae, Scorpaenidae and Plesiopidae, and detected 12 families that KelpCam did not detect at all: Blennidae, Chironemidae, Trachichthyidae, Muraenidae, Pomacanthidae, Aracanidae, Rachycentridae, Siganidae, Tetraodontidae, Aulopidae, Fistulariidae and Urolophidae.

Finally, between KelpCam and SPC, results showed that the underwater video method generally recorded more species per family, with a few exceptions where SPC recorded more: Pomacentridae, Apogonidae, Serranidae, Pempheridae, Scorpaenidae, Plesiopidae. And in other cases SPC recorded families when KelpCam failed to: Muraenidae, Clinidae, Chironemidae, Blennidae, Siganidae, Platycephalidae, Aulopidae and Dasyatididae. However, that also happened the opposite way: some families that were recorded by the use of KelpCam were not recorded by SPC. These were Myliobatidae, Diodontidae, Hemiramphidae, Pinguipedidae, Lutjanidae and Narkidae.

### **2.5.2 Fish assemblages. Species composition.**

Fish assemblages were found to be significantly different ( $P < 0.05$ ) between methods, locations and months (Table 3). I carried out pair-wise tests for those factors and interactions showing significant differences, and given that the factor ‘Mo’ (Month) was driving the significant differences in the interactions ‘LoxMo’ and ‘LoxMoxMe’, ), I ran a PERMANOVA for each separate month (JUN14,

AUG14, JUN15). This time, the experimental design consisted only of 2 factors: Location (random with 3 levels) and Method (fixed with 3 levels).

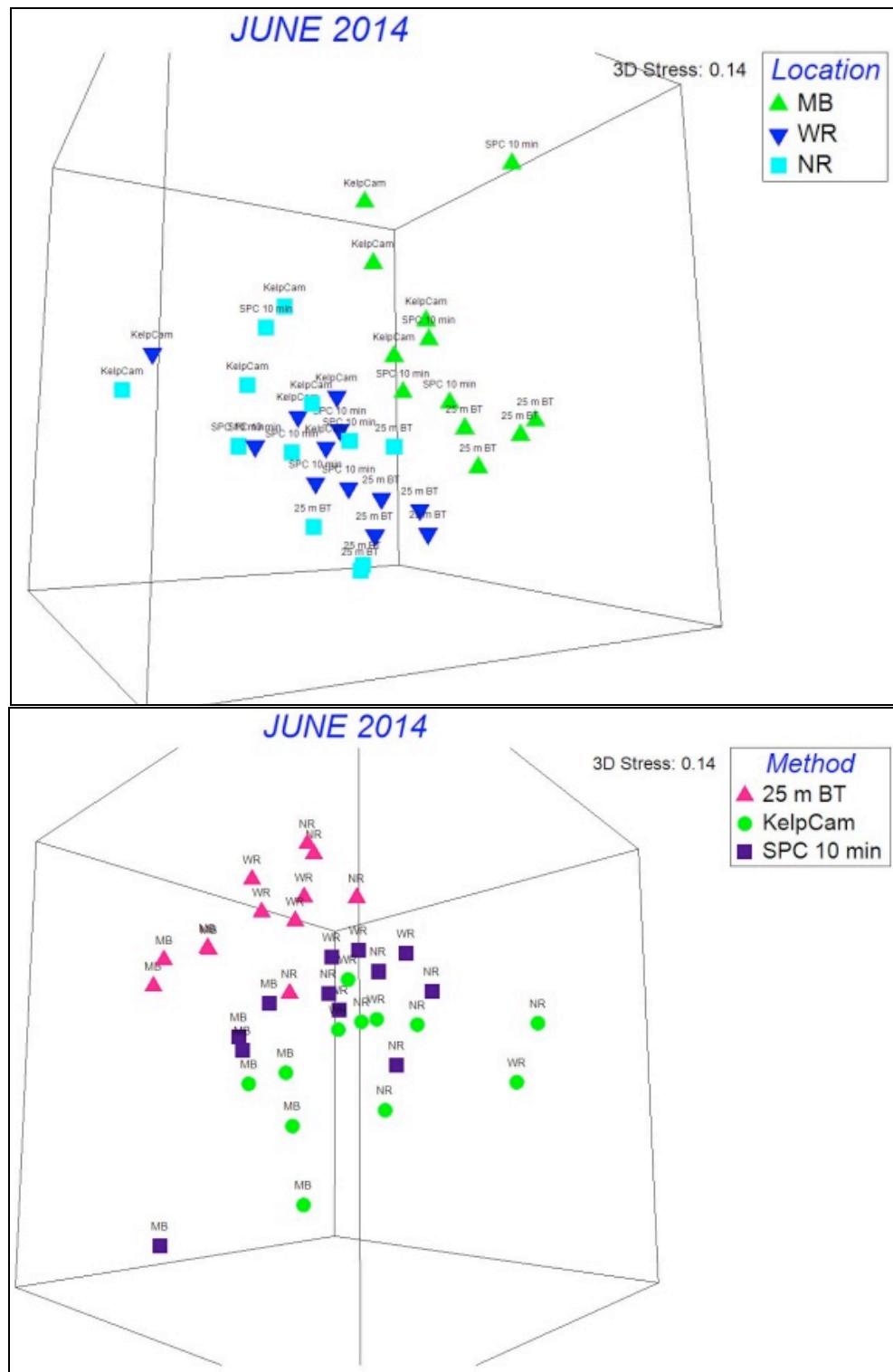
**Table 3.** Summary of results of the 3-way PERMANOVA, showing the significance of differences in fish assemblage structure between Location (Lo), Month (Mo) and Method (Me).

**PERMANOVA results All Methods merged**

Source of variation	df	MS	F	P
Lo	2	9219.2	29.816	0.001
Mo	2	6251.5	20.218	0.031
Me	2	14411	54.205	0.001
LoxMo	4	3092.1	2.288	0.001
LoxMe	4	1818.2	10.415	0.434
MoxMe	4	1162.4	0.66587	0.945
LoxMoxMe	8	1745.7	12.917	0.023
Res	81	1351.4		

Separation of locations and methods in June 2014 is apparently clear on the nMDS ordination (Figure 2), although fish communities in Woolgoolga Reef (WR) and North Rock (NR) show some overlapping. The PERMANOVA test for June 2014 (Table 4) showed significant differences between locations and methods. The pair-wise tests (Table 5) showed that all locations were significantly different to each other the greatest dissimilarities occurring between Muttonbird Island (MBI) and North Rock (NR) (67.60% diss.). The pair-wise tests for methods showed significant differences between the three methods (Table 6), the greatest dissimilarities occurring between 25 m BT and KelpCam (66.47% diss.).

Results from the PERMANOVA on the DIVERSE June 2014 data also showed significant differences between methods and locations (Table 11), but the pair-wise tests indicated that these differences were driven by the groups MB-WR (Table 12), and by BT-SPC (Table 13).

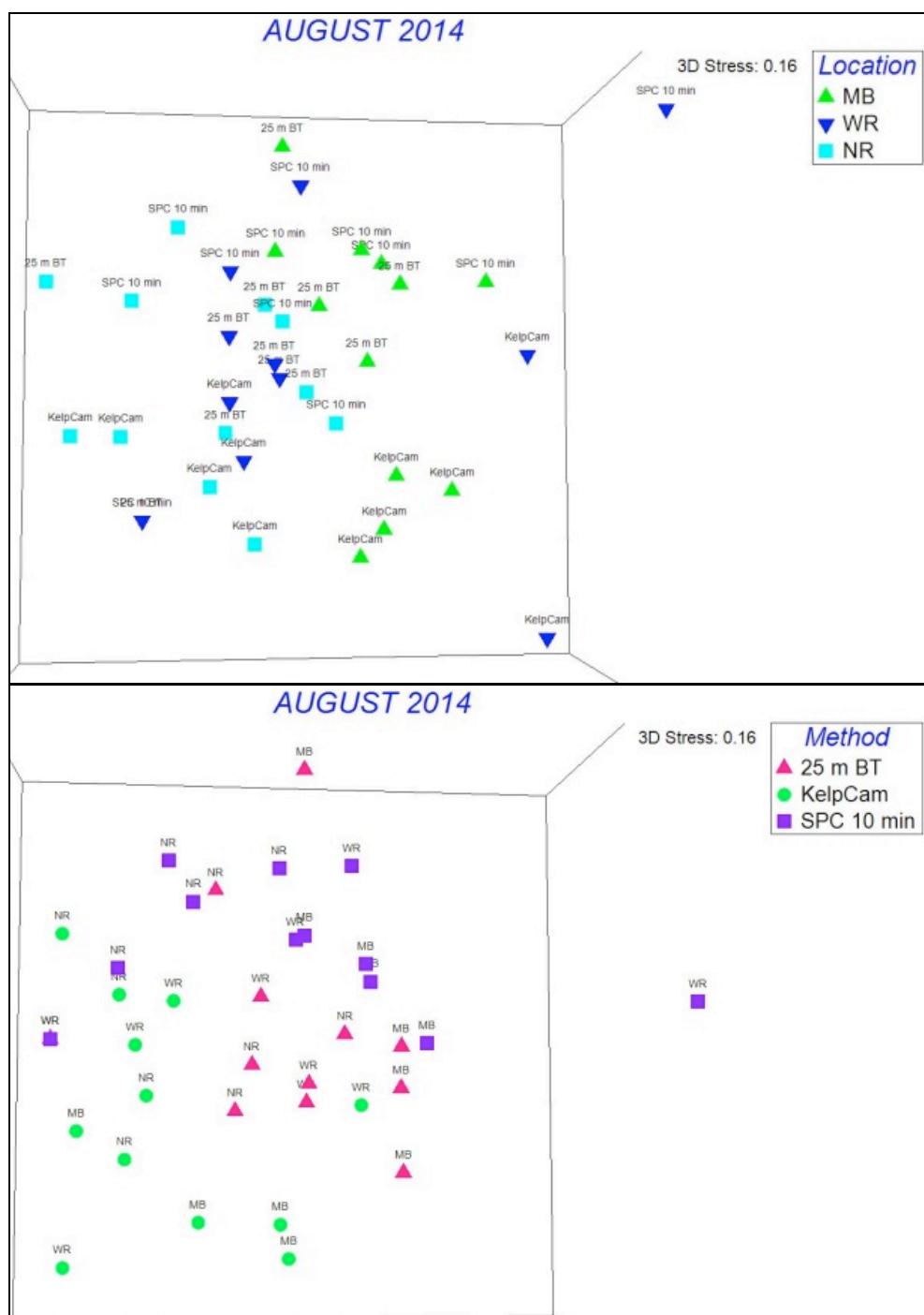


**Figure 2.** Three-dimensional nMDS plots for the factors Location (MB = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock) and Method (25 m Belt-Transects, KelpCam and SPC 10 min) in June 2014.

Separation of methods in August 2014 is clear on the nMDS ordination and fish communities in the three locations show some overlapping, with MBI appearing to form a clear group for the KelpCam method (Figure 3). The PERMANOVA test for August 2014 resulted in significant differences between locations and methods (Table 4). The pair-wise tests (Table 5) showed that all locations were significantly

different to each other, the greatest dissimilarities occurring between MBI and NR (64.62% diss.). The pair-wise tests for methods showed significant differences between the three methods (Table 6), the greatest dissimilarities occurring between 25 m BT and KelpCam (64.52% diss.).

In terms of species richness, results from the PERMANOVA on the DIVERSE August 2014 data showed only significant differences between methods (Table 11), but the pair-wise test indicated that these differences were driven by the groups BT-SPC (Table 13).

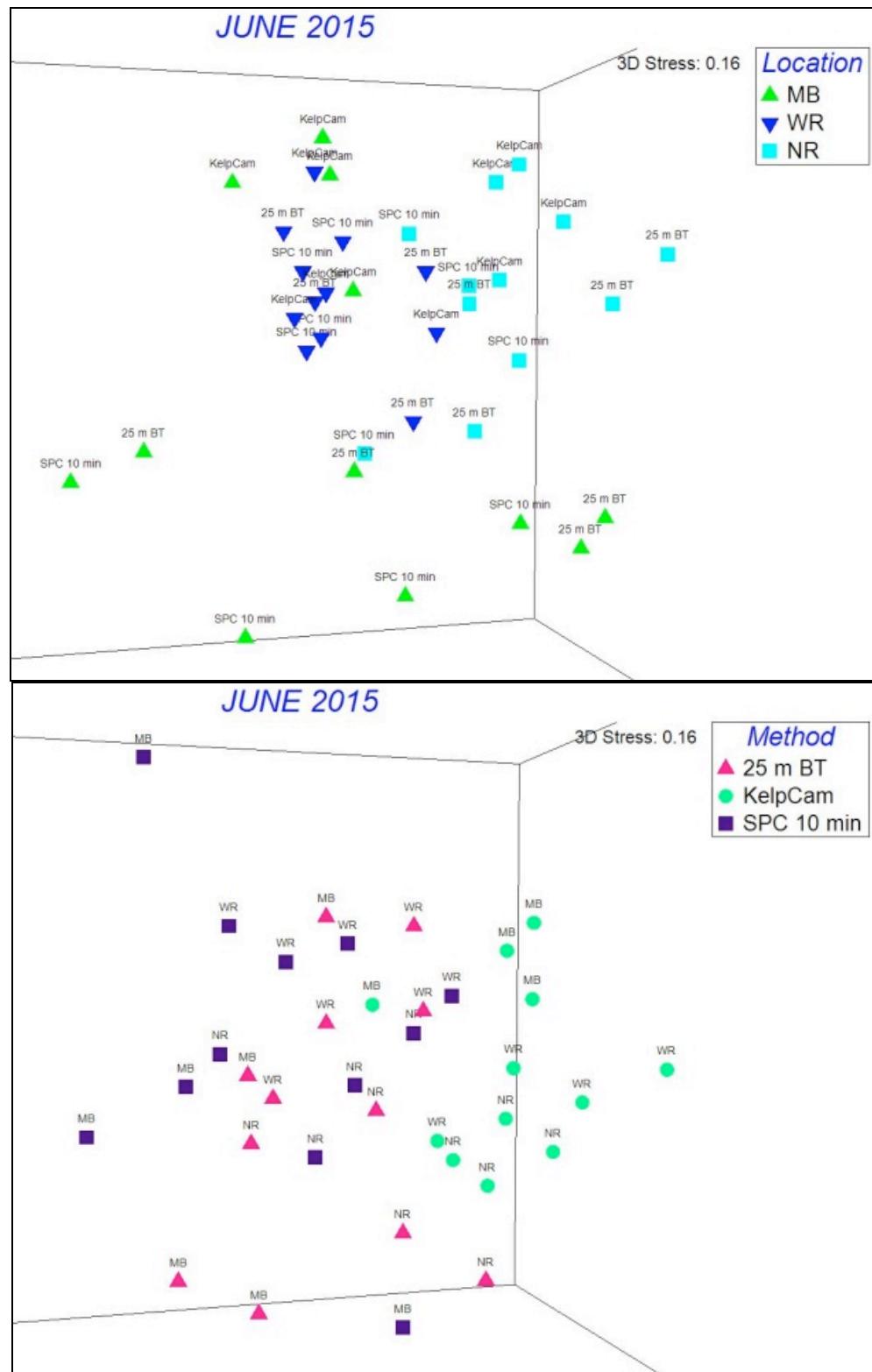


**Figure 3.** Three-dimensional nMDS plots for the factors Location (MB = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock) and Method (25 m Belt-Transects, KelpCam and SPC 10 min) in August 2014.

There is no clear separation of groups for locations and methods in June 2015 on the nMDS ordination, except for NR and KelpCam groups (Figure 4). Fish communities in the MBI and the WR groups show overlapping, which also happens with the groups of methods BT and the SPC. The PERMANOVA test for June 2015 resulted in significant differences between locations and between methods (Table 4). The pair-wise tests (Table 5) showed that all locations were significantly different to each other, the greatest dissimilarities occurring between MBI and NR (57.87% diss.). The pair-wise tests for methods showed significant differences between the three methods (Table 6), the greatest dissimilarities occurring between 25 m BT and KelpCam (57.82% diss.).

For June 2015, The pair-wise test on the interaction ‘LoxMe’ for pairs of levels of factor ‘Method’ showed significant differences between method KelpCam and the other two methods within all levels of location, and within level NR all methods showed significant differences between each other. The pair-wise test on the interaction ‘LoxMe’ for pairs of levels of factor ‘Location’ showed significant differences between WR and NR within both level BT and level SPC, and significant differences between all locations within level KelpCam.

In terms of species richness, results from the PERMANOVA on the DIVERSE June 2015 data also showed only significant differences between locations and methods (Table 11), and the pair-wise test indicated that these differences were driven by the groups NR-WR and NR-MB (Table 12), and the group KelpCam-SPC (Table 13).



**Figure 4.** Three-dimensional nMDS plots for the factors Location (MB = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock) and Method (25 m Belt-Transects, KelpCam and SPC 10 min) in June 2015.

**Table 4.** Summary of results of the 2-way PERMANOVA showing the significance of differences (P values) in fish assemblage structure between Locations (Lo) and Methods (Me) in June 2014, August 2014 and June 2015.**PERMANOVA results by Month**

Source of variation	June 2014	August 2014	June 2015
Lo	0.001	0.001	0.001
Me	0.001	0.002	0.041
LoxMe	0.789	0.231	0.001

**Table 5.** Pair-wise comparisons between Locations (Lo) in June 2014, August 2014 and June 2015.**Pair-wise test results for term Location ‘Lo’**

Source of variation	June 2014	August 2014	June 2015
MB, WR	0.001	0.017	0.268
WR, NR	0.002	0.002	0.001
NR, MB	0.001	0.001	0.001

**Table 6.** Pair-wise comparisons between Methods (Me) in June 2014, August 2014 and June 2015.**Pair-wise test results for term Method ‘Me’**

Source of variation	June 2014	August 2014	June 2015
25 m BT, KelpCam	0.013	0.024	0.117
25 m BT, SPC	0.022	0.012	0.018
KelpCam, SPC	0.018	0.03	0.229

After calculating similarity and dissimilarity percentages between groups (SIMPER), I found which individual species contributed to the observed differences amongst locations and methods. Those species that provided higher percentage of dissimilarities were tested separately on PERMANOVAs to test significant differences between methods and locations. Table 7 and 8 provide a summary of the results of these PERMANOVAs. Tables 9 and 10 and provide the result for the pair-wise test for all those species found to provide significant differences.

**Table 7.** Summary of PERMANOVA results for the term Location (Lo) for the species found to provide the highest dissimilarities between locations and methods.

**PERMANOVA for species contributing to differences by month for the term ‘Lo’**

	June 2014	August 2014	June 2015
<i>Cheilodactylus fuscus</i>	0.003	0.044	0.523
<i>Achoerodus viridus</i>	-	0.023	0.91
<i>Austrolabrus maculatus</i>	0.006	-	0.049
<i>Eupetrichthys angustipes</i>	0.022	-	0.002
<i>Labroides dimidiatus</i>	0.711	0.048	0.016
<i>Notolabrus gymnogenis</i>	0.505	0.358	0.88
<i>Ophthalmolepis lineolata</i>	0.006	0.087	-
<i>Pseudolabrus guentheri</i>	0.024	0.085	0.022
<i>Thalassoma lunare</i>	0.003	0.005	-
<i>Parupeneus spilurus</i>	0.004	0.004	-
<i>Upeneichthys lineatus</i>	0.02	0.048	-
<i>Odax cyanomelas</i>	0.109	0.051	-
<i>Mechaenichthys immaculatus</i>	0.003	0.001	-
<i>Parma oligolepis</i>	-	0.004	0.004
<i>Parma unifasciata</i>	0.518	0.471	0.02
<i>Pomacentrus coelestis</i>	0.179	0.843	0.038
<i>Plectorhinchus flavomaculatus</i>	0.001	-	-
<i>Scorpaena cardinalis</i>	-	0.006	-
<i>Anampsese neoguinaicus</i>	-	-	0.016
<i>Meuchemia trachylepis</i>	-	-	0.05

**Table 8.** Summary of PERMANOVA results for the term Method (Me) for the species found to provide the highest dissimilarities between locations and methods.

**PERMANOVA for species contributing to differences by month for the term ‘Me’**

Source of variation	June 2014	August 2014	June 2015
<i>Cheilodactylus fuscus</i>	0.053	0.029	0.009
<i>Apogon limenus</i>	0.004	0.056	-
<i>Achoerodus viridus</i>	-	0.481	0.032
<i>Austrolabrus maculatus</i>	0.212	-	0.213
<i>Eupetrichthys angustipes</i>	-	-	0.262
<i>Labroides dimidiatus</i>	0.009	0.027	0.338
<i>Notolabrus gymnogenis</i>	0.029	0.004	0.014
<i>Ophthalmocephalus lineolatus</i>	0.031	0.004	-
<i>Pseudolabrus guentheri</i>	0.002	0.025	0.031
<i>Thalassoma lunare</i>	0.428	0.325	-
<i>Brachalutereres jacksonianus</i>	-	0.508	0.04
<i>Parupeneus spilurus</i>	0.291	0.14	-
<i>Upeneichthys lineatus</i>	0.295	0.505	-
<i>Odax cyanomelas</i>	0.051	0.012	-
<i>Machaenichthys immaculatus</i>	0.295	0.136	-
<i>Parma oligolepis</i>	-	0.357	0.779
<i>Parma unifasciata</i>	0.017	0.002	0.098
<i>Pomacentrus coelestis</i>	0.005	0.01	0.253

**Table 9.** Species contributing to significant differences between locations.  
**Pair-wise test results from SIMPER for term Location ‘Lo’**

Source of variation	June 2014	August 2014	June 2015
MB, WR	<i>C. fuscus</i> , <i>A. maculatus</i> , <i>O. lineolata</i> , <i>P. guentheri</i> , <i>T. lunare</i> , <i>P. spilurus</i> , <i>U. lineatus</i> , <i>P. flavomaculatus</i>	<i>L. dimidiatus</i> , <i>P. spilurus</i> , <i>U. lineatus</i> , <i>P. oligolepis</i> , <i>S. cardinalis</i>	<i>P. oligolepis</i> , <i>P. unifasciata</i>
MB, NR	<i>C. fuscus</i> , <i>A. maculatus</i> , <i>T. lunare</i> , <i>P. spilurus</i> , <i>M. immaculatus</i> , <i>E. angustipes</i>	<i>C. fuscus</i> , <i>A. viridus</i> , <i>T. lunare</i> , <i>B. jacksonianus</i> , <i>P. spilurus</i> , <i>M. immaculatus</i> , <i>P. oligolepis</i>	<i>E. angustipes</i> , <i>L. dimidiatus</i> , <i>P. guentheri</i> , <i>P. oligolepis</i> , <i>P. unifasciata</i> , <i>P. coelestis</i>
WR, NR	<i>O. lineolata</i> , <i>T. lunare</i>	<i>C. fuscus</i> , <i>T. lunare</i>	<i>A. maculatus</i> , <i>E. angustipes</i> , <i>L. dimidiatus</i> , <i>A. neoguinaicus</i> , <i>M. trachylepis</i>

**Table 10.** Species contributing to significant differences between methods.  
**Pair-wise test results from SIMPER for term Method ‘Me’**

Source of variation	June 2014	August 2014	June 2015
25 m BT, KelpCam	<i>P. guentheri</i> , <i>P. unifasciata</i>	<i>O. lineolata</i> , <i>P. guentheri</i> , <i>P. unifasciata</i> ,	-
25 m BT, SPC	<i>L. dimidiatus</i> , <i>N. gymnogenis</i> , <i>P. guentheri</i> , <i>P. coelestis</i>	<i>N. gymnogenis</i> , <i>O. lineolata</i> , <i>P. unifasciata</i>	<i>C. fuscus</i>
KelpCam, SPC	-	<i>O. cyanomelas</i>	-

**Table 11.** Summary of results of the PERMANOVA from the DIVERSE resemblance matrix based on Euclidian Distance of the untransformed data, showing the significance of differences in fish species richness between Locations (Lo) and Methods (Me) in June 2014, August 2014 and June 2015.

**PERMANOVA DIVERSE results by Months ‘Mo’**

Source of variation	June 2014	August 2014	June 2015
Lo	0.018	0.124	0.018
Me	0.013	0.010	0.013
LoxMe	0.449	0.912	0.449

**Table 12.** Pair-wise comparisons between Locations (Lo) for DIVERSE in June 2014 and June 2015. August 2014 was not tested as no significant differences were found between Lo (Table 11).**Pair-wise test results from DIVERSE for term Location ‘Lo’**

Source of variation	June 2014	August 2014	June 2015
MB, WR	0.001	-	0.711
WR, NR	0.08	-	0.01
NR, MB	0.398	-	0.005

**Table 13.** Pair-wise comparisons between Methods (Me) for DIVERSE in June 2014, August 2014 and June 2015.**Pair-wise test results from DIVERSE for term Method ‘Me’**

Source of variation	June 2014	August 2014	June 2015
25 m BT, KelpCam	0.061	0.132	0.5
25 m BT, SPC	0.013	0.037	0.112
KelpCam, SPC	0.055	0.161	0.016

The results showed that there were significant differences in species richness between the study sites MBI and WR in June 2014, and between NR and the other two locations in June 2015 (Table 12). Regarding the whole fish community composition, significant differences were found between all locations, for the three sampling times, except for June 2015, between MBI and WR. The species driving the differences between locations (Table 9) were not the same in the three months of survey.

The results showed that 25 m BT and KelpCam recorded more species than SPC in all locations and time. The first two methods recorded similar number of species in all locations. The DIVERSE analysis indicated that there were significant differences between methods in terms of the number of species recorded (Table 11), and the pair-wise tests indicated that these differences were driven by SPC (Table 13). These pair-wise tests showed that there were no significant differences between 25 m BT and KelpCam when recording species richness.

All methods showed significant differences between each other when explaining fish community composition in the three survey times, except in June 2015, when significant differences were only found between 25 m BT and SPC. These significant differences between methods were driven by the same species in most cases (Table 10). The PERMANOVA tests for individual species revealed that the wrasse *Pseudolabrus guentheri* (gunthers wrasse) and the damselfish *Parma unifasciata* drove the differences between 25 m BT and KelpCam in June 2014 and August 2014. Also, another wrasse, *Ophthalmolepis lineolata* (maori wrasse) contributed to the differences between these two methods in August 2014. Differences between 25 m BT and SPC in June 2014 were driven by the wrasses *Notolabrus gymnogenis* (crimson-banded wrasse), *Pseudolabrus guentheri*, *Labroides dimidiatus* (bluestreak cleaner wrasse), and by the damselfish *Pomacentrus coelestis*. In August 2014 these differences were driven by the wrasses *N. gymnogenis* and *O. lineolata* and the damselfish *P. unifasciata*. Finally, differences between these two methods in June 2015 were driven only by the red morwong *Cheilodactylus fuscus*.

In terms of composition of fish families (Appendix 1), 25 m BT recorded more species of Labridae (wrasses) and Pomacentridae (damselfish), Serranidae (cods and basslets), Mullidae (goatfish), Acanthuridae (surgeonfish), Chaetodontidae (butterflyfish), Cheilodactylidae (morwongs), the schooling Plesiopodae (hulafish), Pempheridae (sweepers) and common sharks like Orectolobidae (wobbegongs). 25 m BT also recorded more of the cryptic family Scorpaenidae. Some families detected by 25 m BT were not detected by KelpCam at all, such as the cryptic kelpfish (Chironemidae), small blennies (Blennidae), and families that are commonly found in crevices and caves, such as roughies (Trachichthyidae) and cardinalfish (Apogonidae) and borrows (moray eels - Muraneidae). Finally, some families were only recorded once by 25 m BT (and not by KelpCam) in the length of this study: the rare Pomacanthidae (angelfish), Aracanidae (temperate boxfishes), Rachycentridae (cobia), Siganidae (rabbitfish), Tetradontidae (pufferfish), the bottom-dwelling Aulopidae, Fistulariidae (flute-mouth) and bottom-dweller Urolophidae (stingaree).

On the other hand, KelpCam detected more Monacanthidae (leatherjackets), Myliobatidae (eagle-rays), Odacidae (herring cale), Aplodactylidae (rock cale) and Haemulidae (sweetlips) than 25 m BT. KelpCam also counted more of the schooling Carangidae (trevallies, yellowtail scad, kingfish), Monodactylidae (pomfrets), Sparidae (bream and tarwhine), Scorpidae (silver sweep), Microcanthidae (mado), Dinolestidae (pikes). KelpCam also counted fish from the family Lutjanidae, the rare Diodontidae (porcupinefish), the rare Hemiraphidae (garfish), Narkidae (numb ray) and Ostraciidae (boxfish), when 25 m BT did not detect these families at all.

When I compared KelpCam and SPC, the last method detected many of the families BT had detected and KelpCam had not, although SPC did not detect as many families as BT did. However, apart from those families that BT detected and KelpCam did not (moray-eels, kelpfish, blennies, rabbitfish and the bottom-dwelling Aulopidae), SPC detected three other families that KelpCam did not detect: the small, well camouflaged, bottom-dweller Clinidae; the cryptic Platycephalidae (flathead); and a sting ray of the family Dasyatidae. These three families were only counted once during the surveys with all methods.

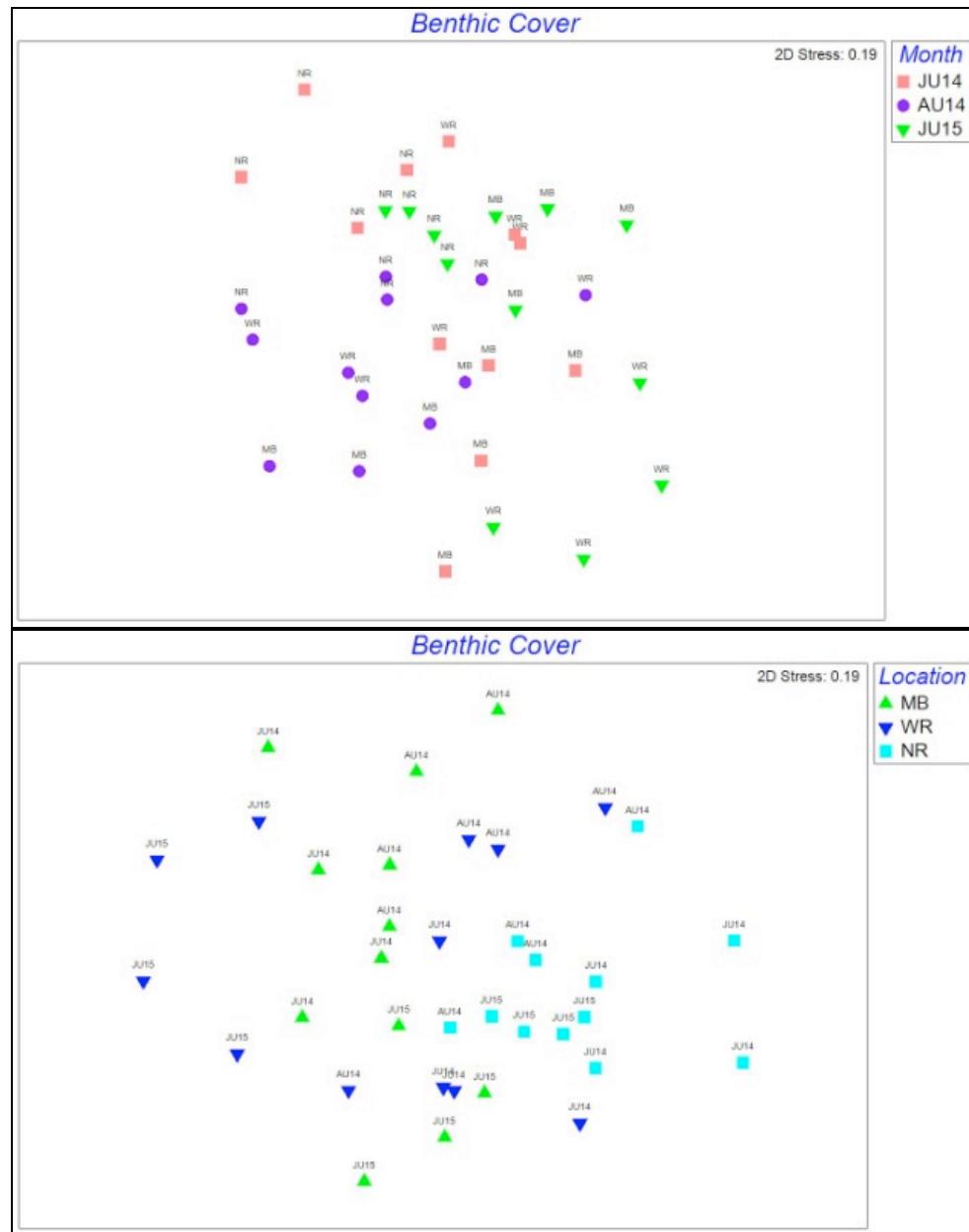
### **2.5.3 Benthic community structure and environmental variables**

Appendix 2 shows the data for benthic organisms and types of benthos found in the three locations in the three survey times. Brown algae and red algae were the most abundant taxa recorded, with *Ecklonia radiata* dominating the Benthic Cover counts. This species was followed by turfing algae, the brown algae *Sargassum*, the red algae *Corallina*, *Phymatolithon*, *Amphiora*, the brown algae *Dictyotacea*, species of the green algae *Caulerpa*, encrusting or sub-massive sponges, and encrusting and plate corals. Other invertebrates commonly found along the 25 m transects were solitary ascidians. Furthermore, sand, rubble or bare rocks were very common (up to 18% of abundance) in some transects.

There is no clear separation of groups for locations and times for benthic composition in the nMDS ordination: all show some overlapping, except for a slight pattern in NR (Figure 5). The PERMANOVA test (Table 14) resulted in significant differences only in the interaction ‘LoxMo’. The pair-wise test on the interaction ‘LoxMo’ for pairs of levels of factor Month ‘Mo’ showed significant differences between months JU14 and JU15 and between AU14 and JU15 within

levels of location MBI and WR. However, within level NR, AU14 was significantly different from the other months, while JU14 and JU15 did not show significant differences.

The pair-wise test on the interaction ‘LoxMe’ for pairs of levels of factor Location ‘Lo’ showed significant differences between all locations within both levels JU14 and JU15, but within level AU14, MB and WR did not show significant differences with each other.



**Figure 5.** nMDS plots for the factors Location (MB = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock) and Month (June 2014, August 2014, Junes 2015) for Benthic Cover.

**Table 14.** Summary of results of the 2-way PERMANOVA test showing the significance of differences in Benthic Cover between Locations (Lo) and Months (Mo).**PERMANOVA results Benthic Cover**

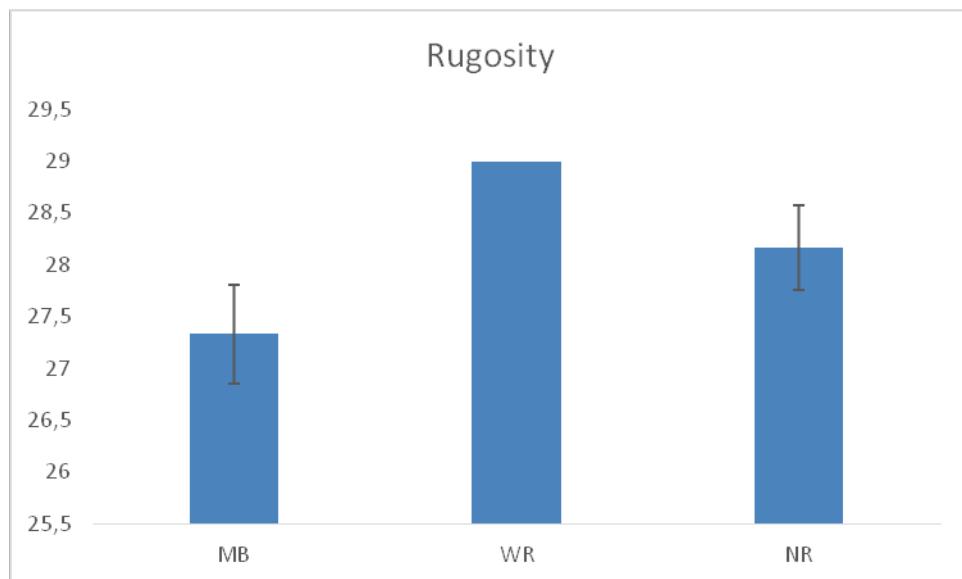
Source of variation	df	MS	F	P
Lo	2	1763.7	14.699	0.18
Mo	2	1614.8	13.458	0.262
LoxMo	4	1199.9	35.719	0.001
Res	27	335.92		

The results for the RELATE test indicated that Rho was closest to zero than to 1 ( $\rho = 0.147$ ) (Table 15), however the significance level of sample statistic was  $<0.05\%$ , therefore I concluded that there was a concordance between the 25 m Belt-Transects dissimilarity matrix and the Benthic Cover similarity matrix.

**Table 15.** Summary of RELATE analysis from the two Bray-Curtis similarity matrixes (25 m BT and Benthic Cover).**RELATE results**

Sample statistic (Rho)	0.147
Significance level of sample statistic	3.7%
Number of permutations	999
Number of permutations statistics greater than or equal to Rho	36

Rugosity was greater in Woolgoolga Reef (Average = 29 m, Standard Error = 0) than in Muttonbird Island (Av. = 27.33, St. Err. =  $+/- 1.65$ ) and North Rock (Av. = 28.17, St. Err. =  $+/- 1.43$ ). However, the DistLM test (Table 17) did not identify Rugosity as a key variable explaining fish community composition.

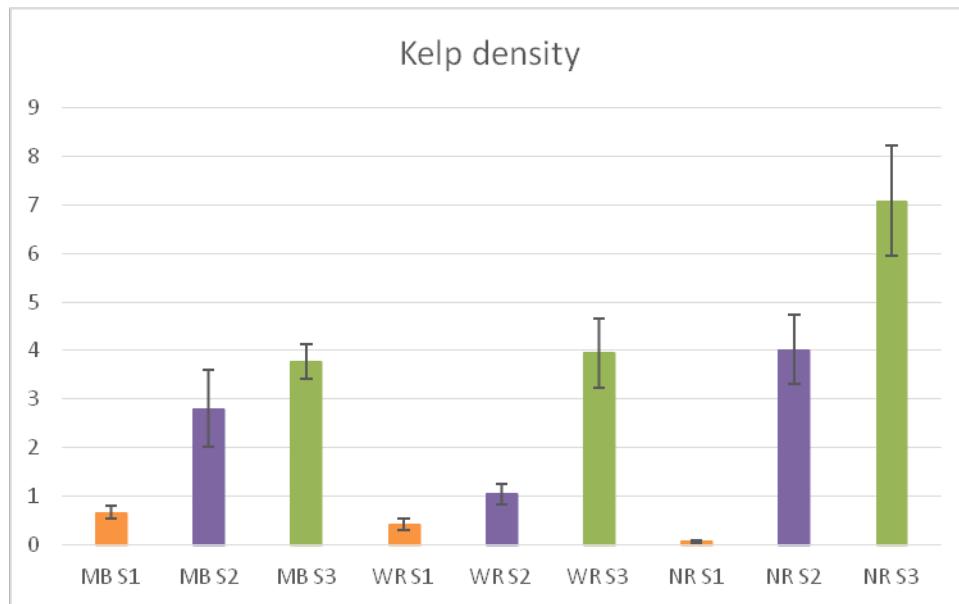


**Figure 6.** Average Rugosity measurements in the three locations (MB = Muttonbird Island, WR = Woolgoolga Reef, NR = North Rock), with standard error (+/-).

**Table 16.** Total kelp density in the three locations for each survey time

	MB JU14	MB AU14	MB JU15	WR JU14	WR AU14	WR JU15	NR JU14	NR AU14	NR JU15
<i>Ecklonia radiata</i> density	25.33%	15.75%	8.58%	15.08%	24.17%	5.67%	32.75%	7.25%	13.92%

Kelp Density appeared to be different in the three months of this study (Figure 7). Its abundance showed a reduction in all locations during time (Table 16, Appendix 2). In general, the presence of S1 kelps was very low in all locations. S2 was common in MBI and NR, but not as much in WR. Finally, the adult Stage (S3) was the most common Stage in all locations, being more abundant in NR than in the other locations. Overall, NR showed more Kelp Density than the other locations (Figure 8), which was also found in the Benthic Cover analyses (Appendix 2). DistLM marginal test resulted in the identification of the all Kelp Stages as significant environmental factors explaining the fish community composition, and the sequential test resulted in S3 being the best as the best variable explaining the fish community composition in all locations and times, although it only explained a 6.22% of the variation (Table 17).



**Figure 7.** Average Kelp Density for the three stages of kelp (S1 = Stage 1, S2 = Stage 2, S3 = Stage 3) in the three locations, with standard error (+/-).

**Table 17.** Summary of DistLM of Environmental data ‘Rugosity’ and ‘Kelp Density’ with 25 m BT.  
**DistLM results**

Variable	F	P	Prop.
Rugosity	15.684	0.096	4.41%
Kelp Density S1	17.251	0.048	4.82%
Kelp Density S2	16.976	0.041	4.76%
Kelp Density S3	22.561	0.006	6.22%

## 2.6 Discussion

### 2.6.1 Underwater methods to study fish communities in *Ecklonia radiata* dominated rocky reefs

The use of 25 m Belt-Transects has been proven to be effective to study fish community structure in near-shore reefs of eastern Australia (Smith et al. 2011, Davis et al. 2014b). In this study, I implemented this technique, along with two more methodologies (Stationary Point Counts and KelpCam), in order determine if there are more effective ways to study the fish community structure in *Ecklonia radiata* dominated rocky reefs.

The results showed that 25 m BT and KelpCam recorded more species in all locations than SPC. These differences may be related to the length of the surveys

rather than the area surveyed: KelpCam and SPC cover the same area, but surveys with the underwater video technique take four times longer than the SPC and do not require the presence of a diver.

None of the abundant species of fish recorded in this study are known to be diver-averse or to be affected by the presence of divers. In fact, previous work with divers in the region showed high abundances of all these species in this type of ecosystem (Malcolm et al. 2007, Malcolm and Smith 2010). Therefore, the significant differences found between methods are possibly due to differences in the number of records of common species, as the area covered by each method and the time spent for each type of survey are different. For example, BT covers more area ( $125\text{ m}^2$ ) than KelpCam and SPC ( $27\text{ m}^2$ ), therefore more counts were expected using BT. However, KelpCam and SPC recorded a few species that BT did not record. Therefore, even though it was expected for BT to count more species, (including cryptic species) the comparison between methods was crucial because some of the species were not recorded by this method but they were by the two others.

In terms of composition of fish families, 25 m BT recorded more species of the common families in the region (Malcolm and Smith 2010), such as the Labridae (wrasses) and Pomacentridae (damselfish), Serranidae (cods and basslets), Mullidae (goatfish), Acanthuridae (surgeonfish), Chaetodontidae (butterflyfish), Cheilodactylidae (morwongs), the schooling Plesiopodae (hulafish), Pempheridae (sweepers) and common sharks like Orectolobidae (wobbegongs). 25 m BT also recorded more of the cryptic family Scorpaenidae. Again, these differences are likely related to the area covered by each method and the time spent in the different survey method, and with the probability of coming across species of the most common families while surveying these differently sized areas. Some families detected by 25 m BT were not detected by KelpCam at all, such as the cryptic kelpfish, small blennies, and families that are commonly found in crevices and caves (Trachichthyidae and Apogonidae) and borrows (moray eels). These findings are associated with the fact that divers performing underwater visual censuses could research the habitat in a way that the static KelpCam camera could not (Lowry et al. 2012), thus finding more of the small and cryptic families.

On the other hand, KelpCam detected more Monacanthidae (leatherjackets), Myliobatidae (eagle-rays), Odacidae (herring cale), Aplodactylidae (rock cale) and

Haemulidae (sweetlips) than 25 m BT. This method seems to be more effective at detecting families that may be quick swimmers, semi-cryptic (blend in colours) and that swim around and above the kelp canopy, hiding and coming out of it when there are no predators or threatening elements (like a diver).

As reviewed, kelp *E. radiata* is a conspicuous feature of the inshore reefs in subtropical and temperate Australia, and were found to be highly abundant in the results of Benthic Cover and Kelp Density measurements in the present study. In these surveys, remote underwater video helped overcome the limitations imposed by kelp canopies, which move with the surge and often make fish identification and counts challenging. Having data permanently recorded means that it can be stored for future consideration or secondary analyses (Longo and Floeter 2012). Furthermore, KelpCam helped overcome the surveying difficulties associated with fish behaviour, as it provided an increased coverage of fast-swimming, kelp canopy-associated fish species. Therefore, these results are an example of how underwater video allows a non-invasive, effective monitoring of fish assemblages (Pelletier et al. 2011, Longo and Floeter 2012, Assis et al. 2013, Mallet and Pelletier 2014) and minimises the diver effect in underwater surveys (Cappo et al. 2003). Furthermore, this method does not require the same level of expertise in fish ID than BT and SPC, as the surveyor can stop the footage and use fish identification resources to determine the species with exactitude. However, this means that the surveyor takes longer to complete the data collection. It is recommendable that the person carrying out this step is already familiar with the common species in the area.

KelpCam also counted more of the schooling Carangidae (trevallies, yellowtail scad, kingfish), Monodactylidae (pomfrets), Sparidae (bream and tarwhine), Scorpidae (silver sweep), Microcanthidae (mado), Dinolestidae (pikes). This can be associated with the angle at which the cameras pointed (towards the surface), thus detecting more schooling families that may swim in front or on top of the canopy, during the 45 minutes of survey, as opposed to the 20-25 minutes BT take to be completed. During the BT surveys, the diver is mostly looking to the front or below, searching for cryptic species, which reduces the chance of recording fast-swimming or schooling species.

Stationary Point Counts recorded a few more families of bottom-dwelling, well-camouflaged fish, as well those ones counted during the 25 m BT surveys. This suggests it could be worth extending the length of the BT surveys, spending a few more minutes in areas equivalent to that covered by SPC along the BT transects, looking for cryptic species only. However, despite the likelihood of recording more cryptic species and higher abundances of these, a major disadvantage would be the addition of minutes to the 20-25 min that BT already take to be completed. Consequently, only one transect would be completed per diver during the length of a dive. Nevertheless, both 25 m BT and SPC recorded more cryptic and small species than the KelpCam did, which has occurred in other studies using similar methodologies (Cappo et al. 2006, Colton and Swearer 2010, Longo and Floeter 2012, Lowry et al. 2012).

Nonetheless, the three sampling methods may be useful tools for sampling in Marine Protected Areas (MPAs), where non-destructive methodologies are essential (Cappo et al. 2003, Willis and Anderson 2003, Cappo et al. 2006, Langlois et al. 2006, Heagney et al. 2007). As reviewed and discussed, each one of these three observational approaches may have strengths and limitations. Therefore, it has been useful to compare these three different techniques in order to detect the kind of biases inherent in each one of them. My conclusion is that adopting this multi-method approach, using more than one standardised underwater visual technique, has helped me obtain a better understanding of the structure of fish assemblages in the three locations of this study, where kelp is a conspicuous feature of the ecosystem.

## **2.6.2 Fish community structure in *Ecklonia radiata* dominated rocky reefs in subtropical eastern Australia**

The results of this study were similar to those obtained by past comprehensive datasets on reef fish assemblages within the Solitary Islands Marine Park (SIMP) (Malcolm et al. 2007, Malcolm and Smith 2010). My study of these near-shore kelp-dominated rocky reefs provided me with a list of 121 species from 47 different species. Malcolm and Smith (2010) surveyed 68 sites within the SIMP and recorded a total of 254 species from 66 families. The present study was therefore successful in recording a big number of fish species, taking into account that only 3 locations within the SIMP were surveyed at three different times.

The results for the representation of fish families were also similar to those obtained by previous studies: wrasses were the most abundant family, followed by damselfish, butterflyfish and leatherjackets. The most abundant species in *Ecklonia radiata* dominated rocky reefs is the damselfish *Parma unifasciata*, which has been found to be one of the most abundant species in the SIMP in previous studies (Malcolm et al. 2007, Malcolm et al. 2010). This species is followed by the wrasses *Notolabrus gymnogenis* and *Ophthalmolepis lineolata*, which have been found to have greater abundances in near-shore reefs (Malcolm et al. 2010a, Davis et al. 2014b), and the wrasse *Pseudolabrus guentheri*, common in both near-shore reefs and off-shore, coral-dominated reefs (pers. obs.).

My results showed that there were significant differences in species richness between the study sites MBI and WR in June 2014, and between NR and the other two locations in June 2015. Furthermore, regarding the whole fish community composition, significant differences were found between all locations, for the three sampling times, except for June 2015 (no significant differences between MBI and WR). These variations may be due to the highly different abundances of the most common species found in the surveys. For instance, the red morwong (*Cheilodactylus fuscus*) was four times more abundant in MBI than in NR. The black-spotted wrasse (*Austrolabrus maculatus*) was three times more abundant in NR than in MBI. And the abundance of the gunthers wrasse (*Pseudolabrus guentheri*) in NR and WR was twice that of MBI. However, the species contributing to differences between locations varied so much in the three times of survey, that it is difficult to describe the community structure in any of the three locations as a definite. This is because there was not a species driving the differences between locations in all the three times of this study. However, I observed that the differences in distribution of some species in different times determined the significant differences between locations at those times. For instance, the moon wrasse (*Thalassoma lunare*) explained differences in fish community structure between MBI-NR, and WR-NR, as it was a very common species in North Rock but not in the other two locations, in the two survey times of 2014. This was also the case of the goatfish (*Parupeneus spilurus*) between MBI and the two other locations in 2014, and the red morwong (*Cheilodactylus fuscus*) between MBI and the other two locations, in 2014. In June 2015, differences between locations were mostly driven by the damselfish *Parma oligolepis*, which

was much more abundant in MBI than in the other two locations (this also had occurred in August 2014). Another damselfish, *Parma unifasciata*, drove differences between locations in June 2015, with bigger numbers in WR and NR than in MBI. Furthermore, the snakeskin wrasse (*Eupetrichthys angustipes*) contributed to the differences between NR and the other two locations in the first and third round of surveys. In fact, this species was barely recorded in WR and not recorded at all in MBI. This same situation happened with the blue-streak cleaner wrasse (*Labroides dimidiatus*).

Differences in fish assemblages at spatial scales have been reported in previous studies of reef fish communities (Curley et al. 2003, Anderson and Millar 2004, García-Charton et al. 2004, Gladstone 2007, Malcolm et al. 2007). Spatial variation of fish assemblages at large spatial scales may be influenced by factors such as biogeography (Edgar et al. 2004a), latitude (Bouchon-Navaro et al. 2005), geology (Guidetti et al. 2004), temperature, depth and productivity (Leathwick et al. 2006). However, at relatively small scales (100s to a few 1000s of metres), spatial variation in fish assemblage structure may be determined by a combination of factors, including differences in habitat structure (Connell and Jones 1991, Willis and Anderson 2003), recruitment (Connell and Jones 1991, Smith et al. 1991) and local hydrodynamics (Warner et al. 2000). The spatial differences between the locations of this study in each of the sampling months may be due to these factors combined and may be independent of the distance between them (Gladstone 2007, Malcolm et al. 2007). The differences in the results between the sampling times within the year of the study may also be the result of a range of factors, including non-examined environmental variables that influenced fish communities in those times, resulting in short term variations. However variability within sites in the Solitary Islands Marine Park was expected, following the results from previous studies on fish communities in the region (Malcolm et al. 2007). Given such different results for each month of survey, I conclude that more replication throughout time is needed to determine the species and the environmental factors that may be driving the differences in the fish community structure between these locations.

Previous studies have determined that habitat plays a very important role in the distribution of rocky reef fish (Anderson and Millar 2004, Tuya et al. 2009, Morton and Gladstone 2011, José de Anchieta et al. 2013). Furthermore, heterogeneity

within habitats generally promotes higher fish abundances than those reefs dominated exclusively by one feature (Tuya et al. 2009). My analyses determined that there was a slight concordance between fish community structure and Benthic Cover. However, the results did not determine Rugosity (habitat complexity) as a main predictor variable explaining differences in the fish community structure. On the other hand, kelp was one of the most abundant features covering the benthos in the areas of the study, showing differences in its density between locations. Although benthic composition showed no significant differences between locations and times, a decrease in kelp abundance was observed in all locations. Furthermore, the results showed that density of Stage 3 of kelp (adults) was the best variable explaining fish community composition in all locations and times in this study, although it only explained 6% of the variance. Previous studies have found a correlation between canopy height and fish density and fish species richness, where higher canopies (i.e. adult kelp) support more complex fish community assemblages (van Lier et al. 2017). Thus, Kelp Density in the present study may explain some differences between fish community structure in the three locations in the three survey times. More replication in the locations of this study is needed to determine whether kelp reduction in these locations is driving the differences in the fish community structure between these locations throughout time. Kelp reduction during the time of this study could be associated with storms and wave action throughout the year, so it would be important to carry out further analysis to obtain more data sets of the same kind, to find out whether these results are consistent throughout longer periods of time.

Kelp canopies promote conditions for the development of understorey macroalgal assemblages, which are mainly dominated by red algae (Clark et al. 2004, Flukes et al. 2014). In this study, several species of red algae were the predominant feature under *E. radiata* canopies. These macrophytes provide a buffer for benthic macroalgal species from high wave energy (Almanza and Buschmann 2013), regulate the shading of the habitat (Kennelly 1987b, Arkema et al. 2009), and alter the water flow (Eckman et al. 1989). These understorey assemblages of macroalgae increase the heterogeneity of the habitat and create suitable environmental conditions (Teagle et al. 2017) for both sessile and motile invertebrate fauna to live (Flukes et al. 2014, Lavender et al. 2017). Thus, differences in kelp density has been shown to result in different assemblages of prey species (Anderson et al.

2005) which ultimately leads to differences in fish assemblages between habitats (Anderson and Millar 2004). The distribution of invertebrate fauna influenced by different kelp density in the locations of this study may be playing an important role structuring fish assemblages in these locations.

As revised in Chapter 1, *Ecklonia radiata* is an engineering and foundation species in subtropical and temperate near-shore reefs of eastern Australia, and it appears to be vulnerable to increasing temperature (Bearham et al. 2013). This results in a reduced capacity to respond to physical perturbations (Wernberg et al. 2010). With climate change, storm events (Stocker et al. 2013) and wave height and speed (Young et al. 2011) will increase. The EAC is already becoming warmer and stronger (Cai et al. 2005, Pearce and Feng 2007, Ridgway 2007, Hobday and Pecl 2014), and more coral-macroalgae competition in subtropical and temperate regions of eastern Australia is expected (Beger et al. 2014, Verges et al. 2014). Newly arrived tropical herbivores will be crucial in the mediation of these interactions (McCook et al. 2001). All these perturbations will have important implications for the architecture of the habitat *Ecklonia radiata* provides, and may have strong impacts on the kelp-associated species, including macroalgae, invertebrates and fish. As a consequence, the trophic ecology of these communities may also be altered (Byrnes et al. 2011, Johnson et al. 2011b).

One of the families of fish that may be affected by the fragmentation and loss of kelp canopies is the family Labridae, which was commonly recorded in this study and previous studies in the region (Malcolm et al. 2010a, Davis et al. 2014b). Wrasses are known to shelter beneath algal canopies (Curley et al. 2003, Graham 2004, Russell et al. 2008), which provide refuge and lower the risk of predation, and protect them from wave action. These canopies also provide them with areas for foraging activity, nocturnal retreats and nesting sites (Graham 2004, Russell et al. 2008). Therefore, the perturbation of the habitat provided by these macrophytes may potentially have strong impacts on the ecology and the distribution of *E. radiata*-associated wrasse species, as well as on the kelp-associated invertebrates (Smith et al. 1996a, Coleman et al. 2007) these wrasse may prey on.

Sea temperature has also been found to be an important factor influencing the distribution of fish assemblages (Booth et al. 2011, Malcolm et al. 2011a, Morton and Gladstone 2011). At present, taxonomic and functional diversity of herbivorous

fish in kelp habitats is generally low (Floeter et al. 2005). But the addition of new species of tropical fish arriving with the stronger EAC, which feed directly upon the macroalgae, may result in strong impacts in *E. radiata* forests of subtropical and temperate Australia. This may result in a decrease of the resilience of these ecosystems and may prevent the recovery of kelp beds after physical disturbances (Wernberg et al. 2013, Verges et al. 2014), having negative effects on the whole community.

In conclusion, this component of my study has succeeded in documenting fish species that use the habitat provided by *E. radiata* and that may be negatively affected or may be lost by contraction of kelp, in an already recognized hot spot for climate change. These results are also important for management purposes, as this study has provided a comparison of the results obtained by the use of different methods to study fish communities in this type of ecosystems. My conclusion is that, given the complexity of the habitat created by kelp canopies, more than one methodology should be used to study fish community composition in *Ecklonia radiata* dominated rocky reefs.

# Chapter 3 – Dietary studies of three species of generalist fish (fam. Labridae)

## 3.1 Abstract

The wrasse family Labridae is one of the most common, species-rich families in fish assemblages of subtropical eastern Australia. Their foraging behaviour, high diversity and abundance make them an important group to study their role as predators and their potential to modify prey assemblages in an ecosystem threatened by climate change. The main aim of this study was to determine prey diversity of three abundant carnivorous species of wrasse (*Notolabrus gymnogenis*, *Pseudolabrus guentheri* and *Ophthalmolepis lineolata*) in three *Ecklonia radiata* dominated rocky reefs off the Coffs Coast of New South Wales. Dietary analyses were undertaken using a cutting edge technique, DNA metabarcoding, in order to test the effectiveness of this technique in the study of food webs in this type of ecosystems. Species-specific blocking primers were designed, tested and applied. In total, 70 different Operational Taxonomic Units were identified across the gut content samples of 14 fish. Previous reports of wrasse as generalist, opportunistic feeders are supported by the findings of this study. The results also provide novel information on prey diversity and indicate that these species may occupy a high trophic level in the food web, given they feed on carnivorous fish. Small sample sizes limited the ability to draw conclusions on competition or food partitioning among these species. However, this study enabled the identification of a wide range of prey from highly digested gut contents, indicating that DNA metabarcoding is a powerful technique for dietary analysis of generalist predators in kelp forests.

## 3.2 Introduction

### 3.2.1 Most abundant carnivorous fish in *Ecklonia radiata* dominated rocky reefs

Assessments of fish community assemblages in *Ecklonia radiata*-dominated rocky reefs in the Coffs Coast region (subtropical eastern Australia), showed that wrasses are the most conspicuous fish in the three study locations examined (Table 18). In order to understand the likely consequences of habitat reduction or species loss, for

example, by climate change-related kelp loss there is a need to understand the distribution, functional role and ecological importance of these conspicuous fish species within their rocky reef ecosystems.

For the purpose of this study, the three most abundant carnivorous fish species were selected for dietary analysis. These are the wrasses: *Notolabrus gymnogenis*, *Pseudolabrus guentheri* and *Ophthalmolespis lineolata*. These species are of different size, and therefore could provide an opportunity to study food partitioning amongst wrasses in *Ecklonia radiata* dominated rocky reefs.

**Table 18.** Most common carnivorous fish species recorded in this study of *Ecklonia radiata*-dominated rocky reefs off Coffs Harbour, NSW. n = number of records. MBI = Muttonbird Island, WR = Woolgollga Reef, NR = North Rock.

Family	Common name	Species	n	MBI	WR	NR
Cheilodactylidae	Red Morwong	<i>Cheilodactylus fuscus</i>	94	50	32	12
Labridae	Black-spotted Wrasse	<i>Austrolabrus maculatus</i>	119	22	38	59
	Crimson-banded Wrasse	<i>Notolabrus gymnogenis</i>	547	159	200	188
	Maori Wrasse	<i>Ophthalmolespis lineolata</i>	201	66	36	99
	Gunther's Wrasse	<i>Pseudolabrus guentheri</i>	249	47	101	101
Mullidae	Black-saddled Goatfish	<i>Parupeneus spilurus</i>	56	44	7	5

### 3.2.2 Distribution and habitat preferences of wrasses

The wrasse family Labridae is one of the most conspicuous, species-rich and abundant families in tropical and temperate fish assemblages (Morton and Gladstone 2011). They prey on a variety of benthic invertebrates (Denny and Schiel 2001, Shepherd and Brook 2005, Morton et al. 2008). Their foraging behaviour, high abundance and diversity make them an important group to study in order to understand the role of predators and their potential to modify prey assemblages within these ecosystems.

Sea temperature is an important factor influencing the distribution and the dynamics of fish assemblages (Booth et al. 2007, Morton and Gladstone 2011). However, habitat also plays a major role in determining the spatial distribution of rocky reef fish (Anderson and Millar 2004, Morton and Gladstone 2011, José de Anchieta et al. 2013). Habitat heterogeneity promotes higher labrid abundance in comparison to that in reefs dominated exclusively by one feature (e.g. kelps) as it facilitates the access to prey (Tuya et al. 2009). Differences in kelp density may also result in different assemblages of prey species (Anderson et al. 2005) which

ultimately leads to differences in fish assemblages between habitats (Anderson and Millar 2004).

Habitat associations of wrasses seem to be partly influenced by the benthic invertebrate assemblages represented within each habitat (Morton et al. 2008). For example, juvenile wrasses have a relatively reduced mouth size and crushing strength of the pharyngeal jaws (Clifton and Motta 1998). Accordingly, their diet may be restricted to amphipods and small molluscs (Morton et al. 2008) which are common in shallow algal habitats (Smith et al. 1996a, Edgar 2001). The association between recruits and juveniles of this family and algal habitats in eastern Australia (Curley et al. 2003, Morton et al. 2008) is likely to be related to the availability of prey preferences in these habitats (Morton and Gladstone 2011).

Ontogenetic shifts in habitat preference were assessed by Morton and Gladstone (2011) and they found that small, recruiting wrasse show greater habitat specialization than larger adult individuals. José de Anchieta et al. (2013) demonstrated in their study that both density and foraging activity of wrasses is influenced by habitat and that the functional roles of wrasses change according to ontogenetic shifts. Habitat association may also be a result of the structure and the availability of refuges (Anderson and Millar 2004). Wrasses are known to shelter beneath algal canopies (Curley et al. 2003), which provide refuge and lower the risk of predation, protection from wave action, areas for foraging activity, nocturnal retreats and nesting sites (Graham 2004, Russell et al. 2008).

Distribution, density of individuals and changes in wrasse family assemblages are also season-dependant. Species richness and density of individuals is normally higher at the end of summer and start of autumn in temperate ecosystems (Pihl and Wennhage 2002). This is due to the recruitment of juveniles of tropical species which expand their distribution in response to seasonally warm sea temperatures and the recruitment of temperate fish stocks after a dispersive planktonic larval stage (Fontes et al. 2011). The northern region of New South Wales, where the sites included in this study are located, relies on the south-flowing Eastern Australian Current, which transports larvae of many species of fish from lower latitudes and provides optimal sea temperatures for them to recruit and survive, maintaining a relatively high fish biodiversity in the region (Booth et al. 2007, Syahailatua et al. 2011).

### 3.2.3 Distribution and habitat preferences of the wrasse species in this study

#### *Notolabrus gymnogenis, Ophthalmolepis lineolata*

*N. gymnogenis* occurs along the Australian east coast from southern Queensland to eastern Victoria, while *O. lineolata* is distributed from southern Queensland along the east and south coast to south-western Western Australia (Kuiter and Kuiter 1996, Edgar 2001). Both species are conspicuous, habitat generalists as adults, and are commonly found in temperate and subtropical reefs, within kelp, urchin barrens, fringe habitats and sponge gardens (Curley et al. 2003, Malcolm et al. 2007, Kingsford and Carlson 2010, Morton and Gladstone 2011, Harvey et al. 2013, Davis et al. 2014b). However, juvenile individuals of both species reportedly prefer habitats rich in algae (Curley et al. 2003, Kingsford and Carlson 2010). Adults of both species do not exhibit cryptic behaviour and thus they are amenable to visual surveys (Davis et al. 2014b). However, *N.gymnogenis* juveniles exhibit cryptic behaviour and have drab coloration that may lead to biases when surveying this species.

Both species have large mouths and the great crushing power of their pharyngeal teeth (Clifton and Motta 1998) allows them to incorporate a broad range of hard-shelled prey into their diet (Morton et al. 2008). Their large fins also provide them with enhanced swimming abilities that allow them to roam over large areas over the reef and into various habitats, including areas affected by tides and waves (Fulton and Bellwood 2004, Kingsford and Carlson 2010).

These species feed in diverse microhabitats (Morton et al. 2008) within broad areas of reef (Morton 2007, Kingsford and Carlson 2010). Adults seem to be more habitat generalists whereas habitat association in these species is primarily seen in juvenile individuals (e.g. *N.gymnogenis* recruits mostly in kelp-covered habitats) (Morton and Gladstone 2011). Large individuals of both species reportedly coexist, which suggests that inter-specific competition is likely to be minimised by differences in the use of resources. For example, adults of *O. lineolata* forage opportunistically in a variety of microhabitats over extensive areas of reef whereas *N. gymnogenis* prefers bare hard-structure microhabitats within relative small areas of reef (Morton et al. 2008). Intra-specific competition among co-occurring smaller and larger individuals is reduced by the shifts in diet related to growth (Morton et al. 2008)

and changes in behaviour (Morton 2007), resulting in the partitioning of rocky reef resources.

In temperate eastern Australia, these wrasse species' richness and densities seem to experience a significant temporal variation between the autumn months of April and May (higher) and the early spring months of August to December (lower) (Morton and Gladstone 2011). Recruitment of wrasses during late summer and autumn coincides with the settlement of prey species (Morton et al. 2008).

### **Pseudolabrus guentheri**

This species occurs in tropical, subtropical and temperate reefs of eastern Australia (Kuiter and Kuiter 1996, Hoese et al. 2006, Goatley and Bellwood 2014). It is abundant amongst the kelp *Ecklonia radiata*, but may not be as readily observed as the other species included in this study due to the frequent use of shelter (Morton 2007). Its distribution reportedly responds to the abundance of prey in algal habitats such as amphipods and small molluscs.

#### **3.2.4 Dietary preferences of wrasses**

Wrasses are predominantly benthic carnivores with diurnal activity, that employ their powerful jaws and associated pharyngeal teeth to crush shells and hard exoskeletons of their invertebrate prey (Clifton and Motta 1998). They have broad diets and employ diverse foraging strategies, which suggest that they exert a strong influence on the regulation on invertebrate populations in rocky-reefs systems (Morton et al. 2008). The wide variety of invertebrates consumed includes cnidarians, sponges, polychaetes, molluscs, echinoderms, crustaceans, sipunculids, bryozoans and ascidians (Shepherd 2006, Morton et al. 2008). The species of wrasse selected for this study present significant intra- and inter-specific differences in feeding strategies and dietary composition (Morton et al. 2008). These feeding, dietary and, associated microhabitat differences (Morton and Gladstone 2011), likely enable the co-existence of these conspicuous species and help to maintain the high species richness of wrasse in *Ecklonia radiata* dominated rocky reefs.

### **Notolabrus gymnogenis**

Small individuals use ambush-hunting regularly to forage on amphipods, decapods and small gastropods under algal canopy. Larger individuals forage

opportunistically over a bigger area of the reef and make greater use of habitats that offer less refuge (e.g. sand/rubble, bare rock), where in particular decapods are obtained (Morton et al. 2008).

### **Ophthalmolespis lineolata**

This species feeds on high volumes of polychaetes, polyplacophorans, marginellid gastropods, bivalves and echinoids, which it finds opportunistically in different microhabitats, but with more frequency in sand/rubble substrates. Previous research suggests that this species may play an important role in the regulation of the echinoid densities in rocky reef habitats (Morton et al. 2008).

### **Pseudolabrus guentheri**

This species feeds on amphipods and small molluscs, commonly found under algal canopies, that can represent up to 60% of the dietary volume (Morton et al. 2008).

#### **3.2.5 Ecological role of wrasses**

Wrasses can play an important role in reducing prey abundances and modifying subtidal invertebrate assemblages (Choat 1982, Edgar and Shaw 1995a, b, Andrew 1999). For example, wrasses have been recorded feeding on echinoids (Sala 1997, Shepherd and Brook 2005, Morton et al. 2008) that are considered to be “ecosystem engineers” responsible for overgrazing on kelp, thus resulting in barrens of lower biodiversity (Ling 2008, Norderhaug and Christie 2009). Therefore, predation of urchins by labrids may have positive benefits for these habitats and the organisms that rely on them. Furthermore, labrids feed on other small prey that causes kelp mortality, such as amphipods (Duffy and Hay 2000, Haggitt and Babcock 2003). Thus, labrids may deserve “key-species” status as they seem to control kelp-dominated rocky reefs ecosystems (Morton et al. 2008). However, further studies on the feeding ecology of this family need to be carried out in order to confirm these assumptions.

#### **3.2.6 Dietary studies in fish**

In section 1.4 (Chapter 1) I have reviewed several approaches to study diet and food webs in marine ecosystems, from visual gut content analysis to recording feeding behaviour, and stable isotope analyses to decipher trophic hierarchy.

Dietary studies on wrasse species in particular have been carried out for decades, on different species, at different locations, and by different researchers. The most common technique used has been the visual analysis of gut content (Dipper et al. 1977, Sayer et al. 1996, Denny and Schiel 2001, Shepherd and Clarkson 2001, Figueiredo et al. 2005, Shepherd and Brook 2005, Muñoz and Cribb 2006, Shepherd 2006, Morton et al. 2008, Kramer et al. 2015), but some studies have used *in situ* observational methods of feeding behaviour (Jones 1984, Shepherd and Clarkson 2001, Sazima et al. 2005, Cole et al. 2010) or stable isotopes (Pinnegar and Polunin 2000, Aguilar et al. 2008, Vizzini and Mazzola 2009).

In the following section, I expand on a cutting-edge technique that has been applied in the present study.

### 3.2.6.1 DNA barcoding of gut content

It has been fourteen years since DNA barcoding was initially proposed as a fast and effective tool to identify species and to assess biodiversity (Hebert et al. 2003). Since this time, millions of DNA barcode sequences from thousands of species have become available in a number of accessible databases (Ratnasingham and Hebert 2007, Geer et al. 2009). Initially DNA barcoding focused on the description and documentation of biodiversity. However, more recently authors have highlighted the potential of DNA barcoding data in ecological studies, and have promoted it as a tool to study trophic interactions and food webs in both terrestrial and aquatic ecosystems (Valentini et al. 2009, Carreon- martinez and Heath 2010, Pompanon et al. 2012, Joly et al. 2014, Bartley et al. 2015).

*Sensu stricto* DNA barcoding is the identification of species from a single standardised DNA region. *Sensu lato*, the definition is less restrictive, as it includes identification to any taxonomical level using a range of DNA fragments. For example, assigning individuals to genera, families or orders using a short DNA fragment can be considered as DNA barcoding (Valentini et al. 2009). Regardless, this technique follows the following criteria:

- (i) the region of the gene sequence needs to have low variability among the individuals of the same species and higher variability between species,
- (ii) it needs to be standardised, using the same DNA region for different taxonomic groups,

- (iii) the target DNA fragment needs to provide sufficient phylogenetic information to enable the assignment of species that have not been previously barcoded to the appropriate taxonomic group (genus, family, order etc.),
- (iv) the DNA region needs to be robust, with priming sites that are highly conserved, enabling reliable PCR amplification and sequencing,
- (v) the target DNA fragment should be short enough so highly degraded DNA can be amplified (DNA regions longer than 150 bp present difficulties to be amplified from degraded DNA),
- (vi) a curate database of reference taxa is available for the target DNA region.

Thus, the ideal marker for DNA barcoding needs to be variable between species, standardised, contain phylogenetic information, robust and short.

Cytochrome C Oxidase subunit 1 (CO1) is a mitochondrial gene which was selected by the Consortium for the Barcode of Life (CBOL) as the ideal gene for DNA barcoding of animal species (Ratnasingham and Hebert 2007). This gene is present in most eukaryotes and it encodes a protein with a crucial role in cell respiration, where food molecules such as glucose, are broken down to release CO<sub>2</sub> and water to generate energy. This gene is an ideal target for species discrimination, as it is present across higher eukaryotes such as insects, fish, birds and mammals. Although the number of mitochondrial organelles varies depending on cellular energy requirements, each cell contains many identical copies of the mitochondrial genome. Total genomic DNA extracts, therefore, contain relatively high proportions of mitochondrial DNA for successful PCR amplification of mitochondrial loci.

At present, a global DNA-based system of barcode identification is being developed for all organisms: this is the Barcode of Life System (BOLD [www.boldsystems.org](http://www.boldsystems.org)) (Ratnasingham and Hebert 2007). This system and its publicly available databases are designed to support the generation and application of DNA barcode data. To this date, there are a total of 5,252,163 barcodes, which cover (formally described species) 174,646 animal species, 65,160 plant species and 20,786 fungi and other life.

Therefore, DNA barcoding is becoming an extended universal tool for vertebrate and invertebrate species identification, and has further applications including dietary analysis of an organism from its gut contents (Deagle et al. 2005a, Smith et al. 2005, Barnett et al. 2010, Dunn et al. 2010, Braid et al. 2012, Hargrove et al. 2012, Valdez-Moreno et al. 2012, Cote et al. 2013, Jung 2014, Paquin et al. 2014, Moran et al. 2015, Sakaguchi et al. 2017), or feces (Deagle et al. 2005b, Deagle et al. 2009, Clare et al. 2011, De Barba et al. 2014) and regurgitates (Alonso et al. 2014). This is particularly useful when prey cannot be identified by morphological characteristics or because it has been partially or totally digested. This technique is also useful when feeding behavior cannot be directly observed.

Several dietary studies targeting organisms from aquatic ecosystems have used DNA barcoding as a tool for the identification of prey in gut contents (Smith et al. 2005, Barnett et al. 2010, Dunn et al. 2010, Braid et al. 2012, Hargrove et al. 2012, Valdez-Moreno et al. 2012, Cote et al. 2013, Arroyave and Stiassny 2014, Jung 2014, Bartley et al. 2015, Moran et al. 2015, Aguilar et al. 2016). In the process, DNA is extracted from the semi-digested prey. Then, the species-specific standardised DNA region (DNA barcode, e.g. COI) is PCR amplified using universal primers (Folmer et al. 1994, Leray et al. 2013b) followed by sequencing of PCR amplicons. Unknown samples are then identified by comparing the sequences against a database of known and curate COI sequences (e.g. BOLD) derived from reference specimens (Ratnasingham and Hebert 2007). This technique has the power to detect prey-specific DNA fragments from the gut contents of vertebrates, even several hours after digestion (Deagle et al. 2005a, Sheppard and Harwood 2005).

Several previous studies have used DNA barcoding of gut content to reveal fish diet. Prey DNA extraction procedure usually involved several steps including: rinsing of the prey items after separation from the complete gut content matter, morphological examination, grouping (by shape, colour, etc.), identification where possible, individual storage, and sub-sampling of prey items to select tissue for DNA extraction using the recommended protocols (Smith et al. 2005, Barnett et al. 2010, Dunn et al. 2010, Valdez-Moreno et al. 2012, Cote et al. 2013, Paquin et al. 2014, Bartley et al. 2015, Moran et al. 2015, Aguilar et al. 2016). To reduce contamination, samples are rinsed with ethanol and the first layers of cells likely to be exposed to predator stomach tissues (therefore, predator DNA) are removed.

These studies demonstrated that DNA barcoding is a very successful technique for identifying obvious prey items. However, there was a limitation in that degraded prey items or those in a liquid form presented a challenge and could not be isolated for identification.

In many cases, predatory species such as wrasses feed either on soft-bodied metazoans or consume small preys that are digested quickly (Randall 1967, Denny and Schiel 2001, Morton et al. 2008). Therefore, digested items cannot be easily separated, identified, stored and sub-sampled. Thus, the gut content available for analysis is semi-digested, homogenised tissue that may contain DNA traces from a large pool of consumed prey and DNA from the predator together.

### **3.2.6.2 Next Generation Sequencing and DNA metabarcoding**

The introduction of next generation sequencing (NGS) and its application in DNA barcoding has enabled the development of DNA metabarcoding. This is a powerful tool that allows the direct characterisation of dozens of samples containing thousands of sequences per PCR product. DNA metabarcoding has been applied to samples from the gut of generalist predators or environmental samples containing heavily degraded DNA (soil, water, faeces, etc.). Metabarcoding has great potential to boost the acquisition of data in biodiversity research (Taberlet et al. 2012) and in the study of food webs (Pompanon et al. 2012), and has been applied in a few dietary studies in marine ecosystems (Leray et al. 2013b, Leray et al. 2015, Harms-Tuohy et al. 2016, Sakaguchi et al. 2017).

In one of the most successful studies to date using DNA metabarcoding, Leray et al. (2013b) designed and used new universal, degenerate PCR primers to amplify a short subsection of the CO1 barcode locus to examine the gut contents of coral reef fish. Novel, degenerate primers were designed (mICO1intF in combination with jgHCO2198) and were more successful in amplifying DNA from degraded gut samples than previous universal primer sets traditionally used for DNA barcoding (Folmer et al. 1994), offering a new powerful tool for metazoan metabarcoding studies. From their study, Leray et al. (2013b) identified 337 prey Operational Taxonomic Units (OTUs) belonging to 14 phyla, from the gut contents of 16 fish from three different species. Of these, 52.5% were identified to species level, as they matched reference barcodes (>98% sequence similarity), and an additional 32% could be classified to a higher taxonomic level. The same authors used this

DNA metabarcoding approach to reveal the diet and functional role of predatory fish feeding on invertebrates in Moorea, French Polynesia (Leray et al. 2015). In total 292 OTUs were identified in the guts of three species of carnivorous fish. Of these, 51% were identified to species level and 26% to higher taxonomical levels.

In a more recent study, Harms-Tuohy et al. (2016) used NGS and Leray et al. (2013b) primers to identify the prey composition of Puerto Rican lionfish gut content. This study identified 39 species from 16 different families from digested liquid-form material many of which may have been undetected with more traditional methods. Sakaguchi et al. (2017) studied the prey richness of juvenile chum salmon using both observational techniques and DNA metabarcoding and proved that DNA-based analyses successfully identified many more taxa than morphological observations.

Nonetheless, a challenge presented when carrying out DNA barcoding or metabarcoding of gut contents is that semi-digested tissue homogenates often contain small amounts of highly degraded DNA along with high quality DNA from the predator whose gut content is being analysed (Jarman et al. 2004). The higher concentration of one DNA template in a sample can potentially interfere with downstream molecular analyses. Total DNA extracted from gut content from the digestive tube usually contains a great amount of DNA from the predator-host as well as DNA from the prey species (O’Rorke et al. 2012).

Several methods are proposed to avoid bias caused by the co-amplification of predator DNA (Leray et al. 2013a). The removal of predator DNA was reviewed in O’Rorke et al. (2012). In a study of two generalist fish species, Leray et al. (2013a) compared two commonly used approaches to inhibit PCR amplification of predator DNA. Their results suggested that blocking primers are preferable to restriction digestion, as the latter recovers lower prey diversity. In order to avoid detecting DNA from the target predator and preferentially amplify DNA from prey species, highly versatile universal PCR primers can be used in conjunction with blocking primers, which specifically block PCR amplification of predator DNA (Vestheim and Jarman 2008, Leray et al. 2013a). Predator-specific blocking primers are modified at the 3’ end with a SPACER C3 CPG (3 hydrocarbons) addition which prevents elongation without affecting annealing properties. Blocking primers compete with the versatile primers in a mix of PCR reactors and restrict the amplification of predator DNA.

In conclusion, DNA metabarcoding combined with the use of blocking primers may help overcome limitations of other techniques to identify dietary items from gut content, reducing the time and cost of sampling, and increasing the accuracy of the results. Where other techniques fail to detect and identify prey items to species or higher taxonomic levels, this technique may improve detection sensitivity and help to clarify questions that remained unanswered. For example, marine ecologists may be able to understand the potential role of labrids as “keystone” species controlling organisms that cause loss of biodiversity (Morton et al. 2008), such as the sea urchin *Centrostephanus rorgersii* (Underwood et al. 1991). Furthermore, because DNA metabarcoding has the potential to assess highly digested consumed prey that could otherwise be unidentifiable, it is becoming a powerful tool for ecologists in food web studies (Bartley et al. 2015). The capacity to quantify the complexity of food webs and to understand energy flow can therefore be considerably enhanced by the use of this cutting-edge technique (Yoccoz 2012). Furthermore, DNA barcoding can potentially increase the rate of data accumulation in studies of consumer diets (Dunn et al. 2010) and food webs (Leray et al. 2013b, Bartley et al. 2015). It has been estimated that the time required to identify prey from gut contents can be halved through the use of DNA techniques (Dunn et al. 2010).

### 3.3 Aims

The main aim of this study was to determine the prey diversity of three generalist, conspicuous species of wrasse in the *Ecklonia radiata*-dominated rocky reef habitat of the north-east coast of New South Wales, Australia. The fish species, *N. gymnogenis*, *P. guentheri* and *O. lineolata* are the most abundant at the study locations Muttonbird Island, Woolgoolga Reef and North Rock, off the Coffs Coast of NSW.

Dietary analyses were undertaken using a cutting edge technique, DNA metabarcoding, in a ‘proof of concept’ pilot study. The aim was to determine if this technique can potentially be useful in the study of food webs in this ecosystem. This tool may be able to provide valuable information on the role that wrasses play

in *Ecklonia radiata*-dominated rocky reefs, through the identification of prey taxa to low taxonomic levels , in order to assess the diet of these species.

For the purpose of this study, species-specific blocking primers will be designed, tested and applied using a metabarcoding approach to assess the diet of three species of wrasse from near-shore, kelp-dominated rocky reefs in subtropical eastern Australia.

## 3.4 Materials and Methods

### 3.4.1 Blocking primer design: sequencing of COI gene in predator species

In order to design species-specific blocking primers for each of the species included in this study, sequence data for the entire CO1 barcode was required. DNA was extracted from fin muscle tissue of the predator fish species *P. guentheri* and *O. lineolata*. A 710 bp region of the CO1 gene was PCR amplified with universal primers (Folmer et al. 1994) and sequenced using Sanger sequencing. Full-length CO1 sequence for *N. gymnogenis* was already available in the iBOL (International Barcode of Life) database. The CO1 sequence data for the three predator-host species included in this study were used to design species-specific blocking primers to be used in PCR amplification of gut content DNA.

### 3.4.2 Sample collection

One individual for each selected species of wrasse (*N. gymnogenis*, *O. lineolata*, *P. guentheri*) was speared by a professional spearfishermen. Fish collection was approved by the Animal Ethics Committee (Southern Cross University) and it was carried out under a scientific collection permit from the Department of Primary Industries of New South Wales (DPI NSW).

Fin muscle samples were excised using small dissection scissors and a scalpel blade on board of the vessel immediately after capture of the fish. About 2 cm<sup>3</sup> of tissue was stored in ethanol 90% in a vial and kept cool on ice during the trip back to the laboratory. It is important to note that dissection instruments were sterilised with ethanol 100% and then burned under a flame before starting the dissection of every individual, in order to avoid cross-contamination.

### 3.4.3 DNA extraction

Total DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Valencia, California, USA) following manufacturer's protocols. The procedure followed is described here briefly: fin tissue from each specimen was cut into small pieces, and approximately 50 mg was placed into a 1.5 ml microcentrifuge tube. Then 180 µl of Buffer ATL was added, followed by 20 µl of proteinase K and mixed thoroughly by vortexing. Samples were incubated at 56°C in a ThermoMixer overnight (approximately 15 h) until the tissue was completely lysed. After incubation, the lysate appeared viscous and samples were vortexed for 15 s. A Master Mix of 200 µl of Buffer AL and 200 µl ethanol (100%) was prepared for each sample, and vortexed thoroughly.

The mix of sample and Buffer AL and ethanol was pipetted (including any precipitate) into a DNeasy Mini spin column placed in a 2 ml collection tube, and then centrifuged at 6000 x g (8000 rpm) for 1 min. The flow-through and the collection tubes were discarded before placing the column in a new 2 ml collection tube and adding 500 µl Buffer AW1 then centrifugation for 1 min at 6000 x g (8000 rpm). The DNeasy Mini spin column was placed into a new 2 ml collection tube before adding 500 µl Buffer AW2, and centrifugation for 3 min at 20,000 x g (14,000 rpm) to dry the membrane.

During the final step of this process, it was important to dry the membrane of the spin column, since residual ethanol may interfere with subsequent reactions. Following centrifugation, the DNeasy Mini spin column was removed carefully to avoid contact between the column and the flow-through. Finally the DNeasy Mini spin column was placed into a clean 2 ml microcentrifuge tube and 200 µl of elution buffer Buffer AE was pipetted directly onto the DNeasy membrane and incubated at room temperature for 1 min, before centrifugation for 1 min at 6000 x g (8000 rpm) to recover purified DNA.

Along all these steps, an extraction negative was also run, which included all the reactors but no tissue.

### 3.4.4 DNA Quantification

To determine the genomic DNA concentration and quality, UV-Vis spectrophotometry and fluorometric quantitation (Qubit, ThermoFisher) and gel agrose electrophoresis methods were used.

#### 3.4.4.1 UV-Vis Spectrophotometry

Spectrophotometry is a simple method for quantifying and evaluating the quality of nucleic acids. Absorbance readings are performed at 260 nm, where DNA has its maximal absorbance, with an extinction coefficient of 50 and this measurement is used to estimate DNA concentration. Using a Nanodrop 2000 instrument the absorbance of DNA extracts at 260 nm was measured and used to estimate DNA concentration. For each extract, 2 µl was pipetted onto the pedestal to estimate concentration. The A260/A280 ratio provided an estimate of the purity of DNA, with values between 1.7-2.0 indicating pure DNA (Ahn et al. 1996).

#### 3.4.4.2 Fluorometric DNA quantification

Fluorometric DNA quantification methods are more sensitive than absorbance methods, especially for samples with low concentration (Demeke and Jenkins 2010). Fluorescent dyes bind specifically to nucleic acids, allowing a more accurate DNA measurement than UV-Vis spectrophotometry. Sample concentration is calculated based on comparison to a standard curve generated from samples of known concentration.

Invitrogen<sup>TM</sup> Qubit<sup>TM</sup> assays and a Qubit Fluorometer were used to estimate concentrations of DNA extracts in this study. The dye used emits fluorescence only when it specifically binds with the target molecule, reducing the effects of possible contaminants on concentration estimates. As the aim was to quantify genomic DNA (quantification range of 2-1,000 ng), a Qubit dsDNA BR (broad range) kit was used.

For each sample, 1 µl of DNA template, 6x loading buffer (ThermoFisher) and water were loaded per well. To determine relative size in base pairs of DNA a molecular weight ladder (Fermentas) was included. To estimate DNA concentration, lambda DNA standards of known quantity (25 and 100 ng) were loaded in separate wells. The gel was place in a gel rig (Biorad) with 0.5x TBE and

subject to electrophoresis for 35 min at 70 W. Results were visualised under UV light with a gel documentation system, Biorad GelDoc XR+.

#### 3.4.4.3 Gel electrophoresis

Gel electrophoresis is a methodology used to visualise the results of genomic DNA extraction and PCR amplification based on the separation of negatively-charged DNA molecules according to size. The migration of DNA molecules through an agarose gel under an electrical current is inversely proportional to their molecular weight.

A 1.5 % gel was prepared using 100 ml of 0.5 TBE and 1.5 g agarose. Agarose was dissolved by heating it a microwave 1 minute at medium-high intensity, then mixed by hand (with a safety glove), followed by heating in the microwave for a further 30 seconds. Prior to pouring the gel 1 µl of gel red (Biotium) was added. When gel red is exposed to UV light it fluoresces with an orange colour that intensifies after creating a bond with DNA enabling visualisation of genomic DNA and PCR amplicons.

The principle and the methodology followed were the same for both electrophoresis of genomic DNA and PCR amplicons, with the exception that 5 µl of PCR amplicons were loaded per well with 6 X loading buffer and water. To estimate the size of PCR amplicons a Fermentas low range ladder was used.

#### 3.4.5 Polymerase Chain Reaction (PCR)

Conditions for PCR amplification of CO1 from fin clip DNA were as described in Folmer et al. (1994). The universal metazoan primers LCO1490 (Forward Primer) 5'-GGTCAACAAATCATAAAGATATTGG-3', and HCO2198 (Reverse Primer) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' were used (Table 19).

Each 25 µl PCR reaction contained 2.5 µl of 10 X Platinum Taq PCR buffer (Thermofisher), 0.75 µl of (50 mM) MgCl<sub>2</sub>, 0.25 µl of (10 µM) forward and reverse primers, 2.5 µl of (1mM) dNTPs, 17.5 µl of sterile distilled water, and 0.25 µl of (5 U/µl) Platinum Taq DNA Polymerase. An extraction negative from the DNA extraction procedure and PCR negative with sterile water in place of DNA template were used to test for contamination.

Thermal cycling conditions were: initial denaturation for 60s at 95°, followed by 35 cycles of denaturation for 10s at 95°, annealing for 10s at 40° and extension for 60 s at 72°.

**Table 19.** Primers used in this study.

Primer label	Sequence (5'-3')
LCO1490 (forward primer) Folmer et al. (1994)	5'-GGTCAACAAATCATAAAGATATTGG-3'
HCO2198 (reverse primer) Folmer et al. (1994)	5'- TAAACTTCAGGGTGACCAAAAAATCA-3'
mlCOIintF (forward primer) Leray et al. (2013)	5'- GGWACWGGWTGAACWGTWTAYCCYCC -3'
jgHCO2198 (reverse primer) Leray et al. (2013)	5'-TAIACYTCIGGRTGICCRAARAAYCA-3'

### 3.4.6 Sanger sequencing

Sanger sequencing in both directions using the forward and reverse primers was used to obtain CO1 sequence data for blocking primer design. PCR amplicons were cycle sequenced using BigDye 3.1 (Applied Biosystems) for 25 cycles of 92°C for 10 s and 60°C for 4 min. Sequences were cleaned by ethanol precipitation and run on a ABI PRISM 3730 genetic analyser (Life Technologies).

During this process of Sanger sequencing, DNA polymerases copy single-stranded DNA templates by adding nucleotides to the extension products (growing DNA chains). Elongation happens at the 3' end of the primer that anneals to the template chain. The deoxynucleotides added to the growing chains are selected by base-pair matching to the template chain. The growth of the new DNA chain occurs by the formation of a phosphodiester bridge between the 3'-hydroxyl group on the primer and the 5'-phosphate group of the added deoxynucleotide (Watson et al. 1987).

### 3.4.7 Blocking primer design

Full-length CO1 sequence data was used to design predator-specific blocking primers for *N. gymnotogenis*, *P. guentheri* and *O. lineolata* using the Primer 3 software in Geneious 9.1.5 (Biomatters), following the methods described previously (Vestheim and Jarman 2008, Leray et al. 2013a).

For primer design, the CO1 sequences for each species were initially aligned to the HCO2198 reverse primer (Folmer et al. 1994). A unique blocking primer was

designed for each of the three species that included a portion of the universal CO1 primer but also extended into species-specific sequence. During primer synthesis, a C3 CPG spacer modification was incorporated on the 3'-end of each primer in order to prevent elongation of the predator species DNA during PCR amplification (Vestheim and Jarman 2008).

When designing blocking primers, stable primer modifications are required to ensure no degradation or enzymatic removal of the modification after synthesis. If even a small percentage of unmodified blocking primers were to anneal this would prime amplification of the predator DNA making the blocking procedure ineffective (Vestheim and Jarman 2008).

To assess effectiveness of blocking primers, PCR amplification of DNA from fin clip and gut content were conducted with and without the addition of blocking primers.

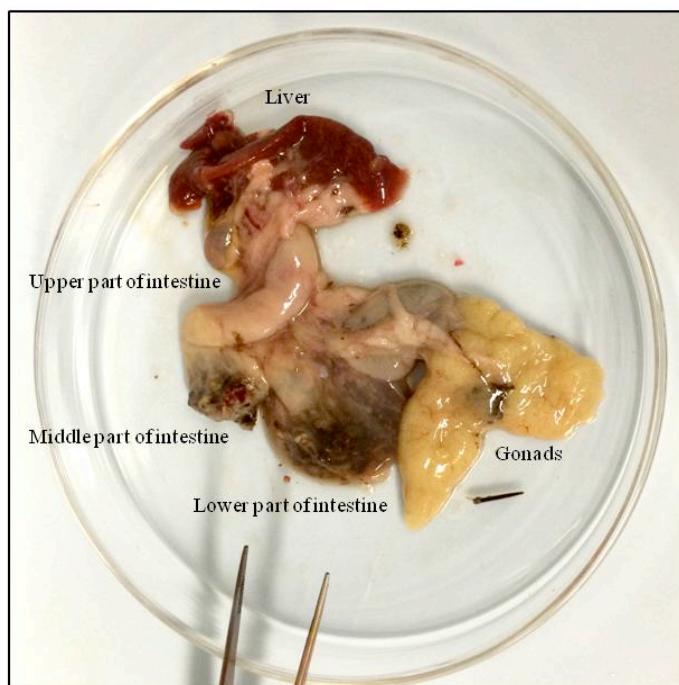
### **3.4.8 Gut content analysis**

#### **3.4.8.1 Collection methods**

In the early stage of this study, a single adult specimen of *N. gymnogenis* was collected in Woolgoolga Reef to test the effectiveness of its species-specific blocking primer in a pilot PCR. Following this, ten adult specimens of each of the three selected species of wrasse (*N. gymnogenis*, *O. lineolata*, *P. guentheri*) were speared by a professional spearfishermen in two different locations (five fish per species in each location), North Rock and Muttonbird Island. The collection was approved by the Animal Ethics Committee (number 14/42) (Southern Cross University) and was carried out under a scientific collection permit (number P14/0040-1.0) from the Department of Primary Industries of New South Wales (DPI NSW). In previous studies of fish communities in *Ecklonia radiata* dominated rocky reefs in the Coffs Coast abundances of these species in these locations have been demonstrated to be high (Table 21). Total fish length (TL, mm) was measured *in situ*, and specimens were placed in separate bags and preserved in a portable fridge with ice before returning to the laboratory.

### 3.4.8.2 Laboratory dissection

Most fish were dissected on the same day of collection, less than 4 hours after capture, in the laboratory at the National Marine Science Centre (Southern Cross University). Each fish was weighed and dissected to obtain the digestive track. Wrasses do not have a morphologically differentiated stomach, but a long intestine (Gillanders 1995) (Figure 8). Dissecting tools were flame sterilised before each dissection. Each intestine was cut open, removed and preserved in 90% ethanol. In the case of a few large individuals where the intestine was too big to fit comfortably in one 50 ml vial only, additional vials were used and labelled as ‘upper intestine’, ‘mid intestine’ and ‘lower intestine’ accordingly.



**Figure 8.** Digestive track organs from one of the dissected individuals of wrasse, a crimson-banded wrasse (*N. gymnogenis*).

A stereoscopic microscope was used in some cases for identification where prey items were potentially identifiable. The larger of these items were preserved separately. Samples were maintained in 90% ethanol at room temperature until extraction.

### 3.4.8.3 Gut content preparation and DNA extraction

With the use of a scalpel, gut content was removed from the walls of the intestines, placed in a 50 ml container and stored in 90% ethanol. Where more than one 50 ml

jar per intestine was required for sample collection, a subsample of gut content was taken from each and combined to make up to 50 mg of tissue in total for each fish, which was cut very finely. The residual ethanol was left to evaporate for one minute, in order to minimise its PCR inhibitory effect (Demeke and Jenkins 2010), and the gut content sample was placed into a 1.5 ml microcentrifuge tube. From this point, the method followed is as described for the DNA extraction of fin muscle above (Section 3.4.3). DNA was quantified using gel electrophoresis, UV-Vis spectrophotometry and fluorometric quantitation as described above (Section 3.4.4).

#### **3.4.8.4 PCR amplification of DNA from fish gut content using degenerate primers and blocking primers.**

A degenerate primer is a mix of sequences of oligonucleotides in which some positions have different possible bases. For example, in the sequence ATCGTT(G/C)AAGT(A/G/C)ATC, the seventh and the twelfth nucleotides can vary between different metazoan species, so degenerate primers are required to ensure that all metazoan species within the gut content are amplified.

In order to test amplification of potentially degraded gut content DNA using the degenerate primers of Leray et al. (2013) mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC) and jgHCO2198 (TAIACYTCIGGRTGICCRAARAAYCA) PCR was conducted without blocking primers on gut content DNA of the three species of wrasse, prior to proceeding with PCR amplification with blocking primers. Blocking primers were included in PCR at 10 times the concentration of the CO1 versatile primers, in order to limit predator co-amplification which could impede prey detection (Vestheim and Jarman 2008).

The optimal amount of genomic DNA required for PCR was tested by amplifying 20 ng and 50 ng of DNA from gut contents in a test with gut content from one individual of each species *N. gymnogenis*, *P. guentheri* and *O. lineolata* (samples from North Rock).

PCR conditions followed those of Leray et al. (2013b). Each 25 µl reaction contained 10 µl of (5 ng/µl) genomic DNA, 2.5 µl of 10 X Platinum Taq PCR buffer (Thermofisher), 0.75 µl of (50 mM) MgCl<sub>2</sub>, 0.25 µl of (10 µM) forward (mlCOIintF) and reverse (jgHCO2198) primers, 2.5 µl of (10 µM) specific

blocking primer , 2.5  $\mu$ l of (1mM) dNTPs, and 0.25  $\mu$ l of (5 U/ $\mu$ l) Platinum Taq DNA Polymerase. Sterile water was added to a final volume of 25  $\mu$ l.

Due to the level of degeneracy in primer sequences, a ‘touchdown’ PCR procedure was used, following the conditions and temperatures established by Leray et al. (2013b). Thermal cycling conditions were: 16 initial cycles of denaturation for 10s at 95°C, annealing for 30s at 62°C (-1°C per cycle) and extension for 60s at 72°C; followed by 25 cycles of denaturation for 10s at 95°C, annealing for 30s at 46°C and extension for 60s at 72°C. PCR amplicons were visualised using gel electrophoresis.

#### **3.4.8.5 Fluorescence quantification**

PCR amplicons from gut content were quantified with an Invitrogen<sup>TM</sup> Qubit<sup>TM</sup> assay and Qubit Fluorometer for dye detection. Methods followed were as described above for genomic DNA quantification, except that a high sensitivity (HS) assay kit, for low concentration samples (10pg/ $\mu$ l -100ng/ $\mu$ l) was used.

#### **3.4.8.6 Sample selection for DNA metabarcoding**

In total, 10 individuals for each species were collected. A subsample of 15 were used for DNA metabarcoding based on PCR amplification success including the presence of an PCR amplicon band or smear of the expected size and post-PCR DNA concentration, ranging from 5.73-39.5  $\mu$ g/ml. The number of samples was determined by the quantity of the reagents needed to perform sequencing. As mentioned, this was a proof of concept type-of-study, if the outcome was to be successful, then we would apply for more funds to carry out sampling and amplification for more samples, and therefore have more replicas per species and locations.

#### **3.4.8.7 Library preparation**

Library preparation of PCR amplicons followed the Nextera XT DNA Library Preparation protocol (Illumina, <http://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/nextera-xt-dna.html>). Firstly, the input post-PCR DNA was labelled with a combination of two indexes, with a unique combination for each sample. A PCR was performed that included 5  $\mu$ l of DNA template, 10  $\mu$ l of TD Buffer, 5  $\mu$ l of ATM Buffer and 5  $\mu$ l of NT Buffer (Nextera

PCR buffers): 3 min at 72°C, 30s at 95°C; then 12 cycles of 10s at 95°C, 30s at 55°C and 30s 72°C; 5 min at 72°C; and a final temperature hold at 10°C. A PCR clean-up was then performed to isolate the DNA from the supernatant, following attachment to magnetic beads. This step was followed by two ethanol washes, and resuspension in a buffer to detach the DNA from the beads. The supernatant containing the DNA was then pipetted into a new plate, carefully avoiding the transfer of beads. Library normalisation was the next step, where samples were resuspended with reagents containing formamide, and therefore this was performed under a fume cabinet. The final step was to pool the libraries, in order to prepare the samples for sequencing. This included the use of heat blocks to denature the DNA, an ice-water bath. Finally, 5 µl of each library were pooled into the same 1.5 ml tube (pooled amplicon library, PAL). prior to loading into an Illumina MiSeq NGS sequencing instrument.

#### 3.4.8.8 Illumina MiSeq sequencing

For next generation sequencing of pooled, indexed libraries, a 600-cycle MiSeq Reagent Kit was used, following procedures recommended by the manufacturer (Illumina). The sequencing instrument used was an Illumina Miseq desktop sequencer.

Pooled amplicon libraries (PAL) were diluted by adding 24 µl PAL to 576 µl of Buffer HT1 to produce a diluted amplicon library (DAL). Using a heatblock, the DAL was incubated at 92°C for 2 minutes, in order to denature the DNA amplicons followed by cooling in an iced-water bath for 5 minutes. Heat denaturation was required before loading the sample into the machine cartridge to ensure efficient template loading on the MiSeq flowcell. The DAL was then loaded into a thawed MiSeq sequencing reagent cartridge and placed into the MiSeq instrument.

Before starting the run, a sample sheet was prepared detailing the run parameters of the 300bp paired-end sequencing run and the specific indices for each sample.

### 3.4.8.9 Bioinformatics Pipeline

#### 1. Denoising and Quality Control of Sequence Reads

##### Trimming and quality control

This pipeline step included trimming for quality control, merging and mapping of sequences against a reference sequence, and was carried out using ‘NGS Core Tools’ in the program CLC Genomics Workbench 9.5.1.

Prior to analysis, paired-end sequencing reads for each sample were separated into individual files within the Illumina MiSeq BaseSpace pipeline based on the unique indices. Paired-end reads were imported into CLC Genomics Workbench for quality control including removal (trimming) of Illumina sequencing adapters, indexes, low quality base calls and repetitive sequence data prior to further analysis.

Initially, adapters and indexes were removed from the sequence reads for each sample, specifying a search on both strands. In addition, reads were trimmed to remove low-quality base calls ( $< Q20$ ,  $P < 0.05$  or 95% base call accuracy) and filtered by length with reads less than 50 bp discarded.

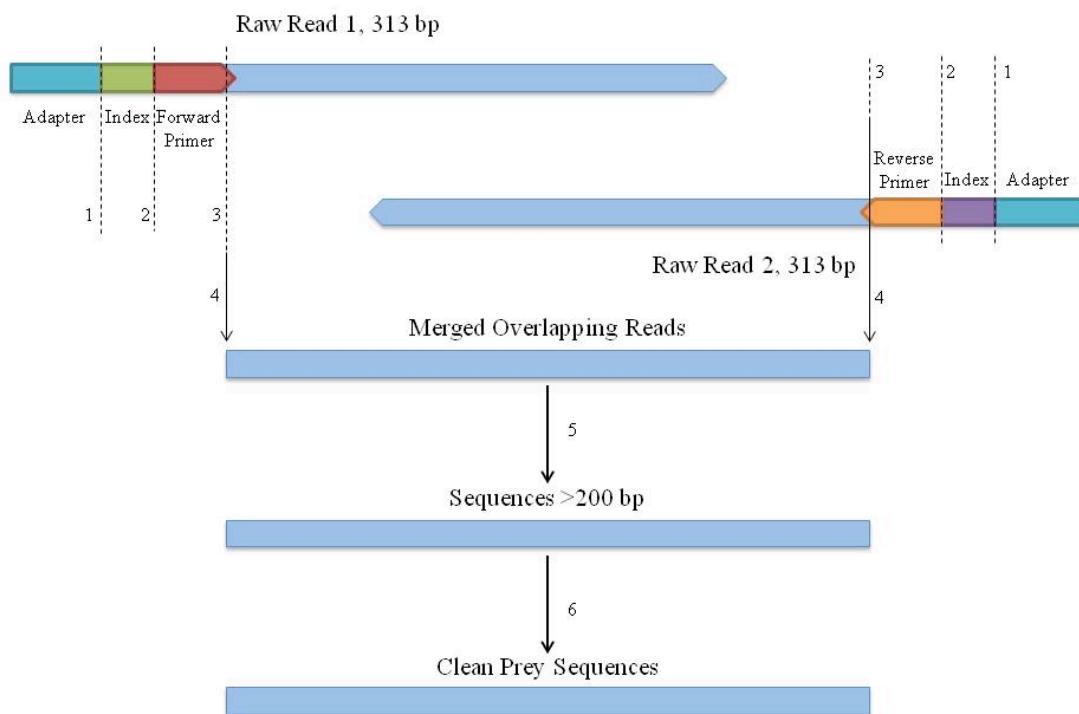
Sequence reads were also filtered to remove repetitive sequence including microsatellite regions (e.g. CTCTCTCTCT) greater than 10 bp in length and homopolymers greater than 8 bp in length (e.g. AAAAAAAA). Finally, the primer sequences used in PCR amplification: mlCOIintF (forward, GGWACGGWTGAACWGTWTAYCCYCC) and jgHCO2198 (reverse, TAIACYTCIGGRTGICCRAARAAYCA) were trimmed from reads.

##### Merging overlapping paired reads

During MiSeq NGS sequencing, paired-end reads were generated for each cluster. Overlapping reads were merged to produce a single sequence using default alignment parameters: mismatch cost, 2; gap cost, 3; max. unaligned end mismatches, 0; minimum score, 8. The target CO1 region amplified in this study was 313 bp in length. Following read merging, sequences were filtered based on length specifying removal of those below 200 bp in length.

## Mapping sequences against reference predator sequence

In order to identify and remove predator-specific COI sequence, sequence reads were mapped against the full length COI sequences for the specific predator fish species from which the gut content was obtained for metabarcoding analysis. Unmapped sequences were retained for further analysis.



**Figure 9.** Quality control of sequence reads through the denoising process.

## **2. BLAST search against BOLD database**

A custom reference nucleotide database was compiled comprising all Australian records in the BOLD (Barcode of Life) database (Ratnasingham and Hebert 2007). The custom database included 204,761 records grouped into 32,503 BINS, or clusters representing operational taxonomic units (OTUs). Following quality control, a file of cleaned sequence reads in ‘fasta’ format was prepared for each sample. A reference database file containing CO1 sequences and specimen data providing taxonomic assignment were downloaded from the Public Data Portal BIN list database (<http://www.boldsystems.org>). The final cleaned reads for each sample were subject to a BLAST (Basic Local Alignment Search Tool) command line search using a nucleotide query (BLASTN). The parameters used for the search were identification of the best hit with a minimum E-value of 1e-10. Following the

BLAST search, the best match ('top hit') was retained as the best estimate of taxonomic identity for each metabarcode sequence analysed.

### **3. Data analysis**

BLAST output files for each sample were imported into Excel (Microsoft) and filtered to remove query (gut content metabarcode) sequences with alignments to the best match sequence (BOLD) sequence that were shorter than 100 bp in length as short sequences were unlikely to provide accurate taxonomic assignment. A 'best' match result was delivered by the search even when there was limited similarity between the query sequence and any sequence in the custom database used as the reference database in the search. Therefore, BLAST match results with percentage identity (percentage of bases identical to the query sequence) lower than 80% were therefore removed (Leray et al. 2013b, Leray et al. 2015, Albaina et al. 2016).

Sequence reads with sequence similarity of greater than 80% were retained and details of the best match to the BOLD reference database were compiled. In order to minimise misidentification at lower taxonomic levels, if the sequence identity was lower than 95%, assignments were reported to the phylum, class, and order levels. Those hits above 95% were considered a successful match and were recorded at the taxonomic level of the match sequence in the BOLD database (Carreon- martinez and Heath 2010, Leray et al. 2013b), in some cases including family and species level (Appendix 3).

Downstream analysis was recorded into a summary table. This included information per sample on the number of sequences after next generation sequencing, after quality control and after BLAST search with >80% and 95% sequence identity, and the number of OTUs identified for each sample.

## **3.5 Results**

### **3.5.1 DNA Extraction and PCR amplification of COI DNA barcode locus from fin muscle**

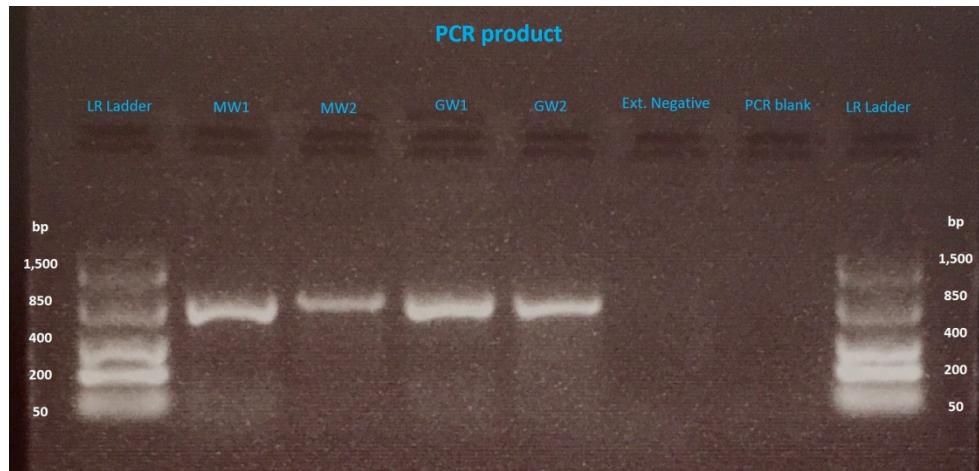
DNA was extracted from replicate fin muscle samples of the two wrasse species included in this study for which a full-length CO1 DNA barcode sequence was not

available in the BOLD database. An average genomic DNA (gDNA) concentration of 40 ng/ $\mu$ l was obtained for both species of fish: *P. guentheri* (GW) and *O. lineolata* (MW). The majority of the DNA recovered from fin muscle was of high concentration and molecular weight although gel electrophoresis provided evidence for degradation in some extracts (Figure 10).

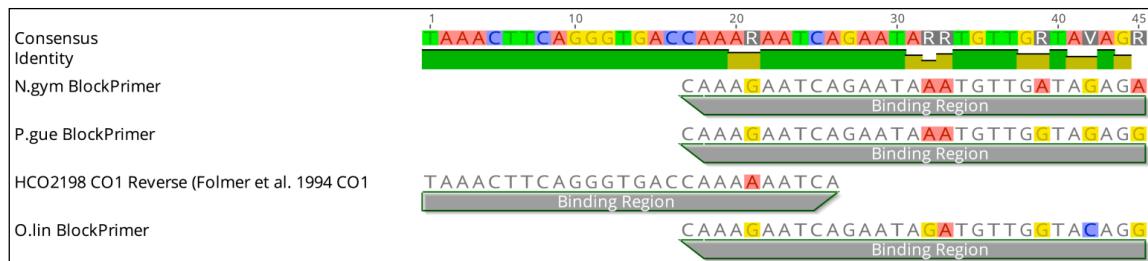


**Figure 10.** Gel electrophoresis results for DNA extracts from fin muscle of *O. lineolata* (Maori Wrasse, MW) and *P. guentheri* (Gunthers Wrasse, GW). There were two replicates per specimen. HR ladder = Fermentas high range molecular weight ladder, bp = base pairs, Ext Neg = extraction negative, lmbda25 = 25ng Lambda DNA, lmbda100 = 10ng Lambda DNA.

Following successful PCR amplification with 20 ng of gDNA and the universal barcode primers of Folmer et al. 1994 (Figure 11), sequence data for a 710 bp region of the CO1 barcode locus was obtained for *O. lineolata* and *P. guentheri* by Sanger sequencing. These sequences and a *N. gymnopterus* CO1 sequence downloaded from the BOLD database were used to design species-specific blocking primers for each species (Table 20). Blocking primers incorporating a C3 CPG spacer modification on the 3' end were synthesised by Sigma-Aldrich. Figure 12 shows the alignment of the designed blocking primers to the Folmer reverse primer (Folmer et al. 1994).



**Figure 11.** Gel electrophoresis results for CO1 PCR amplicons from fin muscle gDNA of *O. lineolata* (Maori Wrasse, MW) and *P. guentheri* (Gunthers Wrasse, GW). Ext. Negative = extraction negative, PCR blank = no template control, LR Ladder = Fermentas low range molecular weight ladder.



**Figure 12.** Alignment of the designed blocking primer with the universal CO1 reverse primer (Folmer et al. 1994).

**Table 20.** Blocking primers designed for three wrasse species.

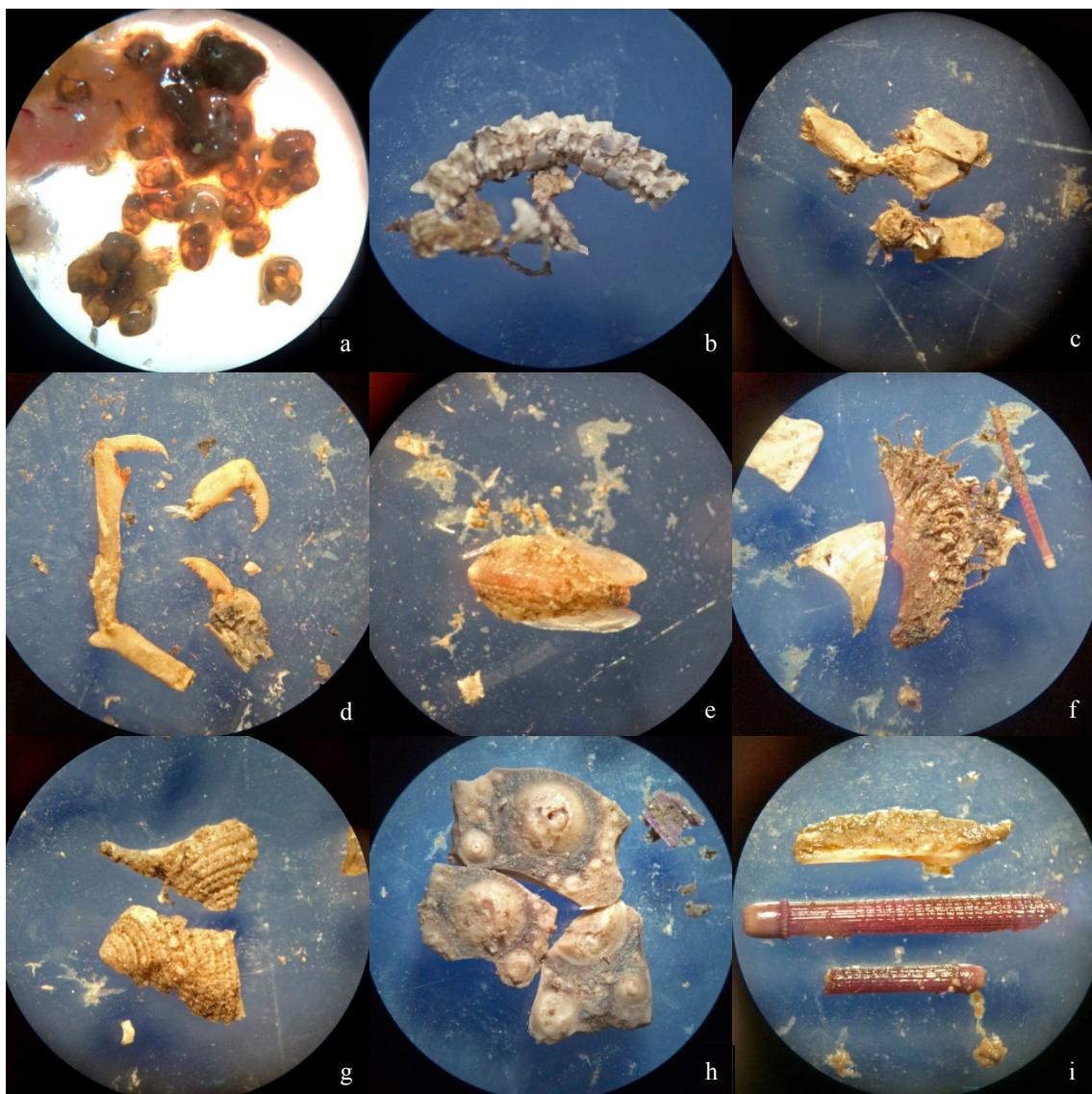
Primer Name	Primer Sequence (5'-3')
N.gym_Block	5'-CAAAGAACATCAGAATAATGTTGATAGAGA(C3 CPG spacer)-3'
P.gue_Block	5'-CAAAGAACATCAGAATAATGTTGGTAGAGG(C3 CPG spacer)-3'
O.lin_Block	5'-CAAAGAACATCAGAATAGATGTTGGTACAGG(C3 CPG spacer)-3'

### 3.5.2 Fish dissection results

When performing dissection on the fish specimens of this study, rubble and sand with no nutritional value were found along with the liquefied digests of prey items. However, in some cases, hard prey fragments were also found in the intestines of some of the largest individuals. Fragments of shells and spines were often mixed through liquefied digests (e.g. Figure 13a, specimen 11, an *O. lineolata* from MBI). Although some of the hard parts were identifiable, it was not possible to assign these to a taxonomical level lower than Class, or Order in some cases. Figure 13

shows some examples of identifiable items in the gut contents of wrasse in this study. These included:

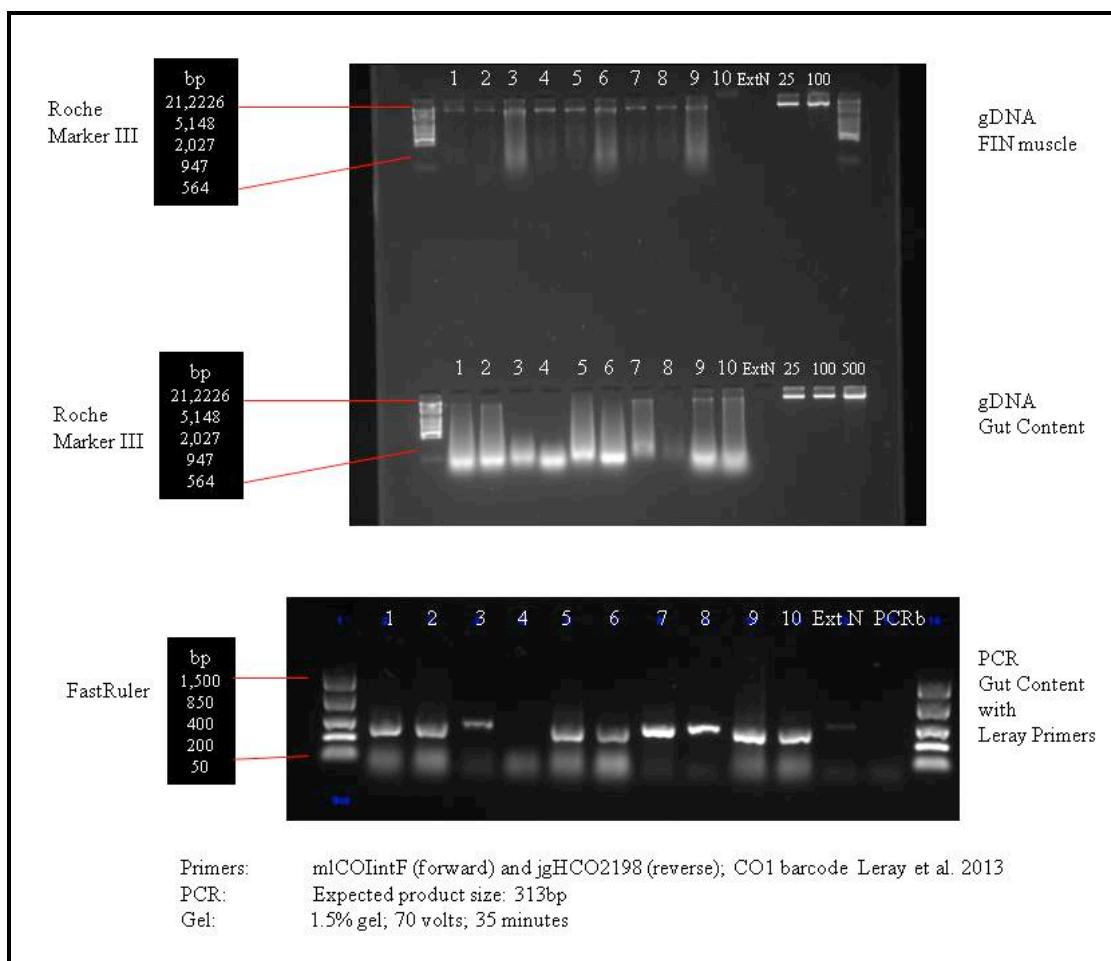
- juvenile archeogastropods in *O. lineolata* specimen 11 from MBI (Fig. 13a)
- fish vertebrae in *O. lineolata* specimen 9 from MBI (Fig. 13b)
- decapod chela (legs) and part of decapod abdomen in *N. gymnogenis* specimen 1 *N. gymnogenis* from NR (Fig. 13c,d)
- a juvenile bivalve in *O. lineolata* specimen 9 from MBI (Fig. 13e)
- parts of bivalve shells in *O. lineolata* specimen 11 from MBI (Fig. 13f)
- shell fragments of an archeogastropod in *N. gymnogenis* specimen 2 *N. gymnogenis* from NR (Fig. 13g)
- echinoid shell items and spines in *O. lineolata* specimen 11 from MBI (Fig. 13h,i)



**Figure 13.** Shell items and spines found in the gut content of some fish specimens in the study. a, f, h and i belong to specimen 11, an *O. lineolata* from MBI. b and e belong to specimen 9, another *O. lineolata* from MBI. c and d belong to specimen 1, a *N. gymnogenis* from NR. g belongs to specimen 2, another *N. gymnogenis* from NR.

### 3.5.3 DNA extraction and PCR amplification of a subsection of the COI barcode locus from gut content gDNA

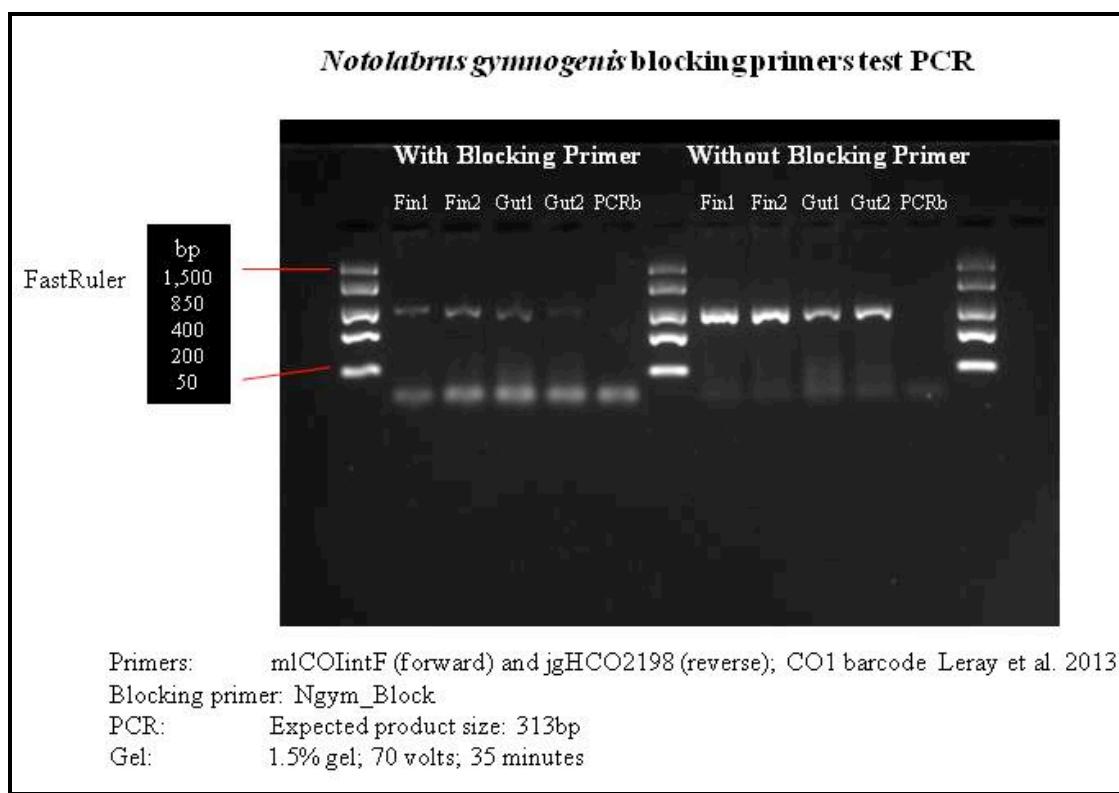
DNA extracts, ranging in concentration from 19.8 to 144 ng/μl, were recovered from gut content samples. For each extract, an initial test PCR amplification was conducted using degenerate universal metazoan barcode primers (Leray et al. 2013b) to amplify a short 313 bp fragment of the COI barcode locus. Figure 14 illustrates the degradation of DNA recovered from the gut contents compared to that from the fin muscle for the three species of wrasse. DNA degradation was expected, as the prey items have been exposed to digestion by gastric juices. However, the test PCR of DNA in gut contents showed amplification of a product of expected size from the majority of samples and confirmed the utility of the primers, PCR and touchdown thermal cycling conditions.



**Figure 14.** Gel electrophoresis results for DNA extraction and PCR amplification of gut content DNA. First row shows a comparison of genomic DNA extracts from fin muscle of three species of wrasse: *N. gymnonogenis* (1,2,3,4), *O. lineolata* (5,6,7) and *P. guentheri* (8,9,10). Second row shows DNA extracts from gut content of the same individuals. Third row shows results of PCR amplicons from gut content DNA with the use of the degenerate barcode primers of Leray et al. (2013). Ext N = Extraction negative, PCRb = no template control. Lambda DNA standards 25ng, 100ng and 500ng.

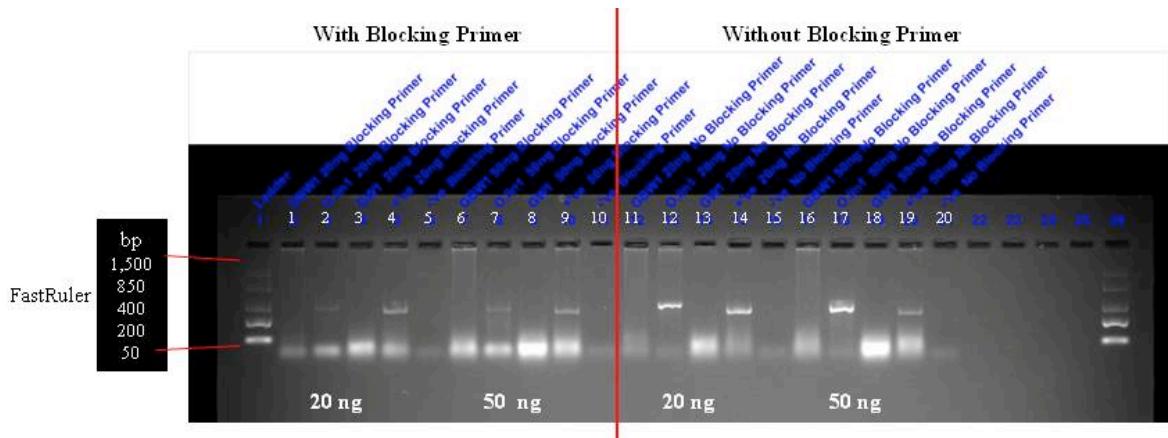
### 3.5.4 DNA amplification of a subsection of the COI barcode locus from gut content gDNA using species-specific blocking primers

In order to test the effectiveness of the species-specific blocking primers in restricting PCR amplification of the predator species, test PCR reactions were conducted with and without blocking primers for two *N. gymnogenis* fin and gut DNA extracts (Figure 15). Blocking primers clearly suppressed PCR amplification of most DNA from both the fin muscle and the gut content samples. These results, however, indicate that there was amplification of predator DNA (*N. gymnogenis*) given the faint bands in the gel image for fin tissue and gut content samples with blocking primers. The PCR amplicons for gut content samples Gut1 and Gut2 are, therefore, likely to include sequences of both prey and the predator species *N. gymnogenis*. For further PCR analyses, the sample *N. gymnogenis* Gut2 (Ngymgut2) was used as a PCR positive control as it produced a clear PCR amplicon in this test.



**Figure 15.** Gel electrophoresis results for test PCR comparisons using degenerate CO1 barcode primers with and without the blocking primer Ngym\_Block (*N. gymnogenis* blocking primer). Samples were from the same specimen (Fin muscle 1, Fin muscle 2, Gut content 1 and Gut Content 2). PCRb = no template control.

Following initial testing, a touchdown PCR was conducted on a wider range of gut content DNA samples from all three species in order to optimise the quantity of genomic DNA required for PCR amplification using blocking primers (Figure 16). Based on these results, 50 ng of input DNA was used for all subsequent PCR reactions (Figure 17) as this quantity produced more concentrated PCR amplicons of the expected size.



Primers: mlCOIntF (forward) and jgHCO2198 (reverse); CO1 barcode Leray et al. 2013

Blocking primers: Ngym\_Block, Pgue\_Block, Olin\_Block

PCR: Expected product size: 313bp

Gel: 1.5% gel; 70 volts; 35 minutes

**Figure 16.** Gel electrophoresis results for PCR amplification of gut content DNA from one individual of each species for reactions with 20 and 50 ng DNA template with and without blocking primers. Samples 1, 6, 11, 16 from *N. gymnonogenis*, samples 2, 7, 12, 17 from *O. lineolata* and samples 3, 8, 13, 18 from *P. guentheri*, all from North Rock. Reactions of samples 1-10 included blocking primers and of samples 11-20 were without blocking primers. Samples 5, 10, 15, 20 are PCR blanks (no template controls).



Primers: mlCOIntF (forward) and jgHCO2198 (reverse); CO1 barcode Leray et al. 2013

Blocking primers: Ngym\_Block, Pgue\_Block, Olin\_Block

PCR: Expected product size: 313bp

Gel: 1.5% gel; 70 volts; 35 minutes

**Figure 17.** Gel electrophoresis results for PCR amplification of gut content DNA from four individuals of each species of wrasse (CBW = *N. gymnonogenis*; GB = *P. guentheri*; Olin = *O. lineolata*) from North Rock, with and without blocking primers. -ve = no template control (PCR blanks).

Following PCR amplification with blocking primers for samples from North Rock and Muttonbird Island, 15 samples were selected for further analysis based on the presence of a clear band, or smear of the expected size, on the gel and post-PCR DNA concentration (Table 21). PCR replicates - i.e. replicate PCR amplifications from the same DNA extractions - were included for two samples: CBW1 and CBW2 had replicates Ngym1 and Ngym2 respectively.

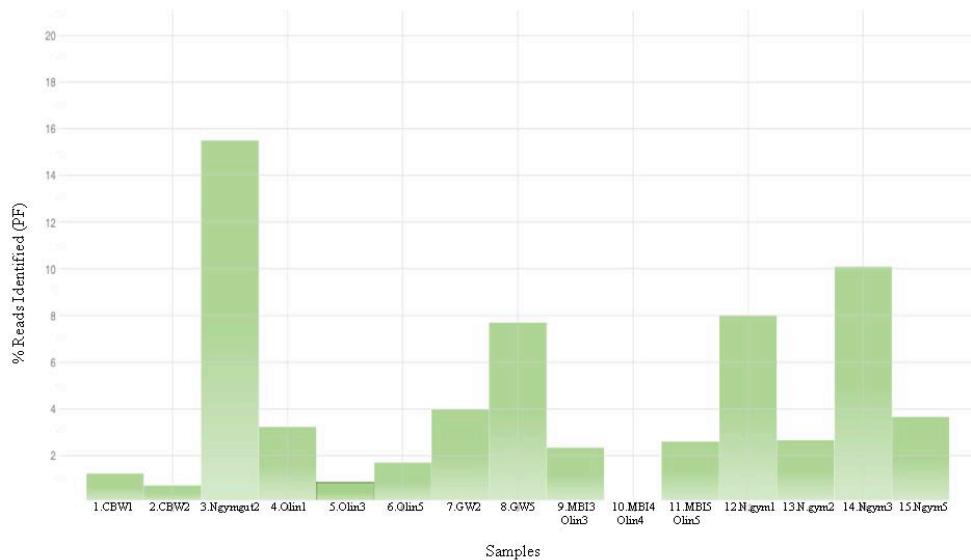
**Table 21.** PCR amplicon concentrations for the 15 samples selected for Next Generation Sequencing including amplicon and NGS sample names and total length (mm) for each individual. PCR replicates of the same DNA extracts are designated as ‘a’ and ‘b’.

PCR amplicon sample name	NGS sample name	Qubit concentration ng/µl	Total Length mm
<i>Notolabrus gymnogenis</i>			
CBW1	NGym1a	35.5	225
Ngym1	Ngym1b	9.8	-
CBW2	Ngym2a	10.6	256
Ngym2	Ngym2b	14.6	-
Ngymgut2	Ngym3	39.1	260
Ngym3	Ngym4	4.62	242
Ngym5	Ngym5	6.12	223
<i>Ophthalmolespis lineolata</i>			
Olin1	Olin1	8.55	165
Olin3	Olin2	8.49	258
Olin5	Olin3	9.57	268
MBI3-Olin3	Olin4	6.75	150
MBI5-Olin5	Olin5	5.73	146
MBI4-Olin4	Olin6	27.3	205
<i>Pseudolabrus guentheri</i>			
GW2	Pgue1	8.88	173
GW5	Pgue2	17.2	155

### 3.5.5 Next Generation Sequencing of DNA from fish gut content

Illumina MiSeq next generation sequencing (NGS) of indexed fish gut samples produced 8.44 million reads (5 Gbp) in total. Of these, 64.23% had identifiable indexes and were assigned to one of the 15 samples included in the pooled sequencing run. The proportion of reads generated per sample was uneven and ranged from 0.01% of indexed reads (2,330 paired sequence reads) for sample

Olin6 to 15.51% (2,533,622 paired sequence reads) for sample Ngymgut2 (Figure 18, Table 22).



**Figure 18.** Percentage of Illumina MiSeq sequence reads identified per sample according to unique indexes.

Following trimming and quality control steps included in the denoising pipeline an average of 11,241 merged sequence reads (30 to 26,508) per sample were used for BLAST analysis to identify and classify dietary items detected in fish gut content (Appendix 3).

To simplify further interpretation of the results, the samples were renamed according to the predator taxa rather than the original sample order and naming system used during extraction, amplification and sequencing (Table 21).

Details of sequences obtained by NGS and results for each step in the denoising (quality control) process are provided in the Appendix 4 and these results are summarised in Table 22. Following the denoising process, between 0.02 and 8.63% sequences per sample were retained for BLAST analysis. Samples with the greatest proportion of retained reads were the *O. lineolata* samples (Olin1-Olin5) with 5.03%, 3.08%, 2.56%, 8.63% and 3.44% respectively. Some samples that produced high numbers of raw sequence reads retained few through the denoising process, including Ngym1b, with 0.28%; Ngym3 with 1.45%, Ngym4 with 1.85% left and Pgue2 with 0.18% retained (Appendix 4, Table 22).

**Table 22.** Summary of NGS, quality control and BLAST search results with >80% and 95% sequence identity, and number of OTUs identified for each sample. See Appendix 4 for denoising steps.

	Raw paired sequence reads	Cleaned sequence reads	% of original sequences remaining	BLAST sequence matches	matches >80% sequence identity	matches >95% sequence identity	OTUs >80% Identity	OTUs >95% Identity
Ngym1a	200,594	34	0.02%	1	1	1	1	1
Ngym1b	1,306,958	3,625	0.28%	197	190	7	13	2
Ngym2a	117,438	382	0.33%	54	54	8	5	4
Ngym2b	434,948	3,234	0.74%	405	404	60	12	4
Ngym3	2,533,622	36,784	1.45%	23,776	23,724	235	37	10
Ngym4	1,648,410	30,565	1.85%	187	155	7	16	2
Ngym5	597,914	1,649	0.28%	824	823	389	8	4
Olin1	526,982	26,508	5.03%	6,002	5,952	1,298	24	3
Olin2	137,164	4,219	3.08%	11	7	-	4	-
Olin3	273,88	7,010	2.56%	354	350	200	14	3
Olin4	381,216	32,898	8.63%	183	95	3	21	2
Olin5	424,066	14,574	3.44%	1,219	1,181	-	27	-
Olin6	2,330	30	1.29%	0	-	-	-	-
Pgue1	650,196	4,844	0.75%	260	251	147	13	2
Pgue2	1,257,700	2,253	0.18%	33	31	-	3	-

### 3.5.6 BLAST search of gut content sequence reads against a custom reference database

The metabarcoding approach used in this study identified at least one taxon (operational taxonomic unit, OTU) in each gut content sample with the exception of Olin6, the sample for which the fewest sequence reads were generated. A maximum of 23,724 and 1,298 sequences per sample matched sequences in the custom reference database at >80% and >95% sequence similarity respectively. This resulted in the identification of between one and 37 OTU prey taxa per gut content sample (Table 3). Complete details of the significant matches against the reference database and results of data analysis are provided in Appendix 3. The samples with the greatest proportion of hits relative to the number of sequences used in the BLAST search were Ngym3 (64.64%), Ngym5 (49.97%) and Olin1 (22.64%). However, for other samples, few matching sequences were recovered compared to the number of cleaned sequences available for the BLAST search. For example,

187 BLAST sequence matches were obtained from 30,565 cleaned sequences for sample Ngym4. Despite this, 16 (>80% similarity) and two (>90% similarity) OTUs were identified in this sample (Table 22).

In total, 70 different OTUs above 80% identity and 16 above 95% identity were identified across all samples. The highest numbers of OTUs were found in samples Ngym3 (37), Olin1 (24), Olin4 (21) and Olin5 (27), while few OTUs were identified in the gut content of samples Ngym1a (1), Ngym2a (5), Ngym5 (8), Olin2 (4) and Pgue2 (3). Although no OTUs were identified using metabarcoding for sample Olin6 during dissection of this fish, hard pieces of a fissurellid gastropod and a piece of a polychaete were identified visually.

PCR replicates (different PCR amplifications from a single DNA extraction) from samples Ngym1 and Ngym2 differed in terms of the number of raw and cleaned reads as well as the number of BLAST hits. For sample Ngym1a, a single BLAST hit above 80% against the reference database was recovered, whereas for Ngym1b there were 190 significant hits. For sample Ngym2a there were 54 hits, while for sample Ngym2b there were 404 hits above 80% identity.

Among species, the highest numbers of OTUs were identified in *O. lineolata* samples with 42 different taxa (ordinal level classification) in total at 80% sequence identity. This compared to 37 OTUs in *N. gymnogenis* and 14 in *P. guentheri*. This difference, however, may be due to differences in sample sizes.

In terms of the specific OTUs identified, decapods were a very common prey item and were found in the specimens of each of the three species included in this study (Table 23, Appendix 3). In terms of numbers of hits, decapods sequences were the most abundant in most samples including Ngym1b, Ngym2a, Ngym2b, Ngym3, Ngym4 and Ngym5; Olin3 and Olin5; Pgue2. Thus, decapods were a common prey item found in the gut content, regardless the species and size of the individuals.

Other common taxa identified at relative high abundances in all three wrasse species in terms of sequence hits were gastropods (Olin1, Olin4; Pgue1; Ngym4, Ngym5), polychaetes (Ngym1b, Ngym3, Ngym4, Ngym5; Pgue1, Pgue2; Olin4), fish (Olin1, Olin3, Olin4; Pgue1, Pgue2) and acorn barnacles (Ngym3, Ngym5) (Table 23, Appendix 3). Some taxa, however, were relatively abundant in only some specimens, for example chitons (Polyplacophora) in Olin3, holothurians in

Olin3, bryozoans in Olin4, Annelida Clitellata in Olin5, Mysid shrimps in Olin5, copepods (Maxillopoda Harpacticoida) in Ngym2b and ophiuroids in Ngym2b (Appendix 3).

These results suggest that *N. gymnogenis* and *O. lineolata* feed on a wider range of invertebrate orders than *P. guentheri*, although this may be biased by the low number of specimens (2) of *P. guentheri*. Interestingly, the results indicate that all three species of wrasse prey on other fish, and *O. lineolata* and *P. guentheri* were found to prey on fish of their own family Labridae (Table 23).

For all samples except Ngym1a and Ngym2a (which had PCR replicas Ngym1b and Ngym2b respectively), there were sequence matches to insects and/or collembolans, however, these were usually present at relatively low abundances (Table 22). The exceptions were Lepidoptera (Olin4, 8 hits, 8.42%) and (Ngym1b, 28 hits, 14.74%), Mantodea in (Ngym5, 15 hits, 1.27%), Diptera (Ngym4, 14 hits, 7.37%), Trichoptera (Ngym1b, 828 hits, 3.49%) and (Ngym3, 7 hits, 3.68%) and Ephemeroptera (Ngym4, 6 hits, 3.87 %) and Ngym5 (24 hits, 2.92%). For one sample, Olin2, which had very few hits, sequences from insects comprised the majority of taxa recovered (Appendix 3).

Algae and fungi were also found in most gut content samples (Table 23). However, in general, most samples presented few algal or fungal sequence matches with the exception of Pgue1 (28 hits, 11.16%) and Olin4 (8 hits, 8.42%) (Appendix 3).

**Table 23.** List of taxa (Operational Taxonomic Unit, OTU) identified in the gut content of the three species of wrasse for BLAST hits above 80% percentage identity.

*N. gymnogenis*      *O. lineolata*      *P. guentheri*

INVERTEBRATA				
Annelida	Clitellata	Haplotaxida	x	x
Annelida	Polychaeta	Eunicida	x	x
Annelida	Polychaeta	Phyllodocida	x	x
Annelida	Polychaeta	Terebellidae	x	x
Decapoda	Malacostraca	Decapoda	x	x
Arthropoda	Brachipoda	Diplostraca	x	
Arthropoda	Malacostraca	Amphipoda	x	x
Arthropoda	Malacostraca	Isopoda	x	x
Arthropoda	Malacostraca	Mysida		x
Arthropoda	Maxillopoda	Calanoida	x	
Arthropoda	Maxillopoda	Harpacticoida	x	

Arthropoda	Maxillopoda	Sessilia	x	x
Bryozoa	Gymnolaemata	Cheilostomatida	x	x
Cnidaria	Hydrozoa	Anthoathecata	x	
Echinodermata	Holothuroidea	Dendrochirotida	x	x
Echinodermata	Ophiuroidea	Ophiurida	x	x
Mollusca	Bivalvia	Mytiloida		x
Mollusca	Gastropoda	Archaeogastropoda	x	x
Mollusca	Gastropoda	Cephalaspidea		x
Mollusca	Gastropoda	Littorinimorpha	x	x
Mollusca	Gastropoda	Neogastropoda	x	
Mollusca	Gastropoda	Pulmonata		x
Mollusca	Gastropoda	Sacoglossa		x
Mollusca	Gastropoda	Sorbeoconcha	x	x
Mollusca	Gastropoda	Vetigastropoda	x	
Mollusca	Polyplacophora			x
Mollusca	Polyplacophora	Chitonida	x	
Nematoda	Secernentea	Spirurida	x	
Nematoda	Secernentea	Tylenchida	x	x
Nemertea			x	
Nemertea	Enopla	Monostilifera	x	
Platyhelminthes	Trematoda	Plagiorchiida		x
Rotifera	Bdelloidea	Adinetida		x

**Other ARTHROPODA**

Arthropoda	Arachnida	Araneae		x
Arthropoda	Collembola	Poduromorpha	x	x
Arthropoda	Collembola	Entomobryomorpha	x	x
Arthropoda	Insecta	Coleoptera	x	x
Arthropoda	Insecta	Diptera	x	x
Arthropoda	Insecta	Ephemeroptera	x	x
Arthropoda	Insecta	Hemiptera		x
Arthropoda	Insecta	Lepidoptera	x	x
Arthropoda	Insecta	Mantodea		x
Arthropoda	Insecta	Orthoptera		x
Arthropoda	Insecta	Psocodea		x
Arthropoda	Insecta	Trichoptera	x	x

**VERTEBRATA**

Chordata	Actinopterygii	Bleniformes	x	x	x
Chordata	Actinopterygii	Gobiiformes		x	
Chordata	Actinopterygii	Labriformes		x	x
Chordata	Actinopterygii	Perciformes	x	x	
Chordata	Actinopterygii	Scombriformes		x	

**FUNGI**

Ascomycota	x	x	x
Basidiomycota		x	
<b>ALGAE</b>			
Heterokontophyta		x	
Rodophyta	x	x	x
Ochrophyta	x	x	x

### 3.6 Discussion

In order to understand how an ecosystem functions, biodiversity studies need to be accompanied with the studies of the diets of its components and its trophic pathways. Understanding trophic interactions may help to explain patterns of habitat use and nutrient flow throughout and between ecosystems (Nagelkerken et al. 2000). Revealing the contribution of different groups of organisms to processes within ecosystems is crucial to predicting how changes in the habitat, for example loss of kelp due to climate change, may affect the communities of invertebrates and vertebrates, and the ecosystem as a whole.

Previous studies have concluded that energy is channeled by fish through aquatic ecosystems, from benthic invertebrates to higher trophic levels (Norderhaug et al. 2005, Depczynski et al. 2007, Kramer et al. 2015). These predators control populations of benthic organisms in lower trophic levels, thus playing a major factor structuring marine communities (Shears and Babcock 2002). The family Labridae is one of the most conspicuous, species-rich and abundant families in tropical and temperate fish assemblages (Morton and Gladstone 2011). Species within the family have been reported to prey on a variety of benthic invertebrates (Morton et al. 2008). Their foraging behaviour, high abundance and diversity make them key species for understanding the role of predators in these ecosystems. Detailed studies of prey preference can also be used to assess the possible effects of predation on prey populations, and the modification of prey assemblages (Shepherd and Clarkson 2001).

The central focus of this study was to reveal prey diversity of three generalist conspicuous species of wrasse in *Ecklonia radiata*-dominated rocky reefs of the Coffs Coast of New South Wales, Australia, a region considered a hot spot for climate and biological change (Hobday and Lough 2011, Wernberg et al. 2011a, Wu et al. 2012, Stocker et al. 2013, Verges et al. 2014). The aims of this pilot study

were to test the feasibility and utility of DNA metabarcoding as a technique to provide information on the diet of these species of fish, to compare results to those of previous studies based on visual techniques, and to obtain further insight on the role wrasses play in *Ecklonia radiata*-dominated rocky reefs.

An understanding of the contribution of these wrasse species to the trophic processes in their ecosystem underpins an understanding of the entire food web. Furthermore, this information may enable predictions on the effects of habitat loss on these species, their prey and ultimately the ecosystem.

With the exception of a few plankton-feeding species (e.g. *Clepticus* sp.) wrasse swim near the benthos and their diet reportedly includes a broad range of benthic invertebrates (Denny and Schiel 2001, Shepherd and Clarkson 2001, Shepherd and Brook 2005, Shepherd 2006, Morton et al. 2008, Cole et al. 2010). Anterior canine teeth protrude to detach molluscs and sessile invertebrates from rocks and pharyngeal teeth are used to crush the hard parts of organisms such as shelled molluscs and crustaceans (Clifton and Motta 1998). When performing dissections during this study, some specimens contained large amounts of hard prey fragments in the intestine and suggests that some prey are swallowed whole. Furthermore, shells, spines, rubble and sand with no apparent nutritional value were found during gut dissections. These were mixed through liquefied digests of prey items that usually could not be identified visually. In fact, most fish gut samples contained no undigested and identifiable prey items.

In an exceptional case, hard pieces of a fissurellid gastropod and a polychaete were identified visually in gut content. In another specimen, sea urchin shell fragments were identified during dissection although a BLAST search did not recover any sequence match to an echinoid in the reference database above 80% identity. Previous studies implementing DNA barcoding as a method to study the diet of generalist fish also found some prey by using visual methods that could not be revealed in the results of the barcoding process (Hargrove et al. 2012, Arroyave and Stiassny 2014, Bartley et al. 2015, Sakaguchi et al. 2017).

The observed differences in abundance of OTUs between samples are unlikely to be due to differences in DNA recovery during extraction as the collection and extraction methods were standardised. However, it is possible that DNA extraction and amplification success may be affected by the extent of prey degradation at the

time of collection (Moran et al. 2015). Gel electrophoresis of DNA extracts indicated varying levels of degradation of gut content extracts in comparison to that from fin muscle samples. Furthermore, the observed variations in the taxa (OTUs) found amongst samples are most likely due to the opportunistic and generalist nature of wrasses (Dipper et al. 1977, Russell 1983, Wainwright 1988, Clifton and Motta 1998, Shepherd and Clarkson 2001, Shepherd 2006, Morton et al. 2008, Gerking 2014).

In future studies, the inclusion of DNA extraction replicates, would test whether DNA extracts from the same gut content samples recover a comparable number and composition of taxa. In this study, there were clear differences within PCR replicate pairs from the same DNA extract. For one *N. gymnogenus* specimen there was a single BLAST hit over 80% to one Decapoda OTU for one PCR replicate, and 190 significant hits to different OTUs (Polychaeta, Decapoda, several Insecta, Isopoda, Ophiuroidea, several Nematoda, Gastropoda, Collembila, Maxillopoda and Fungi) for the other PCR replicate. Similarly, for the second replicated sample, there were 54 compared to 404 sequence matches above 80% identity (Appendix 3). The PCR replicates in each case included the same DNA extract and each reaction followed the same protocol. These results highlight the importance of replication in metabarcoding experiments in order to improve recovery of dietary items and to increase the confidence and reliability of rare sequences, which may help assessing and interpreting dietary variations among individuals and their samples and may provide an effective way to uncover rare food items (De Barba et al. 2014).

Previous studies have reported the limitations of DNA-based techniques to detect some taxa, either because prey DNA was not successfully extracted (Pawlowski 2000), or there were difficulties in amplification (Halanych and Janosik 2006) or during sequencing (Clare et al. 2011). While it is possible that some samples were subject to one of more of these problems, this study demonstrates that a metabarcoding approach can be applied to provide detailed information on the prey diversity of generalist species of fish, such as wrasses. For example, in this study, with only a few samples per each species, up to 42 different orders were recorded in the gut content of *O. lineolata*, 37 in *N. gymnogenis* and 14 in *P. guentheri*. The differences in the numbers of OTUs detected between species may be due to differences in sample size as only two *P. guentheri* individuals in this study were available for metabarcoding. Potentially, with more replicates of the same species,

the numbers of OTUs found would increase. Despite some limitations, this method of dietary analysis is less time consuming than visual methods such as microscopy, a technique which relies on the state of digestion of prey items and requires skilled identifiers (Schooley et al. 2008, Baker et al. 2014, Sakaguchi et al. 2017). This method also reduces the sampling effort, obtaining great amounts of information with only a few samples (Harms-Tuohy et al. 2016).

Crustaceans are widely reported as an important component of the wrasse diet and were the most abundant prey items detected in previous studies (Dipper et al. 1977, Russell 1983, Wainwright 1988, Denny and Schiel 2001, Figueiredo et al. 2005, Muñoz and Cribb 2006, Shepherd 2006, Kramer et al. 2015). *N. gymnogenis* adults reportedly have a preference for decapods while decapods, small gastropods and amphipods were the preferred prey for small individuals of this species (Westneat 2001, Morton et al. 2008). *O. lineolata* (Westneat 2001, Morton et al. 2008) has previously been reported to feed on polychaetes, polyplacophorans, marginellid gastropods, bivalves and echinoids. Similarly, *P. guentheri* reportedly has a preference for small crustaceans, such as amphipods, and small molluscs (Westneat 2001).

Although differences in the relative abundances of prey OTUs between individuals and species were detected in this study, decapods were commonly found in relatively high abundance in the gut contents of all three species. As expected based on previous dietary studies of these species of wrasse, gastropods and polychaetes were also common prey items (Morton et al. 2008). However, some prey items that were expected to be more common had surprisingly low relative abundances. For example, amphipods were found in only 2 *N. gymnogenis* and 3 *O. lineolata* individuals at very low relative abundances, and isopods were detected in one individual of each species, also at low relative abundance. Polyplacophorans are reportedly consumed in high abundances by these two species; however, the relative abundance of these taxa in specimens included in this study was low.

Echinoids, previously reported to be abundant in the gut content of *N. gymnogenis* and *O. lineolata* (Morton et al. 2008), were not detected using DNA metabarcoding. There were, however, sequence matches for other echinoderm classes including holothurians and ophiuroids in gut content samples of both species Only the latter have been previously reported in the gut contents of these species before (Morton et

al. 2008) and other species of wrasse (Dipper et al. 1977, Russell 1983, Shepherd and Clarkson 2001, Shepherd 2006). Wrasse have been previously reported to forage on holothurians (Shepherd and Brook 2005), however these are found in relative low abundances compared to other prey items in the gut content of predatory fish (Francour 1997, Sala and Ballesteros 1997). This may be due to the fact that their spicules are smaller than those in the other echinoderm taxa, and therefore may have been missed during visual analyses of gut content. The reference database used in this study included 71 echinoid sequences. However, it is possible that the echinoid prey taxa of the wrasse species included in this study were not represented. If the sequence similarity to other echinoids in the database was lower than the 80% threshold applied, this would explain the failure to detect echinoid OTUs despite visual confirmation that sea urchin shell and spine fragments were present in the gut content of some samples.

Interestingly, prey taxa that have not been reported previously for these species, were found in the gut contents of the individuals of this study. These included orders of fish, five in *O. lineolata*, two in *N. gymnogenis* and two in *P. guentheri*. In *O. lineolata* and *P. guentheri*, these prey included fish of the same family Labridae. It is possible they may have come from smaller individuals or juveniles, and suggests that these wrasse species occupy a high level in the food web of *Ecklonia radiata* ecosystems.

The presence of some sessile invertebrates such as Bryozoans and Hydrozoans, as well as algae in the samples may be accidental rather than targeted, as these species of wrasse forage in microhabitats that provide refuge for cryptic prey (Morton 2007), such as *Ecklonia radiata* holdfasts and macroalgae bases, which are conspicuous beneath the kelp canopy (Smith 1996, Edgar 2001, Flukes et al. 2014). These wrasse species also forage in microhabitats with sand and rubble (Morton 2007), which would explain the presence of sand items in the guts observed when performing dissection on some individuals.

Although wrasse have been recorded to eat insects (Morton et al. 2008), it is unlikely that the species included in this study rely on an abundance of insect prey. The presence of these arthropods in the gut content of these wrasses may be the result of opportunistic feeding behavior. Samples for this study were collected during the winter months of 2015. At this time of the year, conditions are normally

prime to access the locations of collection on a small research vessel (light off-shore winds, flat seas) and the visibility is optimum for spear-fishing. Westerly off-shore winds in winter bring debris, vegetation and organisms (including insects) from land to the shore (pers. obs). This may also determine the presence of fungi in the samples: spores from land could have been carried by the off-shore winds. However, a more probable explanation is contamination of the samples in the laboratory when carrying out DNA extraction, as fungal spores can be very small and airborne.

Despite the advantages DNA metabarcoding provides for prey identification, this methodology is not without caveats. The initial investment to capture of the specimens, to obtain reagents for all the steps in the methodology (DNA extraction, amplification, results visualisation and NGS), and to purchase the primers and blocking primers, is significantly high. Furthermore, while relative proportion of different prey taxa can be calculated from the resulting DNA metabarcoding data, the quantification of prey composition is limited to the number of sequences (hits) assigned to each OTU found (Deagle et al. 2010, Bowles et al. 2011, Deagle et al. 2013, Harms-Tuohy et al. 2016). The number of sequence matches is not representative of the quantity of DNA within the gut and provides no information on the number of individuals and the frequency of each taxa consumed by the predator. In addition, the DNA of different prey items may degrade in different rates, and prey items may be in different digestion stages at the time of collection (Deagle and Tollit 2007, Schooley et al. 2008, Valentini et al. 2009, Baker et al. 2014, Moran et al. 2015). Therefore, the abundance of sequences assigned to each OTUs may not necessarily reflect the original quantity of prey taxa consumed (Harms-Tuohy et al. 2016, Sakaguchi et al. 2017). Nevertheless, prey species that are poorly represented in the diet may be identified using DNA metabarcoding that would difficult to detect using when other methods (Shepherd and Clarkson 2001, Shepherd and Brook 2005, Morton et al. 2008).

Although the results of this study can be used to describe the diversity of prey in a small sample, they cannot be used to generalise about the diet of *N. gymnogenis*, *O. lineolata*, and *P. guentheri*. Furthermore, it cannot be concluded that there are clear differences within each species depending on the size of the individuals, and it is not possible to determine if there is competition or food partitioning among these species. Due to the small number of samples and the lack of replicates, only

assumptions can be made. Future dietary studies of these species using DNA metabarcoding would benefit from larger sample sizes and the inclusion of additional replicates for each species in each location. For example, Leray et al. (2015) used DNA metabarcoding to examine the gut contents of 25 individuals of three fish species and was able to draw conclusions on food partitioning among these species in a coral reef.

Identification of prey provides crucial information for understanding the ecological role of a predator within an ecosystem. In this study, utilising DNA metabarcoding, substantial information was obtained from few individuals (5 *N. gymnogenis*, 6 *O. lineolata* and 2 *P. guentheri*). To obtain similar prey diversity information for wrasse species, Morton et al. (2008) obtained and visually analysed the gut content of 193 *O. lineolata*, 186 *N. gymnogenis*, and another species (*Pictilabrus laticlavius*, 87 individuals). Following analyses, approximately 75 taxa were identified in total. In this study, very few prey items were identified by morphological observations, while 70 taxa were identified in total using DNA metabarcoding from the same samples.

*Ecklonia radiata* kelp forests support a wide range of algal and invertebrate species (Smith et al. 1996a, Clark et al. 2004, Coleman et al. 2007, Flukes et al. 2014), some of which are small and cryptic. Smith (1996) studied *Ecklonia radiata* holdfast macrofaunal communities, through the time-consuming dissection of holdfasts samples and identification of invertebrate taxa. DNA metabarcoding of gut content has the capacity to reveal many taxa in a single sample, and could, therefore, be used to provide information more broadly about species richness within the ecosystem (Bartley et al. 2015, Leray et al. 2015), provided that the barcodes for these organisms where available on the database.

The results of this study support previous evidence that DNA metabarcoding and the use of next generation sequencing are very powerful, efficient techniques for dietary analysis (Leray et al. 2013b, De Barba et al. 2014, Leray et al. 2015, Albaina et al. 2016, Gebremedhin et al. 2016, Granquist et al. 2016, Harms-Tuohy et al. 2016, Kasapidis et al. 2016, Guilleraud et al. 2017, Kamenova et al. 2017). An important advantage is the ability to analyse highly and partially digested prey items that are unidentifiable using visual techniques, or when little remains are left in the guts. This is particularly evident in studies of generalist predators such as

wrasses, which may have many different prey items belonging to different taxa in their gut contents at one time.

Previous reports of wrasse as generalist, opportunistic feeders, are supported by the findings of this study. High numbers of different OTUs, up to 37 per sample, were detected in the gut contents of only a few individuals. This was only possible by employing the cutting-edge technique of DNA metabarcoding.

### The importance of a BOLD

It is important to note that, unlike previous metabarcoding studies (Leray et al. 2012, Leray et al. 2015), a comprehensive barcode library for the organisms present in *Ecklonia radiata* dominated rocky reefs in the Coffs Coast region was not developed prior to this study. Instead, a reference database was compiled from all Australian records available in the publicly accessible BOLD (Barcode of Life) nucleotide database, [www.boldsystems.org](http://www.boldsystems.org).

As such, taxonomic identification of prey was limited by the incomplete coverage of temperate and subtropical eastern Australia marine species in the BOLD database, especially among invertebrates. Fewer prey OTUs were identified in comparison to previous studies utilising project-specific local genetic databases (Clare et al. 2011, Leray et al. 2012, Leray et al. 2015). Inadequate taxonomic sequence data in reference databases represents a major limitation in DNA-based prey identification (Deagle et al. 2005b, Hargrove et al. 2012, Sakaguchi et al. 2017). It is expected that as the number of curated DNA sequences in reference databases increases so will the success and the accuracy of trophic studies and the accurate characterisation of prey.

In conclusion, the technique of DNA metabarcoding has allowed the identification of a great number prey taxa and to lower taxonomic levels than previous studies, only using the gut contents of a few specimens, therefore it has been proven to be useful in the study of fish diets in *Ecklonia radiata* dominated rocky reefs in this type of ecosystems, providing valuable information on the role *Notolabrus gymnogenys*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* may play in these ecosystems. Further studies should include the gut content analysis by the use of DNA metabarcoding of more individuals from each species, a range of sizes and

possibly temporal variation, and they should include a few PCR replicates for each of the gut contents.

# Chapter 4 – Synthesis

## 4.1 Background

The eastern-coast of Australia has been identified as a hotspot for climate change, with water temperatures predicted to be 1.5-3.0 °C higher by 2070 (Ridgway 2007, Hobday and Lough 2011, Wernberg et al. 2011a, Stocker et al. 2013). Current predictions in relation with climate change within subtropical eastern Australia are for *Ecklonia radiata* kelp forests to become more fragmented (Wernberg et al. 2011b) and eventually disappear from shallow reefs (Connell and Irving 2008, Wernberg et al. 2010, Verges et al. 2014). This may be the result of combined effects of warmer water temperatures (Staehr and Wernberg 2009, Wernberg 2009, Wernberg et al. 2010, Wernberg et al. 2012, Bearham et al. 2013, Mabin et al. 2013), increased frequency of storms and wave action (Wernberg et al. 2010), the arrival and increasing abundance of new herbivorous species of urchins and fish to *Ecklonia radiata* dominated rocky reefs (Verges et al. 2014, Vergés et al. 2016), and local anthropogenic stressors such as pollution (Russell and Connell 2009). All these may have consequences for the distribution of key species within these ecosystems (Ling 2008), which may lead to cascading effects (Johnson et al. 2011a) threatening ecological dynamics and fisheries productivity (Ling et al. 2009b). However, it is still unclear how species interactions and energy flow through these ecosystems will be affected by the loss of habitat. An important challenge will be the assessment of how these changes alter interactions such as predation, grazing and competition.

It is important to understand how these kelp communities and the ecosystems in this region function at the present moment. Understanding the present trophic pathways and inter-specific interactions may provide insight into how populations and communities of invertebrates and fish will be affected by the changes in the habitats, and whether communities may survive future natural and anthropogenic perturbations. Furthermore, such knowledge will make it easier to predict the likely biological impacts of climate change and inform adaptive management, especially with respect to habitat loss. Previous studies in these ecosystems have found that ocean warming may result in trophic alterations (Sanford 1999, O'Connor 2009,

Provost et al. 2017), driving changes in the structure and the function of marine ecosystems (Harley et al. 2012a, Nagelkerken and Connell 2015). In *Ecklonia radiata* kelp forests, these changes are concerning because there may be a reduction of consumption in higher trophic levels. For example, in a recent study, Provost et al. (2017) found that rock lobsters are affected by increasing water temperatures, thus reducing their predation on sea urchins, which may increase their consumption pressure on kelp, eventually thinning the canopies.

As reviewed, food webs are a key feature of ecosystems, defining the interactions between species and the energy flow through the systems. This study had the goal of documenting the fish species that are likely to be dependent on the habitat and the processes provided by *Ecklonia radiata* forests, which may be affected by future contraction of kelp. In this context, I aimed to study the role of three conspicuous carnivorous fish in *Ecklonia radiata* dominated rocky reefs, with the hope of being able to further hypothesise how these species will survive the predicted changes, and what implications kelp loss may have for the whole ecosystem. Fish are key organisms in marine ecosystems: they channel energy through aquatic ecosystems, from benthic invertebrates to higher trophic levels (Norderhaug et al. 2003, Depczynski et al. 2007, Gerking 2014, Kramer et al. 2015), they reportedly can control populations of benthic organisms, and they play a major role in structuring marine communities (Shears and Babcock 2002).

## 4.2 Goals of this study

The first component of this study had the goal of documenting the fish species that are already present in *Ecklonia radiata* ecosystems, and that therefore may be affected by the contraction of kelp forests. The objective was to reveal any significant differences in the structure of fish assemblages amongst three locations on the Coffs Coast, in the Solitary Islands Marine Park, by quantifying spatial variation and, where there were significant differences, to find out whether these differences were consistent over time. For the purpose of the study of fish communities, I also aimed to find out what type of observational technique would be the most suitable and cost-effective in the this type of ecosystem. As a result of this first study, I aimed to provide myself with a list of carnivorous fish species

commonly occurring in the three locations, in order to further carry out dietary studies.

In the second component of my study, I aimed to perform dietary analyses of the chosen conspicuous species of carnivorous fish (3 common species of wrasse) by the use of a cutting-edge technique, DNA metabarcoding. The purpose of this was to reveal dietary preferences of these fish and provide sufficient information on their prey diversity to assess the potential effects of changes in the food web resulting from the projected loss of *Ecklonia radiata* canopies in subtropical eastern-Australia.

## 4.3 The findings

### 4.3.1 Fish communities in *Ecklonia radiata* dominated rocky reefs

The results of the first component showed that wrasses are the most abundant family of fish in the *Ecklonia radiata*-dominated rocky reefs in the three locations of the study (Muttonbird Island, Woolgoolga Reef and North Rock), and that *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* are the most abundant species of this family overall. These results agree with those of similar studies carried out in the region (Malcolm et al. 2010a, Davis et al. 2014b).

Habitat heterogeneity reportedly plays a crucial role in the distribution of rocky reef fish, promoting high abundances (Tuya et al. 2014), due to the increased number of refuges and feeding microhabitats (Anderson and Millar 2004, Morton and Gladstone 2011, José de Anchieta et al. 2013). However, in this study, habitat complexity (rugosity) provided limited explanation for patterns of distribution of reef fish. On the other hand, kelp density, and in particular the density of adult kelp (S3), was strongly correlated with fish community composition between locations and times. These differences in kelp density were the main drivers of the observed differences in fish assemblages between locations and sample times. Furthermore, when carrying out analyses of benthic cover, it was noticed that kelp density in all locations had decreased over the duration of the study. These differences in this short period of time may be associated with storms rather than climate change, but may provide an idea of how fish assemblages might change with a decline in kelp canopies.

As seen in many studies, *Ecklonia radiata* is an engineering and foundation species (Dayton 1975), conspicuous in the near-shore reefs of subtropical and temperate eastern Australia (Wernberg et al. 2010, Coleman 2013, Mabin et al. 2013) and it contributes to the high biodiversity (Smith et al. 1996a, Coleman et al. 2007) and the ecological functioning of most coastal rocky reefs in temperate Australasia (Irving and Connell 2006). Rising temperatures lower the capability of this species to respond to physical perturbations, such as storm events, and pollution (Schiel et al. 2004, Ling 2008, Wernberg et al. 2010). At the same time, kelp growth is impeded by the increasing presence of species that graze on macroalgae and that are expanding their distribution range pole-wards, ‘tropicalising’ temperate and subtropical reefs due to warmer water temperatures (Ling 2008, Vergés et al. 2014, Ling et al. 2015, Vergés et al. 2016, Wernberg et al. 2016, Lenanton et al. 2017). Furthermore, because turfing algae are able to take advantage of the increased availability of carbon in a more acidic environment, they can grow faster than kelp in warmer water and be more competitive for space (Connell et al. 2013, Provost et al. 2017).

All these disturbances may be causing a decline in the extent of the canopies of kelp forests (Steneck and Johnson 2013), which may possibly have strong impacts on kelp-associated species and their trophic ecology (Byrnes et al. 2011, Verges et al. 2014, Vergés et al. 2016, Provost et al. 2017). Wrasses are known to find shelter beneath kelp canopies, which provide refuge and lower the risk of predation (Curley et al. 2003, Graham 2004, Russell et al. 2008). Kelp forests also provide them with protection from wave action, areas for foraging activity, nocturnal retreats and nesting sites (Graham 2004, Russell et al. 2008). It is not clear yet how the loss of habitat will affect the diversity, distribution and trophic ecology of fish communities in an already recognized hot spot for climate change. However, documenting the present species richness and community composition of fish that use these habitats is crucial for a better understanding of the ecosystem. This was achieved in the first component of this study.

In terms of the best methodology to study fish communities in these type of ecosystems, I compared the results obtained by the three different methods. I concluded that, given the complexity of the habitat created by kelp canopies and the differences in the behavior of different fish species, more than one method should be used to study fish assemblages in *Ecklonia radiata* kelp forests. In particular, the

combined use of underwater transects (25-m belt transects), carried out by experienced divers with background in fish identification, and underwater remote cameras (KelpCam) can help overcome the disadvantages of one another, being able to assess both cryptic species and diver-averse species, respectively. I believe that using this two-method approach provides a more comprehensive quantification of the fish community structure in *Ecklonia radiata*-dominated rocky reefs.

#### 4.3.2 Dietary studies of three species of generalist fish (fam. Labridae)

After assessing fish communities in *Ecklonia radiata* dominated rocky reefs, the next step in this study was to try to understand how the loss of kelp canopies may affect the trophic ecology of these fish and, in particular, what effect it will have on the most abundant predatory fish species found in the ecosystem (*Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri*). There are two primary hypotheses about how the disappearance of kelp may affect these fish species: 1) there will be a decrease in the protection from predation due to the loss of canopy, shelter from wave action, areas for foraging activity, nocturnal retreats and nesting sites, and therefore populations will be reduced; 2) there will be a loss of the kelp-associated prey that they forage upon and this will cause a decrease in the populations due to food limitation.

Determining the key dietary resources is a key pre-requisite for any assessment of the second hypothesis. This project provided some preliminary information by examining trophic pathways in *Ecklonia radiata* ecosystems through studying the diet of the most abundant predatory fish species (*Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri*). This component focused on proof of concept, by analyzing fish gut contents using a cutting-edge technique - DNA metabarcoding. The aims were to: reveal prey diversity and dietary preferences for the three species of wrasse and compare these across species to explore possible competition and food partitioning; and, based on the primary prey species, explore the implications of habitat loss for availability of key prey taxa. A secondary aim was to try this new methodology and test its adequacy for studying the diet of small-sized, generalist carnivorous fish.

Only 10 individuals per species (30 in total) were captured, and the DNA contained in their gut-contents was extracted and amplified. Species-specific primers had to be designed and tested for each species. All the steps in these processes worked

well, and a subsample of the best 15 amplified gut contents was sequenced and blast-searched against a public database (Barcode of Life). The results were outstanding: up to 37 Operational Taxonomical Units (OTUs) were found in the gut-contents of one single wrasse and, although some samples did not show many OTUs (samples 1, 3, 4, 5), most samples had a considerable number of them (between 12-37 OTUs). Previous studies of gut content of these species of fish needed hundreds of individuals (Morton et al. 2008) to come up with the similar numbers of OTUs, involving laborious processes of dissection and microscopical identification of undigested prey items, a technique which relies on the state of digestion of prey items (Baker et al. 2014). In those studies, there was the limitation of not being able to identify liquid-form components of the gut contents (Shepherd and Clarkson 2001, Morton et al. 2008), and therefore some OTUs are likely to have been missed. DNA metabarcoding and the power of next generation sequencing have provided me with clear and valuable information on the diet of these three species of fish by using only a few samples of gut contents, which mostly comprised digested, liquid-form mix of prey items.

Nonetheless, we found some prey by using visual methods (undigested items) that were not revealed in the results of the DNA metabarcoding process. This has also been observed in previous studies that implemented barcoding techniques (Hargrove et al. 2012). This may have been due to issues with DNA extraction (Pawlowski 2000), DNA amplification (Halanych and Janosik 2006), or issues in the sequencing step (Clare et al. 2011). These results indicate that there are limitations when using DNA-based techniques. It is not possible to know the amount of data lost during these processes in the present study. There may have been issues with certain organisms, possibly because different prey items were in different digestion stages at the time of collection (Deagle and Tollit 2007, Valentini et al. 2009) or that DNA degradation rates may vary among taxa (e.g. Layton et al. (2014)).

Even though the number of hits per Operational Taxonomical Units (OTUs) may not necessarily reflect the real abundance of prey taxa (Sakaguchi et al. 2017), some prey species that are poorly represented in the diet may be indentified using this method, when other methods would completely miss them (Shepherd and Clarkson 2001, Morton et al. 2008) (e.g. the insect sequences found in the guts of

our specimens, the fish sequences, the sequences of soft-bodied metazoans like sacoglossan heterobranchs or polychaetes).

I conclude that, because of its high resolution, this technique should be considered in further dietary analysis. DNA metabarcoding of gut contents and the possibility of revealing dozens of taxa in one single sample provides a powerful, highly efficient tool, to predator diets with confidence. This technique has demonstrated it can identify prey items to a high level of taxonomical discrimination, including in samples where material is highly digested or when little remains are left in the gut. This will allow researchers to obtain substantial information from a snapshot of the diet of one fish.

In this proof-of-concept study, I have been able to reveal a large amount of information on the prey diversity of the three species of wrasse by the use of DNA metabarcoding. Furthermore, the analysis of their diet has involved relatively low effort, as it has not required hours under the microscope sorting prey items. In general terms, I concluded that the steps of DNA extraction, amplification and sequencing, as well as the design and usage of species-specific blocking primers were successful, as they provided me with a large amount of information for most samples. Nevertheless, some information was lost in one, or more than one, step in the second component of this study. Thus, in future studies, I recommend that samples are replicated during extraction and amplification to potentially reveal more information in the BLAST step. This may also provide some improved understanding of the processes leading to information loss, which would help improve the methodology.

In the meantime, while there is an incomplete understanding of the loss of genetic information, and based on the results obtained in this study, I recommend using both observational methods and DNA metabarcoding in the same dietary studies. These techniques provide information on the same time frame, therefore being compatible methods, whereas other powerful tools to track the flow of nutrients within and between ecosystems, such as stable isotope analyses, do not provide information about the diet in a short time frame at species level (Iverson et al. 2004).

Also, in further studies of the diet of these species of wrasse, gut contents from more individuals should be amplified, including fish of different size ranges, to

assess ontogenetic differences and intra-species competition and food partitioning. Furthermore, there should be more extraction replicates and more PCR replicates for each gut contents, and possibly the factor ‘time’ should be taken into the design, as fish may feed on different prey in different seasons.

After obtaining the results in this study, I also conclude that DNA metabarcoding could be considered a tool to obtain inventories of small-sized, arduous to sample, invertebrates, by using predator gut-content, assuming barcodes for these species exist. So, not only can the method be used as a tool to study the role of predators and the food web structure in an ecosystem, but also as a tool to study biodiversity in the ecosystem at the same time, with the caveat that only dietary items would normally be found.

One clear limitation I found in this study is the lack of comprehensive coverage of biota from temperate and subtropical eastern Australia, especially among marine invertebrates, in the Barcode of Life database. Even though I could identify the genera of some organisms, most hits could not be identified to a greater resolution than ‘order’. DNA metabarcoding has been proven by this study, and other studies (Leray et al. 2013b, Leray et al. 2015, Harms-Tuohy et al. 2016, Sakaguchi et al. 2017), to be a powerful tool in the study of trophic pathways in aquatic ecosystems. Therefore, more resources should be put into barcoding marine organisms in the Great Southern Reef (GSR) (Bennett et al. 2015) in Australia, an entity made up of thousands of reefs dominated by *E. radiata* forests and connected to each other through oceanographic (Coleman et al. 2011, Wernberg et al. 2013) ecological (Irving and Connell 2006, Connell and Irving 2008, Vanderklift and Wernberg 2010) and evolutionary processes (Phillips 2001). This would enable the study of its biodiversity with more accuracy and to use those barcodes in ecological studies like this one. The more barcodes, the more resolution ecologists will obtain in dietary analysis using this technique, supporting former conclusions about trophic pathways.

Despite the limitations of this study (only 15 samples and the lack of barcodes for the region), a large number of taxa was revealed in only a few gut contents. However, at this stage I am unable to use this information to generalise about the dietary preferences of the three species, due to the lack of replicate fish. This also impedes an adequate comparison of the diets of the three species with respect to

potential competition, or food partitioning, amongst the three species. Nonetheless, a snapshot of prey biodiversity of three generalist predators was obtained in this study, revealing up to 70 different taxa in total. Some of the prey items were of particular interest as they included sacoglossan heterobranchs, which are thought to be protected from predation through the storage of secondary metabolites from their algal hosts (Wägele et al. 2008). I also found insects, suggesting some food subsidies from terrestrial sources. It is likely that the latter is dependent on supply, which is highly seasonal and associated with offshore winds in the cooler months. These observations clearly suggest additional work to look at the relative importance of terrestrial subsidies more generally.

Based on the results of this study, it cannot be concluded that there are clear differences in the dietary preferences between the species of wrasse in this study, or within each species depending on the size of the individuals (ontogenetic differences). It cannot be concluded that there is competition for resources or food partitioning among these species. However, other authors that assessed the diet of these wrasses suggested food partitioning, which may be associated with differences in foraging behaviour (Hyndes et al., 2007; Platell et al., 1998).

Two conclusions can be drawn from the dietary component of the study. The first is that the three wrasse species in our study are generalist predators, which agrees with the findings of previous studies (Clifton and Motta, 1988; (Figueiredo et al. 2005, Muñoz and Cribb 2006, Kramer et al. 2015). My results indicate that the species of this study feed on many different taxa, from decapods and molluscs to predatory fish, including fish in their own family (Labridae). These results suggest that wrasse may not only deserve a “key-species” status, as suggested by other authors (Morton et al. 2008, Holmes et al. 2012), as they feed on ecosystem engineering species (e.g. amphipods and urchins) and have a strong effect on the recruitment of other species of fish, but also they may occupy a high trophic level in the food web of *Ecklonia radiata* ecosystems, as they feed on carnivorous fish.

The second conclusion is that DNA metabarcoding is a very powerful technique for studying the diets of generalist predators in this type of ecosystem, especially when guts are small (like in our wrasses) and most prey items are highly digested, or when there are few remains in the gut, mixed through with high levels of DNA from the predator.

## 4.4 Discussion

### 4.4.1 How kelp loss may affect these species of wrasse: food limitation

The hypotheses that the second component of this study was set out to test was ‘these three conspicuous species of wrasse are going to be food limited with the disappearance of kelp, thus affecting and reducing their populations’. It has been reported that invertebrate taxa recorded amongst kelp holdfasts are normally found in the surrounding habitat, beneath other macroalgae, rather than being associated only with kelp holdfast habitats (Smith et al. 1996a, Christie et al. 2003). From the results in this study, it would appear unlikely that *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* would be food limited by the loss of kelp canopies, given that the prey found in their guts in this study include taxa that are also common in habitats nearby (e.g intertidal habitat - Smith et al. (1996b), soft sediments Smith and Rule (2001)).

The three-dimensional structure provided by kelp canopies acts as a physical barrier that reduces water flow (Eckman et al. 1989), efficiently trapping and retaining sediment particles and organic material, which will be consequently deposited under the canopy, on the benthos (Moore 1972, Smith 2000, Connell 2003a, Arroyo et al. 2004). Therefore, kelp holdfast communities are often dominated by filter feeders and deposit feeders (Smith et al. 1996a, Schaal et al. 2012), even though grazers, scavengers and predators are also present (McKenzie and Moore 1981, Smith et al. 1996a). Food supply for these filter and deposit feeders comes mainly from detrital sources (kelp, macroalgae, deposited fitoplankton) that is rarely limiting (Schaal et al. 2012), as the food web in these ecosystems is mainly detrital-based.

Furthermore, kelp canopies promote conditions for the development of understorey macroalgal assemblages, mainly dominated by red algae (Clark et al. 2004, Flukes et al. 2014), which were also a predominant feature under the kelp in this study. Kelp provides a buffer for benthic macroalgal species from high wave energy (Almanza and Buschmann 2013), regulates the shading of the habitat (Kennelly 1987b, Arkema et al. 2009), and alters the water flow (Eckman et al. 1989). The understorey assemblages of macroalgae increases the heterogeneity of the habitat and creates suitable environmental conditions (Teagle et al. 2017) for both sessile and motile invertebrate fauna (Flukes et al. 2014, Lavender et al. 2017). The

composition of this fauna and the rate in which recruits rely on the dispersal stages in the water column above the understorey and the kelp canopy (Marzinelli 2012), but also depend on turbidity and sedimentation rates. For example, suspension feeders are susceptible to smothering by particles (Moore 1973), and kelp canopies play a major role in the dispersion of these particles. Therefore, kelp may be crucial for the maintenance of the assemblages of macroalgae and invertebrates living not only in kelp holdfasts but also in the surrounding areas, which include organisms the wrasse species in this study prey on.

With *Ecklonia radiata* forests becoming more fragmented and eventually disappearing from shallow waters due to the increasing water temperatures, less detritus may accumulate in the area where this study took place. Furthermore, the development of other macroalgae may also be negatively affected by the disappearance of kelp, and the assemblages of invertebrates that are benefited by these macroalgae and the conditions and services they provide may be severely reduced. Furthermore, changes in these habitats may be accelerated and may have stronger effects on invertebrates in depths where grazers (urchins) are more abundant and more likely to overgraze macroalgae, and in areas that are closer to pollution sources (Goodsell and Connell 2002, Coleman et al. 2007).

Furthermore, it has been reported that diversity and abundance of invertebrates in holdfast of *Ecklonia radiata* is greater in shallow waters than in deeper waters (Coleman et al. 2007), and that up to 60% of the taxa is unique to the holdfast of *Ecklonia radiata* in shallower reefs. Therefore, in the context of future kelp loss (Steneck et al. 2002, Marzinelli et al. 2015b, Provost et al. 2017), where kelp distribution is being pushed pole-wards and into deeper waters (limited by the availability of nutrients, light and hard substrata) (Marzinelli et al. 2015b), it is suggested that these habitats will be less productive and less biodiverse, due to the combined effects of climate change, pollution and overfishing (Connell et al. 2008, Ling et al. 2009b, Johnson et al. 2011b). Comparisons of depth-related patterns in previous studies in the holdfast invertebrate assemblages in *Ecklonia radiata* have showed inconsistencies (Edgar 1983, Smith et al. 1996a, Smith 1996, Coleman et al. 2007). The differences in the results could be due to biogeographical variation or to the fact these studies sampled different depths. Rule and Smith (2007) studied the differences of epifaunal assemblages across a depth gradient between two island sites within the SIMP, where *E. radiata* is absent, using artificial substratum units

anchored to rocky reef. The authors found that although species richness increased with depth, overall diversity of invertebrates decreased slightly with depth.

Based on these observations, further research should be carried out in this region comparing the distribution and abundance of invertebrates in kelp forests and in nearby habitats (e.g. subtropical coral reefs, soft sediments, intertidal habitats), with depth as a factor. This would provide valuable information on the present assemblages of invertebrates (and potential prey for generalist carnivorous fish) in *Ecklonia radiata* forests and nearby habitats, and would help compare the invertebrate community composition in kelp forests in deeper waters as opposed to shallower waters. This information would be crucial to predict changes in the diversity of invertebrates in threatened kelp forest in this area, and it would help researchers to better understand how benthic trophic pathways may change when kelp forests become fragmented and when they are confined to deeper waters as kelp disappears from shallower rocky reefs.

#### **4.4.2 How kelp loss may affect these species of wrasse: habitat limitation**

It has been reported in previous studies that the species of wrasse in our study are habitat generalist as adults (Curley et al. 2003, Malcolm et al. 2007, Kingsford and Carlson 2010, Morton and Gladstone 2011, Harvey et al. 2013, Davis et al. 2014b). They have large fins that enhance their swimming abilities (Morton 2007) and allow them to roam over large areas and into different habitats. As adults, *Notolabrus gymnogenys* and *Ophthalmolepis lineolata* have been found in kelp forests, but also in urchin barrens (Curley et al. 2003), sponge gardens and subtropical coral reefs (Malcolm et al. 2010a, Morton and Gladstone 2011, van Lier et al. 2017). However, as juveniles, both species reportedly prefer algal habitats and the shelter canopies provide (Curley et al. 2003), which reduce the risk of predation, protect them from wave action, and provide them with areas for foraging activity, nocturnal retreats and nesting sites (Graham 2004, Russell et al. 2008). Benthic invertebrate assemblages seem to partly influence the presence of wrasses within each habitat (Morton et al. 2008). For example, the diet of juvenile wrasses may be restricted to amphipods and small molluscs (Morton et al. 2008), which are abundant in shallow algal habitats (Smith et al. 1996, Edgar 2001). This preference seems to be due to the fact they have a relatively small mouth and limited crushing strength of the pharyngeal jaws (Clifton and Motta 1998). The association between

recruits and juveniles of this family and algal habitats in eastern Australia (Curley et al. 2003, Morton et al. 2008) is likely to be related to the availability of prey in these habitats (Morton and Gladstone 2011). Past studies in the region where this study took place revealed that both *Notolabrus gymnogenis* and *Ophthalmolepis lineolata* are more abundant in the inshore, kelp-dominated areas of the Solitary Islands Marine Park off the Coffs Coast rather than in the offshore, coral-dominated reefs (Malcolm et al. 2010a, Davis et al. 2014a).

*Pseudolabrus guentheri* is abundant amongst the kelp *Ecklonia radiata*, where it uses the shelter provided by the canopy (Morton 2007, van Lier et al. 2017). Its distribution reportedly correlates with the abundance of prey in algal habitats such as *Ecklonia radiata* forests (Morton and Gladstone 2014), but it can also be found in tropical and subtropical coral reefs that lack this macroalgae (Westneat 2001, Malcolm et al. 2007, Davis et al. 2014a, Fry and Davis 2015, van Lier et al. 2017) and in other microhabitats, such as sponge gardens, and amongst soft corals, but with less abundance (van Lier et al. 2017). The diet of this species has not been studied as extensively as that of the other two species of wrasse in this study. Indeed, the present study is the first study of prey diversity in the guts of this species. There are no comparisons between inshore and offshore reefs for *Pseudolabrus guentheri* in the region of this study, but this species has been reported in both kelp dominated inshore reefs and coral-dominated offshore reefs in the area of the Solitary Islands Marine Park.

I have not managed to answer how climate change and the loss of kelp is going to affect these generalist predatory species of fish. Fish caught in kelp forests in this study may feed in other habitats too, and they may not be fully dependent on organisms that occur only in kelp forests. There is a potential for the development of new surveys and designs that focus on the study of predation rates inside and outside kelp forests. Further studies should target the same species in other habitats in the same region, and should study fish movement and feeding amongst inshore *Ecklonia radiata*-dominated rocky reefs and other habitats, such as coral-dominated patches or urchin barrens, abundant in the offshore areas of the Coffs Coast (Harriott et al. 1994, Malcolm et al. 2010b, Byrne and Andrew 2013, Vergés et al. 2016).

One way of studying fish movement could be comparing stomach contents of these wrasse species with their isotopic signatures ( $d^{13}C$  and  $d^{15}N$ ) in different habitats in the region (Davis et al. 2015). To explore fish movement, firstly a geographic isotope seascape (isoscape) of the area should be developed to find out the gradient of  $d^{13}C$  and  $d^{15}N$  values for invertebrates and fishes (Connolly et al. 2009). Potentially, based on previous studies, fish that spent more time feeding in coastal habitats would have lower values of  $d^{13}C$  and higher values of  $d^{15}N$ , and fish that feed in coral areas near the open ocean would have higher values of  $d^{13}C$  and lower values of  $d^{15}N$  (Wyatt et al. 2012, Davis et al. 2014a). However, fish in inshore habitats feed on a wide range of prey and variation within population is a possibility, and some individuals within the same species and population may use larger areas than others, which would be reflected in the  $d^{13}C$  values of the wrasses (Hammerschlag-Peyer and Layman 2010). A large range in carbon stable isotopes could indicate variation in prey preferences amongst the species and would make it difficult to reveal consistent long-term diet and residency (Davis et al. 2015), confounding the ability to detect whether individuals move between inshore and offshore reefs to feed. Nonetheless, it is understood that these wrasse species are opportunistic, generalist predators as well as habitat generalists (Curley et al. 2003, Malcolm et al. 2007, Kingsford and Carlson 2010, Morton and Gladstone 2011, Harvey et al. 2013, Davis et al. 2014b) and that they have fins that enhance their swimming abilities (Morton 2007), thus allowing them to swim long distances. Therefore differences in isotopic values amongst individuals of the same species could be reflecting these generalist behaviour.

Perhaps kelp forest loss will affect other factors rather than limiting these wrasses food-wise. It is also possible that these species may rely on unique chemical cues from *E. radiata* for the settlement of their planktonic larvae (Kingsford et al. 2002). Or maybe the limiting factor is the shelter that kelp canopies provide, which allows them to persist because they are avoiding predation. Juveniles of these species reportedly prefer habitats rich in macroalgae (Curley et al. 2003, Morton 2007, Morton et al. 2008, Russell et al. 2008, Morton and Gladstone 2011). So maybe the disappearance of kelp would affect these species in the settlement stage or by reducing the protection kelp canopies provide to them at the early stages of their life. Or it could be both. The results in the first component of my study showed that density of Stage 3 of kelp (adults) explained some variation (6%) in fish community

composition in all locations and times of this study. Other studies have found a correlation between canopy height and fish density and fish species richness, where higher canopies (i.e. adult kelp) are associated with more complex community structure (van Lier et al. 2017). Therefore, disappearance of kelp and, in particular, reduction in density of Stage 3 plants, may not only lead to a decline in the abundance of juveniles of the 3 species of wrasse in this study, but also changes in the whole fish community.

## 4.5 Conclusions

- a) Labridae is the most abundant family of fish in *Ecklonia radiata* dominated rocky reefs in the three locations of my study (Muttonbird Island, Woolgoolga Reef and North Rock), and *Notolabrus gymnogenis*, *Ophthalmolepis lineolata* and *Pseudolabrus guentheri* are the most abundant species of this family in these ecosystems in this area.
- b) Adult kelp S3 (Stage 3) is correlated with fish community composition. Of all the environmental variables analysed, differences in kelp density are the primary drivers of the observed differences in fish assemblages in this study. Disappearance of kelp and, in particular, reduction in density of S3 kelp, may therefore lead to a decline in the abundance of juveniles of the three species of wrasse in this study, and it may change the whole fish community composition to a less diverse one.
- c) Given the complexity of the habitat and the differences in behaviour of different fish species, more than one method should be used to study fish assemblages in *Ecklonia radiata* kelp forests. I recommend the combined use of transects using underwater visual census and remote cameras to assess both cryptic species and diver-averse species, respectively, for a more comprehensive quantification of the fish community structure in these ecosystems.
- d) The three wrasse species in this study are generalist predators and may occupy a high trophic level in the food web of *Ecklonia radiata* ecosystems, given they feed on carnivorous fish, even of their same family Labridae.
- e) DNA metabarcoding is a very powerful technique for studying the diets of generalist predators in kelp forests, especially when guts are small and most prey

items are highly digested, or when few remains are present in the gut. Therefore, more resources should be put into barcoding marine organisms in these ecosystems.

- f) DNA metabarcoding could be used as a tool to obtain inventories of small-sized, difficult-to-sample invertebrates, by using gut contents from predators, and assuming barcodes for these species already exist.
- g) Further dietary studies of generalist predatory species of fish should include gut contents from more individuals, of different size ranges, to assess ontogenetic differences and inter-species competition and food partitioning. Factor ‘time’ should be included in the design, as these three species may feed on different prey in different seasons.
- h) Further dietary analyses of generalist predatory species of fish should include more extraction replicates and more PCR replicates from each gut content sample.
- i) Further research should be carried out on the distribution and abundance of invertebrates (and potential prey for the generalist carnivorous fish in this study) in these habitats and in surrounding areas, with depth as a factor. The information obtained from these studies could be crucial to predict changes in the diversity of invertebrates in threatened kelp forest in this area and it would give researchers have a better understanding of how trophic pathways may change when kelp forests become fragmented and/or confined to deeper waters.
- j) It is not possible at this stage to predict how climate change and the loss of kelp are going to affect the diet of these generalist predatory species of fish. Fish caught in kelp forests in this study may also feed in other habitats, and they may not be fully dependent on organisms that only occur in kelp forests. Further studies should target the same species in other habitats in the region. These other habitats should include offshore coral-dominated reefs and urchin barrens, as well as estuaries.
- k) Having a better understanding on the residency in kelp forests of these three species of fish will help researchers to predict how their diet may be affected by the loss of *Ecklonia radiata* canopies. The assessment of fish movement between habitats could be carried out by including stable isotope analyses of these wrasse species amongst different habitats in the region. These would help determine if they have consistent long-term diet or inconsistent diet, depending on how much they move to feed.

## Reference list

- Abdullah, M. I., and S. Fredriksen. 2004. Production, respiration and exudation of dissolved organic matter by the kelp *Laminaria hyperborea* along the west coast of Norway. Journal of the Marine Biological Association of the UK **84**:887-894.
- Aguilar, C., G. Gonzalez-Sanson, I. Faloh, and R. A. Curry. 2008. Spatial variation in stable isotopes (delta C-13 and delta N-15) in marine fish along the coast of Havana City: Evidence of human impacts from Harbor and river waters. Journal of Coastal Research **24**:1281-1288.
- Aguilar, R., M. B. Ogburn, A. C. Driskell, L. A. Weigt, M. C. Groves, and A. H. Hines. 2016. Gutsy genetics: identification of digested piscine prey items in the stomach contents of sympatric native and introduced warmwater catfishes via DNA barcoding. Environmental Biology of Fishes:1-12.
- Ahn, S. J., J. Costa, and J. R. Emanuel. 1996. PicoGreen quantitation of DNA: effective evaluation of samples pre-or post-PCR. Nucleic acids research **24**:2623-2625.
- Albaina, A., M. Aguirre, D. Abad, M. Santos, and A. Estonba. 2016. 18S rRNA V9 metabarcoding for diet characterization: a critical evaluation with two sympatric zooplanktivorous fish species. Ecology and evolution.
- Almanza, V., and A. H. Buschmann. 2013. The ecological importance of *Macrocystis pyrifera* (Phaeophyta) forests towards a sustainable management and exploitation of Chilean coastal benthic co-management areas. International Journal of Environment and Sustainable Development **12**:341-360.
- Alonso, H., J. P. Granadeiro, S. Waap, J. Xavier, W. O. C. Symondson, J. A. Ramos, and P. Catry. 2014. An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. Mol Ecol **23**:3719-3733.
- Anderson, M. J. 2005. Permutational multivariate analysis of variance. Department of Statistics, University of Auckland, Auckland **26**:32-46.
- Anderson, M. J., C. E. Diebel, W. M. Blom, and T. J. Landers. 2005. Consistency and variation in kelp holdfast assemblages: Spatial patterns of biodiversity for the major phyla at different taxonomic resolutions. Journal of Experimental Marine Biology and Ecology **320**:35-56.
- Anderson, M. J., and R. B. Millar. 2004. Spatial variation and effects of habitat on temperate reef fish assemblages in northeastern New Zealand. Journal of Experimental Marine Biology and Ecology **305**:191-221.
- Anderson, R., M. Rothman, A. Share, and H. Drummond. 2006. Harvesting of the kelp *Ecklonia maxima* in South Africa affects its three obligate, red algal epiphytes. Journal of Applied Phycology **18**:343-349.
- Andrew, N. 1999. Under Southern Seas: the ecology of Australia's rocky reefs. UNSW Press.
- Andrew, N., and A. Underwood. 1993. Density-dependent foraging in the sea urchin *Centrostephanus rodgersii* on shallow subtidal reefs in New South Wales, Australia. Marine Ecology Progress Series:89-98.
- Arkema, K. K., D. C. Reed, and S. C. Schroeter. 2009. Direct and indirect effects of giant kelp determine benthic community structure and dynamics. Ecology **90**:3126-3137.
- Arnold, M., H. Teagle, M. P. Brown, and D. A. Smale. 2016. The structure of biogenic habitat and epibiotic assemblages associated with the global invasive kelp *Undaria pinnatifida* in comparison to native macroalgae. Biological invasions **18**:661-676.
- Arroyave, J., and M. L. J. Stiassny. 2014. DNA barcoding reveals novel insights into pterygophagy and prey selection in distichodontid fishes (Characiformes: Distichodontidae). Ecology and evolution **4**:4534-4542.

- Arroyo, N., M. Maldonado, R. Pérez-Portela, and J. Benito. 2004. Distribution patterns of meiofauna associated with a sublittoral *Laminaria* bed in the Cantabrian Sea (north-eastern Atlantic). *Marine Biology* **144**:231-242.
- Assis, J., B. Claro, A. Ramos, J. Boavida, and E. A. Serrão. 2013. Performing fish counts with a wide-angle camera, a promising approach reducing divers' limitations. *Journal of Experimental Marine Biology and Ecology* **445**:93-98.
- Babcock, R. C., S. Kelly, N. T. Shears, J. W. Walker, and T. J. Willis. 1999. Changes in community structure in temperate marine reserves. *Marine Ecology Progress Series* **125**:134.
- Baird, A., B. Sommer, and J. Madin. 2012. Pole-ward range expansion of *Acropora* spp. along the east coast of Australia. *Coral Reefs* **31**.
- Baker, R., A. Buckland, and M. Sheaves. 2014. Fish gut content analysis: robust measures of diet composition. *Fish and Fisheries* **15**:170-177.
- Barnett, A., K. S. Redd, S. D. Frusher, J. D. Stevens, and J. M. Semmens. 2010. Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. *Journal of Experimental Marine Biology and Ecology* **393**:188-192.
- Barrón, C., N. Marbà, C. M. Duarte, M. F. Pedersen, C. Lindblad, K. Kersting, F. Moy, and T. Bokn. 2003. High organic carbon export precludes eutrophication responses in experimental rocky shore communities. *Ecosystems* **6**:0144-0153.
- Barry, J. P., and P. K. Dayton. 1991. Physical heterogeneity and the organization of marine communities. Pages 270-320 *Ecological heterogeneity*. Springer.
- Bartley, T. J., H. E. Braid, K. S. McCann, N. P. Lester, B. J. Shuter, and R. H. Hanner. 2015. DNA barcoding increases resolution and changes structure in Canadian boreal shield lake food webs. *DNA Barcodes* **3**:30-43.
- Bassett, D., and J. Montgomery. 2011. Investigating nocturnal fish populations< i> in situ</i> using baited underwater video: With special reference to their olfactory capabilities. *Journal of Experimental Marine Biology and Ecology* **409**:194-199.
- Bearham, D., M. A. Vanderklift, and J. R. Gunson. 2013. Temperature and light explain spatial variation in growth and productivity of the kelp *Ecklonia radiata*. *Mar. Ecol. Prog. Ser* **476**:59-70.
- Beaumont, N., M. Austen, S. Mangi, and M. Townsend. 2008. Economic valuation for the conservation of marine biodiversity. *Mar Pollut Bull* **56**:386-396.
- Beger, M., B. Sommer, P. L. Harrison, S. D. Smith, and J. M. Pandolfi. 2014. Conserving potential coral reef refuges at high latitudes. *Diversity and Distributions* **20**:245-257.
- Bell, T. W., K. C. Cavanaugh, D. C. Reed, and D. A. Siegel. 2015. Geographical variability in the controls of giant kelp biomass dynamics. *Journal of Biogeography* **42**:2010-2021.
- Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity. *Ecology Letters* **15**:365-377.
- Bennett, S., T. Wernberg, S. D. Connell, A. J. Hobday, C. R. Johnson, and E. S. Poloczanska. 2015. The 'Great Southern Reef': social, ecological and economic value of Australia's neglected kelp forests. *Marine and Freshwater Research* **67**:47-56.
- Bishop, M. J., M. A. Coleman, and B. P. Kelaher. 2010. Cross-habitat impacts of species decline: response of estuarine sediment communities to changing detrital resources. *Oecologia* **163**:517-525.
- Blight, A., and R. Thompson. 2008. Epibiont species richness varies between holdfasts of a northern and a southerly distributed kelp species. *Journal of the Marine Biological Association of the UK* **88**:469-475.
- Bologna, P. A. X., and R. Steneck. 1993. Kelp beds as habitat for American lobster *Homarus americanus*. *Marine Ecology-Progress Series* **100**:127-134.

- Booth, D., W. Figueira, M. Gregson, L. Brown, and G. Beretta. 2007. Occurrence of tropical fishes in temperate southeastern Australia: role of the East Australian Current. *Estuarine, Coastal and Shelf Science* **72**:102-114.
- Booth, D. J., N. Bond, and P. Macreadie. 2011. Detecting range shifts among Australian fishes in response to climate change. *Marine and Freshwater Research* **62**:1027-1042.
- Bouchon-Navaro, Y., C. Bouchon, M. Louis, and P. Legendre. 2005. Biogeographic patterns of coastal fish assemblages in the West Indies. *Journal of Experimental Marine Biology and Ecology* **315**:31-47.
- Bourque, B. J., W. H. Berger, C. H. Peterson, L. W. Botsford, M. X. Kirby, J. A. Estes, R. Cooke, C. B. Lange, S. Kidwell, and J. B. Jackson. 2001. Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *American Association for the Advancement of Science*.
- Bowles, E., P. M. Schulte, D. J. Tollit, B. E. Deagle, and A. W. Trites. 2011. Proportion of prey consumed can be determined from faecal DNA using real- time PCR. *Molecular ecology resources* **11**:530-540.
- Brady, S. M., and R. E. Scheibling. 2005. Repopulation of the shallow subtidal zone by green sea urchins (*Strongylocentrotus droebachiensis*) following mass mortality in Nova Scotia, Canada. *Journal of the Marine Biological Association of the United Kingdom* **85**:1511-1517.
- Braid, H. E., J. Deeds, S. L. DeGrasse, J. J. Wilson, J. Osborne, and R. H. Hanner. 2012. Preying on commercial fisheries and accumulating paralytic shellfish toxins: a dietary analysis of invasive *Dosidicus gigas* (Cephalopoda Ommastrephidae) stranded in Pacific Canada. *Marine Biology* **159**:25-31.
- Branch, G. M. 2008. Trophic interactions in subtidal rocky reefs on the west coast of South Africa. *Food Webs and the Dynamics of Marine Reefs*:50-78.
- Brennand, H. S., N. Soars, S. A. Dworjanyn, A. R. Davis, and M. Byrne. 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *Plos One* **5**:e11372.
- Brierley, A. S., and M. J. Kingsford. 2009. Impacts of Climate Change on Marine Organisms and Ecosystems. *Current Biology* **19**:R602-R614.
- Brodie, J., C. J. Williamson, D. A. Smale, N. A. Kamenos, N. Mieszkowska, R. Santos, M. Cunliffe, M. Steinke, C. Yesson, and K. M. Anderson. 2014. The future of the northeast Atlantic benthic flora in a high CO<sub>2</sub> world. *Ecology and evolution* **4**:2787-2798.
- Bruno, J. F., and M. D. Bertness. 2001. Habitat modification and facilitation in benthic marine communities. *Marine community ecology* **413**:201-218.
- Buschmann, A. H., M. d. C. Hernandez-Gonzalez, and D. Varela. 2008. Seaweed future cultivation in Chile: perspectives and challenges. *International Journal of Environment and Pollution* **33**:432-456.
- Bustamante, R. H., and G. M. Branch. 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of South Africa. *Journal of Experimental Marine Biology and Ecology* **196**:1-28.
- Byrne, M., and N. Andrew. 2013. *Centrostephanus rodgersii*. Sea Urchins: Biology and Ecology:243-254.
- Byrnes, J., K. Krumhansl, D. Okamoto, A. Rassweiler, M. Novak, J. Bolton, K. Cavanaugh, S. Connell, C. Johnson, and B. Konar. 2016. Linking global patterns of kelp forest change and variation in climate over the past half-century. *Page. in 11th International Temperate Reefs Symposium*.
- Byrnes, J. E., D. C. Reed, B. J. Cardinale, K. C. Cavanaugh, S. J. Holbrook, and R. J. Schmitt. 2011. Climate- driven increases in storm frequency simplify kelp forest food webs. *Global Change Biology* **17**:2513-2524.

- Cai, W., G. Shi, T. Cowan, D. Bi, and J. Ribbe. 2005. The response of the Southern Annular Mode, the East Australian Current, and the southern mid- latitude ocean circulation to global warming. *Geophysical Research Letters* **32**.
- Cappo, M., E. Harvey, H. Malcolm, and P. Speare. 2003. Potential of video techniques to monitor diversity, abundance and size of fish in studies of marine protected areas. *Aquatic Protected Areas-what works best and how do we know*:455-464.
- Cappo, M., E. Harvey, and M. Shortis. 2006. Counting and measuring fish with baited video techniques-an overview. Pages 101-114 *in* Australian Society for Fish Biology Workshop Proceedings.
- Carraro, R., and W. Gladstone. 2006. Habitat Preferences and Site Fidelity of the Ornate Wobbegong Shark (*Orectolobus ornatus*) on Rocky Reefs of New South Wales 1. *Pacific Science* **60**:207-223.
- Carreon- martinez, I., and D. Heath. 2010. Revolution in food web analysis and trophic ecology: diet analysis by DNA and stable isotope analysis. *Mol Ecol* **19**:25-27.
- Cetina- Heredia, P., M. Roughan, E. Sebille, M. Feng, and M. A. Coleman. 2015. Strengthened currents override the effect of warming on lobster larval dispersal and survival. *Global Change Biology* **21**:4377-4386.
- Ciais, P., C. Sabine, G. Bala, L. Bopp, V. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, and M. Heimann. 2014. Carbon and other biogeochemical cycles. Pages 465-570 *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.
- Clare, E., B. Barber, B. Sweeney, P. Hebert, and M. Fenton. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Mol Ecol* **20**:1772-1780.
- Clark, R., M. Edwards, and M. Foster. 2004. Effects of shade from multiple kelp canopies on an understory algal assemblage. *Marine Ecology Progress Series* **267**:107-119.
- Clarke, K., and R. Warwick. 1994. An approach to statistical analysis and interpretation. *Change in Marine Communities* **2**.
- Clarke, K., and R. Warwick. 2001. *Change in Marine Communities: An approach to statistical analysis and interpretation*. 2001. PRIMER-E: Plymouth, UK.
- Clarke, K., and R. Warwick. 2005. Primer-6 computer program. Natural Environment Research Council, Plymouth.
- Clifton, K. B., and P. J. Motta. 1998. Feeding morphology, diet, and ecomorphological relationships among five Caribbean labrids (Teleostei, Labridae). *Copeia*:953-966.
- Cole, A., M. Pratchett, and G. Jones. 2010. Corallivory in tubelip wrasses: diet, feeding and trophic importance. *Journal of Fish Biology* **76**:818-835.
- Cole, R., and R. Babcock. 1996. Mass mortality of a dominant kelp (Laminariales) at Goat Island, north-eastern New Zealand. *Marine and Freshwater Research* **47**:907-911.
- Coleman, M. A. 2013. Connectivity of the habitat-forming Kelp, *Ecklonia radiata* within and among estuaries and open coast. *Plos One* **8**:e64667.
- Coleman, M. A., P. Cetina- Heredia, M. Roughan, M. Feng, E. Sebille, and B. P. Kelaher. 2017. Anticipating changes to future connectivity within a network of marine protected areas. *Global Change Biology*.
- Coleman, M. A., M. Feng, M. Roughan, P. Cetina- Heredia, and S. D. Connell. 2013. Temperate shelf water dispersal by Australian boundary currents: implications for population connectivity. *Limnology and Oceanography: Fluids and Environments* **3**:295-309.
- Coleman, M. A., M. Roughan, H. S. Macdonald, S. D. Connell, B. M. Gillanders, B. P. Kelaher, and P. D. Steinberg. 2011. Variation in the strength of continental boundary currents determines continent- wide connectivity in kelp. *Journal of Ecology* **99**:1026-1032.

- Coleman, M. A., E. Vytopil, P. J. Goodsell, B. M. Gillanders, and S. D. Connell. 2007. Diversity and depth-related patterns of mobile invertebrates associated with kelp forests. *Marine and Freshwater Research* **58**:589-595.
- Colton, M. A., and S. E. Swearer. 2010. A comparison of two survey methods: differences between underwater visual census and baited remote underwater video. *Marine Ecology Progress Series* **400**:19-36.
- Collins, M., R. Knutti, J. Arblaster, J.-L. Dufresne, T. Fichefet, P. Friedlingstein, X. Gao, W. Gutowski, T. Johns, and G. Krinner. 2013. Long-term climate change: projections, commitments and irreversibility.
- Connell, S. 2003a. The monopolization of understorey habitat by subtidal encrusting coralline algae: a test of the combined effects of canopy-mediated light and sedimentation. *Marine Biology* **142**:1065-1071.
- Connell, S., and G. Jones. 1991. The influence of habitat complexity on postrecruitment processes in a temperate reef fish population. *Journal of Experimental Marine Biology and Ecology* **151**:271-294.
- Connell, S. D. 2003b. Negative effects overpower the positive of kelp to exclude invertebrates from the understorey community. *Oecologia* **137**:97-103.
- Connell, S. D., and A. D. Irving. 2008. Integrating ecology with biogeography using landscape characteristics: a case study of subtidal habitat across continental Australia. *Journal of Biogeography* **35**:1608-1621.
- Connell, S. D., K. J. Kroeker, K. E. Fabricius, D. I. Kline, and B. D. Russell. 2013. The other ocean acidification problem: CO<sub>2</sub> as a resource among competitors for ecosystem dominance. *Philosophical Transactions of the Royal Society B: Biological Sciences* **368**:20120442.
- Connell, S. D., and B. D. Russell. 2010. The direct effects of increasing CO<sub>2</sub> and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proc Biol Sci* **277**:1409-1415.
- Connell, S. D., B. D. Russell, D. J. Turner, A. J. S. Shepherd, T. N. Kildea, D. Miller, L. Airoldi, and A. Cheshire. 2008. Recovering a lost baseline: missing kelp forests from a metropolitan coast. *Marine Ecology Progress Series* **360**:63-72.
- Connolly, R. M., T. A. Schlacher, and T. F. Gaston. 2009. Stable isotope evidence for trophic subsidy of coastal benthic fisheries by river discharge plumes off small estuaries. *Marine biology research* **5**:164-171.
- Cooke, S. J., and J. F. Schreer. 2002. Determination of fish community composition in the untempered regions of a thermal effluent canal - The efficacy of a fixed underwater videography system. *Environmental Monitoring and Assessment* **73**:109-129.
- Corse, E., C. Costedoat, R. Chappaz, N. Pech, J.-F. Martin, and A. Gilles. 2010. A PCR- based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. *Molecular ecology resources* **10**:96-108.
- Costanza, R., R. de Groot, P. Sutton, S. van der Ploeg, S. J. Anderson, I. Kubiszewski, S. Farber, and R. K. Turner. 2014. Changes in the global value of ecosystem services. *Global environmental change* **26**:152-158.
- Cote, I. M., S. J. Green, J. A. Morris, J. L. Akins, and D. Steinke. 2013. Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. *Marine Ecology Progress Series* **472**:249-256.
- Cresson, P., S. Ruitton, M. Ourgaud, and M. Harmelin-Vivien. 2014. Contrasting perception of fish trophic level from stomach content and stable isotope analyses: A Mediterranean artificial reef experience. *Journal of Experimental Marine Biology and Ecology* **452**:54-62.
- Curley, B. G., M. J. Kingsford, and B. M. Gillanders. 2003. Spatial and habitat-related patterns of temperate reef fish assemblages: implications for the design of Marine Protected Areas. *Marine and Freshwater Research* **53**:1197-1210.

- Chapman, A. 1981. Stability of sea urchin dominated barren grounds following destructive grazing of kelp in St. Margaret's Bay, eastern Canada. *Marine Biology* **62**:307-311.
- Choat, J. 1982. Fish feeding and the structure of benthic communities in temperate waters. *Annual review of ecology and systematics*:423-449.
- Christie, H., S. Fredriksen, and E. Rinde. 1998. Regrowth of kelp and colonization of epiphyte and fauna community after kelp trawling at the coast of Norway. Pages 49-58 *Recruitment, Colonization and Physical-Chemical Forcing in Marine Biological Systems*. Springer.
- Christie, H., N. M. Jørgensen, and K. M. Norderhaug. 2007. Bushy or smooth, high or low; importance of habitat architecture and vertical position for distribution of fauna on kelp. *Journal of Sea Research* **58**:198-208.
- Christie, H., N. M. Jørgensen, K. M. Norderhaug, and E. Waage-Nielsen. 2003. Species distribution and habitat exploitation of fauna associated with kelp (*Laminaria hyperborea*) along the Norwegian coast. *Journal of the Marine Biological Association of the UK* **83**:687-699.
- Christie, H., K. M. Norderhaug, and S. Fredriksen. 2009. Macrophytes as habitat for fauna. *Marine Ecology Progress Series* **396**:221-233.
- Dalben, A., and S. Floeter. 2012. Cryptobenthic reef fishes: depth distribution and correlations with habitat complexity and sea urchins. *Journal of Fish Biology* **80**:852-865.
- Daly, R., P. W. Froneman, and M. J. Smale. 2013. Comparative Feeding Ecology of Bull Sharks (*Carcharhinus leucas*) in the Coastal Waters of the Southwest Indian Ocean Inferred from Stable Isotope Analysis. *Plos One* **8**:11.
- Daly, R., and M. J. Smale. 2013. Evaluation of an underwater biopsy probe for collecting tissue samples from bull sharks *Carcharhinus leucas*. *African Journal of Marine Science* **35**:129-132.
- Davenport, A. C., and T. W. Anderson. 2007. Positive indirect effects of reef fishes on kelp performance: the importance of mesograzers. *Ecology* **88**:1548-1561.
- Davis, J. P., K. A. Pitt, B. Fry, and R. M. Connolly. 2015. Stable isotopes as tracers of residency for fish on inshore coral reefs. *Estuarine, Coastal and Shelf Science* **167**:368-376.
- Davis, J. P., K. A. Pitt, B. Fry, A. D. Olds, and R. M. Connolly. 2014a. Seascape-scale trophic links for fish on inshore coral reefs. *Coral Reefs* **33**:897-907.
- Davis, R., L. Fuiman, T. Williams, S. Collier, W. Hagey, S. Kanatous, S. Kohin, and M. Horning. 1999. Hunting behavior of a marine mammal beneath the Antarctic fast ice. *Science* **283**:993-996.
- Davis, T., D. Harasti, and S. D. Smith. 2014b. Compensating for length biases in underwater visual census of fishes using stereo video measurements. *Marine and Freshwater Research*.
- Dayton, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological monographs* **45**:137-159.
- Dayton, P. K. 1985. Ecology of kelp communities. *Annual review of ecology and systematics*:215-245.
- De Barba, M., C. Miquel, F. Boyer, C. Mercier, D. Rioux, E. Coissac, and P. Taberlet. 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Molecular ecology resources* **14**:306-323.
- de Bettignies, T., T. Wernberg, P. S. Lavery, M. A. Vanderklift, J. R. Gunson, G. Symonds, and N. Collier. 2015. Phenological decoupling of mortality from wave forcing in kelp beds. *Ecology* **96**:850-861.
- Deagle, B., S. Jarman, D. Pemberton, and N. Gales. 2005a. Genetic screening for prey in the gut contents from a giant squid (*Architeuthis* sp.). *Journal of Heredity* **96**:417-423.

- Deagle, B., D. Tollit, S. Jarman, M. Hindell, A. Trites, and N. Gales. 2005b. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Mol Ecol* **14**:1831-1842.
- Deagle, B. E., A. Chiaradia, J. McInnes, and S. N. Jarman. 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? *Conservation Genetics* **11**:2039-2048.
- Deagle, B. E., R. Kirkwood, and S. N. Jarman. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Mol Ecol* **18**:2022-2038.
- Deagle, B. E., A. C. Thomas, A. K. Shaffer, A. W. Trites, and S. N. Jarman. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? *Molecular ecology resources* **13**:620-633.
- Deagle, B. E., and D. J. Tollit. 2007. Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? *Conservation Genetics* **8**:743-747.
- Demeke, T., and G. R. Jenkins. 2010. Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and bioanalytical chemistry* **396**:1977-1990.
- Denny, C. M., and D. R. Schiel. 2001. Feeding ecology of the banded wrasse *Notolabrus fucicola* (Labridae) in southern New Zealand: prey items, seasonal differences, and ontogenetic variation. *New Zealand Journal of Marine and Freshwater Research* **35**:925-933.
- Depczynski, M., C. J. Fulton, M. J. Marnane, and D. R. Bellwood. 2007. Life history patterns shape energy allocation among fishes on coral reefs. *Oecologia* **153**:111-120.
- Díez, I., N. Muguerza, A. Santolaria, U. Ganzledo, and J. M. Gorostiaga. 2012. Seaweed assemblage changes in the eastern Cantabrian Sea and their potential relationship to climate change. *Estuarine, Coastal and Shelf Science* **99**:108-120.
- Dipper, F., C. Bridges, and A. Menz. 1977. Age, growth and feeding in the ballan wrasse *Labrus bergylta* Ascanius 1767. *Journal of Fish Biology* **11**:105-120.
- Dromard, C. R., Y. Bouchon-Navaro, M. Harmelin-Vivien, and C. Bouchon. 2014. Diversity of trophic niches among herbivorous fishes on a Caribbean reef (Guadeloupe, Lesser Antilles), evidenced by stable isotope and gut content analyses. *Journal of Sea Research*.
- Duffy, J. E., and M. E. Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecological monographs* **70**:237-263.
- Duffy, J. E., K. S. Macdonald, J. M. Rhode, and J. D. Parker. 2001. Grazer diversity, functional redundancy, and productivity in seagrass beds: an experimental test. *Ecology* **82**:2417-2434.
- Duggins, D., and J. Eckman. 1997. Is kelp detritus a good food for suspension feeders? Effects of kelp species, age and secondary metabolites. *Marine Biology* **128**:489-495.
- Duggins, D., C. Simenstad, and J. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. *Science(Washington)* **245**:170-173.
- Dunn, M. R., A. Szabo, M. S. McVeagh, and P. J. Smith. 2010. The diet of deepwater sharks and the benefits of using DNA identification of prey. *Deep Sea Research Part I: Oceanographic Research Papers* **57**:923-930.
- Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of under story kelp environments. I. Effects of kelps on flow and particle transport near the bottom. *Journal of Experimental Marine Biology and Ecology* **129**:173-187.
- Edgar, G. 1983. The ecology of south-east Tasmanian phytal animal communities. I. Spatial organization on a local scale. *Journal of Experimental Marine Biology and Ecology* **70**:129-157.
- Edgar, G., S. Banks, J. Fariña, M. Calvopiña, and C. Martínez. 2004a. Regional biogeography of shallow reef fish and macro-invertebrate communities in the Galapagos archipelago. *Journal of Biogeography* **31**:1107-1124.

- Edgar, G. J. 2001. Australian marine habitats in temperate waters. New Holland.
- Edgar, G. J., N. S. Barrett, A. J. Morton, and C. R. Samson. 2004b. Effects of algal canopy clearance on plant, fish and macroinvertebrate communities on eastern Tasmanian reefs. *Journal of Experimental Marine Biology and Ecology* **312**:67-87.
- Edgar, G. J., and C. Shaw. 1995a. The production and trophic ecology of shallow-water fish assemblages in southern Australia I. Species richness, size-structure and production of fishes in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194**:53-81.
- Edgar, G. J., and C. Shaw. 1995b. The production and trophic ecology of shallow-water fish assemblages in southern Australia II. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194**:83-106.
- Egan, S., T. Harder, C. Burke, P. Steinberg, S. Kjelleberg, and T. Thomas. 2013. The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews* **37**:462-476.
- Elahi, R., and K. P. Sebens. 2013. Experimental Removal and Recovery of Subtidal Grazers Highlights the Importance of Functional Redundancy and Temporal Context. *Plos One* **8**:10.
- Elner, R., and R. Vadas. 1990. Inference in ecology: the sea urchin phenomenon in the northwestern Atlantic. *American Naturalist* **136**:108.
- Estes, J., E. Danner, D. Doak, B. Konar, A. Springer, P. Steinberg, M. Tinker, and T. Williams. 2004. Complex trophic interactions in kelp forest ecosystems. *Bulletin of Marine Science* **74**:621-638.
- Estes, J. A., and D. O. Duggins. 1995. Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological monographs* **65**:75-100.
- Falkenberg, L. J., S. D. Connell, and B. D. Russell. 2013a. Disrupting the effects of synergies between stressors: improved water quality dampens the effects of future CO<sub>2</sub> on a marine habitat. *Journal of Applied Ecology* **50**:51-58.
- Falkenberg, L. J., B. D. Russell, and S. D. Connell. 2013b. Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO<sub>2</sub> and nutrient regimes. *Oecologia* **172**:575-583.
- Fielding, P., and C. Davis. 1989. Carbon and nitrogen resources available to kelp bed filter feeders in an upwelling environment. *Marine Ecology Progress Series*:181-189.
- Figueira, W. F., and D. J. Booth. 2010. Increasing ocean temperatures allow tropical fishes to survive overwinter in temperate waters. *Glob Chang Biol* **16**.
- Figueiredo, M., T. Morato, J. P. Barreiros, P. Afonso, and R. S. Santos. 2005. Feeding ecology of the white seabream, *Diplodus sargus*, and the ballan wrasse, *Labrus bergylta*, in the Azores. *Fisheries Research* **75**:107-119.
- Filbee-Dexter, K., and R. E. Scheibling. 2012. Hurricane-mediated defoliation of kelp beds and pulsed delivery of kelp detritus to offshore sedimentary habitats. *Marine Ecology Progress Series* **455**:51-64.
- Filbee-Dexter, K., and R. E. Scheibling. 2014. Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Marine Ecology Progress Series* **495**:1-25.
- Fletcher, W. 1987. Interactions among subtidal Australian sea urchins, gastropods, and algae: effects of experimental removals. *Ecological monographs* **57**:89-109.
- Floeter, S. R., M. Behrens, C. Ferreira, M. Paddock, and M. Horn. 2005. Geographical gradients of marine herbivorous fishes: patterns and processes. *Marine Biology* **147**:1435-1447.
- Flukes, E., C. Johnson, and J. Wright. 2014. Thinning of kelp canopy modifies understory assemblages: the importance of canopy density. *Marine Ecology Progress Series* **514**:57-70.

- Folmer, O., W. Hoeh, M. Black, and R. Vrijenhoek. 1994. Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology* **3**:294-299.
- Fontes, J., R. S. Santos, P. Afonso, and J. E. Caselle. 2011. Larval growth, size, stage duration and recruitment success of a temperate reef fish. *Journal of Sea Research* **65**:1-7.
- Foster, M. S., and D. R. Schiel. 1985. Ecology of giant kelp forests in California: a community profile. San Jose State Univ., Moss Landing, CA (USA). Moss Landing Marine Labs.
- Foster, M. S., and D. R. Schiel. 2010. Loss of predators and the collapse of southern California kelp forests (?): Alternatives, explanations and generalizations. *Journal of Experimental Marine Biology and Ecology* **393**:59-70.
- Fowler-Walker, M. J., and S. D. Connell. 2002. Opposing states of subtidal habitat across temperate Australia: consistency and predictability in kelp canopy-benthic associations. *Marine Ecology-Progress Series* **240**:49-56.
- Fox, R. J., and D. R. Bellwood. 2007. Quantifying herbivory across a coral reef depth gradient. *Marine Ecology Progress Series* **339**:49-59.
- Fox, R. J., and D. R. Bellwood. 2008. Remote video bioassays reveal the potential feeding impact of the rabbitfish *Siganus canaliculatus* (f: Siganidae) on an inner-shelf reef of the Great Barrier Reef. *Coral Reefs* **27**:605-615.
- Francour, P. 1997. Predation on holothurians: a literature review. *Invertebrate Biology*:52-60.
- French, B., K. R. Clarke, M. E. Platell, and I. C. Potter. 2013. An innovative statistical approach to constructing a readily comprehensible food web for a demersal fish community. *Estuarine, Coastal and Shelf Science* **125**:43-56.
- Fry, B., and J. Davis. 2015. Rescaling stable isotope data for standardized evaluations of food webs and species niches. *Marine Ecology Progress Series* **528**:7-17.
- Fuiman, L., R. Davis, and T. Williams. 2002. Behavior of midwater fishes under the Antarctic ice: observations by a predator. *Marine Biology* **140**:815-822.
- Fulton, C., and D. Bellwood. 2004. Wave exposure, swimming performance, and the structure of tropical and temperate reef fish assemblages. *Marine Biology* **144**:429-437.
- García-Charton, J., A. Pérez-Ruzafa, P. Sánchez-Jerez, J. Bayle-Sempere, O. Reñones, and D. Moreno. 2004. Multi-scale spatial heterogeneity, habitat structure, and the effect of marine reserves on Western Mediterranean rocky reef fish assemblages. *Marine Biology* **144**:161-182.
- Gebremedhin, B., Ø. Flagstad, A. Bekele, D. Chala, V. Bakkestuen, S. Boessenkool, M. Popp, G. Gussarova, A. Schrøder-Nielsen, and S. Nemomissa. 2016. DNA metabarcoding reveals diet overlap between the endangered walia ibex and domestic goats- implications for conservation. *Plos One* **11**:e0159133.
- Geer, L. Y., A. Marchler-Bauer, R. C. Geer, L. Han, J. He, S. He, C. Liu, W. Shi, and S. H. Bryant. 2009. The NCBI biosystems database. *Nucleic acids research*:gkp858.
- Gerking, S. D. 2014. Feeding ecology of fish. Elsevier.
- Ghedini, G., B. D. Russell, and S. D. Connell. 2015. Trophic compensation reinforces resistance: herbivory absorbs the increasing effects of multiple disturbances. *Ecology Letters* **18**:182-187.
- Gillanders, B. 1995. Reproductive biology of the protogynous hermaphrodite *Achoerodus viridis* (Labridae) from south-eastern Australia. *Marine and Freshwater Research* **46**:999-1008.
- Gillanders, B. M., T. S. Elsdon, I. A. Halliday, G. P. Jenkins, J. B. Robins, and F. J. Valesini. 2011. Potential effects of climate change on Australian estuaries and fish utilising estuaries: a review. *Marine and Freshwater Research* **62**:1115-1131.
- Gillies, C. L. 2012. Trophic ecology of the nearshore zone in East Antarctica: a stable isotope approach.

- Gladstone, W. 2007. Requirements for marine protected areas to conserve the biodiversity of rocky reef fishes. *Aquat Conserv Mar Freshwat Ecosyst* **17**.
- Goatley, C. H., and D. R. Bellwood. 2014. Moving towards the equator: reverse range shifts in two subtropical reef fish species, *Chromis nitida* (Pomacentridae) and *Pseudolabrus guentheri* (Labridae). *Marine Biodiversity Records* **7**:e12.
- Goodsell, P., and S. Connell. 2002. Can habitat loss be treated independently of habitat configuration? Implications for rare and common taxa in fragmented landscapes.
- Goodsell, P., and S. Connell. 2008. Complexity in the relationship between matrix composition and inter-patch distance in fragmented habitats. *Marine Biology* **154**:117-125.
- Graham, M., B. Halpern, and M. Carr. 2008. Diversity and dynamics of Californian subtidal kelp forests. *Food webs and the dynamics of marine reefs*. Oxford University Press, New York:103-134.
- Graham, M. H. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. *Ecosystems* **7**:341-357.
- Graham, M. H., B. P. Kinlan, L. D. Druehl, L. E. Garske, and S. Banks. 2007a. Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proceedings of the National Academy of Sciences* **104**:16576-16580.
- Graham, M. H., J. A. Vasquez, and A. H. Buschmann. 2007b. Global ecology of the giant kelp *Macrocystis*: From ecotypes to ecosystems. Pages 39-88 in R. N. Gibson, R. J. A. Atkinson, and J. D. M. Gordon, editors. *Oceanography and Marine Biology*, Vol 45.
- Granquist, S., R. Esparza-Sala, E. Hauksson, O. Karlsson, I. G. Jónsdóttir, and A. Angerbjörn. 2016. Prey consumption of Harbour seals (*Phoca vitulina*) in north western Iceland:: Comparing DNA metabarcoding and morphological analyses.
- Gratwicke, B., and M. R. Speight. 2005. Effects of habitat complexity on Caribbean marine fish assemblages. *Marine ecology. Progress series* **292**:301-310.
- Guidetti, P., C. N. Bianchi, M. Chiantore, S. Schiaparelli, C. Morri, and R. Cattaneo-Vietti. 2004. Living on the rocks: substrate mineralogy and the structure of subtidal rocky substrate communities in the Mediterranean Sea. *Marine Ecology Progress Series* **274**:57-68.
- Guillerault, N., S. Bouletreau, A. Iribar, A. Valentini, and F. Santoul. 2017. Application of DNA metabarcoding on faeces to identify European catfish *Silurus glanis* diet. *Journal of Fish Biology* **90**:2214-2219.
- Gutierrez, A., T. Correa, V. Munoz, A. Santibanez, R. Marcos, C. Cáceres, and A. H. Buschmann. 2006. Farming of the giant kelp *Macrocystis pyrifera* in southern Chile for development of novel food products. *Journal of Applied Phycology* **18**:259-267.
- Haggitt, T., and R. Babcock. 2003. The role of grazing by the lysianassid amphipod *Orchomenella aahu* in dieback of the kelp *Ecklonia radiata* in north-eastern New Zealand. *Marine Biology* **143**:1201-1211.
- Halanych, K. M., and A. M. Janosik. 2006. A review of molecular markers used for Annelid phylogenetics. *Integrative and Comparative Biology* **46**:533-543.
- Hammerschlag-Peyer, C. M., and C. A. Layman. 2010. Intrapopulation variation in habitat use by two abundant coastal fish species. *Marine Ecology Progress Series* **415**:211-220.
- Hargrove, J. S., D. C. Parkyn, D. J. Murie, A. W. Demopoulos, and J. D. Austin. 2012. Augmentation of French grunt diet description using combined visual and DNA-based analyses. *Marine and Freshwater Research* **63**:740-750.
- Harley, C. D., K. M. Anderson, K. W. Demes, J. P. Jorve, R. L. Kordas, T. A. Coyle, and M. H. Graham. 2012a. Effects of climate change on global seaweed communities. *Journal of Phycology* **48**:1064-1078.

- Harley, C. D. G., K. M. Anderson, K. W. Demes, J. P. Jorve, R. L. Kordas, T. A. Coyle, and M. H. Graham. 2012b. Effects of climate change on global seaweed communities. *Journal of Phycology* **48**:1064-1078.
- Harms-Tuohy, C. A., N. V. Schizas, and R. S. Appeldoorn. 2016. Use of DNA metabarcoding for stomach content analysis in the invasive lionfish *Pterois volitans* in Puerto Rico. *Marine Ecology Progress Series* **558**:181-191.
- Harriott, V., S. Smith, and P. L. Harrison. 1994. Patterns of coral community structure of subtropical reefs in the Solitary Islands Marine Reserve, Eastern Australia. *Marine Ecology Progress Series* **67**-76.
- Harrold, C., K. Light, and S. Lisin. 1998. Organic enrichment of submarine-canyon and continental-shelf benthic communities by macroalgal drift imported from nearshore kelp forests. *Limnology and Oceanography* **43**:669-678.
- Harvey, E. S., M. Cappo, J. J. Butler, N. Hall, and G. A. Kendrick. 2007. Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. *Marine Ecology-Progress Series* **350**:245.
- Harvey, E. S., M. Cappo, G. A. Kendrick, and D. L. McLean. 2013. Coastal Fish Assemblages Reflect Geological and Oceanographic Gradients Within An Australian Zootone. *Plos One* **8**:e80955.
- Hawkins, S. J., P. Moore, M. T. Burrows, E. Poloczanska, N. Mieszkowska, R. J. Herbert, S. R. Jenkins, R. C. Thompson, M. J. Genner, and A. J. Southward. 2008. Complex interactions in a rapidly changing world: responses of rocky shore communities to recent climate change. *Climate Research* **37**:123-133.
- Heagney, E. C., T. P. Lynch, R. C. Babcock, and I. M. Suthers. 2007. Pelagic fish assemblages assessed using mid-water baited video: standardising fish counts using bait plume size. *Marine Ecology-Progress Series* **350**:255.
- Hebert, P. D., S. Ratnasingham, and J. R. de Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **270**:S96-S99.
- Held, I. M., and B. J. Soden. 2006. Robust responses of the hydrological cycle to global warming. *Journal of Climate* **19**:5686-5699.
- Henríquez, L. A., A. H. Buschmann, M. A. Maldonado, M. H. Graham, M. C. Hernández- González, S. V. Pereda, and M. I. Bobadilla. 2011. Grazing on giant kelp microscopic phases and the recruitment success of annual populations of *Macrocystis pyrifera* (Laminariales, Phaeophyta) in southern Chile. *Journal of Phycology* **47**:252-258.
- Henry, G. W., and J. M. Lyle. 2003. National recreational and indigenous fishing survey.
- Hobday, A. J., and J. M. Lough. 2011. Projected climate change in Australian marine and freshwater environments. *Marine and Freshwater Research* **62**:1000-1014.
- Hobday, A. J., T. A. Okey, E. S. Poloczanska, T. J. Kunz, and A. J. Richardson. 2007. Impacts of climate change on Australian marine life. Australian Government, Department of the Environment and Water Resources, Australian Greenhouse Office.
- Hobday, A. J., and G. T. Pecl. 2014. Identification of global marine hotspots: sentinels for change and vanguards for adaptation action. *Reviews in Fish Biology and Fisheries* **24**:415-425.
- Hoegh-Guldberg, O., P. Mumby, A. Hooten, R. Steneck, P. Greenfield, E. Gomez, C. Harvell, P. Sale, A. Edwards, and K. Caldeira. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* **318**:1737-1742.
- Hoese, D., D. Bray, J. Paxton, and G. Allen. 2006. Fishes. In: *Zoological Catalogue of Australia*, Beasley, PL and A. Wells (Eds.) **85**.
- Holmes, T., S. Wilson, M. Vanderklift, R. Babcock, and M. Fraser. 2012. The role of *Thalassoma lunare* as a predator of juvenile fish on a sub-tropical coral reef. *Coral Reefs* **31**:1113-1123.

- Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* **486**:105-108.
- Hudson, I. R., and B. D. Wigham. 2003. In situ observations of predatory feeding behaviour of the galatheid squat lobster *Munida sarsi* using a remotely operated vehicle. *Journal of the Marine Biological Association of the United Kingdom* **83**:463-464.
- Hussey, N., M. MacNeil, J. Olin, B. McMeans, M. Kinney, D. Chapman, and A. Fisk. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology* **80**:1449-1484.
- Hussey, N. E., M. A. MacNeil, B. C. McMeans, J. A. Olin, S. F. J. Dudley, G. Cliff, S. P. Wintner, S. T. Fennessy, and A. T. Fisk. 2014. Rescaling the trophic structure of marine food webs. *Ecology Letters* **17**:239-250.
- Irving, A. D., and S. D. Connell. 2006. Predicting understorey structure from the presence and composition of canopies: an assembly rule for marine algae. *Oecologia* **148**:491-502.
- Iverson, S. J., C. Field, W. Don Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological monographs* **74**:211-235.
- Jackson, J. B. 2008. Ecological extinction and evolution in the brave new ocean. *Proceedings of the National Academy of Sciences* **105**:11458-11465.
- Jaime-Rivera, M., J. Caraveo-Patino, M. Hoyos-Padilla, and F. Galvan-Magana. 2013. Evaluation of biopsy systems for sampling white shark *Carcharodon carcharias* (Lamniformes: Lamnidae) muscle for stable isotope analysis. *Revista De Biología Marina Y Oceanografía* **48**:345-351.
- Jarman, S., B. Deagle, and N. Gales. 2004. Group- specific polymerase chain reaction for DNA- based analysis of species diversity and identity in dietary samples. *Mol Ecol* **13**:1313-1322.
- Johnson, C. R., S. C. Banks, N. S. Barrett, F. Cazassus, P. K. Dunstan, G. J. Edgar, S. D. Frusher, C. Gardner, M. Haddon, and F. Helidoniotis. 2011a. Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. *Journal of Experimental Marine Biology and Ecology* **400**:17-32.
- Johnson, C. R., S. C. Banks, N. S. Barrett, F. Cazassus, P. K. Dunstan, G. J. Edgar, S. D. Frusher, C. Gardner, M. Haddon, F. Helidoniotis, K. L. Hill, N. J. Holbrook, G. W. Hosie, P. R. Last, S. D. Ling, J. Melbourne-Thomas, K. Miller, G. T. Pecl, A. J. Richardson, K. R. Ridgway, S. R. Rintoul, D. A. Ritz, D. J. Ross, J. C. Sanderson, S. A. Shepherd, A. Slotwinski, K. M. Swadling, and N. Taw. 2011b. Climate change cascades: Shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. *Journal of Experimental Marine Biology and Ecology* **400**:17-32.
- Johnson, C. R., S. Ling, D. Ross, S. Shepherd, and K. Miller. 2005. Establishment of the long-spined sea urchin (*Centrostephanus rodgersii*) in Tasmania: first assessment of potential threats to fisheries.
- Joly, S., T. J. Davies, A. Archambault, A. Bruneau, A. Derry, S. W. Kembel, P. Peres- Neto, J. Vamosi, and T. A. Wheeler. 2014. Ecology in the age of DNA barcoding: the resource, the promise and the challenges ahead. *Molecular ecology resources* **14**:221-232.
- Jones, D. 1972. Changes in the ecological balance of invertebrate communities in kelp holdfast habitats of some polluted North Sea waters. *Helgoländer Wissenschaftliche Meeresuntersuchungen* **23**:248-260.
- Jones, G. P. 1984. The influence of habitat and behavioural interactions on the local distribution of the wrasse, *Pseudolabrus celidotus*. *Environmental Biology of Fishes* **10**:43-57.

- Jones, W., E. S. DENT, and D. Crisp. 1971. The effect of light on the growth of algal spores. Pages 363-374 in Fourth European Marine Biology Symposium. Cambridge University Press Cambridge.
- José de Anchieta, C., C. L. Sampaio, and F. Barros. 2013. How wave exposure, group size and habitat complexity influence foraging and population densities in fishes of the genus *Halichoeres* (Perciformes: Labridae) on tropical rocky shores. *Marine Biology* **160**:2383-2394.
- Jung, J.-L. 2014. DNA barcoding for the identification of soft remains of prey in the stomach contents of grey seals (*Halichoerus grypus*) and harbour.
- Kamenova, S., R. Mayer, E. Coissac, M. Plantegenest, and M. Traugott. 2017. Comparing three types of dietary samples for prey DNA decay in an insect generalist predator. *bioRxiv*:098806.
- Kasapidis, P., F. Boyer, A. Christidis, J. Kristoffersen, A. Oulas, N. Nikolioudakis, and J. Fric. 2016. Using next generation sequencing technologies to assess the diet of the Mediterranean Shag (*Phalacrocorax aristotelis*) and implication of these technologies for high-throughput study and monitoring of marine biodiversity.
- Kendrick, G. A., P. S. Lavery, and J. C. Phillips. 1999. Influence of *Ecklonia radiata* kelp canopy on structure of macro-algal assemblages in Marmion Lagoon, Western Australia. Pages 275-283 in Sixteenth International Seaweed Symposium. Springer.
- Kennelly, S. 1987a. Physical disturbances in an Australian kelp. *Mar. Ecol. Prog. Ser* **40**:145-153.
- Kennelly, S. J. 1987b. Physical disturbances in an Australian kelp community. 2. Effects on understorey species due to differences in kelp cover. *Marine ecology progress series*. Oldendorf **40**:155-165.
- Kennelly, S. J. 1989. Effects of kelp canopies on understory species due to shade and scour. *Marine ecology progress series*. Oldendorf **50**:215-224.
- Kerswell, A. P. 2006. Global biodiversity patterns of benthic marine algae. *Ecology* **87**:2479-2488.
- Kingsford, M. 1998. Reef fishes. Studying temperate marine environments. A handbook for ecologists. Canterbury University Press, Christchurch, NZ:132-166.
- Kingsford, M. J., and I. J. Carlson. 2010. Patterns of distribution and movement of fishes, *Ophthalmodon lineolatus* and *Hypoplectrodes maccullochi*, on temperate rocky reefs of south eastern Australia. *Environmental Biology of Fishes* **88**:105-118.
- Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. *Bulletin of Marine Science* **70**:309-340.
- Kirkman, H. 1981. The first year in the life history and the survival of the juvenile marine macrophyte, *Ecklonia radiata* (Turn.) J. Agardh. *Journal of Experimental Marine Biology and Ecology* **55**:243-254.
- Klages, J., A. Broad, B. P. Kelaher, and A. Davis. 2014. The influence of gummy sharks, *Mustelus antarcticus*, on observed fish assemblage structure. *Environmental Biology of Fishes* **97**:215-222.
- Kohler, K. E., and S. M. Gill. 2006. Coral Point Count with Excel extensions (CPCE): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Computers & Geosciences* **32**:1259-1269.
- Kordas, R. L., C. D. G. Harley, and M. I. O'Connor. 2011. Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology* **400**:218-226.
- Krajewski, J. P., and S. R. Floeter. 2011. Reef fish community structure of the Fernando de Noronha Archipelago (Equatorial Western Atlantic): the influence of exposure and benthic composition. *Environmental Biology of Fishes* **92**:25.

- Kramer, M. J., O. Bellwood, C. J. Fulton, and D. R. Bellwood. 2015. Refining the invertivore: diversity and specialisation in fish predation on coral reef crustaceans. *Marine Biology* **162**:1779-1786.
- Krumhansl, K. A., and R. E. Scheibling. 2012. Production and fate of kelp detritus. *Marine Ecology Progress Series* **467**:281-302.
- Kuiter, R. H., and R. H. Kuiter. 1996. Guide to sea fishes of Australia. New Holland Pub Pty Limited.
- Lafferty, K. D. 2004. Fishing for lobsters indirectly increases epidemics in sea urchins. *Ecological Applications* **14**:1566-1573.
- Langlois, T., P. Chabane, D. Pelletier, and E. Harvey. 2006. Baited underwater video for assessing reef fish populations in marine reserves. *FISHERIES NEWSLETTER-SOUTH PACIFIC COMMISSION* **118**:53.
- Last, P. R., W. T. White, D. C. Gledhill, A. J. Hobday, R. Brown, G. J. Edgar, and G. Pecl. 2011. Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography* **20**:58-72.
- Lauzon-Guay, J.-S., and R. E. Scheibling. 2007. Seasonal variation in movement, aggregation and destructive grazing of the green sea urchin (*Strongylocentrotus droebachiensis*) in relation to wave action and sea temperature. *Marine Biology* **151**:2109-2118.
- Lavender, J. T., K. A. Dafforn, M. J. Bishop, and E. L. Johnston. 2017. Small scale habitat complexity of artificial turf influences the development of associated invertebrate assemblages. *Journal of Experimental Marine Biology and Ecology*.
- Layton, K. K., A. L. Martel, and P. D. Hebert. 2014. Patterns of DNA barcode variation in Canadian marine molluscs. *Plos One* **9**:e95003.
- Leathwick, J., J. Elith, M. Francis, T. Hastie, and P. Taylor. 2006. Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. *Marine Ecology Progress Series* **321**:267-281.
- Leclerc, J. C., P. Riera, C. Leroux, L. Levesue, and D. Davout. 2013. Temporal variation in organic matter supply in kelp forests: linking structure to trophic functioning. *Marine Ecology Progress Series* **494**:87-105.
- Leet, W. S. 2001. California's living marine resources: A Status Report. UCANR Publications.
- Legendre, P., and M. J. Anderson. 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological monographs* **69**:1-24.
- Lenanton, R., C. Dowling, K. Smith, D. Fairclough, and G. Jackson. 2017. Potential influence of a marine heatwave on range extensions of tropical fishes in the eastern Indian Ocean—Invaluable contributions from amateur observers. *Regional Studies in Marine Science*.
- Leray, M., N. Agudelo, S. C. Mills, and C. P. Meyer. 2013a. Effectiveness of annealing blocking primers versus restriction enzymes for characterization of generalist diets: unexpected prey revealed in the gut contents of two coral reef fish species. *Plos One* **8**:e58076.
- Leray, M., J. Boehm, S. C. Mills, and C. Meyer. 2012. Moorea BIOCODE barcode library as a tool for understanding predator-prey interactions: insights into the diet of common predatory coral reef fishes. *Coral Reefs* **31**:383-388.
- Leray, M., C. P. Meyer, and S. C. Mills. 2015. Metabarcoding dietary analysis of coral dwelling predatory fish demonstrates the minor contribution of coral mutualists to their highly partitioned, generalist diet. *PeerJ* **3**:e1047.
- Leray, M., J. Y. Yang, C. P. Meyer, S. C. Mills, N. Agudelo, V. Ranwez, J. T. Boehm, and R. J. Machida. 2013b. A new versatile primer set targeting a short fragment of the

- mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front Zool* **10**:34.
- Lima, F. P., P. A. Ribeiro, N. Queiroz, S. J. Hawkins, and A. M. Santos. 2007. Do distributional shifts of northern and southern species of algae match the warming pattern? *Global Change Biology* **13**:2592-2604.
- Ling, S. 2008. Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. *Oecologia* **156**:883-894.
- Ling, S., C. Johnson, K. Ridgway, A. Hobday, and M. Haddon. 2009a. Climate- driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. *Global Change Biology* **15**:719-731.
- Ling, S., R. Scheibling, A. Rassweiler, C. Johnson, N. Shears, S. Connell, A. Salomon, K. Norderhaug, A. Pérez-Matus, and J. Hernández. 2015. Global regime shift dynamics of catastrophic sea urchin overgrazing. *Phil. Trans. R. Soc. B* **370**:20130269.
- Ling, S. D., C. R. Johnson, S. D. Frusher, and K. R. Ridgway. 2009b. Overfishing reduces resilience of kelp beds to climate-driven catastrophic phase shift. *Proceedings of the National Academy of Sciences of the United States of America* **106**:22341-22345.
- Lingo, M. E., and S. T. Szedlmayer. 2006. The influence of habitat complexity on reef fish communities in the northeastern Gulf of Mexico. *Environmental Biology of Fishes* **76**:71-80.
- Longo, G., and S. Floeter. 2012. Comparison of remote video and diver's direct observations to quantify reef fishes feeding on benthos in coral and rocky reefs. *Journal of Fish Biology* **81**:1773-1780.
- Lorentsen, S.-H., K. Sjøtun, and D. Grémillet. 2010. Multi-trophic consequences of kelp harvest. *Biological Conservation* **143**:2054-2062.
- Lowry, M., H. Folpp, M. Gregson, and I. Suthers. 2012. Comparison of baited remote underwater video (BRUV) and underwater visual census (UVC) for assessment of artificial reefs in estuaries. *Journal of Experimental Marine Biology and Ecology* **416–417**:243-253.
- Lucas, A. 1935. The marine algae of Lord Howe Island. Pages 194-232 in *Proceedings of the Linnean Society of New South Wales*.
- Luckhurst, B. E., and K. Luckhurst. 1978. Analysis of influence of substrate variables on coral-reef fish communities. *Marine Biology* **49**:317-323.
- Lüning, K., C. Yarish, and H. Kirkman. 1990. *Seaweeds : their environment, biogeography, and ecophysiology*. Wiley, New York.
- Mabin, C. J. T., P. E. Gribben, A. Fischer, and J. T. Wright. 2013. Variation in the morphology, reproduction and development of the habitat-forming kelp *Ecklonia radiata* with changing temperature and nutrients. *Marine Ecology Progress Series* **483**:117-131.
- Malcolm, H., S. Lindfield, J. Wraith, T. Lynch, and W. Gladstone. 2007. Spatial and temporal variation in reef fish assemblages of marine parks in New South Wales, Australia- baited video observations.
- Malcolm, H. A., P. L. Davies, A. Jordan, and S. D. A. Smith. 2011a. Variation in sea temperature and the East Australian Current in the Solitary Islands region between 2001–2008. *Deep Sea Research Part II: Topical Studies in Oceanography* **58**:616-627.
- Malcolm, H. A., A. Jordan, and S. D. Smith. 2010a. Biogeographical and cross-shelf patterns of reef fish assemblages in a transition zone. *Marine Biodiversity* **40**:181-193.
- Malcolm, H. A., A. Jordan, and S. D. Smith. 2011b. Testing a depth- based Habitat Classification System against reef fish assemblage patterns in a subtropical marine park. *Aquatic Conservation: Marine and Freshwater Ecosystems* **21**:173-185.
- Malcolm, H. A., and S. D. Smith. 2010. Objective selection of surrogate families to describe reef fish assemblages in a subtropical marine park. *Biodiversity and conservation* **19**:3611-3618.

- Malcolm, H. A., S. D. A. Smith, and A. Jordan. 2010b. Using patterns of reef fish assemblages to refine a Habitat Classification System for marine parks in NSW, Australia. *Aquatic Conservation-Marine and Freshwater Ecosystems* **20**:83-92.
- Malpica-Cruz, L., S. Z. Herzka, O. Sosa-Nishizaki, and M. A. Escobedo-Olvera. 2013. Tissue-specific stable isotope ratios of shortfin mako (*Isurus oxyrinchus*) and white (*Carcharodon carcharias*) sharks as indicators of size-based differences in foraging habitat and trophic level. *Fisheries Oceanography* **22**:429-445.
- Mallet, D., and D. Pelletier. 2014. Underwater video techniques for observing coastal marine biodiversity: A review of sixty years of publications (1952–2012). *Fisheries Research* **154**:44-62.
- Mann, K. 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnology and Oceanography* **33**:910-930.
- Mann, K. H. 1973a. Seaweeds: Their Productivity and Strategy for Growth The role of large marine algae in coastal productivity is far more important than has been suspected. *Science* **182**:975-981.
- Mann, K. H. 1973b. Seaweeds: Their Productivity and Strategy for Growth: The role of large marine algae in coastal productivity is far more important than has been suspected. *Science* (New York, N.Y.) **182**:975-981.
- Mann, K. H. 2009. Ecology of coastal waters: with implications for management. John Wiley & Sons.
- Marzinelli, E. M. 2012. Artificial structures influence fouling on habitat-forming kelps. *Biofouling* **28**:339-349.
- Marzinelli, E. M., A. H. Campbell, E. Zozaya Valdes, A. Vergés, S. Nielsen, T. Wernberg, T. Bettignies, S. Bennett, J. G. Caporaso, and T. Thomas. 2015a. Continental- scale variation in seaweed host- associated bacterial communities is a function of host condition, not geography. *Environmental microbiology* **17**:4078-4088.
- Marzinelli, E. M., S. B. Williams, R. C. Babcock, N. S. Barrett, C. R. Johnson, A. Jordan, G. A. Kendrick, O. R. Pizarro, D. A. Smale, and P. D. Steinberg. 2015b. Large-scale geographic variation in distribution and abundance of Australian deep-water kelp forests. *Plos One* **10**:e0118390.
- Mau, R., T. Byrnes, J. Wilson, and L. Zann. 1998. The distribution of selected continental shelf habitats and biotic communities in the Solitary Islands Marine Park. Southern Cross University Report to the NSW Marine Parks Authority. Lismore NSW.
- McArdle, B. H., and M. J. Anderson. 2001. Fitting multivariate models to community data: a comment on distance- based redundancy analysis. *Ecology* **82**:290-297.
- McCauley, D. J., K. A. McLean, J. Bauer, H. S. Young, and F. Micheli. 2012. Evaluating the performance of methods for estimating the abundance of rapidly declining coastal shark populations. *Ecological Applications* **22**:385-392.
- McCook, L., J. Jompa, and G. Diaz-Pulido. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* **19**:400-417.
- McCormick, M. I. 1994. Comparison of field methods for measuring surface topography and their associations with a tropical reef fish assemblage. *Marine ecology progress series*. Oldendorf **112**:87-96.
- McGrath, D. 2001. Inter-algal movement of marked blue-rayed limpets, *Patella pellucida* L., between kelps on the lower shore. *Journal of Molluscan Studies* **67**:398-400.
- McKenzie, J., and P. Moore. 1981. The microdistribution of animals associated with the bulbous holdfasts of *Saccorhiza polyschides* (Phaeophyta). *Ophelia* **20**:201-213.
- Meehl, G. A., F. Zwiers, J. Evans, T. Knutson, L. Mearns, and P. Whetton. 2000. Trends in extreme weather and climate events: issues related to modeling extremes in projections of future climate change. *Bulletin of the American Meteorological Society* **81**:427-436.

- Melville, A., and S. Connell. 2001. Experimental effects of kelp canopies on subtidal coralline algae. *Austral Ecology* **26**:102-108.
- Merzouk, A., and L. E. Johnson. 2011. Kelp distribution in the northwest Atlantic Ocean under a changing climate. *Journal of Experimental Marine Biology and Ecology* **400**:90-98.
- Mezaki, T., and S. Kubota. 2012. Changes of hermatypic coral community in coastal sea area of Kochi, high-latitude, Japan. *Aquabiology* **201**:332-337.
- Millar, A., and G. Kraft. 1994. Catalogue of marine brown algae (Phaeophyta) of New South Wales, including Lord Howe Island, south-western Pacific. *Australian Systematic Botany* **7**:1-47.
- Millar, A. J. 2007. The Flindersian and Peronian Provinces. *Algae of Australia: introduction*:554-559.
- Miller, M. W., and M. E. Hay. 1998. Effects of fish predation and seaweed competition on the survival and growth of corals. *Oecologia* **113**:231-238.
- Moe, R. L., and P. C. Silva. 1977. Antarctic marine flora: uniquely devoid of kelps. *Science* **196**:1206-1208.
- Moksnes, P. O., M. Gullström, K. Tryman, and S. Baden. 2008. Trophic cascades in a temperate seagrass community. *Oikos* **117**:763-777.
- Moore, P. 1972. Particulate matter in the sublittoral zone of an exposed coast and its ecological significance with special reference to the fauna inhabiting kelp holdfasts. *Journal of Experimental Marine Biology and Ecology* **10**:59-80.
- Moore, P. 1973. The kelp fauna of northeast Britain. I. Introduction and the physical environment. *Journal of Experimental Marine Biology and Ecology* **13**:97-125.
- Moran, Z., D. Orth, J. Schmitt, E. Hallerman, and R. Aguilar. 2015. Effectiveness of DNA barcoding for identifying piscine prey items in stomach contents of piscivorous catfishes. *Environmental Biology of Fishes* **99**:161-167.
- Morton, J. K. 2007. The ecology of three species of wrasse (Pisces: Labridae) on temperate rocky reefs of New South Wales, Australia. University of Newcastle.
- Morton, J. K., and W. Gladstone. 2011. Spatial, temporal and ontogenetic variation in the association of fishes (family Labridae) with rocky-reef habitats. *Marine and Freshwater Research* **62**:870-884.
- Morton, J. K., and W. Gladstone. 2014. Changes in rocky reef fish assemblages throughout an estuary with a restricted inlet. *Hydrobiologia* **724**:235-253.
- Morton, J. K., M. E. Platell, and W. Gladstone. 2008. Differences in feeding ecology among three co-occurring species of wrasse (Teleostei: Labridae) on rocky reefs of temperate Australia. *Marine Biology* **154**:577-592.
- Moy, F., H. Christie, H. Steen, and m. authors. 2008. Final Report from the Sugar Kelp Project 2005e2008. SFT Report TA-2467/2008, NIVA Report 5709, 131 pp.(in Norwegian).
- Muñoz, G., and T. Cribb. 2006. Parasite communities and diet of *Coris batuensis* (Pisces: Labridae) from lizard island, Great Barrier Reef. *Memoirs of the Queensland Museum* **52**:191-198.
- Nagelkerken, I., and S. D. Connell. 2015. Global alteration of ocean ecosystem functioning due to increasing human CO<sub>2</sub> emissions. *Proceedings of the National Academy of Sciences* **112**:13272-13277.
- Nagelkerken, I., M. Dorenbosch, W. Verberk, E. C. De La Morinière, and G. Van Der Velde. 2000. Day-night shifts of fishes between shallow-water biotopes of a Caribbean bay, with emphasis on the nocturnal feeding of Haemulidae and Lutjanidae. *Marine Ecology Progress Series* **194**:55-64.
- Nasrolahi, A., S. B. Stratil, K. J. Jacob, and M. Wahl. 2012. A protective coat of microorganisms on macroalgae: inhibitory effects of bacterial biofilms and epibiotic

- microbial assemblages on barnacle attachment. FEMS microbiology ecology **81**:583-595.
- Nimbs, M. J., M. Larkin, T. R. Davis, D. Harasti, R. C. Willan, and S. D. A. Smith. 2016. Southern range extensions for twelve heterobranch sea slugs (Gastropoda: Heterobranchia) on the eastern coast of Australia. Marine Biodiversity Records **9**:27.
- Nimbs, M. J., and S. D. A. Smith. 2016. Welcome strangers: Southern range extensions for seven heterobranch sea slugs (Mollusca: Gastropoda) on the subtropical east Australian coast, a climate change hot spot. Regional Studies in Marine Science **8**, Part 1:27-32.
- Nimbs, M. J., R. C. Willan, and S. D. Smith. 2017. Is Port Stephens, eastern Australia, a global hotspot for biodiversity of Aplysiidae (Gastropoda: Heterobranchia)? Molluscan Research **37**:45-65.
- Nimbs, M. J., R. C. Willan, and S. D. A. Smith. 2015. Range extensions for heterobranch sea slugs (formerly opisthobranch) belonging to the families Diaphanidae, Plakobranchidae and Facelinidae on the eastern coast of Australia. Mar Biodivers Rec **8**.
- Norderhaug, K., H. Christie, J. Fosså, and S. Fredriksen. 2005. Fish-macrofauna interactions in a kelp (*Laminaria hyperborea*) forest. Journal of the Marine Biological Association of the United Kingdom **85**:1279-1286.
- Norderhaug, K., H. Christie, and E. Rinde. 2002. Colonisation of kelp imitations by epiphyte and holdfast fauna; a study of mobility patterns. Marine Biology **141**:965-973.
- Norderhaug, K., S. Fredriksen, and K. Nygaard. 2003. Trophic importance of *Laminaria hyperborea* to kelp forest consumers and the importance of bacterial degradation to food quality. Marine Ecology Progress Series **255**:135-144.
- Norderhaug, K. M., and H. C. Christie. 2009. Sea urchin grazing and kelp re-vegetation in the NE Atlantic. Marine biology research **5**:515-528.
- Norton, T., K. Hiscock, and J. Kitching. 1977. The ecology of lough Ine: XX. The *Laminaria* forest at Carrigathorna. The Journal of Ecology:919-941.
- O'Connor, M. I. 2009. Warming strengthens an herbivore-plant interaction. Ecology **90**:388-398.
- O'hara, T. 2001. Consistency of faunal and floral assemblages within temperate subtidal rocky reef habitats. Marine and Freshwater Research **52**:853-863.
- O'Rorke, R., S. Lavery, and A. Jeffs. 2012. PCR enrichment techniques to identify the diet of predators. Molecular ecology resources **12**:5-17.
- Ojeda, F., and B. Santelices. 1984. Invertebrate communities in holdfasts of the kelp *Macrocystis pyrifera* from southern Chile. Marine ecology progress series. Oldendorf **16**:65-73.
- Pace, M. L., J. J. Cole, S. R. Carpenter, and J. F. Kitchell. 1999. Trophic cascades revealed in diverse ecosystems. Trends in Ecology & Evolution **14**:483-488.
- Page, H. M., A. J. Brooks, M. Kulbicki, R. Galzin, R. J. Miller, D. C. Reed, R. J. Schmitt, S. J. Holbrook, and C. Koenigs. 2013. Stable isotopes reveal trophic relationships and diet of consumers in temperate kelp forest and coral reef ecosystems. Oceanography **26**:180-189.
- Pankhurst, N. W., and P. L. Munday. 2011. Effects of climate change on fish reproduction and early life history stages. Marine and Freshwater Research **62**:1015-1026.
- Paquin, M. M., T. W. Buckley, R. E. Hibshman, and M. F. Canino. 2014. DNA-based identification methods of prey fish from stomach contents of 12 species of eastern North Pacific groundfish. Deep Sea Research Part I: Oceanographic Research Papers **85**:110-117.
- Pawlowski, J. 2000. Introduction to the molecular systematics of foraminifera. Micropaleontology **46**:1-12.

- Pearce, A., and M. Feng. 2007. Observations of warming on the Western Australian continental shelf. *Marine and Freshwater Research* **58**:914-920.
- Pelletier, D., K. Leleu, G. Mou-Tham, N. Guillemot, and P. Chabanet. 2011. Comparison of visual census and high definition video transects for monitoring coral reef fish assemblages. *Fisheries Research* **107**:84-93.
- Phillips, J., and I. Price. 1997. A catalogue of Phaeophyta (brown algae) from Queensland, Australia. *Australian Systematic Botany* **10**:683-721.
- Phillips, J. A. 2001. Marine macroalgal biodiversity hotspots: why is there high species richness and endemism in southern Australian marine benthic flora? *Biodiversity and conservation* **10**:1555-1577.
- Pihl, L., and H. Wennhage. 2002. Structure and diversity of fish assemblages on rocky and soft bottom shores on the Swedish west coast. *Journal of Fish Biology* **61**:148-166.
- Pinnegar, J. K., and N. V. Polunin. 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia* **122**:399-409.
- Poloczanska, E., R. Babcock, A. Butler, A. Hobday, O. Hoegh-Guldberg, T. Kunz, R. Matear, D. Milton, T. Okey, and A. Richardson. 2007. Climate change and Australian marine life. *Oceanography and marine biology* **45**:407.
- Poloczanska, E. S., C. J. Brown, W. J. Sydeman, W. Kiessling, D. S. Schoeman, P. J. Moore, K. Brander, J. F. Bruno, L. B. Buckley, and M. T. Burrows. 2013. Global imprint of climate change on marine life. *Nature Climate Change* **3**:919-925.
- Polunin, N. V. 2008. Aquatic ecosystems: trends and global prospects. Cambridge University Press.
- Pompanon, F., B. E. Deagle, W. O. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* **21**:1931-1950.
- Porzio, L., M. C. Buia, and J. M. Hall-Spencer. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* **400**:278-287.
- Preti, A., C. Soykan, H. Dewar, R. J. D. Wells, N. Spear, and S. Kohin. 2012. Comparative feeding ecology of shortfin mako, blue and thresher sharks in the California Current. *Environmental Biology of Fishes* **95**:127-146.
- Provost, E. J., B. P. Kelaher, S. A. Dworjanyn, B. D. Russell, S. D. Connell, G. Ghedini, B. M. Gillanders, W. Figueira, and M. A. Coleman. 2017. Climate- driven disparities among ecological interactions threaten kelp forest persistence. *Global Change Biology* **23**:353-361.
- Przeslawski, R., S. Ahyong, M. Byrne, G. Woerheide, and P. Hutchings. 2008. Beyond corals and fish: the effects of climate change on noncoral benthic invertebrates of tropical reefs. *Global Change Biology* **14**:2773-2795.
- Randall, J. E. 1967. Food habits of reef fishes of the West Indies. Institute of Marine Sciences, University of Miami.
- Ratnasingham, S., and P. D. Hebert. 2007. BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular ecology notes* **7**:355-364.
- Raybaud, V., G. Beaugrand, E. Goberville, G. Delebecq, C. Destombe, M. Valero, D. Davoult, P. Morin, and F. Gevaert. 2013. Decline in kelp in west Europe and climate. *Plos One* **8**:e66044.
- Reed, D. C., A. Rassweiler, M. H. Carr, K. C. Cavanaugh, D. P. Malone, and D. A. Siegel. 2011. Wave disturbance overwhelms top-down and bottom-up control of primary production in California kelp forests. *Ecology* **92**:2108-2116.
- Ridgeway, K., and K. Hill. 2012. East Australian Current.
- Ridgway, K. R. 2007. Long-term trend and decadal variability of the southward penetration of the East Australian Current. *Geophysical Research Letters* **34**.

- Ríos, C., W. E. Arntz, D. Gerdes, E. Mutschke, and A. Montiel. 2007. Spatial and temporal variability of the benthic assemblages associated to the holdfasts of the kelp *Macrocystis pyrifera* in the Straits of Magellan, Chile. *Polar Biology* **31**:89-100.
- Ripple, W. J., J. A. Estes, O. J. Schmitz, V. Constant, M. J. Kaylor, A. Lenz, J. L. Motley, K. E. Self, D. S. Taylor, and C. Wolf. 2016. What is a trophic cascade? *Trends in Ecology & Evolution* **31**:842-849.
- Rule, M. J., and S. D. Smith. 2007. Depth-associated patterns in the development of benthic assemblages on artificial substrata deployed on shallow, subtropical reefs. *Journal of Experimental Marine Biology and Ecology* **345**:38-51.
- Russell, B. 1983. The food and feeding habits of rocky reef fish of north-eastern New Zealand. *New Zealand Journal of Marine and Freshwater Research* **17**:121-145.
- Russell, B. D., and S. D. Connell. 2009. Eutrophication science: moving into the future. *Trends in Ecology & Evolution* **24**:527-528.
- Russell, B. D., and S. D. Connell. 2012. Origins and consequences of global and local stressors: incorporating climatic and non-climatic phenomena that buffer or accelerate ecological change. *Marine Biology* **159**:2633-2639.
- Russell, B. D. T., Jo-Anne I., L. J. Falkenberg, and S. D. Connell. 2009. Synergistic effects of climate change and local stressors: CO<sub>2</sub> and nutrient- driven change in subtidal rocky habitats. *Global Change Biology* **15**:2153-2162.
- Russell, I., D. Parrott, M. Ives, D. Goldsmith, S. Fox, D. Clifton-Dey, A. Prickett, and T. Drew. 2008. Reducing fish losses to cormorants using artificial fish refuges: an experimental study. *Fisheries Management and Ecology* **15**:189-198.
- Sagarin, R. D., J. P. Barry, S. E. Gilman, and C. H. Baxter. 1999. Climate- related change in an intertidal community over short and long time scales. *Ecological monographs* **69**:465-490.
- Sakaguchi, S. O., S. Shimamura, Y. Shimizu, G. Ogawa, Y. Yamada, K. Shimizu, H. Kasai, H. Kitazato, Y. Fujiwara, K. Fujikura, and K. Takishita. 2017. Comparison of morphological and DNA-based techniques for stomach content analyses in juvenile chum salmon *Oncorhynchus keta*: a case study on diet richness of juvenile fishes. *Fisheries Science* **83**:47-56.
- Sala, E. 1997. Fish predators and scavengers of the sea urchin *Paracentrotus lividus* in protected areas of the north-west Mediterranean Sea. *Marine Biology* **129**:531-539.
- Sala, E., and E. Ballesteros. 1997. Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. *Marine Ecology Progress Series*:273-283.
- Sala, E., C. Boudouresque, and M. Harmelin-Vivien. 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. *Oikos*:425-439.
- Sala, E., and P. K. Dayton. 2011. Predicting strong community impacts using experimental estimates of per capita interaction strength: benthic herbivores and giant kelp recruitment. *Marine Ecology* **32**:300-312.
- Salter, Z. T. H. E. S. K. G. A. M. K. 2010. The effect of kelp bed disturbance on the abundance and feeding behaviour of fishes on high-relief reefs. *Marine & Freshwater Behaviour & Physiology* **43**:109-125.
- Sanford, E. 1999. Regulation of keystone predation by small changes in ocean temperature. *Science* **283**:2095-2097.
- Sayer, M., R. Gibson, and R. Atkinson. 1996. Growth, diet and condition of corkwing wrasse and rock cook on the west coast of Scotland. *Journal of Fish Biology* **49**:76-94.
- Sazima, C., R. M. Bonaldo, J. P. Krajewski, and I. Sazima. 2005. The Noronha wrasse: a jack-of-all-trades follower. *Aqua, Journal of Ichthyology and Aquatic Biology* **9**:97-108.

- Scott, A., D. Harasti, T. Davis, and S. D. Smith. 2015. Southernmost records of the host sea anemone, *Stichodactyla haddoni*, and associated commensal shrimps in a climate change hotspot. *Marine Biodiversity* **45**:145-146.
- Schaal, G., P. Riera, and C. Leroux. 2010. Trophic ecology in a Northern Brittany (Batz Island, France) kelp (*Laminaria digitata*) forest, as investigated through stable isotopes and chemical assays. *Journal of Sea Research* **63**:24-35.
- Schaal, G., P. Riera, and C. Leroux. 2012. Food web structure within kelp holdfasts (*Laminaria*): a stable isotope study. *Marine Ecology* **33**:370-376.
- Scheibling, R. E., A. W. Hennigar, and T. Balch. 1999. Destructive grazing, epiphytism, and disease: the dynamics of sea urchin-kelp interactions in Nova Scotia. *Canadian Journal of Fisheries and Aquatic Sciences* **56**:2300-2314.
- Schiel, D. 1994. Kelp communities. *Marine Biology*:345-361.
- Schiel, D. R. 2011. Biogeographic patterns and long-term changes on New Zealand coastal reefs: Non-trophic cascades from diffuse and local impacts. *Journal of Experimental Marine Biology and Ecology* **400**:33-51.
- Schiel, D. R., and M. S. Foster. 1986. The structure of subtidal algal stands in temperate waters. *Oceanography and Marine Biology Annual Review* **24**:265-307.
- Schiel, D. R., J. R. Steinbeck, and M. S. Foster. 2004. Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology* **85**:1833-1839.
- Schooley, J. D., A. P. Karam, B. R. Kesner, P. C. Marsh, C. A. Pacey, and D. J. Thornbrugh. 2008. Detection of larval remains after consumption by fishes. *Transactions of the American Fisheries Society* **137**:1044-1049.
- Sen Gupta, A., J. Brown, N. Jourdain, E. van Sebille, A. Ganachaud, and A. Vergés. 2013. Episodic and non-uniform shifts of thermal habitats in a warming ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*.
- Serisawa, Y., Z. Imoto, T. Ishikawa, and M. Ohno. 2004. Decline of the *Ecklonia cava* population associated with increased seawater temperatures in Tosa Bay, southern Japan. *Fisheries Science* **70**:189-191.
- Shears, N. T., and R. C. Babcock. 2002. Marine reserves demonstrate top-down control of community structure on temperate reefs. *Oecologia* **132**:131-142.
- Shears, N. T., and R. C. Babcock. 2003. Continuing trophic cascade effects after 25 years of no-take marine reserve protection. *Marine Ecology Progress Series* **246**:1-16.
- Shepherd, S. 2006. Ontogenetic changes in diet, feeding behaviour and activity of the western blue groper, *Achoerodus gouldii*. *The Marine Fauna and Flora of Esperance, Western Australia* **2**:477-494.
- Shepherd, S., and J. Brook. 2005. Foraging ecology of the western blue groper, *Achoerodus gouldii*, at the Althorpe Islands, South Australia. *Transactions of the Royal Society of South Australia* **129**:202-208.
- Shepherd, S., and P. Clarkson. 2001. Diet, feeding behaviour, activity and predation of the temperate blue-throated wrasse, *Notolabrus tetricus*. *Marine and Freshwater Research* **52**:311-322.
- Sheppard, S., and J. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. *Functional Ecology* **19**:751-762.
- Sivertsen, K. 1997. Geographic and environmental factors affecting the distribution of kelp beds and barren grounds and changes in biota associated with kelp reduction at sites along the Norwegian coast. *Canadian Journal of Fisheries and Aquatic Sciences* **54**:2872-2887.
- Smale, D. A., M. T. Burrows, P. Moore, N. O'Connor, and S. J. Hawkins. 2013. Threats and knowledge gaps for ecosystem services provided by kelp forests: a northeast Atlantic perspective. *Ecology and evolution* **3**:4016-4038.

- Smale, D. A., G. A. Kendrick, K. I. Waddington, K. P. Van Niel, J. J. Meeuwig, and E. S. Harvey. 2010. Benthic assemblage composition on subtidal reefs along a latitudinal gradient in Western Australia. *Estuarine, Coastal and Shelf Science* **86**:83-92.
- Smale, D. A., and T. Vance. 2016. Climate-driven shifts in species' distributions may exacerbate the impacts of storm disturbances on North-east Atlantic kelp forests. *Marine and Freshwater Research* **67**:65-74.
- Smale, D. A., and T. Wernberg. 2013. Extreme climatic event drives range contraction of a habitat-forming species. *Proceedings of the Royal Society B: Biological Sciences* **280**:20122829.
- Smith, M. L., J. Bell, and C. Hair. 1991. Spatial variation in abundance of recently settled rocky reef fish in southeastern Australia: implications for detecting change. *Marine Ecology Progress Series*:95-103.
- Smith, P. J., S. M. McVeagh, V. Allain, and C. Sanchez. 2005. DNA identification of gut contents of large pelagic fishes. *Journal of Fish Biology* **67**:1178-1183.
- Smith, S. A. 2000. Evaluating stress in rocky shore and shallow reef habitats using the macrofauna of kelp holdfasts. *Journal of Aquatic Ecosystem Stress and Recovery* **7**:259-272.
- Smith, S. D., S. J. Dalton, R. J. Edgar, A. L. Schultz, and I. V. Shaw. 2011. A long-term program to monitor the health of nearshore reefs: report cards for 2011. Report to the Northern Rivers Catchment Management Authority June.
- Smith, S. D., M. J. Rule, M. Harrison, and S. J. Dalton. 2008. Monitoring the sea change: preliminary assessment of the conservation value of nearshore reefs, and existing impacts, in a high-growth, coastal region of subtropical eastern Australia. *Mar Pollut Bull* **56**:525-534.
- Smith, S. D., R. D. Simpson, and S. C. Cairns. 1996a. The macrofaunal community of *Ecklonia radiata* holdfasts: description of the faunal assemblage and variation associated with differences in holdfast volume. *Australian Journal of Ecology* **21**:81-95.
- Smith, S. D. A. 1996. The macrofaunal community of *Ecklonia radiata* holdfasts: Variation associated with sediment regime, sponge cover and depth. *Australian Journal of Ecology* **21**:144-153.
- Smith, S. D. A., and M. J. Rule. 2001. The Effects of Dredge-Spoil Dumping on a Shallow Water Soft-Sediment Community in the Solitary Islands Marine Park, NSW, Australia. *Mar Pollut Bull* **42**:1040-1048.
- Smith, S. D. A., R. D. Simpson, and S. C. Cairns. 1996b. The macrofaunal community of *Ecklonia radiata* holdfasts: Description of the faunal assemblage and variation associated with differences in holdfast volume. *Australian Journal of Ecology* **21**:81-95.
- Staehr, P. A., and T. Wernberg. 2009. Physiological responses of *ecklonia radiata* (laminariales) to a latitudinal gradient in ocean temperature. *Journal of Phycology* **45**:91-99.
- Steneck, R., and C. Johnson. 2013. Kelp forests: dynamic patterns, processes, and feedbacks. In 'Marine Community Ecology'.(Eds MD Bertness, J. Bruno, BR Silliman and JJ Stachowicz.) pp. 315–336. Sinauer Associates: Sunderland, MA.
- Steneck, R. S. 1998. Human influences on coastal ecosystems: does overfishing create trophic cascades? *Trends in Ecology & Evolution* **13**:429-430.
- Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* **29**:436-459.
- Steneck, R. S., J. Vavrinec, and A. V. Leland. 2004. Accelerating trophic-level dysfunction in kelp forest ecosystems of the western North Atlantic. *Ecosystems* **7**:323-332.

- Stocker, T. F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley. 2013. Climate Change 2013. The Physical Science Basis. Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change-Abstract for decision-makers. Groupe d'experts intergouvernemental sur l'évolution du climat/Intergovernmental Panel on Climate Change-IPCC, C/O World Meteorological Organization, 7bis Avenue de la Paix, CP 2300 CH-1211 Geneva 2 (Switzerland).
- Syahailatua, A., M. Roughan, and I. M. Suthers. 2011. Characteristic ichthyoplankton taxa in the separation zone of the East Australian Current: larval assemblages as tracers of coastal mixing. Deep Sea Research Part II: Topical Studies in Oceanography **58**:678-690.
- Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol Ecol **21**:2045-2050.
- Teagle, H., S. J. Hawkins, P. J. Moore, and D. A. Smale. 2017. The role of kelp species as biogenic habitat formers in coastal marine ecosystems. Journal of Experimental Marine Biology and Ecology.
- Tegner, M., and P. Dayton. 2000. Ecosystem effects of fishing in kelp forest communities. ICES Journal of Marine Science: Journal du Conseil **57**:579-589.
- Thiel, M., and J. A. Vásquez. 2000. Are kelp holdfasts islands on the ocean floor?—Indication for temporarily closed aggregations of peracarid crustaceans. Pages 45-54 Island, Ocean and Deep-Sea Biology. Springer.
- Thomsen, M. S., T. Wernberg, and G. A. Kendrick. 2004. The effect of thallus size, life stage, aggregation, wave exposure and substratum conditions on the forces required to break or dislodge the small kelp *Ecklonia radiata*. Botanica Marina **47**:454-460.
- Toohey, B. D. 2007. The relationship between physical variables on topographically simple and complex reefs and algal assemblage structure beneath an *Ecklonia radiata* canopy. Estuarine, Coastal and Shelf Science **71**:232-240.
- Troell, M., D. Robertson-Andersson, R. J. Anderson, J. J. Bolton, G. Maneveldt, C. Halling, and T. Probyn. 2006. Abalone farming in South Africa: an overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture **257**:266-281.
- Tuya, F., L. Png-Gonzalez, R. Riera, R. Haroun, and F. Espino. 2014. Ecological structure and function differs between habitats dominated by seagrasses and green seaweeds. Marine environmental research **98**:1-13.
- Tuya, F., T. Wernberg, and M. S. Thomsen. 2008. The spatial arrangement of reefs alters the ecological patterns of fauna between interspersed algal habitats. Estuarine, Coastal and Shelf Science **78**:774-782.
- Tuya, F., T. Wernberg, and M. S. Thomsen. 2009. Habitat structure affect abundances of labrid fishes across temperate reefs in south-western Australia. Environmental Biology of Fishes **86**:311-319.
- Underwood, A., M. Chapman, and S. Connell. 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. Journal of Experimental Marine Biology and Ecology **250**:97-115.
- Underwood, A., M. Kingsford, and N. Andrew. 1991. Patterns in shallow subtidal marine assemblages along the coast of New South Wales. Australian Journal of Ecology **16**:231-249.
- Vahl, O. 1983. Mucus drifting in the limpet *Helcion* (= *Patina*) *pellucidus* (Prosobranchia, Patellidae). Sarsia **68**:209-211.
- Valdez-Moreno, M., C. Quintal-Lizama, R. Gomez-Lozano, and M. D. Garcia-Rivas. 2012. Monitoring an Alien Invasion: DNA Barcoding and the Identification of Lionfish and Their Prey on Coral Reefs of the Mexican Caribbean. Plos One **7**.

- Valentine, J. P., and C. R. Johnson. 2005. Persistence of sea urchin (*Heliocidaris erythrogramma*) barrens on the east coast of Tasmania: inhibition of macroalgal recovery in the absence of high densities of sea urchins. *Botanica Marina* **48**:106-115.
- Valentini, A., F. Pompanon, and P. Taberlet. 2009. DNA barcoding for ecologists. *Trends in Ecology & Evolution* **24**:110-117.
- van Lier, J. R., D. Harasti, R. Laird, M. M. Noble, and C. J. Fulton. 2017. Importance of soft canopy structure for labrid fish communities in estuarine mesohabitats. *Marine Biology* **164**:45.
- Vanderklift, M. A., and T. Wernberg. 2010. Stable isotopes reveal a consistent consumer-diet relationship across hundreds of kilometres. *Marine Ecology Progress Series* **403**:53-61.
- Vásquez, J. 2009. Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. Pages 7-17 in M. Borowitzka, A. Critchley, S. Kraan, A. Peters, K. Sjøtun, and M. Notoya, editors. Nineteenth International Seaweed Symposium. Springer Netherlands.
- Vásquez, J. A. 2008. Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery. *Journal of Applied Phycology* **20**:457.
- Vea, J., and E. Ask. 2011. Creating a sustainable commercial harvest of *Laminaria hyperborea*, in Norway. *Journal of Applied Phycology* **23**:489-494.
- Vega, J. M. A., J. A. Vasquez, and A. H. Buschmann. 2005. Population biology of the subtidal kelps *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales, Phaeophyceae) in an upwelling ecosystem of Northern Chile: Interannual variability and El Niño 1997-1998. *Revista Chilena De Historia Natural* **78**:33-50.
- Vergés, A., C. Doropoulos, H. A. Malcolm, M. Skye, M. Garcia-Pizá, E. M. Marzinelli, A. H. Campbell, E. Ballesteros, A. S. Hoey, and A. Vila-Concejo. 2016. Long-term empirical evidence of ocean warming leading to tropicalization of fish communities, increased herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences*:201610725.
- Vergés, A., P. D. Steinberg, M. E. Hay, A. G. B. Poore, A. H. Campbell, E. Ballesteros, K. L. Heck, Jr., D. J. Booth, M. A. Coleman, D. A. Feary, W. Figueira, T. Langlois, E. M. Marzinelli, T. Mizerek, P. J. Mumby, Y. Nakamura, M. Roughan, E. van Sebille, A. Sen Gupta, D. A. Smale, F. Tomas, T. Wernberg, and S. K. Wilson. 2014. The tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and community phase shifts. *Proceedings of the Royal Society B-Biological Sciences* **281**.
- Vergés, A., F. Tomas, E. Cebrian, E. Ballesteros, Z. Kizilkaya, P. Dendrinos, A. A. Karamanlidis, D. Spiegel, and E. Sala. 2014. Tropical rabbitfish and the deforestation of a warming temperate sea. *Journal of Ecology*.
- Vestheim, H., and S. N. Jarman. 2008. Blocking primers to enhance PCR amplification of rare sequences in mixed samples—a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology* **5**:12.
- Villegas, M. J., J. Laudien, W. Sielfeld, and W. E. Arntz. 2008. *Macrocystis integrifolia* and *Lessonia trabeculata* (Laminariales; Phaeophyceae) kelp habitat structures and associated macrobenthic community off northern Chile. *Helgoland Marine Research* **62**:33-43.
- Vizzini, S., and A. Mazzola. 2009. Stable isotopes and trophic positions of littoral fishes from a Mediterranean marine protected area. *Environmental Biology of Fishes* **84**:13-25.
- Voerman, S. E., E. Llera, and J. M. Rico. 2013. Climate driven changes in subtidal kelp forest communities in NW Spain. *Marine environmental research* **90**:119-127.
- Wägele, H., A. Klussmann-Kolb, V. Vonnemann, and M. Medina. 2008. Heterobranchia I: the opisthobranchia. *Phylogeny and Evolution of the Mollusca*:385-408.

- Wahl, M., M. Molis, A. J. Hobday, S. Dudgeon, R. Neumann, P. Steinberg, A. H. Campbell, E. Marzinelli, and S. Connell. 2015. The responses of brown macroalgae to environmental change from local to global scales: direct versus ecologically mediated effects. *Perspectives in Phycology* 2:11-29.
- Wainwright, P. C. 1988. Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. *Ecology* 69:635-645.
- Wańkowski, J., and J. Thorpe. 1979. Spatial distribution and feeding in Atlantic salmon, *Salmo salar* L. juveniles. *Journal of Fish Biology* 14:239-247.
- Warner, R. R., S. E. Swearer, and J. E. Caselle. 2000. Larval accumulation and retention: implications for the design of marine reserves and essential habitat. *Bulletin of Marine Science* 66:821-830.
- Watson, D. L., E. S. Harvey, M. J. Anderson, and G. A. Kendrick. 2005. A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. *Marine Biology* 148:415-425.
- Watson, J. V., S. H. Chambers, and P. J. Smith. 1987. A pragmatic approach to the analysis of DNA histograms with a definable G1 peak. *Cytometry* 8:1-8.
- Wernberg, T. 2009. Spatial variation in juvenile and adult *Ecklonia radiata* (Laminariales) sporophytes. *Aquatic Botany* 90:93-95.
- Wernberg, T., S. Bennett, R. C. Babcock, T. de Bettignies, K. Cure, M. Depczynski, F. Dufois, J. Fromont, C. J. Fulton, and R. K. Hovey. 2016. Climate-driven regime shift of a temperate marine ecosystem. *Science* 353:169-172.
- Wernberg, T., and N. Goldberg. 2008. Short-term temporal dynamics of algal species in a subtidal kelp bed in relation to changes in environmental conditions and canopy biomass. *Estuarine, Coastal and Shelf Science* 76:265-272.
- Wernberg, T., G. A. Kendrick, and B. D. Toohey. 2005. Modification of the physical environment by an *Ecklonia radiata* (Laminariales) canopy and implications for associated foliose algae. *Aquatic Ecology* 39:419-430.
- Wernberg, T., B. D. Russell, P. J. Moore, S. D. Ling, D. A. Smale, A. Campbell, M. A. Coleman, P. D. Steinberg, G. A. Kendrick, and S. D. Connell. 2011a. Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology and Ecology* 400:7-16.
- Wernberg, T., Bayden D. Russell, Mads S. Thomsen, C. Frederico D. Gurgel, Corey J. A. Bradshaw, Elvira S. Poloczanska, and Sean D. Connell. 2011b. Seaweed Communities in Retreat from Ocean Warming. *Current Biology* 21:1828-1832.
- Wernberg, T., D. A. Smale, F. Tuya, M. S. Thomsen, T. J. Langlois, T. de Bettignies, S. Bennett, and C. S. Rousseaux. 2013. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nature Climate Change* 3:78-82.
- Wernberg, T., D. A. Smale, A. Vergés, A. H. Campbell, B. D. Russell, M. A. Coleman, S. D. Ling, P. D. Steinberg, C. R. Johnson, and G. A. Kendrick. 2012. Macroalgae and temperate rocky reefs.
- Wernberg, T., and M. S. Thomsen. 2005. The effect of wave exposure on the morphology of *Ecklonia radiata*. *Aquatic Botany* 83:61-70.
- Wernberg, T., M. S. Thomsen, F. Tuya, and G. A. Kendrick. 2011c. Biogenic habitat structure of seaweeds change along a latitudinal gradient in ocean temperature. *Journal of Experimental Marine Biology and Ecology* 400:264-271.
- Wernberg, T., M. S. Thomsen, F. Tuya, G. A. Kendrick, P. A. Staehr, and B. D. Toohey. 2010. Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. *Ecology Letters* 13:685-694.
- Westneat, M. 2001. Labridae. Wrasses, hogfishes, razorfishes, corises, tuskfishes. FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific 6:3381-3467.

- Willis, T. J., and M. J. Anderson. 2003. Structure of cryptic reef fish assemblages: relationships with habitat characteristics and predator density. *Marine Ecology Progress Series* **257**:209-221.
- Wolf, S. G., M. A. Snyder, W. J. Sydeman, D. F. Doak, and D. A. Croll. 2010. Predicting population consequences of ocean climate change for an ecosystem sentinel, the seabird Cassin's auklet. *Global Change Biology* **16**:1923-1935.
- Worm, B., and H. K. Lotze. 2006. Effects of eutrophication, grazing, and algal blooms on rocky shores. *Limnology and Oceanography* **51**:569-579.
- Wright, J. T., S. A. Dworjanyn, C. N. Rogers, P. D. Steinberg, J. E. Williamson, and A. G. Poore. 2005. Density-dependent sea urchin grazing: differential removal of species, changes in community composition and alternative community states. *Marine Ecology Progress Series* **298**:143-156.
- Wu, L., W. Cai, L. Zhang, H. Nakamura, A. Timmermann, T. Joyce, M. J. McPhaden, M. Alexander, B. Qiu, and M. Visbeck. 2012. Enhanced warming over the global subtropical western boundary currents. *Nature Climate Change* **2**:161-166.
- Würzberg, L., J. Peters, H. Flores, and A. Brandt. 2011. Demersal fishes from the Antarctic shelf and deep sea: A diet study based on fatty acid patterns and gut content analyses. *Deep-sea research. Part 2. Topical studies in oceanography* **58**:2036-2042.
- Wyatt, A., A. Waite, and S. Humphries. 2012. Stable isotope analysis reveals community-level variation in fish trophodynamics across a fringing coral reef. *Coral Reefs* **31**:1029-1044.
- Yoccoz, N. G. 2012. The future of environmental DNA in ecology. *Mol Ecol* **21**:2031-2038.
- Yorke, C. E., R. J. Miller, H. M. Page, and D. C. Reed. 2013. Importance of kelp detritus as a component of suspended particulate organic matter in giant kelp *Macrocystis pyrifera* forests. *Marine Ecology Progress Series* **493**:113-125.
- Young, I., S. Zieger, and A. Babanin. 2011. Global trends in wind speed and wave height. *Science* **332**:451-455.
- Zarnetske, P. L., D. K. Skelly, and M. C. Urban. 2012. Biotic multipliers of climate change. *Science* **336**:1516-1518.

# Appendices

## Appendix 1 Sampling Effort

	Area m <sup>2</sup>	Duration of survey on the area	Time needed per replica to transfer the data from the source to a spreadsheet	People Needed to Complete	Preparation Time (level of expertise in fish ID) 1-10	Total Nº species	Total Nº families	Unique families (found only with this method)
10 min SPC	27 m <sup>2</sup>	10 min	10 min	3-4	9-10	42	19	Weedfish (Clinidae), flathead (Platycephalidae), sting ray (Dasyatidae)
25 m Transects	125 m <sup>2</sup>	20-25 min	10 min	3-4	9-10	89	38	Kelpfish (Chironemidae), blennies (Blennidae), moray eels (Muraenidae), roughies (Trachichthyidae), cardinalfish (Apogonidae), anglefish (Pomacanthidae), boxfish (Acanthidae), cobia (Rachycentridae), rabbitfish (Siganidae), pufferfish (Tetradontidae), sergeant Baker (Aulopidae), flute-mouth (Fistulariidae), stingaree (Urolophidae)
KelpCam	27 m <sup>2</sup>	50 min	2 h	1-2	2-5	84	19	Tropical snapper (Lutjanidae), porcupinefish (Diodontidae), garfish (Hemiraphidae), numb ray (Narkidae), boxfish (Ostraciidae)

## Appendix 2 Benthic Cover

	MB JU14	MB AU14	MB JU15	WR JU14	WR AU14	WR JU15	NR JU14	NR AU14	NR JU15
<b>CORALS</b>									
Coral branching	1.33%	0.33%	1.92%	0.92%	0.08%	1.33%	0.42%	0.83%	1.17%
Acroporidae encrusting	1.58%	2.42%	2.00%	1.92%	0.42%	3.33%	0.75%	0.84%	1.00%
Corals plate	1.75%	0.58%	3.25%	2.92%	0.75%	1.75%	0.17%	1.92%	0.75%
Faviidae	0.42%	0.00%	1.67%	0.67%	0.08%	0.92%	0.00%	1.33%	0.00%
Other corals encrusting	0.17%	0.17%	0.00%	0.66%	0.00%	0.00%	0.25%	0.08%	0.08%
Soft coral encrusting	0.08%	0.17%	1.17%	0.33%	0.08%	0.42%	0.25%	0.00%	0.00%
Soft coral massive	1.50%	0.00%	1.00%	1.17%	0.08%	1.08%	0.00%	0.17%	0.25%
<b>RED ALGAE</b>									
<i>Amphiroa anceps</i>	0.67%	4.25%	5.83%	3.08%	5.67%	0.17%	0.25%	1.92%	3.08%
Other filamentous red algae	0.08%	0.08%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
<i>Peysonnelia</i>	0.00%	0.25%	2.17%	2.75%	1.83%	0.17%	0.50%	1.17%	0.25%
<i>Kallymenia</i>	0.17%	0.67%	0.00%	0.42%	0.83%	0.58%	0.08%	0.42%	0.00%
<i>Delisea</i>	1.33%	1.33%	1.83%	0.33%	0.08%	0.08%	1.33%	0.00%	0.25%
<i>Guiryella</i>	0.00%	0.08%	1.08%	0.08%	0.50%	0.00%	0.00%	0.17%	0.08%
<i>Corallina berteri</i>	7.00%	3.58%	27.33%	30.42%	17.50%	2.00%	26.58%	10.75%	18.50%
<i>Tricleocarpa</i>	0.25%	1.75%	0.00%	0.00%	3.42%	0.17%	0.00%	0.08%	0.00%
<i>Phymatolithon</i>	1.83%	2.92%	12.50%	3.00%	6.92%	7.75%	2.00%	5.17%	6.25%
<i>Sarconema</i>	0.33%	4.00%	0.08%	0.08%	1.25%	0.08%	0.00%	1.33%	0.00%
<b>GREEN ALGAE</b>									
<i>Caulerpa racemosa</i>	0.08%	1.33%	2.17%	0.75%	0.00%	0.00%	2.67%	6.33%	2.92%
<i>Caulerpa spp.</i>	0.67%	3.08%	0.75%	1.08%	0.42%	0.17%	2.25%	1.00%	3.42%
<i>Codium spp.</i>	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.08%	0.00%	0.08%
<i>Halimeda</i>	1.00%	0.33%	0.08%	1.58%	0.17%	0.50%	0.08%	0.08%	0.17%
<b>BROWN ALGAE</b>									
<i>Dictyotaceae</i>	2.08%	4.67%	1.83%	10.50%	5.67%	0.67%	3.33%	8.17%	6.00%
<i>Ecklonia radiata</i>	25.33%	15.75%	8.58%	15.08%	24.17%	5.67%	32.75%	7.25%	13.92%
<i>Sargassum</i>	0.83%	1.25%	0.83%	4.25%	2.25%	0.08%	11.00%	13.67%	5.92%
Turf algae	21.00%	22.42%	5.75%	5.75%	7.33%	35.83%	2.00%	12.00%	3.75%
<b>OTHER INVERTEBRATES</b>									
Ascidian colonial	0.25%	0.75%	0.08%	0.33%	0.75%	0.92%	0.00%	0.00%	0.00%
Ascidian solitary	2.67%	1.42%	4.50%	1.67%	0.50%	3.67%	1.42%	1.33%	4.75%
Asteroid	0.00%	0.08%	0.00%	0.00%	0.00%	0.08%	0.00%	0.00%	0.08%
Bryozoan	0.08%	0.00%	0.00%	0.00%	0.00%	0.33%	0.00%	0.00%	0.00%
Crinoid	0.17%	0.17%	0.00%	0.00%	0.00%	0.17%	0.00%	0.00%	0.00%
Echinoid	0.25%	0.17%	0.00%	0.00%	0.00%	0.17%	0.00%	0.00%	0.00%
Hydroid	0.00%	0.08%	0.08%	0.00%	0.00%	0.50%	0.00%	0.00%	0.08%
Sponge encrusting	5.92%	3.17%	1.58%	0.42%	1.42%	8.92%	0.00%	0.67%	0.50%
Sponge submassive	8.33%	4.00%	2.08%	2.75%	0.83%	7.33%	0.50%	0.25%	1.00%
Tube worm	0.08%	0.08%	0.17%	0.00%	0.17%	0.33%	0.00%	0.00%	0.00%
<b>BENTHOS TYPE</b>									
Bare rock	0.25%	0.83%	4.08%	2.33%	4.42%	1.50%	0.50%	4.92%	5.50%
Rubble	3.08%	4.25%	3.42%	2.58%	3.33%	3.92%	7.25%	12.00%	18.25%
Sand	9.33%	13.50%	2.17%	2.17%	9.08%	9.33%	3.58%	6.08%	1.83%

### Appendix 3 80% and 95% Hits

	Hits >80% Identity	Relative abundance
<b>Ngym1a</b>		
Arthropoda. Malacostraca. Decapoda	1	100%
<b>Ngym1b</b>		
Annelida. Polychaeta. Phyllodocida	65	34.21%
Arthropoda. Malacostraca. Decapoda	53	27.89%
Arthropoda. Insecta. Lepidoptera	28	14.74%
Arthropoda. Insecta. Diptera	14	7.37%
Arthropoda. Malacostraca. Isopoda	8	4.21%
Arthropoda. Insecta. Trichoptera	7	3.68%
Echinodermata. Ophiuroidea. Ophiurida	6	3.16%
Nematoda. Secernentea. Spirurida	3	1.58%
Mollusca. Gastropoda. Neogastropoda	2	1.05%
Arthropoda. Collembola. Entomobryomorpha	1	0.53%
Arthropoda. Maxillopoda. Sessilia	1	0.53%
Ascomycota. Leotiomycetes	1	0.53%
Nematoda. Secernentea. Tylenchida	1	0.53%
<b>Ngym2a</b>		
Arthropoda. Malacostraca. Decapoda	48	88.89%
Mollusca. Gastropoda. Archaeogastropoda	2	3.70%
Ascomycota. Leotiomycetes	2	3.70%
Mollusca. Polyplacophora. Chitonida	1	1.85%
Echinodermata. Ophiuroidea. Ophiurida	1	1.85%
<b>Ngym2b</b>		
Arthropoda. Malacostraca. Decapoda	330	81.68%
Arthropoda. Maxillopoda. Harpacticoida	28	6.93%
Echinodermata. Ophiuroidea. Ophiurida	15	3.71%
Mollusca. Gastropoda. Archaeogastropoda	13	3.22%
Mollusca. Gastropoda. Vetigastropoda	8	1.98%
Arthropoda. Insecta. Diptera	3	0.74%
Arthropoda. Maxillopoda. Calanoida	2	0.50%
Chordata. Actinopterygii. Perciformes	1	0.25%
Bryozoa. Gymnolaemata. Cheilostomatida	1	0.25%
Ascomycota. Leotiomycetes	1	0.25%
Arthropoda. Malacostraca. Amphipoda	1	0.25%
Ochrophyta. Phaeophyceae. Fucales	1	0.25%
<b>Ngym3</b>		
Arthropoda. Malacostraca. Decapoda	11174	47.10%
Annelida. Polychaeta. Phyllodocida	6464	27.25%
Arthropoda. Maxillopoda. Sessilia	4766	20.09%
Arthropoda. Insecta. Trichoptera	828	3.49%
Annelida. Clitellata. Haplotauxida	107	0.45%
Arthropoda. Collembola. Entomobryomorpha	77	0.32%
Arthropoda. Malacostraca. Amphipoda	75	0.32%
Ochrophyta. Phaeophyceae. Ectocarpales	65	0.27%
Arthropoda. Insecta. Ephemeroptera	41	0.17%

Bryozoa. Gymnolaemata. Cheilostomatida	23	0.10%
Annelida. Polychaeta. Terebellida	21	0.09%
Rhodophyta. Florideophyceae. Corallinales	12	0.05%
Nemertea (order unknown)	7	0.03%
Arthropoda. Insecta. Lepidoptera	7	0.03%
Rhodophyta. Florideophyceae. Gigartinales	7	0.03%
Rhodophyta. Florideophyceae. Peyssonneliales	7	0.03%
Ochrophyta. Phaeophyceae. Laminariales	5	0.02%
Mollusca. Gastropoda. Littorinimorpha	4	0.02%
Mollusca. Gastropoda. Archaeogastropoda	3	0.01%
Cnidaria. Hydrozoa. Anthoathecata	3	0.01%
Nemertea. Enopla. Monostilifera.	3	0.01%
Rhodophyta. Florideophyceae. Ceramiales	3	0.01%
Arthropoda. Collembola. Poduromorpha	2	0.01%
Arthropoda. Insecta. Diptera	2	0.01%
Ascomycota. Dothideomycetes. Capnodiales.	2	0.01%
Ascomycota. Leotiomycetes	2	0.01%
Mollusca. Gastropoda. Vetigastropoda	2	0.01%
Mollusca. Gastropoda. Sorbeoconcha	2	0.01%
Rhodophyta. Florideophyceae. Colaconematales	2	0.01%
Arthropoda. Brachipoda. Diplostraca	1	0.00%
Arthropoda. Insecta. Coleoptera	1	0.00%
Chordata. Actinopterygii. Blenniiformes	1	0.00%
Echinodermata. Holothuroidea. Dendrochirotida	1	0.00%
Echinodermata. Ophiuroidea. Ophiurida	1	0.00%
Ochrophyta. Phaeophyceae. Fucales	1	0.00%
Ochrophyta. Phaeophyceae. Sphacelariales	1	0.00%
Rhodophyta. Florideophyceae. Sporolithales	1	0.00%

#### Ngym4

Arthropoda. Malacostraca. Decapoda	79	50.97%
Mollusca. Gastropoda. Archaeogastropoda	26	16.77%
Annelida. Polychaeta. Eunicida	23	14.84%
Arthropoda. Insecta. Ephemeroptera	6	3.87%
Ascomycota. Dothideomycetes. Capnodiales	3	1.94%
Echinodermata. Ophiuroidea. Ophiurida	3	1.94%
Mollusca. Gastropoda. Sorbeoconcha	3	1.94%
Annelida. Polychaeta. Phyllodocida	2	1.29%
Arthropoda. Insecta. Diptera	2	1.29%
Rhodophyta. Florideophyceae. Rhodymeniales	2	1.29%
Chordata. Actinopterygii. Blenniiformes	1	0.65%
Arthropoda. Insecta. Lepidoptera	1	0.65%
Ascomycota. Leotiomycetes	1	0.65%
Ascomycota. Sordariomycetes	1	0.65%
Chordata. Actinopterygii. Perciformes	1	0.65%
Mollusca. Gastropoda. Vetigastropoda	1	0.65%

#### Ngym5

Mollusca. Gastropoda. Archaeogastropoda	677	82.26%
Arthropoda. Malacostraca. Decapoda	50	6.08%

Annelida. Polychaeta. Eunicida	36	4.37%
Arthropoda. Maxillopoda. Sessilia	32	3.89%
Arthropoda. Insecta. Ephemeroptera	24	2.92%
Annelida. Polychaeta. Phyllodocida	2	0.24%
Chordata. Actinopterygii. Blenniiformes	1	0.12%
Arthropoda. Insecta. Lepidoptera	1	0.12%
Olin1		
Mollusca. Gastropoda. Sacoglossa	3779	63.49%
Chordata. Actinopterygii. Blenniiformes	1261	21.19%
Mollusca. Gastropoda. Cephalaspidea	516	8.67%
Arthropoda. Malacostraca. Amphipoda	138	2.32%
Mollusca. Gastropoda. Pulmonata	76	1.28%
Mollusca. Gastropoda. Archaeogastropoda	67	1.13%
Arthropoda. Collembola. Entomobryomorpha	19	0.32%
Bryozoa. Gymnolaemata. Cheilostomatida	16	0.27%
Arthropoda. Insecta. Lepidoptera	15	0.25%
Annelida. Polychaeta. Phyllodocida	15	0.25%
Arthropoda. Insecta. Diptera	13	0.22%
Annelida. Clitellata. Haplotaxida	10	0.17%
Arthropoda. Insecta. Orthoptera	5	0.08%
Chordata. Actinopterygii. Perciformes	4	0.07%
Arthropoda. Insecta. Ephemeroptera	4	0.07%
Rhodophyta. Florideophyceae. Rhodymeniales	2	0.03%
Ascomycota. Dothideomycetes. Capnodiales	2	0.03%
Ascomycota. Leotiomycetes	2	0.03%
Arthropoda. Insecta. Trichoptera	2	0.03%
Arthropoda. Insecta. Hemiptera	2	0.03%
Arthropoda. Malacostraca. Decapoda	1	0.02%
Mollusca. Polyplacophora	1	0.02%
Heterokontophyta. Oomycota. Pythiales	1	0.02%
Ochrophyta. Phaeophyceae. Fucales	1	0.02%
Olin2		
Arthropoda. Insecta. Lepidoptera	3	42.86%
Arthropoda. Insecta. Diptera	2	28.57%
Rhodophyta. Florideophyceae. Balliales	1	14.29%
Arthropoda. Maxillopoda. Sessilia	1	14.29%
Olin3		
Chordata. Actinopterygii. Blenniiformes	147	42.00%
Arthropoda. Malacostraca. Decapoda	84	24.00%
Mollusca. Polyplacophora. Chitonida	42	12.00%
Echinodermata. Holothuroidea. Dendrochirotida	27	7.71%
Arthropoda. Insecta. Diptera	13	3.71%
Annelida. Polychaeta. Terebellida	12	3.43%
Mollusca. Gastropoda. Archaeogastropoda	11	3.14%
Arthropoda. Insecta. Coleoptera	3	0.86%
Chordata. Actinopterygii. Perciformes	3	0.86%
Arthropoda. Malacostraca. Amphipoda	3	0.86%
Rhodophyta. Florideophyceae. Rhodymeniales	2	0.57%

Ascomycota. Dothideomycetes. Capnodiales	1	0.29%
Arthropoda. Insecta. Lepidoptera	1	0.29%
Chordata. Actinopterygii. Labrifomes	1	0.29%
Olin4		
Chordata. Actinopterygii. Perciformes	26	27.37%
Mollusca. Gastropoda. Sorbeoconcha	8	8.42%
Ascomycota. Dothideomycetes. Capnodiales	8	8.42%
Arthropoda. Insecta. Lepidoptera	8	8.42%
Bryozoa. Gymnolaemata. Cheilostomatida	8	8.42%
Arthropoda. Maxillopoda. Sessilia	7	7.37%
Annelida. Polychaeta. Phyllodocida	5	5.26%
Arthropoda. Insecta. Diptera	4	4.21%
Rhodophyta. Florideophyceae. Rhodymeniales	3	3.16%
Arthropoda. Insecta. Coleoptera	3	3.16%
Rhodophyta. Florideophyceae	3	3.16%
Ascomycota. Leotiomycetes	2	2.11%
Arthropoda. Insecta. Trichoptera	2	2.11%
Rhodophyta. Florideophyceae. Balliales.	1	1.05%
Arthropoda. Malacostraca. Decapoda	1	1.05%
Chordata. Actinopterygii. Scombriformes	1	1.05%
Chordata. Actinopterygii. Gobiiformes	1	1.05%
Rotifera. Bdelloidea. Adinetida	1	1.05%
Nematoda. Secernentea. Tylenchida	1	1.05%
Mollusca. Bivalvia. Mytiloida	1	1.05%
Heterokontophyta. Oomycota. Pythiales	1	1.05%
Olin5		
Arthropoda. Malacostraca. Decapoda	904	76.61%
Annelida. Clitellata. Haplotauxida	150	12.71%
Arthropoda. Malacostraca. Mysida	38	3.22%
Arthropoda. Insecta. Mantodea	15	1.27%
Bryozoa. Gymnolaemata. Cheilostomatida	9	0.76%
Arthropoda. Insecta. Diptera	6	0.51%
Echinodermata. Ophiuroidea. Ophiurida	6	0.51%
Arthropoda. Malacostraca. Amphipoda	6	0.51%
Mollusca. Bivalvia. Mytiloida	6	0.51%
Mollusca. Gastropoda. Sorbeoconcha	6	0.51%
Basidiomycota. Agaricomycetes. Boletales	4	0.34%
Annelida. Polychaeta. Phyllodocida	3	0.25%
Arthropoda. Insecta. Lepidoptera	3	0.25%
Ascomycota. Dothideomycetes. Capnodiales	3	0.25%
Ochrophyta. Phaeophyceae. Fucales	3	0.25%
Rhodophyta. Florideophyceae. Rhodymeniales	3	0.25%
Arthropoda. Insecta. Psocodea	2	0.17%
Arthropoda. Malacostraca. Isopoda	2	0.17%
Arthropoda. Maxillopoda. Sessilia	2	0.17%
Chordata. Actinopterygii. Perciformes	2	0.17%
Annelida. Polychaeta. Terebellida	1	0.08%
Arthropoda. Arachnida. Araneae	1	0.08%

Arthropoda. Collembola. Entomobryomorpha	1	0.08%
Arthropoda. Insecta. Coleoptera	1	0.08%
Arthropoda. Insecta. Trichoptera	1	0.08%
Ochrophyta. Phaeophyceae. Ectocarpales	1	0.08%
Rhodophyta. Florideophyceae. Balliales	1	0.08%
Pgue1		
Annelida. Polychaeta. Eunicida	97	38.65%
Chordata. Actinopterygii. Blenniiformes	59	23.51%
Chordata. Actinopterygii. Labriformes	44	17.53%
Ochrophyta. Phaeophyceae. Fucales	28	11.16%
Mollusca. Gastropoda. Littorinimorpha	12	4.78%
Arthropoda. Insecta. Lepidoptera	2	0.80%
Platyhelminthes. Trematoda. Plagiorchiida	2	0.80%
Arthropoda. Insecta. Coleoptera	2	0.80%
Annelida. Polychaeta. Phyllodocida	1	0.40%
Ascomycota. Dothideomycetes. Botryosphaeriales	1	0.40%
Arthropoda. Insecta. Diptera	1	0.40%
Arthropoda. Malacostraca. Decapoda	1	0.40%
Rhodophyta. Florideophyceae. Rhodymeniales	1	0.40%
Pgue2		
Arthropoda. Malacostraca. Decapoda	22	70.97%
Annelida. Polychaeta. Phyllodocida	8	25.81%
Arthropoda. Insecta. Trichoptera.	1	3.23%

	Hits >95% Identity	Relative abundance
Ngym1a		
Arthropoda. Malacostraca. Decapoda	1	100%
Ngym1b		
Echinodermata. Ophiuroidea. Ophiurida. Ophiocomidae. <i>Ophiocoma</i> sp.	6	85.71%
Arthropoda. Malacostraca. Decapoda	1	14.29%
Ngym2a		
Arthropoda. Malacostraca. Decapoda. Paguridae. <i>Anomura</i> sp.	8	66.67%
Mollusca. Gastropoda. Archaeogastropoda	2	16.67%
Arthropoda. Malacostraca. Decapoda	1	8.33%
Mollusca. Polyplacophora. Chitonida	1	8.33%
Ngym2b		
Arthropoda. Malacostraca. Decapoda. Paguridae. <i>Anomura</i> sp.	33	55.00%
Mollusca. Gastropoda. Archaeogastropoda	13	21.67%
Mollusca. Gastropoda. Vetigastropoda	8	13.33%
Arthropoda. Malacostraca. Decapoda	6	10.00%
Ngym3		
Arthropoda. Malacostraca. Decapoda	190	80.85%
Arthropoda. Malacostraca. Decapoda. Paguridae. <i>Anomura</i> sp.	13	5.53%
Rhodophyta. Florideophyceae. Corallinales	11	4.68%
Ochrophyta. Phaeophyceae. Laminariales	5	2.13%

Rhodophyta. Florideophyceae. Corallinales	5	2.13%
Ochrophyta. Phaeophyceae. Ectocarpales	5	2.13%
Mollusca. Gastropoda. Archaeogastropoda	3	1.28%
Echinodermata. Ophiuroidea. Ophiurida.		
Ophiocomidae. <i>Ophiocoma</i> sp.	1	0.43%
Chordata. Actinopterygii. Blenniiformes	1	0.43%
Rhodophyta. Florideophyceae. Peyssonneliales	1	0.43%
<b>Ngym4</b>		
Annelida. Polychaeta. Eunicida. Onuphidae.		
<i>Hyalinoecia</i> sp.	6	85.71%
Chordata. Actinopterygii. Blenniiformes	1	14.29%
<b>Ngym5</b>		
Mollusca. Gastropoda. Archaeogastropoda	371	95.37%
Annelida. Polychaeta. Eunicida. Onuphidae.		
<i>Hyalinoecia</i> sp.	15	3.86%
Arthropoda. Malacostraca. Decapoda. Paguridae.		
<i>Anomura</i> sp.	2	0.51%
Chordata. Actinopterygii. Blenniiformes	1	0.26%
<b>Olin1</b>		
Chordata. Actinopterygii. Blenniiformes	1260	97.07%
Mollusca. Gastropoda. Archaeogastropoda	37	2.85%
Ochrophyta. Phaeophyceae. Fucales	1	0.08%
<b>Olin3</b>		
Chordata. Actinopterygii. Blenniiformes	147	73.50%
Mollusca. Polyplacophora. Chitonida	42	21.00%
Mollusca. Gastropoda. Archaeogastropoda	11	5.50%
<b>Olin4</b>		
Mollusca. Gastropoda. Sorbeoconcha. Terebridae.		
<i>Duplicaria</i> sp.	2	66.67%
Annelida. Polychaeta. Phyllodocida. Nereididae	1	33.33%
<b>Pgue1</b>		
Annelida. Polychaeta. Eunicida. Onuphidae.		
<i>Hyalinoecia</i> sp.	88	59.86%
Chordata. Actinopterygii. Blenniiformes	59	40.14%

## Appendix 4 Denoising steps

<b>Location</b>	NR	NR	WR	NR	NR	NR	NR	NR	MBI3	MBI4	MBI5	NR	NR	NR	NR
<b>Gut sample</b>	1. CBW1	2. CBW2	3. Ngymgut	4. Olin1	5. Olin3	6. Olin5	7. GW2	8. GW5 9. Olin3	10. Olin4	11. Olin5	12. Ngym1	13. Ngym2	14. Ngym3	15. Ngym5	
<b>Paired original sequences</b>	200,594	117,438	2,533,622	526,982	137,164	273,880	650,196	1,257,700	381,216	2,330	424,066	1,306,958	434,948	1,648,410	597,914
<b>Post trimming adapters, indexes, microsatellites and primers</b>	9,870	47,346	1,213,772	419,820	100,556	214,312	274,284	164,172	304,052	1,106	302,356	351,396	287,728	1,222,330	232,204
<b>Post merging overlapping pairs</b>	2,511	20,483	546,598	180,653	46,041	98,410	126,068	63,542	121,431	222	140,512	155,531	134,934	571,312	100,217
<b>Post trimming sequences below 200 bp</b>	34	759	40,588	33,269	5,515	13,769	6,244	2,887	35,900	31	15,560	5,180	8,332	57,264	2,055

<b>Unmapped sequences against predator sequence</b>	34	382	36,784	26,508	4,219	7,010	4,844	2,253	32,898	30	14,574	3,625	3,234	30,565	1,649
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