Investigating trophic effects of recolonising generalist predators in complex ecosystems

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Attribution of authorship for published and collaborative work contained in this thesis

The work contained in the body of this thesis, except otherwise acknowledged, is the result of my own investigations. For one integrative review chapter and three data chapters, I led and undertook the following: study design, field organization, data collection, data analysis, writing of thesis chapters and manuscripts for publication in peer reviewed journals.

Chapter 2. I collected all the data, and conducted all analyses, and wrote this paper in its entirety.

Chapter 3. Field work and sample collections were done in 2014 with the support of the National Parks and Wildlife Services (NPWS) at Montague Island (NSW, Australia), as well as Booderee National Park in Jervis Bay (ACT, Aus). The study was designed by me, in collaboration with co-authors. Sample processing, genetic analyses and bioinformatics were conducted in collaboration with Tina Berry and Prof. Michael Bunce of the Trend Laboratory at Curtin University, Perth, Western Australia. I performed all of the data analyses and the writing for the manuscript. Co-authors provided reviews for drafts of the manuscript. The final accepted publication appears as chapter 3 of this thesis, after undergoing review by three anonymous reviews and publication by Marine Ecology Progress Series in April 2017:

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Chapters 4 and 5. Field work and data collection were done in 2014 with the support of Booderee National Park (ACT, Aus). The study was designed by me, in collaboration with co-authors. Co-authors Dr. Renata Ferrari, Dr. Will Figueira, Dr. Brendan Kelaher, Dr. Melinda Coleman, Georgina Wood and Gwenael Cadiou were indispensable in the field for data collection and processing on location. I received training and support from Joanne Potts, a statistician from the Analytical Edge, and a co-author on the publications to be derived from this work. I performed all statistical analyses of the data. I prepared two draft manuscripts that appear as Chapters 4 and 5 of this thesis, and are also in preparation for submission to peer reviewed journals. All

co-authors listed above have reviewed draft manuscripts that appear in this thesis as Chapters 4 and 5.

Animal ethics for all research contained herein was administered by the USYD Animal Ethics Committee, project approval number 2013/6056. In addition, all observations of wild seals, the collection of seal faecal samples and observations of wild fish in NSW, Australia, from 2013–2016 were done under the following permits: a Scientific Licence issued by the NSW Office of Environment and Heritage to work in NSW National Parks and Wildlife Service (SL101244); a permit to conduct research in Booderee National Park, ACT, Australia (BDR13/00011); a permit to access biological resources in a Commonwealth Area for non-commercial purposes (AU-COM2013-224); finally, access to Marine Parks was approved by Jervis Bay Marine Park Authority

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Under the Thesis and Examination Policy & Procedures (2014) as amended 2015, both of the supervisor and the student must attest to the above. The following are suggested

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Supervisor Name Signature

Date

To my parents, and family,

Who marvel at anything from genomes to the storms rolling in from a southern ocean.

For the wild places in this world,

That are constant sources of wonder, deserving of protection and that I live for.

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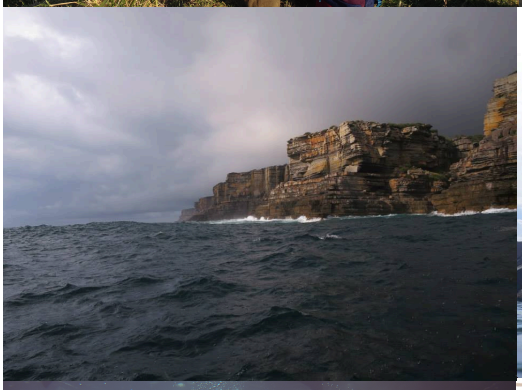


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Abstract

Large-scale changes to predator populations, due mainly to anthropogenic effects, have led to a trophic downgrading of our planet. In many ecosystems, we know this can result in large-scale ecological changes and phase-shift due to the cascading trophic effects between the key players of a food web. However, our knowledge of the ways in which predators interact with ecosystems is patchy, largely focused on relatively simple food webs and is poorly resolved for complex food webs that include large numbers of generalist predator and prey species. Does the return of high-trophic level predators hail trophic effects cascading from prey species down to the lowest trophic levels in complex marine ecosystems?

The recovery of Australian (*Arctocephalus pusillus doriferus*) and long-nosed (*Arctocephalus forsteri*) fur seals after the cessation of sealing in Australia affords a unique opportunity to investigate interactions between recolonising predator species and complex coastal ecosystems. However, prior knowledge on the diets of these recovering predators and of significant trophic interactions involving complex temperate ecosystems was lacking. I first reviewed the current body of work utilising DNA-based methods for the analysis of predator diets and ecological interactions. This review highlights the strength of DNA-based methods for exploratory diet analyses in ecosystems lacking prior information on important trophic interactions, by providing dietary information at high taxonomic sensitivity and resolution. I therefore applied DNA metabarcoding techniques to obtain detailed information on the diets of recovering fur seals in complex ecosystems of eastern Australia.

The diets of both fur seal species at a newly established breeding colony in southeastern Australia yielded patterns that were similar to that of breeding colonies in the centre of their geographic range, in southern Australia, whereby fur seal diet composition varied seasonally with a greater frequency of benthic and demersal prey species in diets in the summer months and of pelagic species in the winter months. However, these patterns were not observed at non-breeding aggregation (haul-out) sites at the species' current geographic range edge in eastern Australia, where the diets of both seal species contained a high prevalence of coastal prey items. The convergence of diets, and thus ecological interactions, of two predator species at their

range edge correlates with known differences in seal population densities and demographics at sites newly recolonised by these predators.

The frequency of coastal prey in the diets of both fur seal species highlighted the need to further assess potential ecological interactions between recolonising predators and coastal communities at their range edge. I therefore surveyed reef fish communities at newly established haul-out sites in eastern Australia, compared to local reference sites that do not harbour fur seal aggregations. Multivariate trait-based analyses of reef fish assemblages identified a significant relationship between the usage of sites by fur seals and the prevalence of fish functional groups. Schooling fish and browsing herbivores were negatively associated with fur seal aggregation sites. Additionally, at one fur seal haul-out site, the total abundance of fish was lower and there was a greater proportional biomass of smaller fish compared to that of reference sites. The potential cascading and indirect effects of coastal predator recolonisations on benthic reef communities were then investigated across multiple functional components of temperate reef ecosystems. The trophic structure of benthic invertebrate assemblages were not significantly different between seal haul-out compared to reference sites. However, the abundance of the ecologically important herbivore (*Centrostephanus rodgersii*) was lowest at one haul-out site. There was also a trend of decreased macro-algal cover observed at haul-out sites.

These results provide some evidence for differences between reef fish and benthic communities adjacent to fur seal aggregation sites compared to local reference sites along the coast. These differences did not, however, correspond to large ecological changes in reef community trophic structure or size structure, and differences were contingent on the location of the haul-out site sampled. At an early stage in the recovery of two predator species, the results of this body of work do provide important baseline information and novel insights with which to assess future trajectories of change in Australian temperate reefs, and also in complex ecosystems with recovering predator populations. Trait-based analyses enabled the development of a framework with which to identify potential trophic effects of recovering predators on complex ecological communities. Finally, this thesis provides a framework with which to continue monitoring trophic interactions and potential trophic cascades linking recovering predator populations and complex ecosystems.

1 General Introduction

1.1 On the importance of ecological interactions

"It was a starting place for untangling the complexity of interactions," said Paine. *"If all species were created equal, you wouldn't know where to start."* – Yong (2013)

A central question in ecology is that of what factors affect the structure and stability of biological communities. This is a question that Darwin himself probably considered, as he suggested that species interactions occur within complex networks or a 'tangled bank' (Darwin 1859). Elton (1927) first introduced the concept of trophically bonded species and ecological connectedness. MacArthur (1955) later connected community diversity, and trophic diversity, with community stability. Menge and Sutherland (1987) proposed a general model of ecological function with an interaction web that incorporated not only trophic linkages but also interactions between species and their physical environment, and in which the concept of a food web is embedded.

Nearly a century of research has revealed that ecological interactions between species occupying particular functional roles are immensely important in shaping ecosystems. Countless natural or anthropogenic-induced examples show that changes in primary resource availability, or increased competition for resources or predation pressure can subsequently alter the structure and function of ecosystems (reviewed by Estes *et al.* 2016; Terborgh & Estes 2010). Ecologists share a duty in documenting and investigating these interactions to enable a deeper understanding of ecosystem function, and to protect of important ecosystem processes. Ever-increasing, anthropogenic pressures on natural systems bring a sense of urgency to our duty to understand and protect important ecological interactions.

1.1.1 From bottom-up to top-down processes, and the discovery of keystone species

Ecological interactions have been categorised according to two often-competing hypotheses. Bottom-up processes occur when resources limit consumers: precisely when the net primary productivity and efficiency of energy transport upward through trophic levels drive the distributions, abundances and diversity of consumers, with flow-on effects up the interaction web (White 1978). Top-down effects occur when higher trophic level consumers control the abundance, biomass and/or diversity of species at lower trophic levels with flow-on effects down the interaction web (Steinberg *et al.* 1995). The relative importance to ecosystem structure and function of both types of processes has been hotly debated for decades. It is now widely accepted that both top-down and bottom-up processes interact in structuring ecological communities.

Within this framework of interaction of top-down and bottom-up processes, some organisms have been found to exert a particularly strong effect on the structure and function of their ecosystem. A major breakthrough in our understanding of how biological communities function was Robert Paine's historic manipulation in the 1960's of the predatory seastar, *Pisaster ochraceus*, on the rocky shores of Makah Bay and Tatoosh Island in Washington State, USA. Paine discovered that like the keystone of an arch, certain species act as the keystone for an ecological community's structure, whereby their activities and abundances determine community integrity and stability (Paine 1969). Keystone species impact their ecosystems in a manner that is disproportionately large compared to their abundance or biomass, and their removal or reintroduction from their ecosystem has been found to produce unexpected and significant consequences for that ecosystem (Paine 1969). Paine also identified that these species were typically of higher trophic standing, thus they exert impacts on ecosystems from the top-down.

Many inadvertent and serendipitous ecological experiments have enabled the identification of a myriad of keystone effects or strong ecological interactions in ecosystems the world over. In addition to Paine's sea stars, famous cases include otters in the northeast Pacific (Estes & Duggins 1995; Estes & Palmisano 1974), fish in prairie streams (Power *et al.* 1985) and in lakes (Carpenter *et al.* 1985), the effects

of cougars and wolves on ungulates and forests (Beschta & Ripple 2009, 2012; McLaren & Peterson 1994), foxes in the Aleutians (Bailey 1993), and, fishes and urchins in coral reefs (Hughes 1994; Lessios *et al.* 1984).

The commonality in these ecosystem-scale experiments was the anthropogenic-induced removal or introduction of a species, hitherto unknown to be ecologically important, thereby setting off a chain reaction through layers of a food web, and destabilising ecosystems in a pattern known as a 'trophic cascade' (Paine 1980; and reviewed by Ripple *et al.* 2016). These removal and reintroduction events essentially served as large-scale perturbation experiments (reviewed by Mills *et al.* 1993) and were instrumental in our discovery of the importance of certain species in their ecosystems (Pace *et al.* 1999). Examples exist where these trophic cascades were reversed through the removal of invasive species or the reintroduction of an important native released from anthropogenic pressure (Beschta & Ripple 2009, 2012; Estes *et al.* 2016; Estes *et al.* 1978).

In some cases, the ecological damage was too great, ecological communities were already too degraded by anthropogenic activities and were pushed to a tipping point beyond which they were driven to ecological melt-down and ecosystem phase shifts (i.e., Caribbean coral reefs, Pacific Northwest) (Estes & Palmisano 1974; Mumby 2009). A kelp forest, for example, may be reduced to urchin barrens following the decline of sea otter populations in the northeastern Pacific (Estes & Palmisano 1974). Coral reefs may become dominated by algae if populations of grazers catastrophically decline due to overfishing and disease on Caribbean reefs (Mumby 2009). The dire warnings from ecosystem phase-shifts around the world (Terborgh & Estes 2010) demonstrated that to conserve ecosystem function, it is crucial to understand and protect ecological processes, and the key players involved (reviewed by Estes *et al.* 2011).

1.1.2 Context-dependence and the nuances of ecological interactions

The concept of keystone species has been useful in demonstrating that under certain conditions some species have particularly strong effects on ecological communities. Several types of keystone effects have been identified since the term's introduction, including keystone predators, prey, mutualists, hosts and modifiers (Mills *et al.* 1993).

However, Mills *et al.* (1993) feared that a keystone vs. non-keystone dualism was too simplistic and that it failed to recognize the complexity of ecosystems, as well as the temporal and spatial dynamics of interactions. Mills *et al.* (1993) advocated for the study of interaction strengths, a concept developed by MacArthur (1972). Indeed, it became apparent that species interactions and trophic cascades varied in strength (reviewed by Polis *et al.* 2000) and sometimes didn't occur at all (Casey *et al.* 2017).

Numerous complementary hypotheses have been proposed to explain the occurrences, strengths and context-dependencies of trophic cascades (Polis *et al.* 2000; Polis & Strong 1996). First, spatial heterogeneity is expected to weaken trophic cascades because prey refugia should reduce predator foraging efficiency (Polis *et al.* 2000). Secondly, the trophic diversity of interacting species and the complexity of the food web can dampen the strength of trophic cascades (Polis *et al.* 2000). Competition between predators within an ecosystem, or intraguild predation, drives niche shifts and therefore adds trophic diversity and complexity to an ecosystem (reviewed by Polis & Holt 1992). Additionally, prey diversity is associated with weaker consumer effects (Edwards *et al.* 2010), and thus generalist foraging strategies by predators could also be associated with weaker consumer effects. Omnivory, which increases trophic diversity and versatility, further dampens the strength of consumer interactions by contributing to community stability (McCann & Hastings 1997; Polis *et al.* 2000). Thus, the ecological effects of trophically diverse predators will be further dissipated throughout a complex and biodiverse food web (Bellwood *et al.* 2006; Polis *et al.* 2000).

Thirdly, trophic cascade strength has been linked back to bottom-up processes or the productivity of an ecosystem. Trophic cascades were found to be more likely where primary productivity and resource quality were strong, and where resources were dominated by few species (reviewed by Polis 1999; Polis *et al.* 2000; Strong 1992), for example in the highly productive coastal ecosystems of the Northeast Pacific. Fourth, the efficiency of energy transfer through an ecosystem, through herbivory or predation, is associated with increased trophic cascade strength (Polis 1999; Polis *et al.* 2000; Strong 1992). Borer *et al.* (2005) added that taxonomic distinctness and physiological characteristics relating to the efficiency of herbivores and predators were the strongest drivers for trophic cascades. In their meta-analysis, the strongest cascades occurred in association with invertebrate herbivores and

energetically inefficient, endothermic vertebrate predators in temperate marine ecosystems (Borer *et al.* 2005). Thus, the combination of the Northeast Pacific's highly productive, cold-water kelp forests, voracious invertebrate herbivores and warm-blooded marine otters bore the characterics for one of the strongest trophic cascade cases known to ecologists. By contrast, terrestrial endothermic predators were associated with the weakest trophic cascades, as were invertebrate predators and also ecosystems where plant defenses decreased herbivore efficiency (Borer *et al.* 2005).

Interaction webs are therefore the product of both bottom-up and top-down processes (Estes *et al.* 2016; Power *et al.* 1985), as well as numerous contextual factors (Borer *et al.* 2005; Casey *et al.* 2017; Polis *et al.* 2000) creating variability in food web structure. Trophic cascades vary in nature due to the relative strength of species interactions, which in turn depend on the diversity and complexity of ecological communities, themselves influenced by the strength and diversity of primary productivity. Strong interactions resulting in trophic cascades may not actually be the rule in complex ecosystems (Casey *et al.* 2017), however relatively few studies have yet explored trophic cascades in complex ecosystems. This thesis will thus explore trophic cascade theory in the case of a complex ecosystem experiencing the recovery of large predators.

1.2 Large predator ecology in the 21st century

"[A] lot of what we study are apex predators. They matter. We should be asking — what are the general consequences going to be when we remove all the sharks from the sea? Or conversely, if the great whales recover, what will those effects be? I have no idea what the answers are, but these are questions we should be asking. The answers change our perception of the whole system from the top down."

– Robert Paine (2013)

Large predators naturally fit the criteria for strong interactors. As a result of their size, lower numbers, metabolic and spatial needs, they often have the highest per capita strength of interaction in a food web (Borer *et al.* 2005; Ripple *et al.* 2016; Terborgh & Estes 2010). A large number of studies have identified that predators are capable of causing strong interactions within ecosystems, both aquatic and terrestrial (Estes *et al.* 2016; Heithaus *et al.* 2012; Ripple *et al.* 2016). Interactions between species can be

trophically direct, or consumptive, between predators and prey, or indirect involving one or more intervening or subsequently affected species (Estes *et al.* 2016). Behavioural responses of prey and competing species to predators can often be as important as the direct trophic interaction itself (Brown & Kotler 2004; Prugh *et al.* 2009). The presence of an apex predator in an ecosystem can alter the distributions and foraging behaviour of prey species, as has been demonstrated in the relationship between wolves, elk foraging behaviour and the recovery of vegetation along stream banks in Yellowstone National Park (Ripple & Beschta 2004, 2007). At an ecosystem scale, predators are capable of creating a landscape of fear, a phenomenon found in both terrestrial and aquatic ecosystems at opposites ends of the world (Madin *et al.* 2011; Ripple & Beschta 2004; Wirsing & Ripple 2010).

Decades of work inspired by the likes of Paine are beginning to bring answers to the question he posited in the above quote. Roman *et al.* (2014) revealed the role of great whales as important ocean ecosystem engineers in their roles as consumers, prey for apex predators, detritus as whale falls, as important forms of material storage, and as physical vectors for nutrient cycling. Evidence for strong ecological effects of large sharks is also emerging. Research across multiple ocean basins is indicating that declines in the abundances of large sharks is associated with mesopredator release, or an increase in abundance of smaller-bodied predators, including smaller sharks and rays (Ferretti *et al.* 2010; Myers *et al.* 2007). Tiger sharks influence the distribution and foraging behaviour of dugongs and sea turtles with flow on effects for seagrass cover (Heithaus *et al.* 2008; Heithaus *et al.* 2012; Wirsing *et al.* 2007a, b), thus playing a role similar to wolves in North American terrestrial ecosystems (Wirsing & Ripple 2010).

The ecological roles of many groups of predators are poorly resolved, despite their potential importance to ecosystem processes (Estes *et al.* 2016). The ecological roles of predators are a relatively recent focus of the scientific community (Ripple *et al.* 2016), and effectively date back to Paine (1969). Well before that time, humans have greatly affected and modified ecosystems globally, and the scientific community is facing a race against time in understanding the natural roles of predators within ecosystems that range from pristine to degraded. This task is becoming even more difficult as predator populations themselves are being negatively impacted by human activities (Estes *et al.* 2011).

1.2.1 On trophic downgrading: the state of the world's large predators

Large carnivores face tremendous threats including range reduction, population reduction or eradication, worldwide (reviewed by Dulvy *et al.* 2014, McCauley *et al.* 2015, and Ripple *et al.* 2014). Large predators are arguably among the most threatened and endangered functional group of taxa across the world's ecosystems (reviewed by Estes *et al.* 2011, Pimm *et al.* 2014, and Ripple *et al.* 2014). Examples span terrestrial and marine ecosystems: from canids, bears, big cats and birds of prey on land to cetaceans, pinnipeds, chondrichthyans and osteichthyans in the water (Ceballos & Ehrlich 2002; IUCN 2017; Myers & Worm 2003; Ripple *et al.* 2014). Large carnivores are the unfortunate victims of targeted elimination either because of perceived threats (eg., sharks, bears, wolves) or for commercial gain (eg., fur from seals or otters, products from whales) (McCauley *et al.* 2015; Ripple *et al.* 2014). Large predators can directly compete with humans for resources (food, space) whereby they suffer from habitat fragmentation and loss, as well as a reduction in preferred food (McCauley *et al.* 2015; Ripple *et al.* 2014). Predators can also become indirectly targeted, by becoming fisheries bycatch in aquatic systems (Dulvy *et al.* 2014).

Large predators occupy the highest levels of food web structure within ecosystems and coupled with their size, require large geographic ranges and freedom of movement across this range in order to meet their energy requirements (Estes *et al.* 2016; Ripple *et al.* 2014). Additionally, species in this ecological group are generally K-selected species, sharing broad characteristics such as being slow-growing, slow-maturing taxa and with slow-population doubling times (Cardillo *et al.* 2005). These characteristics not only bring them in direct contact and competition with humans, but also intensify their vulnerability to human activities (Cardillo *et al.* 2004).

Increasing awareness, education and conservation efforts both local and global bring some hope (i.e., Large Carnivore Initiative, "LCI") (IUCN 2017; LCI 2017; Magera *et al.* 2013; McCauley *et al.* 2015). This is reflected in the conservation and protective legislation of most of the world's developed nations (eg., the United States Congress' *Marine Mammal Protection Act*, 1972; the Australian Government's *Environment Protection and Biodiversity Conservation Act,* 1999) , and in the

signatures of global initiatives such as the IUCN (2017). However, protection efforts are not necessarily ecologically representative (Pimm *et al.* 2014). These efforts are also recent in the context of the life histories of these species, and species protection alone will likely be insufficient in accounting for longer-term anthropogenic and environmental stressors.

Ecological research in the 21st century must contend with a past legacy of anthropogenic impacts on natural systems, ongoing competition for resources, and also a future of potentially critical environmental changes to predator populations and their ecosystems (McCauley *et al.* 2015). The widespread trophic downgrading of ecosystems can nonetheless be reversed or managed through novel and deliberate actions (Ripple *et al.* 2014). Much of our knowledge of natural systems is contingent on specific environmental circumstances that are changing. In a changing world, the accurate identification of key ecosystems processes and players, and of the role of vulnerable functional groups within interactions webs, may provide information that is key to conserving ecosystems and potentially reversing their degradation.

1.2.2 On pinnipeds and trophic upgrading

Pinnipeds, which include seals, sea lions and walruses, are common and large predators across many ecosystems globally, however they have experienced severe population depletion through historical harvesting (IUCN 2017; McCauley *et al.* 2015). Many species are now recovering and populations are recolonising ecosystems that were historically released from these predators (IUCN 2017; Magera *et al.* 2013). Much like the return of wolves to terrestrial ecosystems, the recovery of pinnipeds in marine ecosystems presents opportunities to observe the effects of trophic upgrading, put simply – the return of upper-trophic levels to an ecosystem. Predator recoveries typically result in prey limitation (Beschta & Ripple 2009; Estes *et al.* 2016; Ripple *et al.* 2014). Despite large-scale changes in pinniped populations and distributions, their role in the dynamic function and structure of affected ecosystems is poorly understood (Estes *et al.* 2016).

Only seven published studies are known to investigate the ecological influence of pinnipeds (reviewed by Estes *et al.* 2016). Pinniped activity can negatively impact prey populations across a range of ecosystems (Boveng *et al.* 1998; Kelaher *et al.*

2015; Oliver *et al.* 1983; Power & Gregoire 1978), and can also impact prey behaviour, whereby penguins and fish have been observed to be cautious and reduce foraging time in the presence of pinnipeds (Ainley & Ballard 2012; Connell 2002; Shepherd *et al.* 2010). One pinniped species was found to significantly modify the structure of benthic communities (Oliver *et al.* 1983). Additionally, evidence for localised resource depletion has been found following recolonisation events by northern fur seals in the North Pacific (*Callorhinus ursinus*) (Kuhn *et al.* 2014).

The representation among these ecological studies of the 33 extant species of pinnipeds and the ecosystems investigated are very small. Of the studies investigating direct effects of pinnipeds on prey, they involved two otariid species (Connell 2002; Kelaher *et al.* 2015; Shepherd *et al.* 2010), walrus, the only extant odobenid species (Oliver *et al.* 1983), and two phocid species (Ainley & Ballard 2012; Boveng *et al.* 1998; Power & Gregoire 1978). The results of these studies are also difficult to generalize across ecosystems because they have not been repeated and the literature on the ecological effects of pinnipeds on food webs remains in its infancy.

1.2.3 Recovering fur seal populations in southeastern Australia

In southeastern Australia, two sympatric seal species, Australian fur seals, *Arctocephalus pusillus doriferus*, and long-nosed fur seals (formerly New Zealand fur seals), *A. forsterii*, are undergoing population and range recovery following historical overexploitation and near-extinction (Burleigh *et al.* 2008; Goldsworthy *et al.* 2003; Kirkwood *et al.* 2010; Shaughnessy *et al.* 2001). Australian fur seals are estimated to number over ~120,000 based on the 2007-08 census (Kirkwood *et al.* 2010), however pup production for several colonies in Bass Strait was in decline whilst new colonies have been identified at the northeastern extent of their range, making total population estimates uncertain (McIntosh *et al.* 2014). Long-nosed fur seal abundance was estimated at 97,200 in South Australian waters based on a 2013-14 census (Shaughnessy *et al.* 2015), and these estimates are conservative, as several new colonies and haul-out sites are known for this species (McIntosh *et al.* 2014).

Fur seals have been returning to New South Wales (NSW, eastern Australia) since at least the 1990's (Shaughnessy *et al.* 2001). Breeding colonies for both species

have recently been established and recognized at Montague Island, NSW, as of a 2014 pup census (McIntosh *et al.* 2014), with pup production in NSW accounting for 1% of total pup production across Australia. Prior to the establishment of breeding colonies in NSW, it was extremely difficult to estimate the populations of largely marine and transient species in this location. Thus, accurate fur seal numbers are lacking for this region. Several permanent haul-out sites are now also established in NSW, and these populations represent the first resident seals in NSW for nearly a century (P. Shaughnessy & S. Goldsworthy, SARDI aquatic sciences, pers. comm.), in a region at the frontier of the core geographic range of the species in Australian waters.

Australian and long-nosed fur seals are both generalist and sympatric predators, whose ranges partially overlap in Australian waters (Hoskins *et al.* 2017; Page *et al.* 2005). Both species target benthic and pelagic fish and cephalopods, hunting primarily over the continental shelf and shelf edge (Fea *et al.* 1999; Hoskins *et al.* 2017; Hume *et al.* 2004; Page *et al.* 2005). These predators also exhibit niche partitioning, whereby Australian fur seal diets are reported as benthopelagic (Arnould *et al.* 2011; Hoskins *et al.* 2017; Kirkwood *et al.* 2008), and long-nose fur seal diets are mostly pelagic (Baylis *et al.* 2008; Fea *et al.* 1999; Harcourt *et al.* 2002; Hoskins *et al.* 2017). Both species also exhibit spatially specific foraging patterns across their geographic range in relation to resource availability (Baylis *et al.* 2008; Deagle *et al.* 2009; Hume *et al.* 2004; Page *et al.* 2006). Most diet studies for both fur seal species have been conducted at the centre of their geographic range in Bass Strait and South Australia, and were conducted at established breeding colonies.

Little is known about the diets of fur seals at their recolonisation frontier in NSW, eastern Australia, an area that is biogeographically distinct from Bass Strait and southern Australia (Connell & Irving 2008; Jordan *et al.* 2010). It is more than likely that these species will target similar benthopelagic and pelagic prey according to their seasonal availabilities on the east coast of Australia. However, the coast of NSW is characterized by significant rocky reef systems and contains the narrowest portions of the continental shelf anywhere in Australia (Jordan *et al.* 2010). Coastal ecosystems in this region are thus heavily influenced by the East Australian Current (Suthers *et al.* 2011). The relative importance of coastal versus oceanic sources of prey in the diets of both fur seal species are not known at their northeastern range edge. Reef associated species have been identified in the diets of Australian fur seals

from Bass Strait (Deagle *et al.* 2009). Fur seals are also suspected to affect reef fish assemblages at a colony at their northern range edge, Montague Island, in southeastern Australia (Kelaher *et al.* 2015).

Australian and long-nosed fur seals likely contribute to a significantly large resident biomass of high trophic-level predators in coastal waters of southern Australia (Kirkwood & Arnould 2011). The effect of this biomass on coastal ecosystems is not well understood. Its possible that despite a high biomass, the trophic effects on coastal reefs of these recovering populations could be minimal as they are generalist predator species whose diets are known to contain highly mobile oceanic prey from open marine systems. Fur seals are also perceived as a threat to commercial and recreational fishers, and they have been the subjects of calls for culls, particularly to long-nosed fur seals, due to perceived or potential competition for coastal and oceanic prey resources (Shaughnessy *et al.* 2015). Coastal reef communities typically harbor site-attached species and many are already vulnerable to fishing (Shepherd *et al.* 2010; Kelaher *et al.* 2015). As a result, reef fishes are a particular focus of coastal zone management through networks of marine protected areas (Halpern 2003). The recent and ongoing fur seal recovery in southeastern Australia provides unique experimental conditions involving multiple predators with which to test hypotheses about the ecological effects of pinnipeds on temperate ecosystems. This is particularly salient in a context of rapid recolonisation of the southeastern coast by two large, warm-bodied predators, and where trophic interactions with coastal ecosystems are unknown.

1.3 Thesis aims and outline

The overarching aim of this thesis was to identify any trophic effects resulting from the recovery of two important coastal predators, Australian and long-nosed fur seals, on complex, temperate ecosystems in southeastern Australia (Figure 1.1). What do these predators eat at their northeastern range edge? Does the return of fur seals to coastal ecosystems of southeastern Australia hail direct (consumptive) and indirect (non-consumptive) effects cascading from prey species down to the lowest trophic levels in complex marine ecosystems? I therefore embarked on a project to determine whether the recolonisation of important coastal predators, fur seals, mattered or not to

the reef ecosystems of southeastern Australian. Overall, I hypothesised that the recovery of fur seals in eastern Australia would result in: (1) important consumptive interactions linking fur seals to temperate reefs, which would (2) lead to prey limitation, and subsequently (3) release secondary consumers from pressure by mesopredators, thereby (4) increasing herbivory on temperate reefs (Figure 1.1).

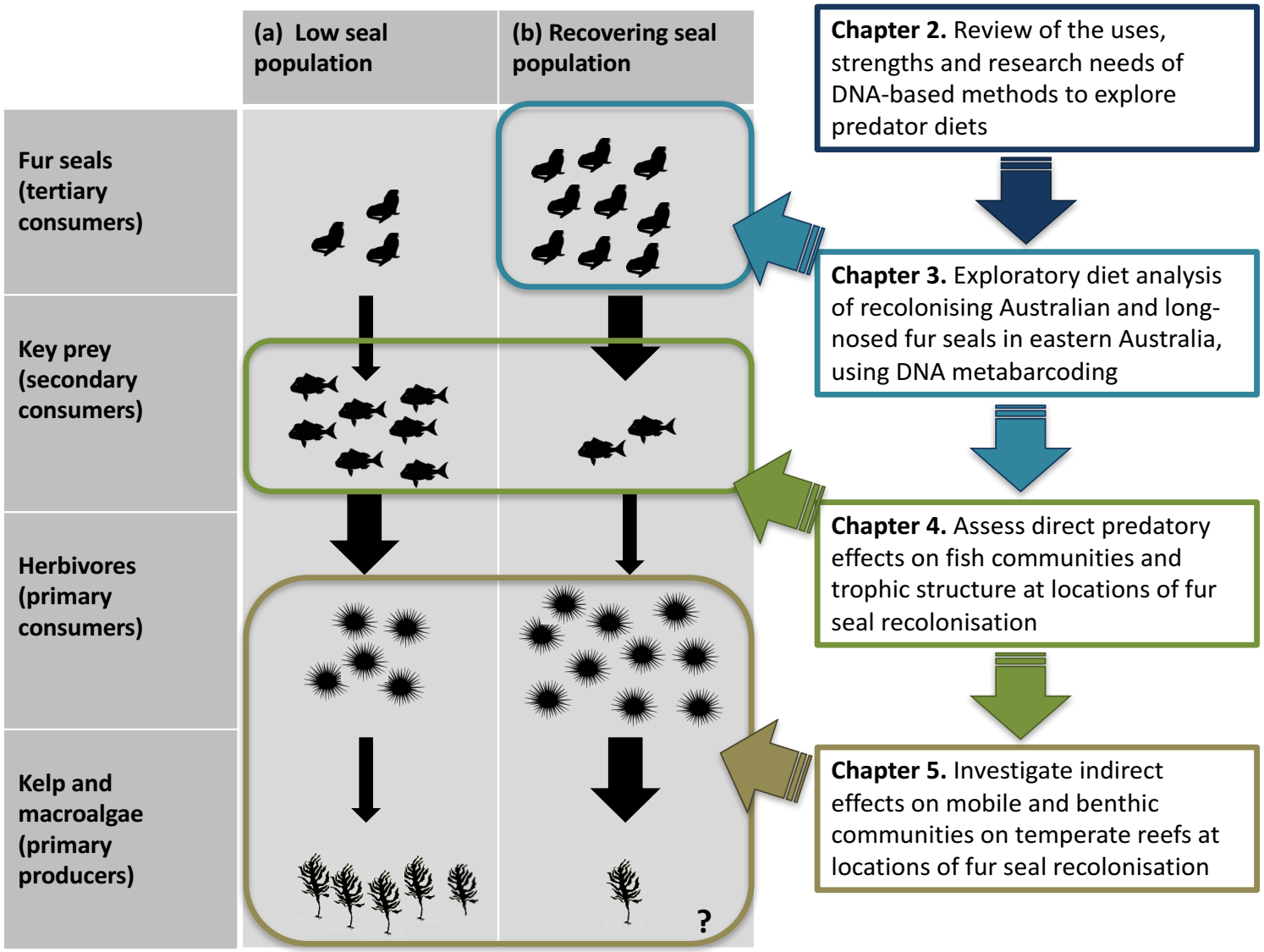


Figure 1.1 Conceptual diagram of the aims and structure of this thesis. Illustrated are hypothetical relationships between different trophic levels from recolonising predators, fur seals, to prey communities, to mobile and sessile benthic communities on temperate reefs. Groups are simplistically symbolized by one figurative animal, that in reality represents more complex prey and benthic communities on temperate Australian reefs. Prey communities are symbolized by fish as these are the likely primary prey of fur seals, however they can also include many predatory invertebrate species.

In this thesis, I present a four-point research plan for identifying ecological interactions between recolonising predators, prey communities and complex ecosystems (Figure 1.1). I : (i) undertook an integrative review of the uses and strengths of DNA-based methods to explore the diets and ecological roles of high-order predators (**Chapter 2**); (ii) investigated the diets of these predators in their novel and recolonised location, in eastern Australia, using DNA metabarcoding techniques (**Chapter 3**); I then aimed to (iii) identify whether recent recolonisations by coastal predators has produced localised functional changes in fish communities (**Chapter 4**); and to (iv) identify signs of indirect and potentially cascading effects on invertebrate and benthic communities at locations of fur seal recolonisation in eastern Australia (**Chapter 5**).

In an ecosystem where prior information on trophic interactions is lacking, the first port of call for understanding species interactions is, commonly, to first identify consumptive or predatory relationships. I needed to undertake a detailed and exploratory study of the diets of recolonising predators in complex temperate ecosystems of eastern Australia. It was important to first assess the appropriate method for this task. The priorities for my research were to use a technique of diet assessment that was taxonomically sensitive and produced data across a range of potentially foraged ecosystems without any prior information about the diets of the study animals in those ecosystems. Additionally, due to working on protected species, I also valued a method of sampling predator diets that is as non-invasive as possible. DNA-based analysis of predator diets satisfies all the aforementioned criteria and is a promising method that may revolutionise the way that we model trophic interactions. In **Chapter 2**, I therefore reviewed the current literature using DNA-based methods for the assessment of predator diets and applications of this method for exploring trophic interactions. This review provides a valuable semi-quantitative synthesis of the uses, strengths and future research needs of DNA-based diet analysis across a broad range of predators and ecosystems. The review highlights DNA-based methods as the most taxonomically sensitive method available and effective for obtaining detailed information on ecological interactions in a complex food web, and I therefore apply DNA-based techniques to my study system.

Chapter 3 contains a detailed analysis of the diets of both recolonising predator species, Australian and long-nosed fur seals, from the frontier of their

expanding range in southeastern Australia. I characterised the diets of both species across two locations of recolonisation, one site an established breeding colony and the other, a new but permanent haul-out site. Next-generation DNA metabarcoding techniques were used to obtain high taxonomic-resolution data on diets and inform ecological trait-based analyses of trophic interactions across time and space. This was the first DNA-based study to analyse the diets of both predator species at the northern-most part of their geographic range, and the first DNA-based analysis of the diets of long-nosed fur seals throughout their geographic range. Detailed information on the diets of these predators from this location identified an important overlap in the diets of these predators at their northeastern range edge, with a particularly high prevalence of coastal and reef-associated prey species.

Chapter 4 investigates direct trophic impacts on reef fish communities adjacent to predator aggregation sites. This study included two spatially distinct haul-out sites each paired with multiple reference sites that do not harbour fur seal aggregations. In this chapter, I investigate localised differences in reef community metrics, as well as size structure of fish communities, between fur seal aggregation sites and local reference sites. This chapter also examines how fish functional traits can explain differences fish community composition at locations of fur seal recovery in temperate reef ecosystems of eastern Australia, using a fourth-corner model.

Chapter 5 investigates potential indirect effects on coastal benthic communities following the natural recolonisation of discrete locations by fur seals on temperate southeast Australian reefs. I aimed to identify and quantify any localised differences in macro-invertebrate, cryptic fish and sessile benthic communities adjacent to newly recolonised fur seal haul-out sites compared to multiple local reference sites without seals. Differences in the trophic structure of invertebrate communities were assessed between locations of fur seal recolonisation and reference locations, as well as the relationship between invertebrate species assemblages and the prevalence of kelps and macroalgae. Additionally, changes in sessile benthic invertebrate and macro-algal communities were assessed at locations of fur seal recovery.

A review paper (Chapter 2) and three primary research papers for this thesis (Chapters 3–5) were written as separate scientific papers. Chapter 3 has been published (Hardy *et al.* 2017). Chapters 2, 4 and 5 are currently being drafted for

submission to scientific peer reviewed journals. These chapters include diverse investigations and focal points across coastal reef ecosystems in southeastern Australia: from large predators, reef fish, benthic invertebrate to macroalgal communities. These chapters are tied together by the common theme of investigating the ecological ramifications of predator recovery in complex coastal ecosystems. This study uses an ecosystem-scale approach to assess interactions between pinnipeds on temperate reefs, providing novel insights at an early stage in the recovery of eastern Australian fur seals with significant implications for understanding the ecological interactions of in recolonising predators globally.

2 Exploring the ecological role of predators through genetic diet analysis

2.1 Abstract

The accurate identification of trophic interactions between predators and their ecosystems enables scientists to understand important ecological processes that structure ecosystems. The genetic analysis of predation, through the recovery and amplification of diagnostic DNA barcodes from the predator and/or their prey, offers taxonomically sensitive techniques for investigating trophic interactions. Genetic techniques for the analysis of degraded DNA have undergone rapid changes and advancement within the last 20 years, and these techniques are now widely available and applicable. Here I reviewed the recent applications of genetic techniques for the analysis of predator diets to investigate common themes and advancements for ecology, as well as significant limitations and research needs that have been highlighted after two decades of direct applications of genetic techniques in the field A total of 205 papers dating from 2002–2017 used DNA-based methods to analyse the diets of predators, either by identifying the predator and/or the prey. Through these studies, genetic techniques and in particular DNA metabarcoding have emerged as an excellent tool for the exploration of trophic interactions, particularly in complex ecosystems, for generalist predators and in ecosystems for which we have little prior knowledge. DNA metabarcoding can inform further analyses of predator trophic and foraging ecology, and genetic techniques can be combined with other methods of analyses to produce powerful ecological interaction models and insights into a predator's true diet. Several ongoing challenges were identified in this review and do currently limit the use of genetic techniques including extracting quantitative information beyond frequency of occurrence, and the current paucity of long-term predator diet monitoring using genetic techniques. These limitations are the future research needs for genetic techniques of diet analysis and their improvement will serve to further strengthen a powerful method for ecologists.

2.2 Introduction

Predators include the most threatened functional groups across the world's ecosystems (Estes *et al.* 2011; Ripple *et al.* 2014) due to range reduction, population reduction and competition with people. Predators shape and are shaped by their ecosystems. However, our understanding of the ecological roles of predators in ecosystems is limited in space and time, there are still entire ecosystems and taxa for which little is known about important trophic processes (reviewed by Estes *et al.* 2016). Humans also continue to alter interaction webs directly through the removal of predators (Ripple *et al.* 2014) or indirectly through climate change (Hamilton *et al.* 2017). Understanding consumptive relationships linking predators to their ecosystem enables scientists to assemble food webs, to study and predict the ecological consequences of predation and the influence of resources on food webs (Estes *et al.* 2013; Goldsworthy *et al.* 2013). Central to this question is understanding the diets of predators, for which a large array of methods and entire disciplines in science have been developed to suit the needs of the study systems: from morphological analyses of prey hard parts to molecular analyses of predator tissues using biological tracers (reviewed by Bowen & Iverson 2013). The trophic "holy grail" is accurate techniques for determining prey identities, proportions and ideally biomass consumed by a predator (reviewed by Pompanon *et al.* 2012).

Genetic markers are arguably the most universal biological tracers as every living organism is made up of them. Technological advances in the study of ancient DNA have vastly advanced our ability to extract useable DNA from even the most degraded environmental substrates (e.g. "coprolites" or ancient faeces) (Bon *et al.* 2012). DNA-based methods thus present powerful and objective tools for exploratory diet analysis and the identification of ecological interactions. DNA represents directly what is present in the diet (Hargrove *et al.* 2012), has greater taxonomic resolution and sensitivity than traditional morphological techniques (Bowen & Iverson 2013; Deagle *et al.* 2009), requires less prior knowledge about the study system compared to other methods and molecular tools (e.g. taxonomic expertise, stable isotopes) (Valentini *et al.* 2009) and can be thus applied to a broad suite of study systems. Additionally, DNA-based methods that estimate the relative contribution of prey in

predator diets have also very recently been developed (Thomas *et al.* 2016; Thomas *et al.* 2014).

The literature on the genetic analysis of predator diets now spans over 20 years since genetic methods were first applied in the 1990's to this purpose (reviewed by Symondson 2002). There are several thorough reviews on the technical advances and past or current limitations in molecular methods of diet analysis (Table 2.1). Several reviews also appraise the advantages and disadvantages of DNA-based methods in comparisons with other methods of diet analysis for specific groups of predators (Table 2.1). DNA-based methods of diet analysis have evolved rapidly, from early bacterial cloning techniques to quantitative polymerase chain reaction (qPCR) (Jarman *et al.* 2002; Sheppard & Harwood 2005; Symondson 2002). The field of environmental DNA experienced a dizzying succession of high-throughput sequencing (HTS) or next generation sequencing (NGS) technologies in under 20 years (reviewed by King *et al.* 2008, Pompanon *et al.* 2012, Taberlet *et al.* 2012). The development of new HTS and NGS platforms has dramatically improved sequencing capacity, enabling effective and rapid sequencing of mixed environmental samples (reviewed by Staats *et al.* 2016).

There has been a recent surge in the number of publications using DNA-based methods to assess predator diets. Beyond methodological reviews, there has been very little recent synthesis of the direct applications of DNA-based methods of diet analysis (Figure 2.1), and of the contribution of this body of work towards a deeper understanding of the ecological roles of predators. The majority of published papers are of primary research studies (Figure 2.1). In the context of rapid growth and technological development of molecular tools for ecologists, it is all the more important to take stock of what has been achieved in the recent development and applications of molecular methods for predator diet analysis.

In this study, I therefore aim to provide an integrative review of the current body of work using DNA-based methods for the assessment of predator diets. The study aims are threefold: (i) to provide an overview of trends in the recent uptake of DNA-based methods for predator diet analysis; (ii) summarise progress to date and to appraise the contribution this work has made to understanding the ecological roles of predators; and (iii) to identify from recent practical applications of DNA-based predator diet analyses, what are critical limitations, advancements and future

directions needed to harness the full potential of genetic methods to revolutionise trophic ecology.

Table 2.1 Reviews (n = 14) of DNA-based methods for the analysis of predator diets and trophic interactions from 2002–2017. They were categorised either as (i) *methodological* for broad reviews of technical and applied aspects of DNA-based analysis of predator diets, or (ii) *group-specific* for reviews of the methods of analysing diets pertaining to particular taxonomic groups.

Type	Author/s (Year)	Paper title
Methodological	Symondson (2002)	Molecular identification of prey in predator diets
	Sheppard and Harwood (2005)	Advances in molecular ecology: tracking trophic links through predator-prey food-webs
	Hudson (2008)	Sequencing breakthroughs for genomic ecology and evolutionary biology
	King *et al.* (2008)	Molecular analysis of predation: a review of best practice for DNA-based approaches
	Valentini *et al.* (2009)	DNA barcoding for ecologists
	Bucklin *et al.* (2011)	DNA Barcoding of Marine Metazoa
	Pompanon *et al.* (2012)	Who is eating what: diet assessment using next generation sequencing
	Yoccoz (2012)	The future of environmental DNA in ecology
Group-specific	O'Rorke *et al.* (2012)	PCR enrichment techniques to identify the diet of predators
	Tollit *et al.* (2006)	Estimating diet composition in sea lions: Which technique to choose?
	Barrett *et al.* (2007b)	Diet studies of seabirds: a review and recommendations
	Bowen and Iverson (2013)	Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty
	Furlong (2015)	Knowing your enemies: Integrating molecular and ecological methods to assess the impact of arthropod predators on crop pests
	Adams *et al.* (2016)	A century of Chinook salmon consumption by marine mammal predators in the Northeast Pacific Ocean

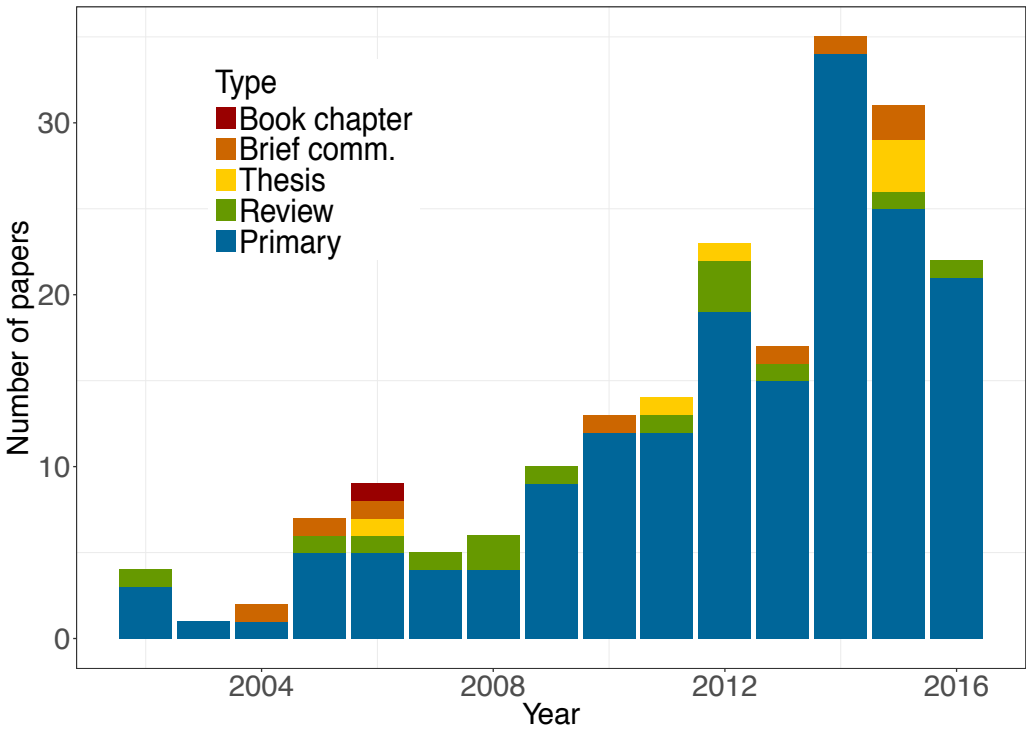


Figure 2.1 Trends in the number of research articles published from 2002–2016 on DNA-based analyses of predator diets by type of paper (n = 199 papers). Brief communications included mini-reviews, opinion pieces and letters on the topic of genetic analysis of predator diets, typically less than 5 pages. Papers from 2017 (n = 6) have been excluded from graphical summaries of the data by date because the year had not yet ended at the time of writing and thus trends for 2017 were not yet representative.

2.3 Data collection and terminology

2.3.1 Definitions

DNA-based methods are used to identify predator and prey through the targeted amplification of unique genetic markers or "barcodes" from predator or prey tissues (reviewed by King *et al.* 2008). DNA barcoding is the process by which species can be identified from short DNA sequences (Hebert *et al.* 2003a). When DNA based identification of taxa is applied to the mass amplification of DNA barcodes using NGS technologies, it is referred to as DNA metabarcoding (Taberlet *et al.* 2012). DNA metabarcoding techniques enable the identification of multiple taxa from a single bulk environmental sample containing degraded DNA (reviewed by Staats *et al.* 2016 and Taberlet *et al.* 2012).

The use of the term ‘DNA metabarcoding’ throughout this review refers to the bulk amplification and sequencing of group-specific or universal barcodes that are then compared to local or global databases. By contrast, I refer to species-specific assays or primers for the amplification of DNA from single taxa, and these samples can be individually sequenced and verified, or samples can be screened using probes or diagnostic PCR. As an alternative to the terms ‘DNA-based methods’ or ‘DNA metabarcoding’, these terms are often referred to in the literature as ‘molecular’ or ‘genetic’ methods/analyses (King *et al.* 2008; Pompanon *et al.* 2012; Symondson 2002). Here, I use the alternative term ‘genetic methods/analysis’ to avoid potential confusion with other molecular methods such as fatty acid or stable isotope techniques.

True predators kill and consume other animals, and here I have taken predators to be at least third-order through to fifth-order consumers, that is they must consume at least herbivores or above (Terborgh & Estes 2010). For the purposes of this review, predators belong to the kingdom Animalia and typically consume organisms from that kingdom. Predators from the papers collated in this review belonged to the phyla Mollusca, Arthropoda and Chordata. For this review, studies were considered if they investigated predation using DNA-based methods primarily to genetically identify the prey, but also the predator. Additionally, predator DNA can also be amplified to identify the predator, either from their faeces or from the remains of their prey.

2.3.2 Literature search

Literature on the analyses of predator diets using DNA-based methods were compiled by searching the ISI Web of Science database (2002–July 2017) using the following criteria: TOPIC (diet analysis), *AND* TOPIC (DNA) *AND* TOPIC (predator or carnivore). The literature was searched from 2002 until present, following the early review of DNA-based diet assessment by Symondson (2002) and since the initial application of quantitative PCR for predator diet analysis by Jarman *et al.* (2002). We selected every article that reviewed or performed diet analyses for predators using DNA-based methods to identify the predator and/or prey, and also papers that reviewed or undertook any combination of methods of diet analysis that included

DNA-based methods. The compilation was supplemented by subsequently searching material cited within those articles including published journal articles, namely the special issue by *Molecular Ecology* on the "molecular detection of trophic interactions" (2014, vol. 23), as well as unpublished theses. Information was gathered from published reviews on DNA-based methods for diet analysis (Bowen & Iverson 2013; King *et al.* 2008; Pompanon *et al.* 2012; Valentini *et al.* 2009) and the references cited therein were searched. A total of 205 papers were collated in this review and these are listed in Appendix A.

The information extracted from the literature first included coarse detail, such as the date of the study and the type of paper (i.e., primary research article, review) (Figure 2.1). Primary research papers were then searched using a framework of questions (Table 2.2). These questions relate to the broad aims of the studies (i.e., methodological development/validation or application); to specific details of a study's design, use of comparative methods of diet analysis, method of sampling and also the duration of the study (Table 2.2). The specific genetic technique and aim of that technique were noted. Additionally, I recorded the predator targeted by a study, as well as the location for field-based studies and the method of sampling used (Table 2.2). These questions aimed to quantify broad trends in the recent applications of DNA-based methods for the analysis of predator diets. It must be noted that papers from 2017 ($n = 6$) have been excluded from graphical summaries of the data by date because the year had not yet ended at the time of writing and thus trends for 2017 were not yet representative. These papers were, however, included in any figures that illustrated trends irrespective of the date of publication.

Primary research articles were also qualitatively searched for specific conclusions drawn from the development or application of genetic techniques and their utility in predator diet analysis. Additionally, the research output was searched for discussion of technical limitations and recommendations stemming from the use of genetic techniques to answer their research questions.

Table 2.2 Framework of queries systematically applied to the reviewed primary research articles.

Query	Categories	Additional description
Aim (Figure 2.3)	(i) Methods development/validation	Primarily develops, optimises and/or validates methods, including assays, workflow and/or primer development. Methods are typically validated and compared in the laboratory and/or field through small-scale trials.
	Method application:	Primarily applies a pre-existing method, and can include some study-specific optimisation and methods comparisons
	(ii) exploratory diet description	For the exploratory descriptive analysis of predator diets, often in novel species or systems
	(iii) ecological investigation	Application of DNA-based methods in studies where the main aim is investigating an ecological question, often in addition to diet description. Includes: detection of invasive species, species of conservation value, targeted fisheries, food web parameters and modelling.
Technical strategy (Figure 2.2)	DNA Metabarcoding	Uses universal and group-specific primers to recover the biodiversity of a predator's diet
	Targeted predator/prey assay	Uses targeted primers or assays to recover the DNA of specific predators and specific prey
	Targeted predator	Uses targeted primers or assays to identify the predator
	Targeted prey	Uses targeted primers or assays to identify specific prey of interest

Table 2.2 Queries for review continued.

Query	Categories	Additional description
Study design (Figure 2.4)	Captive	Primarily conducts experiments in captivity or a laboratory using samples generated from these experiments
	Wild	Primarily field-based study using samples from wild animals
	Combination	Usess a combination of captive-based experiments and wild-derived samples
Latitudinal region of study (Figure 2.5)	Tropical, subtropical, temperate, alpine*, subpolar, polar and combination of regions	The latitudinal region studied is ascribed based on the latitude and geographic region reported in the study. *Alpine environments largely refer to studies from the Himalaya, the high Alps and those conducted in high mountain environments at temperate and polar latitudes.
Study taxa (Figure 2.5)	Class, order, genus and species	Data for Class are reported in this review
Number of predators studied (Figure 2.6)	1–5 and >5	The number of predators that are the object of the study and of dietary analysis
Duration of study (Figure 2.6)	Single sample or sampling event, seasons (1–3), years (1–5), >5 years	For field-based studies only
Method of sampling	Faeces, regurgitates, whole predator, prey tissues, gut content (lethal/non-lethal)	

Table 2.2 Queries for review continued.

Query	Categories	Additional description
Comparison of diet analysis methods (Figure 2.7)	Direct*	Refers to studies that apply genetic and non-genetic methods of diet analysis to the same sampling units in order to directly compare the resulting output
	Complementary*	Studies using both genetic and non-genetic methods to analyse predator diets, but usually in a complementary strategy where genetic methods are used to identify tissues that cannot be identified morphologically, or where two methods are applied to different sampling units and are not directly comparable
	None	Studies using only genetic methods to analyse predator diets
*Comparative method (Figure 2.7)	Morphology (hard-part analysis), stable isotope analysis (SIA), acoustic tracking, observation	For studies using a direct or complementary strategy for integrating DNA-based and other methods of diet analysis

2.4 The rise of DNA metabarcoding for predation ecology and overview of applications

2.4.1 Summary of recent advances and research trends

The earliest applications of genetic techniques for diet analysis in vertebrate systems were typically for the identification of predator DNA from faeces, the remains of which were assessed using morphological or histological techniques (reviewed by Symondson 2002 and Sheppard & Harwood 2005). For invertebrate study systems, early applications involved targeting the specific species or genus of prey (Symondson 2002). DNA-based methods of diet analyses have since evolved from targeted and group-specific PCR-based techniques (Jarman *et al.* 2002) to DNA metabarcoding methods capable of processing complex environmental samples and producing large volumes of genetic data (these methods and advances are reviewed by Staats *et al.* 2016 and Taberlet *et al.* 2012). The most significant recent progress relevant to molecular diet analysis includes: advances in sequencing technologies, DNA metabarcoding methods and in cost benefit of DNA-based methods (reviewed by Hudson 2008, King *et al.* 2008, and Pompanon *et al.* 2012). These advances have made molecular techniques vastly more accessible to the scientific community (Staats *et al.* 2016; Taberlet *et al.* 2012; Yoccoz 2012).

Technological and methodological developments alongside global initiatives for biodiversity barcoding and compiling genetic databases ultimately paved the way for large-scale DNA metabarcoding studies (GenBank, http://www.ncbi.nlm.nih.gov; EMBL, http:// www.ebi.ac.uk/embl; DDBJ, http://www.ddbj.nig.ac.jp; Consortium for the Barcode of Life, CBOL, http://barcoding.si.edu; Barcode of Life Data Systems, BOLD, http://www.barcodinglife.org; Moorea BIOCODE http://www.mooreabiocode.org/) (reviewed by Bucklin *et al.* 2011; Valentini *et al.* 2009). These databases provide an unambiguous reference that facilitates species identification. Global databases also expand scientific horizons – given the known number of described species compared with that which are yet to be discovered, genetics offers a rapid method of accounting for them (Bucklin *et al.* 2011).

Naturally, a proliferation of applications by ecologists has followed recent methodological advances. The number of studies using DNA-based methods for the

analysis of predator diets has increased from only a handful in 2002 (Symondson 2002) to over 200 in 2017, included in this review (Figure 2.1). The bulk of these studies have appeared in print within the last decade, roughly tracking the increasing affordability and applicability of these methods (Hudson 2008; Yoccoz 2012). These studies mainly utilized targeted predator or prey assays in the earlier days (prior to 2009). The number of studies utilising species-specific methods of identifying prey from predation events or predator tissues have remained relatively stable at under 7 studies per year since 2002 (Figure 2.2). The recent proliferation of molecular diet studies almost entirely consists of studies using DNA metabarcoding methods (Figure 2.2). Additionally, the recent publication of a special issue on "the molecular detection of trophic interactions" by *Molecular Ecology* (Symondson & Harwood 2014) has no doubt contributed to the recent peak in publications on this topic in 2014.

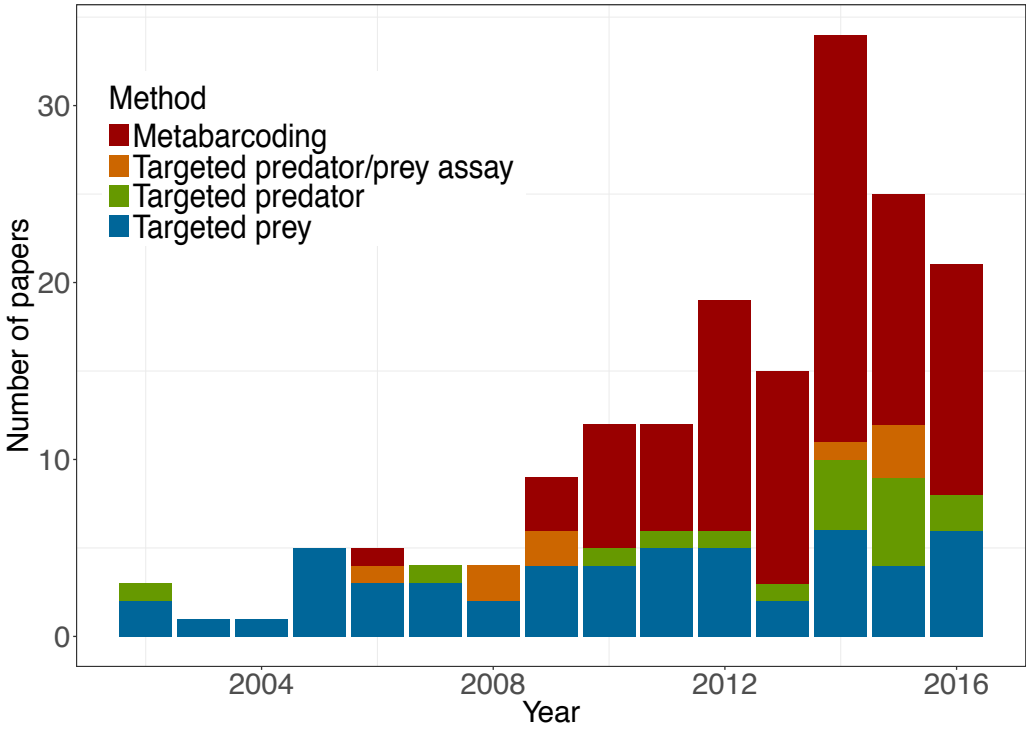


Figure 2.2 Trends in the method of molecular diet analysis used by primary research articles from 2002–2016 (n = 170). Here, the "method" refers to whether studies used DNA metabarcoding techniques or taxon-specific assays to target predator or prey DNA, or both (Table 2.2). Another 6 papers from the first half of 2017 utilised only DNA metabarcoding methods, but are not included in this graph as they may not be representative of 2017 as a whole.

Due to ongoing advances in methods of extracting, amplifying and sequencing DNA, the development and validation of genetic methods for predator diet analysis remains an important contribution of the research output in this field (Fig 2.3). Overall, trends in the aims of primary research papers have not yet stabilised, concerning the development and validation of methods or their application from exploratory diet analysis to more complex ecological questions (Figure 2.3). Studies applying pre-existing or well-developed methods to predator diet analysis have been conducted ever since these methods have been available (Figure 2.3). However, applications towards descriptive diet studies and for complex ecological investigations have consistently dominated primary research publications since 2009 (Figure 2.3).

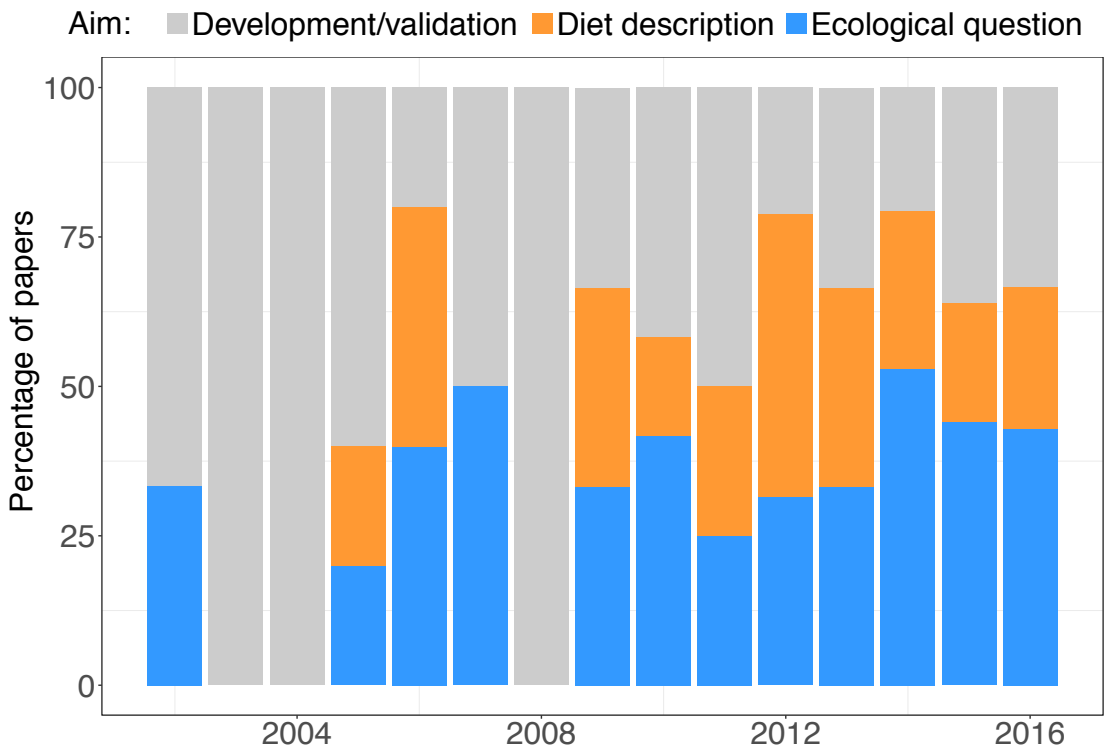


Figure 2.3 The proportional contribution of primary research papers from 2002–2016 according to their primary aims: (i) the development and/or validation of molecular methods for predator diet analysis; (ii) the application of validated methods for exploratory diet analysis; or (iii) the application of validated DNA-based methods in studies where the main aim is investigating an ecological question, in addition to diet description.

Applications of genetic tools for predator diet analysis include: assays for targeted predator or prey species detection (e.g., Jarman & Wilson 2004; Sigler *et al.*

2006); exploring the genetic and biological diversity of predator diets using broad molecular barcodes for modern (e.g., Carreon-Martinez *et al.* 2011; Deagle *et al.* 2009; Peters *et al.* 2014) and ancient predators (Bon *et al.* 2012). Additionally, the genetic analysis of predator diets has been increasingly applied for the monitoring of trophic interactions for conservation and management purposes, involving threatened or endangered species (e.g., Berry *et al.* 2017; Hanson *et al.* 2010; Keskin 2016; Wegge *et al.* 2012), invasive species (e.g, Braid *et al.* 2012; Côté *et al.* 2013; Witczuk *et al.* 2013) and for ecosystem-based modelling (e.g, Bartley *et al.* 2015; Bowser *et al.* 2013; Wirta *et al.* 2015).

2.4.2 From the laboratory to applications in the field

King *et al.* (2008) identified the need for applications of molecular analysis techniques to investigate complex trophic interactions in the field and the authors provided a user-friendly guide to do so, still useful for all predators, years after the technology reviewed has been superseded. Prior to 2002, the molecular detection of prey remains in predator tissues was at the time restricted to arthropod predator-prey systems, and was almost exclusively laboratory-based (reviewed by Symondson 2002, and Sheppard & Harwood 2005). Jarman et al. (2002) were among the first to develop and apply a group-specific PCR-based approach for prey DNA detection in a non-terrestrial arthropod and wild system, for the detection of krill in Adélie penguin and pygmy blue whale faeces.

The development and validation of methods largely involves captive feeding trials, as well as a combination of captive trials and the validation of methods using wild samples (Figure 2.4), and these types of studies dominated the research output largely until 2009. Studies applying validated methods for exploratory diet analysis and the investigation of food webs were almost exclusively based on samples derived from wild predators or ecosystems (Figure 2.4), and studies using wild-sourced samples now account for the majority of the research output on the molecular analysis of predator diets. Several applied studies also used a combination of captive trials and wild sources of sampling, usually in order to develop a primer and test its application prior to undertaking larger-scale ecological studies (Bobrowiec *et al.* 2015; Collier *et al.* 2014; Karp *et al.* 2014; Suzuki *et al.* 2006).

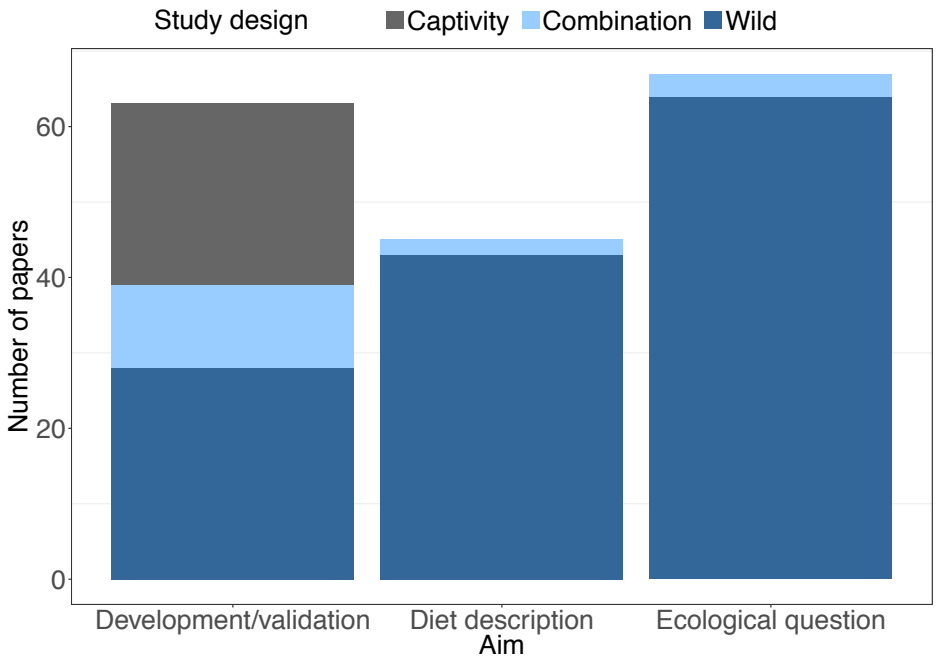


Figure 2.4 The number of primary research papers from 2002–2017 categorised by their primary aims: (i) the development and/or validation of molecular methods for predator diet analysis; (ii) the application of validated methods for exploratory diet analysis; or (iii) the application of validated DNA-based methods in studies where the main aim is investigating an ecological question, in addition to diet description.

Applications of DNA-based methods for diet analysis have been biologically and geographically diverse (Figure 2.5). Studies spanned 11 Classes of the Kingdom Animalia, however, the vast majority of studies involved animals from the Phylum Chordata (Figure 2.5a). Nearly half of all studies were conducted on mammals, which represent only 2% of Vertebrate diversity in an array of studies that also included Arthropods and Molluscs. The coverage of species within the classes recorded is likely very patchy. The numbers of studies conducted in terrestrial compared to aquatic ecosystems were roughly even. However, 50% of primary research studies conducted in the wild were in temperate ecosystems (Figure 2.5b). This review therefore highlights a large contribution of temperate ecosystems, and of mammalian predatory species in the literature on the molecular analysis of predator diets. This trend is likely related to the demand for research in these topic areas. DNA-based methods have been applied to the assessment of specific trophic interactions involving species of commercial or conservation value, including deleterious interactions between native and invasive species (e.g., Kvitrud *et al.* 2005; Marlow *et al.* 2015; Schreier *et al.* 2016).

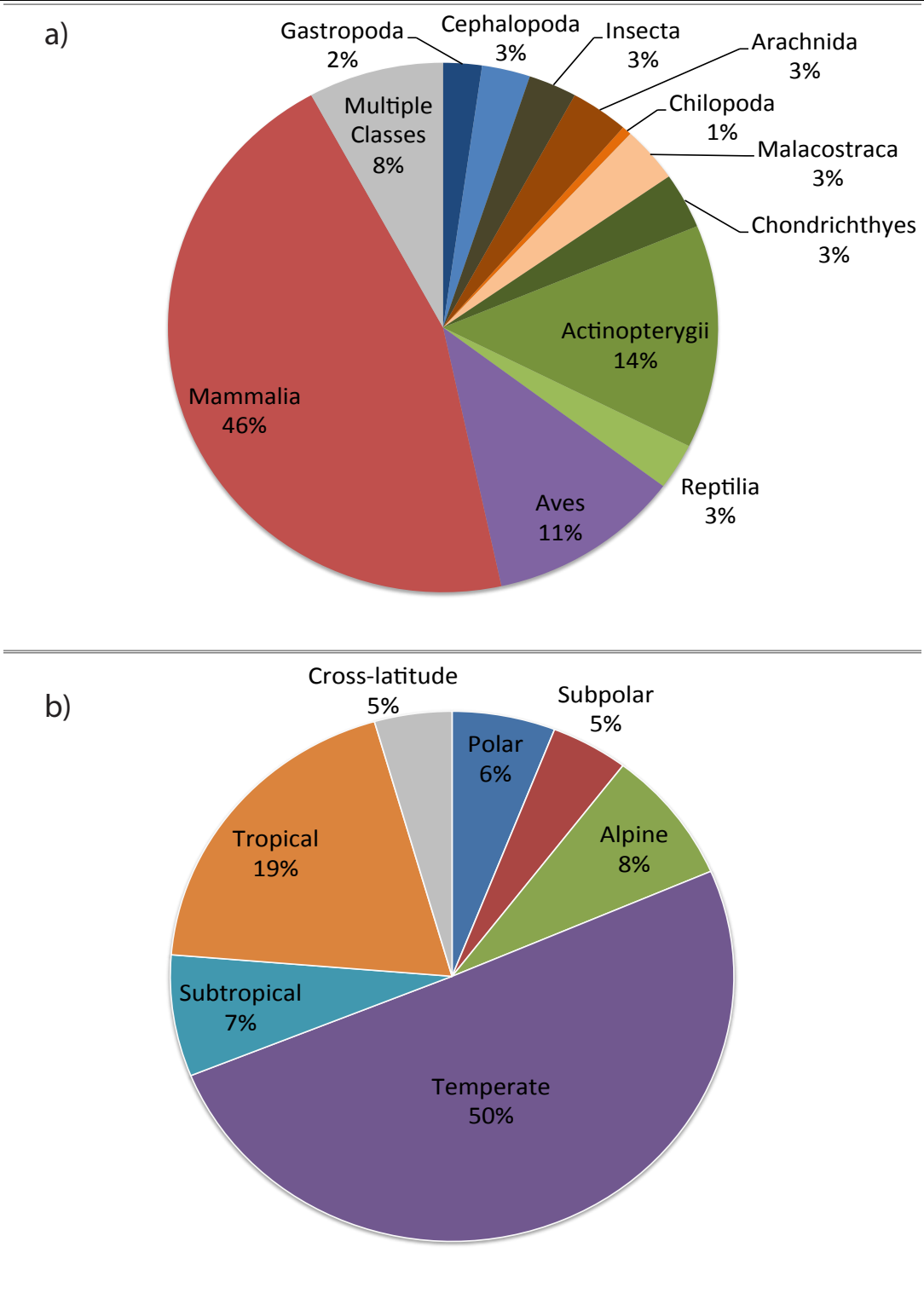


Figure 2.5 Proportions of primary research articles according to a) the Class of the predator investigated using genetic methods of diet analysis, and b) the latitudinal region of the study system for field-based studies only from 2002–2017.

Studies typically focused on a single predatory species and the duration of molecular diet analysis studies reviewed was generally short. Out of 176 primary research articles, 110 involved studying the diet of a single species (Figure 2.6a). Of the studies conducted in the field (n = 135), several were conducted from a single sample or brief sampling event, more than half were conducted over 1-2 seasons (Fig 2.6b). Forty-five studies (33%) involved sampling over multiple years (Figure 2.6b), however the temporal resolution for the longer-term studies became patchy. These details are consistent with the recent development of genetic techniques and thus the recent nature of their application.

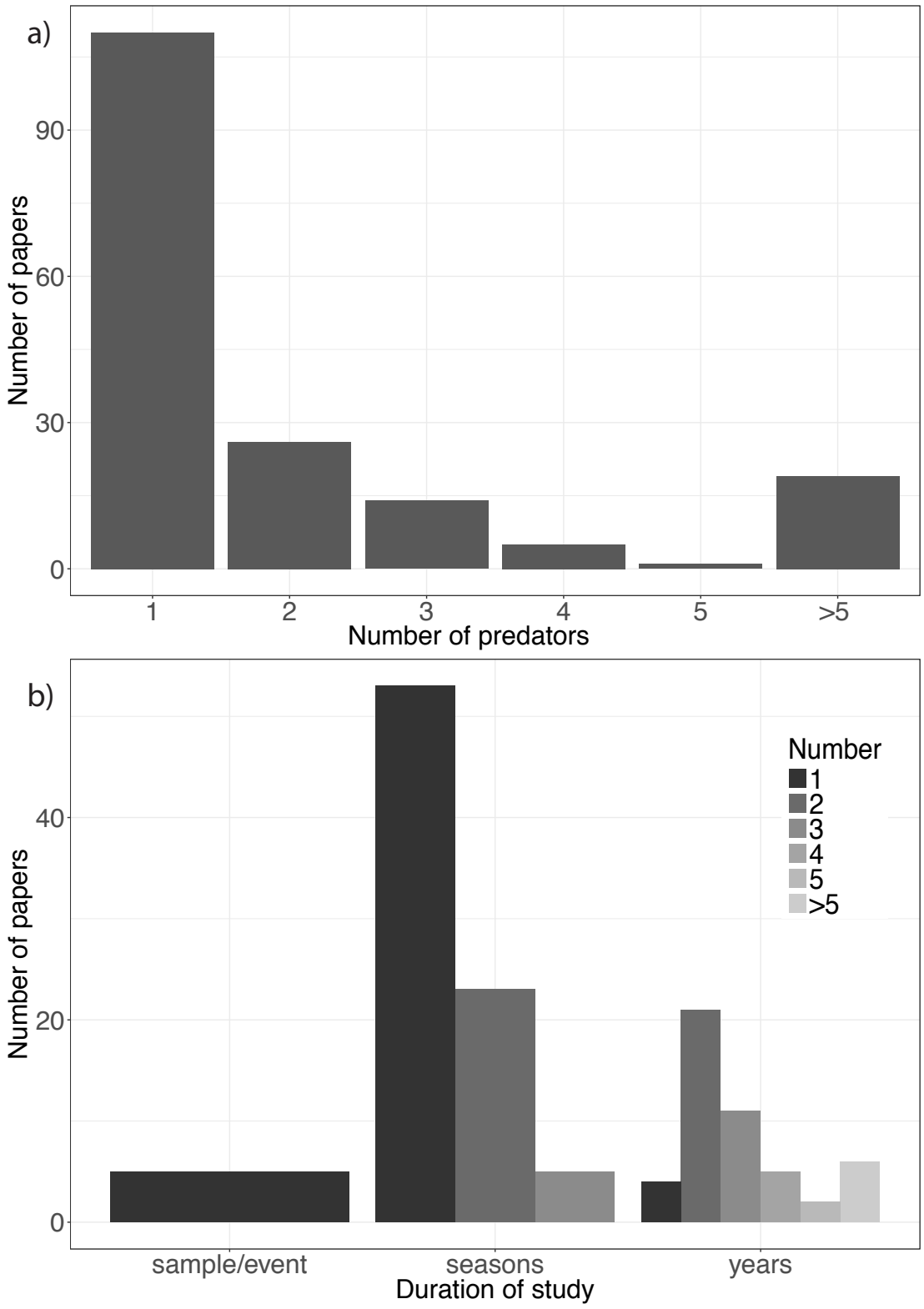


Figure 2.6 The number of primary research articles (from 2002–2017) as a function of a) the number of predatory species investigated within each study; b) the duration of study. Studies with > 5 targeted predatory species included several studies with very large numbers of targeted species, of up 108 targeted predators.

2.5 Findings and future perspectives from two decades of applications of DNA-based diet analysis

> "High-throughput DNA sequencing (HTS) of diets is a rapid way to parameterize food webs at enhanced taxonomic resolution, and potentially, to optimize the functioning of ecosystem models." – Berry *et al.* (2015).

Genetic methods of diet analysis have advantages and disadvantages, many of which are common to other methods of diet analysis, and some are unique to genetic methods. The strengths and weaknesses of genetic methods of diet analysis have been reviewed in particular with regard to methodological advances (King *et al.* 2008 and Pompanon *et al.* 2012) and for particular study systems or taxa (Barrett *et al.* 2007b, Bowen & Iverson 2013, Furlong 2015). Many of the main gains and limitations of DNA-based methods previously highlighted in reviews and primary literature remain relevant to current predator diet analyses. Following the recent proliferation of applied research, I synthesize the mains strengths of genetic methods for trophic ecology in section 2.5.1. The limitations of genetic methods are also the main research needs for this field, and in section 2.5.2, I therefore synthesize the limitations of genetic methods of diet analysis that are commonly reported in current research, and summarise research needs that are outlined in the recent primary literature following a growth in direct applications of this method. An overall summary of research outputs, limitations and recommendations is provided in Box 1.

2.5.1 Genetic methods for exploratory ecology and complementary to other methods

DNA metabarcoding is emerging as a highly suited tool for the exploration of predator diets, particularly in the detection of species where traditional methods are unreliable (Egeter *et al.* 2015; Hargrove *et al.* 2012; King *et al.* 2008; Peters *et al.* 2015). A large number of the reviewed studies applied DNA-based methods alone precisely to explore trophic interactions at high taxonomic resolution. This review also identified close to 40 studies that directly compared DNA-based methods to other

methods of diet analysis, and the latter almost always consisted of morphological analyses of prey remains (Figure 2.7). The majority of these comparative studies corroborated the recommendation of DNA over morphological methods for improved taxonomic resolution and scope of predator diets. Additionally, the vast majority of the 176 primary research articles reviewed herein reported improved taxonomic resolution and sensitivity of predator-prey interactions compared to previous knowledge of those interactions.

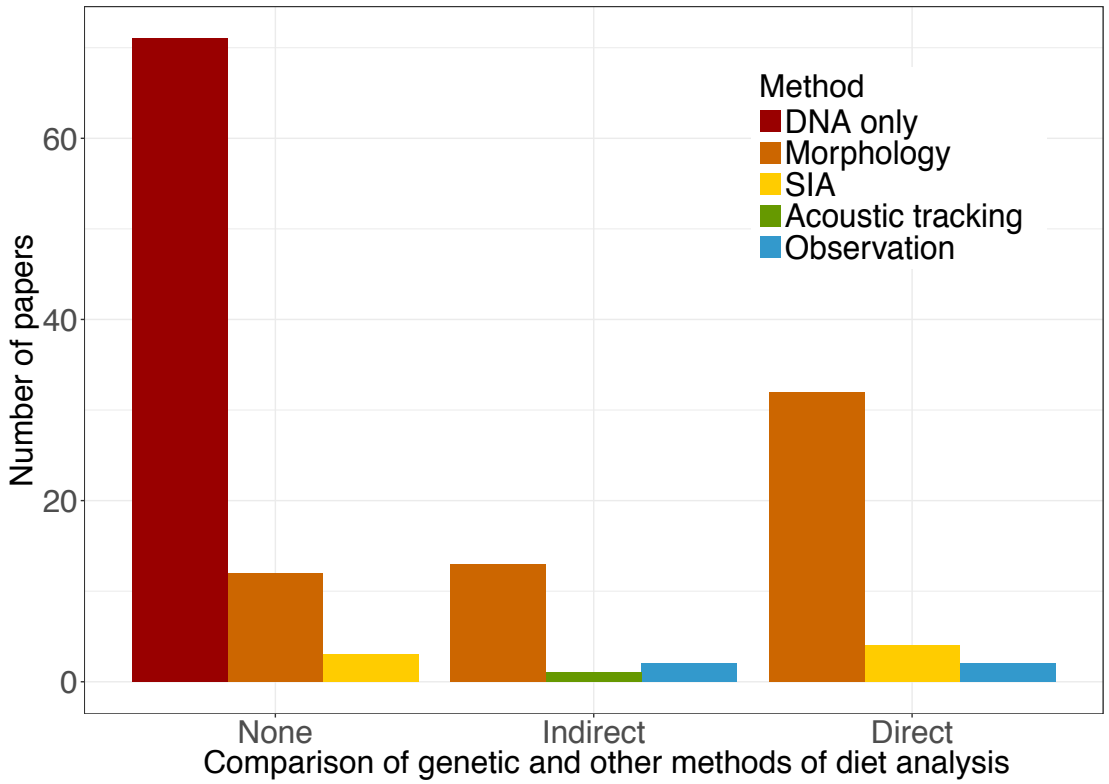


Figure 2.7 Number of studies (from 2002–2017) that conducted comparisons between DNA-based methods and other methods of diet analysis ('directly' or 'indirectly'), or did not undertake a comparison ('none') but used genetic techniques to identify the predator or prey and then used a single method to analyse predator diets. Direct comparisons involved the same methods being applied to all sampling units, whilst indirect comparisons featured the application of methods to different sampling units (Table 2.2c). SIA = stable isotope analysis.

Genetic methods were particularly useful in identifying remains that would otherwise be unidentifiable (i.e., blood, soft tissues, larvae, eggs). This was proven across the breadth of taxa studied, for example in the identification of marine mammal tissues in the stomachs of sharks (Porsmoguer *et al.* 2015; Sigler *et al.* 2006), for the identification of highly digested remains in Australian sea lions (Berry

et al. 2017; Peters *et al.* 2014; Peters *et al.* 2015), in some reptiles (Brown *et al.* 2014b; Goiran *et al.* 2013) and for the identification of blood in bat and spider diets (Ito *et al.* 2016; King *et al.* 2008). Genetic methods were useful in the identification of prey from small arthropod or larval predators (O'Rorke *et al.* 2013; Paula *et al.* 2016; Roura *et al.* 2012; Sint *et al.* 2015).

DNA-based methods were extremely useful in the identification of ecological interactions using either broad metabarcoding approaches or targeted species-specific assays. Numerous studies developed species-specific and cost-effective assays that could be applied to large sample sizes and large numbers of predatory taxa, for the detection of specific species or families of high conservation or commercial interest (Fox *et al.* 2012; Hunter *et al.* 2012; Schreier *et al.* 2016). Fox *et al.* (2012) used a TaqMan probe to identify the predation of plaice *Pleuronectes platessa* eggs in the stomachs of 3373 fish, crustacean and cephalopod individuals, effectively assaying 42 species, representing a monumental effort and largely impossible task had they used virtually any other method. Another study also utilised genetic techniques not only to identify the prey species of interest, but also used several microsatellite markers to identify the origin of prey stock at high spatial resolution (Skaala *et al.* 2014).

With advances in DNA metabarcoding techniques, genetic techniques are emerging as a particularly strong and universal method with which to investigate trophic interactions in the following cases: (i) ecosystems for which we lack prior knowledge, (ii) diverse ecosystems, and (iii) for complex trophic interactions involving generalist predators and omnivores, that are likely to consume a broad variety of prey. DNA metabarcoding is universally applicable to taxa and ecosystems without extensive prior taxonomic knowledge of prey, for example of their identifiable remains, particularly useful for deepsea ecosystems and for understudied predators (Braid & Bolstad 2014; Deagle *et al.* 2005; Dunn *et al.* 2010). DNA metarbarcoding techniques have been instrumental in capturing the diversity of prey and of trophic interactions in complex ecosystems (Hardy *et al.* 2017; Razgour *et al.* 2011; Wirta *et al.* 2015).

A large number of studies across multiple taxa and ecosystems highlighted the benefits of using a combination of methods, including DNA-based methods, as best practice to provide the most comprehensive and ecologically relevant information on predator diets (Chiaradia *et al.* 2014; Taguchi *et al.* 2014; Tollit *et al.* 2006; Tollit *et*

al. 2009). All methods of diet analysis that sample the predator's gastro-intestinal tract suffer a temporal resolution penalty in exchange for detailed information on prey species or abundances (Pompanon *et al.* 2012). By contrast, other methods can provide higher temporal and spatial resolution (e.g., stable isotopes, fatty acids) but often at reduced taxonomic information, especially for complex ecosystems (Barrett *et al.* 2007b; Chiaradia *et al.* 2014). These limitations are thoroughly reviewed and discussed in the literature (see Table 2.1). A total of 54 primary research articles, or 39% of those reviewed, combined DNA-based methods with other methods of predator diet analysis, either using complementary or comparative strategies. Other methods used to assess predator diets and foraging ecology included visual observations, predator tracking using biotelemetry, morphological identification of prey remains, stable isotope and fatty acid analyses. Combining complementary methods can offset the limitations and potential biases of either method used, and is a strong strategy for the investigation of predator diets.

DNA-based diet analysis techniques can be used to inform strategies for predator diet analysis (Tollit *et al.* 2009), particularly in ecosystems with limited prior knowledge of trophic interactions or for complex ecosystems. Two studies directly compared DNA-based analysis of predator diet with a higher-temporal resolution method of diet analysis, one with the analysis of stable isotope signatures (SIAs) from little penguins (*Eudyptula minor*) (Chiaradia *et al.* 2014) and Tollit *et al.* (2006) compared DNA-based methods with morphological, stable isotope and fatty acid techniques in Steller sea lions (*Eumetopias jubatus*). Using a controlled feeding trial, Chiaradia showed that models incorporating combined DNA-based and SIA information provided the best estimates for the known diet consumed by little penguins. DNA metabarcoding studies can objectively contribute to building prey libraries for the development of tissue corrective factors for further quantitative DNA-based studies, to identify important species for hard-part analyses in systems where prey remains are easily recoverable, and to inform species-specific calibrations for fatty acid and stable isotope techniques. Genetic techniques can thus be used to help overcome structural uncertainty in food webs, a key limitation to ecosystem modelling (Gregr & Chan 2014). The opposite is also true, that other methods of diet analysis can also be used to provide additional spatial or temporal context to results obtained from DNA-based methods. For those intending to combine methods of diet

analysis, the choice of combination of dietary analysis methods is necessarily subject to the study system and predator, and the specific aims of the study.

DNA-based methods offer non-invasive approaches to analysing predator diets that are particularly suited to exploratory investigations of species interactions and in particular for protected species. Applications of DNA-based methods for predator diet analysis used both invasive and non-invasive methods of sampling predator tissues (King *et al.* 2008). The majority of primary research papers based on wild-caught samples used predator faeces to analyse their diets (n = 81 of 135). Less than a dozen of those studies reported needing to handle the animals to obtain faeces (Brown *et al.* 2014b; Duda *et al.* 2009; McCracken *et al.* 2012). Sampling methods also included the collection of predator gut contents (n = 46), although 5 studies obtained gut contents from animals that were: bycatch (Méheust *et al.* 2015), stranded (Dunshea *et al.* 2013) or killed by hunters (Oehm *et al.* 2011). Six studies demonstrated non-lethal methods of stomach flushing to obtain gut contents (Barnett *et al.* 2010; Goiran *et al.* 2013). Whilst still invasive, the latter represent an obvious methodological improvement to lethal sampling. Lethal methods were more common in arthropods, in larval phases of animals, and in fishes, compared to other vertebrate taxa. Finally, DNA was also obtained in predator regurgitates (Alonso *et al.* 2014) and from prey tissues (Ford *et al.* 2011; Nystrom *et al.* 2006). Studies are largely aiming to adopt non-invasive and non-lethal methods of sampling from predators and ecosystem.

2.5.2 Challenges and research needs identified through applications of DNA-based diet analysis

The main challenges highlighted in the reviewed studies included: (i) deficiencies in global genetic databases, (ii) lack of accounting for technical and biological biases in quantitative information from DNA sequence data, (iii) the influence of secondary predation on genetic diet information, and (iv) the current paucity of long-term studies mapping trophic interactions, and the temporal resolution of DNA-based methods. The vast majority of studies reviewed herein reported significant advances in understanding predator-prey interactions within their study systems using DNA-based methods, however I would recommend carefully assessing the benefits as well as the limitations of DNA-based methods for each prospective case. Where a study's

priorities quantitative information on prey consumption, or longer-term integration of diet over time, then other methods exist that are fit for those purposes. The following are several limitations to DNA-based methods of diet analysis, some strategies to mitigate those limitations and further research needs to address them.

2.5.2.1 Global sequence reference databases and limitations of barcoding

Bucklin *et al.* (2011) reviewed the global genetic barcoding effort for all marine metazoa, finding that for a ~648 bp region of the mitochondrial cytochrome oxidase c subunit I (COI) gene, only roughly 9.5% of all known marine species have been barcoded. This effort is likely to be even smaller for other genes that have received less attention from barcoding initiatives. Inconsistencies in large-scale barcoding efforts for shorter genes are problematic for predator diet analyses that largely use digested materials or faeces containing degraded DNA, and thus primarily target shorter barcodes and even 'mini-barcodes' (Staats *et al.* 2016). The reviewed primary research articles cited data deficiencies for both terrestrial and marine invertebrates (Berry *et al.* 2015; Deagle *et al.* 2007; Sakaguchi *et al.* 2017). Even groups that are considered well represented in global databases, such as Chordates, have variable representation for the genetic diversity within species (Bucklin *et al.* 2011).

A significant research need is therefore that of large-scale efforts dedicated to improving the coverage of global and local biodiversity, as well as the coverage of genes in sequence reference databases (Dunn *et al.* 2010). Despite deficiencies in global sequence reference databases, DNA-based methods remain taxonomically sensitive. Significant global and local efforts to sequence the genes of organisms across ecosystems are cited in section 2.4.1 and are expected to vastly improve the taxonomic resolution of DNA-based investigations of ecological interactions. The use of microsatellite DNA paired with detailed reference databases, such as that of salmonids, can reveal information at even finer resolution than the species level (Hanson *et al.* 2010; Skaala *et al.* 2014). Skaala *et al.* (2014) were able to track source populations of Atlantic salmon prey consumed by brown trout. Individual predators have been identified from their scats using multiple microsatellite markers (Aryal *et al.* 2014). In the near future, it may be possible to apply next-generation population genetics techniques for the analysis of the genetic diversity of within prey species consumed by predators, affording highly detailed analyses of food webs.

Discrepancies between the taxonomic and genetic classifications of animals also limit the accurate identification of species for some groups of animals, due to potential misclassifications (Packer *et al.* 2009). Vital-Rodríguez *et al.* (2016) were aiming to distinguish several closely related predatory fish species, but were unable to distinguish them morpholohically or genetically, and indicate that this could be due to taxonomic and genetic differences in the classification of their study species. This limitation was overcome by considering the genetic diversity within a group of animals, usually taken to be > 3% for a given gene to distinguish between species and is known as a divergence threshold (Hebert *et al.* 2003b). However, gene loci used to target specific groups or species must be carefully chosen and the gene region must be highly conserved for the targeted taxa in order to differentiate between taxa (Jarman *et al.* 2004). Many studies thus use molecular operational taxonomic units (MOTUs or OTUs), based on known divergence thresholds between taxa, where reference sequences are unavailable but where legitimate prey sequences were identified (Berry *et al.* 2015). Best practice for the accurate differentiation and identification of prey species and taxonomic groups is to use multiple redundant primers based on different regions of an animals' genome, thus targeting multiple loci (Valentini *et al.* 2009).

2.5.2.2 Limitations for certain taxa and ecosystems

There are of course ecosystems and study taxa for which genetic methods of diet analysis are opportunistic at best. Genetic methods rely on both the recovery of predator digesta that are representative of their diet, and that produce useable genetic information following digestion. One study in this review reported failing to identify prey taxa in one reptile (Falk & Reed 2015) and suggested that samples from the gastrointestinal tract of Burmese python could be too digested, a problem that could be common in reptiles. Additionally, sampling is necessarily opportunistic for deep diving marine mammals and cryptic animals, whose digesta are difficult to access and if they are accessible – they are unlikely to be representative of that predator's diet. Combined strategies for diet analyses in these taxa and ecosystems are recommended to obtain as much information as possible from sampling efforts, especially in cases where difficulties in sampling would not bias the ability to retrieve useable DNA from digesta. However, DNA-based methods may not be suited to systems where the recovery of predator digesta are problematic and where any recovery of such digesta

are not likely to be representative of that predator population. Indeed, the aforementioned limitations will also affect any methods that sample from such predator's GI tracts in these cases.

Studies finding non-significant results and problems from applications of DNA-based methods applications are likely to be underrepresented in the literature. Largely, this is because those problems are then tackled by a particular laboratory and solved, thus publishing the solution. Alternatively, if one method of diet analysis failed to produce results then one can assume that another method would have been used and published. Non-significant results are useful for ecologists to know where genetic methods were particularly limited – for what study systems and animals.

Several studies have provided useful group-specific reviews of methods for predator diet analysis, including or exclusively reviewing DNA-based methods, for marine mammals (Tollit *et al.* 2006; Bowen & Iverson 2013), seabirds (Barrett *et al.* 2007b), and arthropods (O'Rorke *et al.* 2012; Furlong 2015) (Table 2.1). Reptiles and fish are notably absent from these reviews (except where they may feature as prey), and prospective users of genetic methods to analyse the diets of predators within these taxonomic groupings would benefit considerably from system-specific studies that could guide them.

2.5.2.3 Towards quantitative genetic analyses of ecological interactions

Uncertainties surrounding the quantitative information that can be extracted from DNA sequence abundance information remain an ongoing challenge to applications of this method (Pompanon *et al.* 2012). Biological and technical biases exist for potentially every step in a genetic analysis workflow, from original DNA copy numbers and preservation in samples through to variations in their amplification and treatment in bioinformatics pipelines (Deagle *et al.* 2010; Deagle *et al.* 2006; Deagle *et al.* 2013; Deagle & Tollit 2006; Thomas *et al.* 2016; Thomas *et al.* 2014). DNA copy number will inevitably vary between different species and primers may vary in their efficiency for amplifying DNA for different species (King *et al.* 2008; Thomas *et al.* 2016). The age of the sample recovered could also affect DNA abundance that is recoverable in samples. At present, the most reliable metric reported by genetic diet analysis studies is that of frequency of occurrence. This is also the most common metric reported by traditional methods of diet analysis in predators.

Information on the magnitude of trophic energy flow, such as the biomass exchanged in trophic interactions (the trophic 'holy grail'), are ultimately necessary to accurately model dynamic trophic interaction webs within ecosystems. In certain circumstances, the morphological analysis of prey remains offers information on the relative size, abundance and biomass of prey (Deagle *et al.* 2009; Fajardo *et al.* 2014; Taguchi *et al.* 2014). However, this information is circumstantial and based on the consumption of taxonomically identifiable prey remains and their survival through digestion (Peters *et al.* 2014; Peters *et al.* 2015; Pompanon *et al.* 2012; Tollit *et al.* 2006). This method is largely unreliable for invertebrates, and is limited for vertebrates (Bowen & Iverson 2013; Furlong 2015). These limitations can currently be overcome by employing diet investigation strategies that combine genetic methods with morphological or biochemical methods of diet analysis.

There is growing evidence that relative and semi-quantitative information can be gleaned from genetic diet analysis. Deagle *et al.* (2005) showed that the prey proportions recovered by genetic screening of samples were roughly consistent with prey proportions consumed for Steller sea lions, but they cautioned that the information based on raw sequence abundance is semi-quantitative, should be treated with caution (Deagle *et al.* 2010) and can be affected by sequence data filtering protocols (Deagle *et al.* 2013). In a comparison between qPCR and HTS techniques, Murray *et al.* (2011) found highly reproducible results between techniques, but recommend sound DNA purification techniques that produce extracts free of inhibitors to obtain reproducible quantitative data between genetic methods used. Thomas *et al.* (2014) investigated technical and biological biases that may affect semi-quantitative and quantitative information from recoverable prey DNA. Thomas *et al.* (2014) identified biological biases in template DNA copy number as a significant source of bias in recoverable quantitative DNA information, and also differential digestion rates of prey as a secondary source of bias. Both issues could be corrected for based on the biochemical composition of tissues, namely the lipid and protein composition of tissues, whereby animals with high muscle density are associated with increased levels of mitochondrial DNA (Fernández-Vizarra *et al.* 2011; Thomas *et al.* 2014).

Further investigation of correction factors for sources of bias in genetic information are needed for the development of quantitative metrics derived from the

genetic analysis predator diets (Thomas *et al.* 2016; Thomas *et al.* 2014). Tissue-based correction factors offer solutions that are transferable across ecosystems (Thomas *et al.* 2014), whilst species-specific correction factors offer targeted solutions for the study of particular trophic interactions of conservation or commercial interest (Thomas *et al.* 2016).

2.5.2.4 Limitations to taxonomic sensitivity: secondary predation, cannibalism and lower trophic-level interactions

The recovery of genetic information in predator stomach or faecal samples resulting from secondary predation, the prey's prey, is a commonly cited caveat for taxonomically-detailed diet information. In arthropod systems, genetic information from secondary predation was recoverable for several hours after the predator consumed its prey (Sheppard *et al.* 2005). In arthropod systems, this issue can be minimised by waiting several hours before collecting scats. This is obviously problematic for vertebrates, and very little research has been conducted to estimate the contribution of secondary predation to the genetic information obtained for predator diet analysis. One study to date has attempted to estimate this in a vertebrate study system by simultaneously sequencing the diets of a high-order predator and an important prey item and mesopredator. Bowser *et al.* (2013) detected the planktonic prey of herring in the faeces of adult puffins that had consumed herring, and also in the faeces of their chicks. Assessing the persistence of DNA from the predators' prey in samples of the higher-order predator is a key recommendation for further research and for analyses of ecological interactions.

Secondary predation is an issue that is common, in one way or another, to all methods of dietary analysis that derive information from predator tissues. The most logical way to mitigate this issue is by obtaining better information on trophic interactions throughout food webs. Predators are typically a vulnerable functional group across the world's ecosystems, and therefore the subject of much management and conservation effort and research attention (Ripple *et al.* 2014). However, information on trophic interactions involving the middle of complex food webs are currently limiting for trophic ecologists. There is therefore a need to conduct genetic dietary analyses on omnivores and herbivores (Valentini *et al.* 2009). For DNA metabarcoding of predator diets, it remains important to treat the identification of

seemingly rare and novel prey DNA with caution, and to carefully investigate the likelihood of these trophic interactions being primary or secondary.

The most abundant DNA typically recovered is that of the host, or predator, when either universal primers are used to explore diets or when the predator belongs to the same taxonomic group as do the target taxa (e.g., fish) (Berry *et al.* 2015). This raises questions surrounding the preferential amplification of predator DNA in such circumstances, either due to quantity or relative freshness in comparison to digested prey remains. Many studies overcame this issue by using a species-specific blocking primer, however they have the potential to introduce biases by screening out portions of the diet (Pompanon *et al.* 2012). Many studies also utilized multiple group-specific primers, in addition to universal primers, building redundancy in the amplification and identification of sequences (Berry *et al.* 2015; Jarman *et al.* 2004; Peters *et al.* 2014). Berry *et al.* (2015) recommended incorporating redundancy in the number of sequences obtained, as well as the post-hoc removal of the host predator sequences.

Lastly, most DNA-based techniques for predator diet analysis do not allow the researcher to positively identify cannibalistic interactions using DNA-based methods of analysis (Braley *et al.* 2010; King *et al.* 2008). Cannibalism is known to occur among some mammals (Iverson *et al.* 2013), fish and particularly among invertebrates (King *et al.* 2008). Techniques are available to assess cannibalistic interactions within species, specifically with the use of DNA microsatellites to identify beyond the species (DeWoody *et al.* 2001; King *et al.* 2008). It is recommended to utilize such techniques where cannibalism is likely to compose a significant portion of a predator's diet or where such interactions are important to elucidate, such as for conservation purposes (Iverson *et al.* 2013).

2.5.2.5 Long-term data and complex ecological investigations

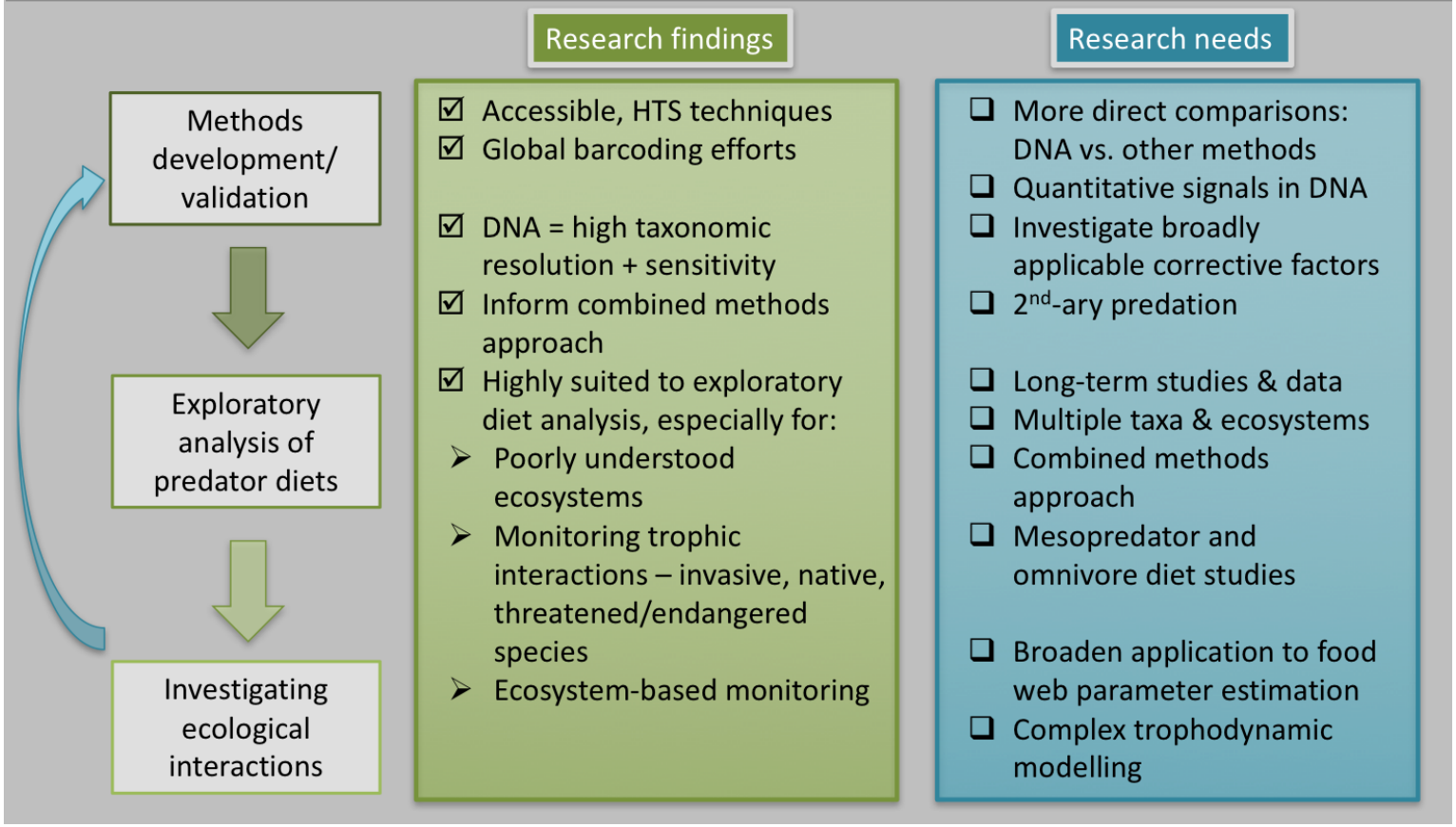
The majority of studies reviewed involved singular species, and given the recent nature of the method, were often the first study to explore that species' diet with this method and most sampled for one or two seasons. These trends are expected of a new investigative method for ecology. However, there is a clear issue of the longevity of genetic diet data for all predators, as most studies do not have previous genetic data with which to compare new results. Long-term datasets for genetic methods of diet

analysis are needed to observe dynamic ecological interactions and that are ultimately important for understanding ecosystem processes.

The temporal scale of current DNA-based studies is further compounded by the fact that genetic information on predator diets are obtained by sampling from an animal's GI tract (discussed in section 2.5.1), also an issue for morphological analysis of prey remains. Sampling from an animal's GI tract naturally limits studies to within days or hours of predation events (Bowen & Iverson 2013; Tollit *et al.* 2006), depending on the study animal. These temporal limitations can be mitigated by implementing a rigorous sampling program that both frequently and extensively samples from the targeted population of predators, to ensure representative sampling of a population across time. Typically, diet studies that use minimally invasive strategies of sampling faeces offer the largest sample sizes and most cost-effective sampling compared to any other methods of sampling for predator diet estimation (Bowen & Iverson 2013). Indeed, taxon-specific probes enable rapid and objective screening of very large sample sizes and are extremely cost-effective for large-scale detections of specific taxa (Fox *et al.* 2012; Hunter *et al.* 2012). Decreasing costs of DNA metabarcoding techniques are also enabling researchers to process increasingly large amounts of information from mixed samples for more complex ecological questions (Taberlet *et al.* 2012).

The next steps are to integrate DNA-based methods into new and existing long-term monitoring projects for ecological interactions. Syntheses of available diet information for certain taxa over long time periods offer important information on dynamic ecological interactions. DNA-based methods are likely to be improving the detection of ecological interactions (Adams *et al.* 2016) over the duration of time that these interactions have been monitored. Therefore, controlled comparative studies also remain important as they will enable researchers to better understand the types of information that can be gleaned from different methods and enable stronger syntheses of diet information across methods and over time.

Box 1. Summary of research findings, limitations and research needs in the genetic analysis of predator diets and ecological interactions. The list is structured from methods development and validation items, through to items relevant to exploratory analysis and ecological investigations.



2.6 Conclusions

Genetic techniques are demonstrably powerful tools for the analysis of ecological interactions, food web mapping at high taxonomic resolution and enhancing ecosystem modelling. Genetic approaches significantly increase the taxonomic scope of dietary analyses and are particularly useful for investigating complex food webs and novel ecological interactions for which there is little prior information. The results of two decades of DNA-based diet analysis also highlight the benefits of combining multiple methods of diet analysis for optimal food web and ecosystem modeling in space and time. Genetic tools can be used to inform further research using different techniques for the investigation of ecological interactions. Further research is required in order to make full use of DNA metabarcoding methods, in particular to obtain quantitative information from DNA sequence data for detailed food web and trophodynamic modeling. Sophisticated, high-throughput and high-yield methods are now widely available for the large-scale sequencing of environmental samples for the analysis of predator diets and application to complex ecological questions. Longer-term applications of these genetic techniques will greatly benefit research on predator ecology.

3 Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets

3.1 Abstract

The populations of many protected, top predator species that were once intensively hunted, are rebounding in size and geographic distribution. The cessation of sealing along coastal Australia and subsequent recovery of Australian (*Arctocephalus pusillus doriferus*) and long-nosed (*Arctocephalus forsteri*) fur seals represents a unique opportunity to investigate trophic linkages at a frontier of predator recolonisation. We characterised the diets of both species across two locations of recolonisation, one site an established breeding colony and a new but permanent haul-out site. Using DNA metabarcoding, high taxonomic resolution data on diets was used to inform ecological trait-based analyses across time and location. Australian and long-nosed fur seals consumed 76 and 73 prey taxa, respectively, a prey diversity greater than previously reported. We found unexpected overlap of prey functional traits in the diets of both seal species at the haul-out site, where we observed strong trophic linkages with coastal ecosystems due to the prevalence of benthic, demersal and reef-associated prey. The diets of both seal species at the breeding colony were consistent with foraging patterns observed in the centre of their geographic range, regarding diet partitioning between predator species and seasonal trends typically observed. The unexpected differences between sites in this region, and the convergence of both predators' effective ecological roles at the range-edge haul-out site, correlate with known differences in seal population densities and demographics at newly recolonised locations. This study provides a baseline for the diets and trophic interactions for recovering fur seal populations, and from which to understand the ecology of predator recolonisation.

3.2 Introduction

Pinnipeds are common high trophic-level predators in many ecosystems globally and may play a key role in structuring temperate food webs. Pinnipeds have experienced severe rates of population depletion globally through historical overexploitation and many species are currently recovering (IUCN 2017; Magera *et al.* 2013; McCauley *et al.* 2015). Nevertheless, most pinniped species are facing new anthropogenic threats through climate change and competition for resources with fisheries (Forcada & Hoffman 2014; Goldsworthy *et al.* 2003). Yet, the role of pinnipeds in the dynamic structure and function of temperate ecosystems remains a key knowledge gap (Connell 2002; Estes *et al.* 2013).

In southeastern Australia, two sympatric seal species, Australian fur seals, *Arctocephalus pusillus doriferus* (hereafter AUFS), and long-nosed fur seals (formerly New Zealand fur seals), *A. forsteri* (LNFS), are undergoing population and range recovery following historical overexploitation and near-extinction (Burleigh *et al.* 2008; Goldsworthy *et al.* 2003; Kirkwood *et al.* 2010; Shaughnessy *et al.* 2001). A breeding colony and several new haul-out sites have recently established in New South Wales (NSW, eastern Australia), and these populations represent the first for nearly a century at their northeastern range-edge (P. Shaughnessy & S. Goldsworthy, SARDI aquatic sciences, pers. comm.). Newly recolonised locations represent a frontier for species range recovery and/or expansion, where predator densities are still low, affording an opportunity to document predator diets and ecological interactions at an early stage of recolonisation. Additionally, frontier populations, due to their low densities, may be especially vulnerable within the greater population as they come into conflict with anthropogenic activities.

Knowledge of the diets of these species is based almost entirely on single predator studies from the central parts of their geographic ranges: Bass Strait for AUFS and, South Australia and New Zealand for LNFS, and the majority are from breeding colonies (Deagle *et al.* 2009; Fea *et al.* 1999; Gales & Pemberton 1994; Harcourt *et al.* 2002; Kirkwood *et al.* 2008; Page *et al.* 2005; Page *et al.* 2006). These studies report a broad diet in both species, and resource partitioning between species, whereby AUFS diets are reported as benthopelagic and LNFS as mostly pelagic. Both

species exhibit seasonal variations in diet that correlate with prey availabilities and fur seal reproductive cycles, namely, a greater prevalence of benthic and demersal prey for both fur seal species in the summer compared to winter when adult fur seals typically forage further offshore (Arnould *et al.* 2011; Harcourt *et al.* 2002; Page *et al.* 2005). Diet studies using morphological analyses of prey remains typically identify between 20 and 50 prey taxa, mostly bony fishes and cephalopods (Fea *et al.* 1999; Gales & Pemberton 1994; Kirkwood *et al.* 2008; Page *et al.* 2005). In contrast, the only other DNA-based study (Deagle *et al.* 2009) from one of the fur seal species studied here (AUFS) revealed a total of 62 prey species in only a single season of sampling. There is currently no published information on the diets of these species at their northern geographic range edge, a frontier for population and range recovery in Australia, and an area distinct in its oceanography and biogeography compared to that of the rest of their range (Connell & Irving 2008).

A predator's diet represents the direct pathway of interaction with their ecosystems and forms the basis for understanding food web structure (Pompanon *et al.* 2012; Tollit *et al.* 2009). Dietary information at high taxonomic resolution (i.e., to genus/species) enables accurate identification of key drivers that underpin food web processes (Eisenberg *et al.* 2013; Pompanon *et al.* 2012). A suite of methods exist to study the diets of predators: from traditional morphological analyses of prey remains extracted from a predator's digestive tract to various molecular methods analysing chemical signals from predator tissues including stable isotope, fatty acid and DNA-based methods (Bowen & Iverson 2013). However, many methods of diet analysis suffer problems and biases that impede fine-scale taxonomic identification of diet components (Bowen & Iverson 2013; Casper *et al.* 2007; Deagle *et al.* 2005; King *et al.* 2008; Tollit *et al.* 1997). DNA-based metabarcoding approaches have proven to be taxonomically sensitive, detecting prey items where traditional methods have not, as well as enabling higher taxonomic resolution identification of prey requiring molecular expertise rather than extensive taxon-specific expertise (Berry *et al.* 2015; Deagle *et al.* 2009; Peters *et al.* 2014; Pompanon *et al.* 2012; Tollit *et al.* 2009). This method is ideally suited to explore predator diets (Leray *et al.* 2012; Pompanon *et al.* 2012), as it enables the identification of the ecological function of prey taxa (i.e., their trophic level and the type of ecosystem from which prey were likely obtained) and thus to characterise the role of predators in ecosystems (Spitz *et al.* 2014).

We investigated trophic interactions in two sympatric fur seal species in the newly recolonised region of eastern Australia, using DNA-based methods to extract high taxonomic resolution data from scats obtained from two main sites in this region. Our aims were to: (i) characterise the diets of these sympatric predators at a frontier of recolonisation and range expansion; (ii) identify important trophic interactions and investigate how the ecological function of prey taxa varies between seal species, sampling sites, and time in these newly recolonised areas. We expected that the broad dietary patterns and prey resource partitioning observed between these seal species in southern Australia, would be reflected in their diets in our study region in eastern Australia. We therefore hypothesized that diet composition would differ between seal species and across time, but that within seal species, diets would be similar across the eastern Australian sites.

3.3 Methods

3.3.1 Study populations, sites and sample collections

The study populations of AUFS and LNFS in NSW are at the northeastern range-edge of these species' geographic distributions – an area experiencing rapid population growth (McIntosh *et al.* 2014). To date, the majority of the NSW population of both seal species occurs at breeding colonies on Montague Island, whereby breeding colonies are defined as locations harbouring the birth of at least 15 or more pups within each species (McIntosh *et al.* 2014). As such, the population of fur seals at Montague Island has a relatively large number of adult females, as well as large breeding males in eastern Australia, similar to the demographic composition of colonies elsewhere in Australia (R. Harcourt, Macquarie University, pers. comm.; N. Hardy, pers. obs). Additionally, growing haul-out sites around Jervis Bay (Burleigh *et al.* 2008), and new haul-out sites at the Five Islands Nature Reserve typically harbour juvenile and sub-adults of either sexes, and some adult seals (G. Ross, Office of Envionrment & Heritage, NSW Government, pers. comm.; N. Hardy, pers. obs) (Figure 3.1). There are no ongoing surveys of seals in these areas so accurate estimates of population size or gender/size/age structure are not available.

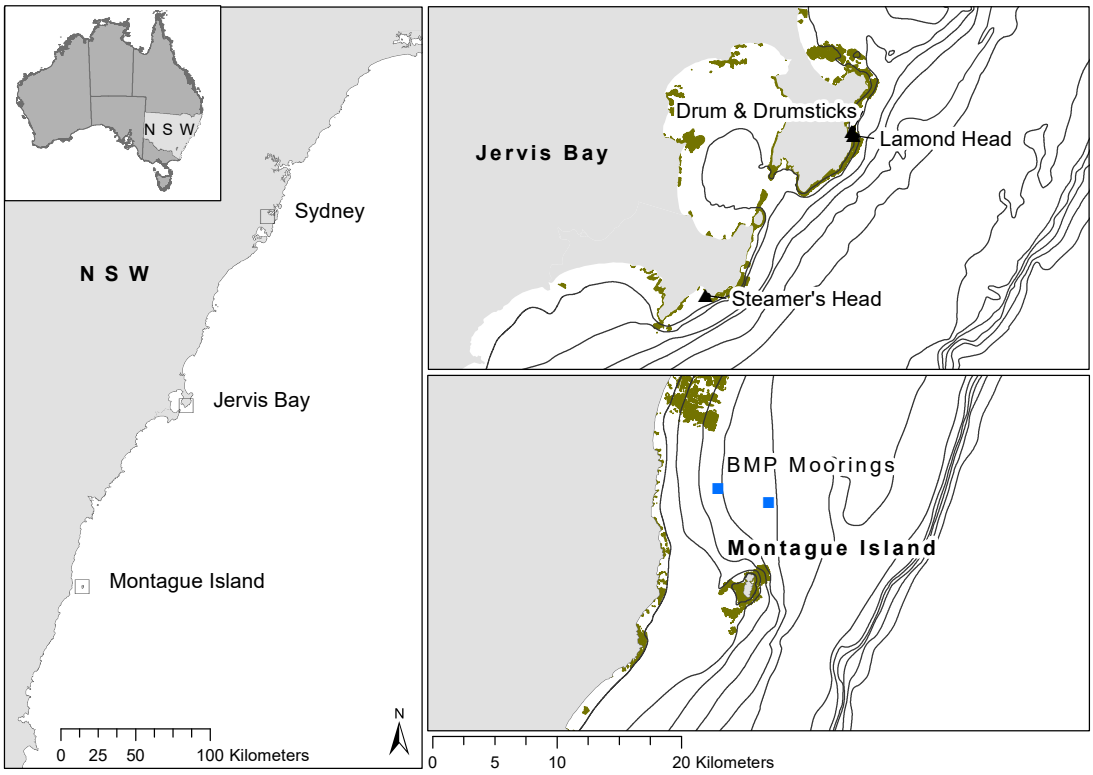


Figure 3.1 Fur seal (*Arctocephalus pusillus doriferus* and *A. forsteri*) haul-out sites at Jervis Bay and Montague Island on the New South Wales (NSW) coast in relation to continental shelf (depth contours displayed every 20 m from 20−300 m) and shallow to intermediate reef habitat (up to 60 m, shaded in green) (OEH 2015). Water temperature data are from the Batemans Marine Park (BMP) moorings north of Montague Island (IMOS 2014). Black triangles indicate the locations of seal haul-out sites at Jervis Bay.

Sampling occurred in January–April and September 2014 (hereafter the austral 'summer' and 'winter' samples, respectively), representing the warmest and coldest months of the year in terms of water temperature (data from Batemans Bay, NSW, Australia, at 20–60 m depth; Figure 3.1) (IMOS 2014). Sampling locations included: colonies at Montague Island (MI, 36° 14.645'S, 150° 13.439'E); and three haul-out sites at Jervis Bay (JB) which were: Steamer's Head (for AUFS, 35° 10.725'S, 150° 43.895'E), Drum & Drumsticks and Lamond Head (for AUFS & LNFS respectively, 35° 2.799'S, 150° 50.552'E) (Table 3.1; Figure 3.1). These sites are adjacent to extensive networks of complex shallow and intermediate depth rocky reefs (MI and JB) (Figure 3.1), a narrow continental shelf influenced by the warm East Australian Current and proximal to pelagic waters (MI and JB); MI is particularly close to one of the deepest sections of the continental shelf (at 132 m depth) and JB to a large shallow and sheltered bay with mixed seasonal estuarine influences (Jordan *et al.* 2010).

We collected a total of 129 faecal samples ($n_{AUFS} = 67$, $n_{LNFS} = 62$) across both fur seal species at two key locations (MI and JB) and times (Table 3.1). However, LNFS are typically absent from JB in the summer months at the time of this study, and only a single LNFS sample from this time point and location was opportunistically obtained in a predominantly AUFS haul-out site (Table 3.1). Fresh faecal samples were collected in zip-lock bags. Each whole scat was homogenised in the field using disposable spatulas, creating a mixed substrate, from which a 2 mL sub-sample was taken. Whole scats and sub-samples were immediately stored at -10˚C in a portable freezer (WAECO) for up to 10 days, and later transferred to -20˚C freezer facilities for longer-term storage (maximum of 6 months) (Murray *et al.* 2011).

Table 3.1 Collection locations, seasons and sample sizes for Australian (AUFS) and long-nosed fur seals (LNFS).

	Seal Species	AUFS	LNFS
Location	*Time period (2014)*		
Montague Island (MI)	Jan-Apr	15	21
	Sept	15	17
Jervis Bay (JB)	Jan-Apr	17	1[†]
	Sept	13	15

[†]Few LNFS are present in JB in the summer; one sample was collected opportunistically at a predominantly AUFS haul-out site and confirmed to belong to LNFS from DNA analyses. This sample was not included in the statistical analyses, but prey items identified in this sample are indicated in Table 3.3 and Table B.1 (Appendix B).

3.3.2 Molecular analyses

Extractions were carried out on 120–200 mg of scat sub-sample using a QIAmp DNA Stool Mini Kit (QIAGEN) as per manufacturer's instructions. As a result of the laboratory optimisation of extraction procedures, we also included an overnight digestion (at 55˚C) prior to extraction and we used half an InhibitEX tablet (an inhibitor absorption reagent, QIAGEN) per sample in accordance with Deagle *et al.* (2005). DNA was eluted in 50 µL of AE buffer (10 mM) at three dilutions in water

(neat, 1:10, 1:100) and stored at -20°C. Quantitative PCR (qPCR) was used to optimise the selection of samples and DNA concentration for subsequent fusion tagging or single-step PCR. DNA extracts were screened using qPCR to assess the DNA quality and quantity, and to detect possible PCR inhibition (Deagle *et al.* 2009; Murray *et al.* 2011). Four previously designed group-specific primers were used to bind directly to and amplify short regions of the 16S mtDNA gene, targeting mammals, fishes, cephalopods and crustaceans, and the 12s mtDNA gene for birds (Table 3.2).

All qPCR reactions were carried out in 25µL: 15.4µL of H_2O, 1X Taq Gold buffer (Applied Biosystems [ABI], USA), 2nM $MgCl_2$ (ABI, USA), 0.4mg/mL BSA (Fisher Biotec, Australia), 0.25mM dNTPs (Astral Scientific, Australia), 0.4µM each of forward and reverse primers (Integrated DNA Technologies, Australia), 0.6µL of 1/10,000 SYBR Green dye (Life Technologies, USA) and 0.05U/µL of Taq polymerase Gold (ABI, USA) with 2µL of DNA. Each qPCR was run on Applied Biosystems® step-ONE qPCR thermocycler (ABI, USA): 95˚C for 5 min, then 40-50 cycles of 95˚C for 30s, 50–57˚C for 30s (as per primer annealing temperature; Table 3.2) and 72°C for 45s. This was followed by a 1s melt curve and a 10 min final extension of 72˚C.

After screening samples, each DNA extract was then assigned a unique MID (Multiplex IDentifier) tag combination along with the next-generation sequencing (NGS) adaptors. Group-specific primers (Table 3.2), MID tags and NGS adaptors were bound to target DNA in a single-step PCR reaction, using the same reaction conditions as for qPCR. The resulting tagged amplicons were combined in pools of up to 5 samples of similar DNA molarity. Amplicon pools were then purified (Agencourt AMPure XP beads, Beckman Coulter Life Sciences, NSW, Australia), combined again in accordance with their DNA concentrations to produce a single DNA library of 60–100 samples for sequencing. Each sequencing library was quantified alongside a set of standard synthetic oligonucleotides of known molarity (Bunce *et al.* 2012) before sequencing. Sequencing was performed on an Illumina MiSeq platform (300bp V2 Nano kit) using single-end sequencing.

3.3.3 Bioinformatics

The sorting, filtering, clustering and identification of sequences were executed using specialised software. Samples were demultiplexed, and sequences were assigned to the correct sample using the unique MID tag combinations, after which identifiers, NGS adaptor sequences and primers were trimmed, in the program Geneious R8.1.5 (Kearse *et al.* 2012) leaving just the target sequences. Any sequences that did not contain exact matches to both the forward and reverse PCR primers, tags and adaptor sequences were discarded, as well as sequences that were significantly shorter than the primer product length. Discarded sequences at this stage typically corresponded to primer dimer or low quality reads.

For each sample, target sequences were filtered with FastQ using a maximum error of 0.5 and dereplicated into clusters of unique sequences, using 97% similarity for clustering, in USEARCH (Edgar 2010). Sequence clusters containing less than 1% of the total number of unique sequences detected in the sample were discarded. This minimises the risk of erroneous sequences and false positives from sequencing and other error, and vastly improves confidence in the subsequent analysis of the remaining sequences. Sequence clusters were then queried against the GenBank database using the algorithm BLASTn (Basic Local Alignment Search Tool).

Table 3.2 Metabarcoding primers for PCR used for dietary analysis of Australian and long-nosed fur seals.

Target amplicon	Gene	Primer name	Sequence (5'-3')	Product size (bp)	Annealing temperature	Reference
Mammal	mtDNA 16S	Mam16S1	CGGTTGGGGTGACCTCGGA	90	57˚C	Taylor et al. (1996)
Mammal	mtDNA 16S	Mam16S2	GCTGTTATCCCTAGGGTAACT			
Fish	mtDNA 16S	Fish16S_F	GACCCTATGGAGCTTTAGAC	200	54˚C	F: Deagle et al. (2007)
Fish	mtDNA 16S	Fish16S_R	CGCTGTTATCCCTADRGTAACT			R: Murray D. (unpublished)
Cephalopod	mtDNA 16S	S_Cephalopoda_F	GCTRGAATGAATGGTTTGAC	70	50˚C	Peters et al. 2015
Cephalopod	mtDNA 16S	S_Cephalopoda_R	TCAWTAGGGTCTTCTCGTCC			
Crustacea	mtDNA 16S	Crust16s_F(short)	GGGACGATAAGACCCTATA	150	51˚C	Berry T. (unpublished)
Crustacea	mtDNA 16S	Crust16S_R(short)	ATTACGCTGTTATCCCTAAAG			
Bird	mtDNA 12S	Bird12sa	CTGGGATTAGATACCCCACTAT	230	57˚C	Cooper et al. (1994)
Bird	mtDNA 12S	Bird12sh	CCTTGACCTGTCTTGTTAGC			

The resulting "blasted" sequences were then assigned to taxa, a part of the analyses that is necessarily done manually and follows a set of criteria outlined below (see also Deagle et al. 2009), and performed in the program MEGAN (MEtaGenome ANalyser) (Huson *et al.* 2007). Reads were reported based on the LCA-assignment algorithm parameters of a minimum bit score of 65.0, reports were limited to the top 10% of matches, and a minimum support of 1 (Huson *et al.* 2007), whereby the program MEGAN returns a shortlist of likely taxonomic assignments based on genetic similarity to the sequence. From that list, an assignment was considered reliable only when the match was made across the whole of the queried sequence. Potential prey identifications were individually investigated by consulting reference resources to assess the likelihood of prey assignments. The factors considered prior to identification include: (i) ensuring that the identified prey's geographic distribution broadly matched that of the likely SE Australian foraging areas for fur seals, and (ii) checking the diversity of closely related species and the presence/absence of voucher sequences for these in GenBank to ensure that any other likely prey species were not overlooked for want of genetic reference information. A broad range of reference databases were consulted and include: FishBase (Froese & Pauly 2016), Atlas of Living Australia (ALA 2016), reference books for coastal and pelagic fishes of southeastern Australia (Hutchins & Swainston 1986; Kuiter 2002), the Australian Museum (2016) reference base and Redmap (2016), the latter to check for out of range species.

In addition to this first assessment of the likelihood of the identified taxon being encountered by the predator, a further qualitative assessment was made on a case-by-case basis to classify the likely pathway of interaction (i.e., primary or secondary consumption) in order to remain conservative in our analyses of ecological interactions. This was largely based on, and limited by, knowledge of the biology of prey, and consisted of a sequential checklist of the following criteria: (i) whether the prey taxon was recorded in the literature either at a family-, genus- or species-level, and if so, previously corroborated records were generally considered sufficient evidence that the prey was likely consumed by the predator. If not, further criteria were examined: (ii) the frequency of detection of the taxon and whether it consistently occurred with a known mesopredator (i.e., likely secondary predation), or whether it appeared as the sole prey item in a sample (i.e., likely primary predation); (iii) the

known maximum size and average size of the species identified (FishBase, The Australian Museum, Hutchins and Swainston 1986, Kuiter 2000). Whilst DNA does not provide information on the actual size of the taxon ingested by the predator, all taxa presented as likely primary prey belonged to species that matched size-based criteria for consideration as potential prey, based on morphological studies that have estimated prey consumed by fur seals can range from 4000 g to 20 g for example (Page *et al.* 2005). Where there was insufficient evidence to support consideration for direct consumption of prey, these were considered likely to be the result of secondary consumption and were excluded from statistical analyses to reduce the risk of false positives influencing the analyses (Table B.2).

3.3.4 Data processing & Statistics

3.3.4.1 Response variables

This study aimed to evaluate trends in both fine-scale diet using species-level data, and secondly to evaluate key trophic interactions for two predator species by analysing data based on prey ecological traits. Prey taxa were assigned to collective trait-based schemes that including traits relating to trophic niche, the known spatial association of prey and a combination of these two traits which we refer to as the prey's "functional trait" (defined in Table 3.3). The spatial attributes do not assume exactly where the predator encountered that prey, but rather where that prey species most commonly occurs to the best available knowledge, and are thus necessarily broad (Table 3.3). Analyses of seal diet composition were then performed at species-level or trait-based groupings of the data, taken as the presence of identified taxa. Additionally, differences in prey species richness were investigated and defined as the number of species in a scat sample.

3.3.4.2 Statistical analyses

All statistical models included three categorical explanatory variables with two levels each: seal species (AUFS, LNFS), location (MI, JB) and time sampled (summer, winter). For the purposes of statistical analyses, each specific combination of the levels of the explanatory variables (species, location and time) can be considered an

independent "group" of seals that were sampled, and for which replicate faecal samples were collected. As we obtained only one sample from LNFS from the JB location in the austral summer, it was not possible to test a fully orthogonal model of location, time and species. Instead, differences in diet composition between groups of seals were tested by running four reduced models that included explanatory variables in combinations where they were replicated: (i) for AUFS, prey assemblage ~ location×time; (ii) for LNFS, prey assemblage ~ group (combination of location and time, i.e., MI-summer vs. MI-winter); (iii) at MI, prey assemblage ~ seal species×time; (iv) at JB, prey assemblage ~ group (combination of seal species and time, i.e., AUFS-winter vs. LNFS-winter) (Table 3.4).

Differences in diet composition were tested using multivariate generalised linear models (mvGLMs) and were fitted using a binomial distribution for multivariate presence/absence data on species-level and trait-based diet assemblages (spatial and functional trait-based grouping of the species-level response variable). The mvGLMs were performed in the *mvabund* package in *R* version 3.2.4 (R Core Team 2017; Wang *et al.* 2012). Broad trends, overdispersion and outliers in multivariate space were checked graphically by non-metric multi-dimensional scaling (nMDS) plots (Field *et al.* 1982) using the *vegan* package in *R* (Oksanen *et al.* 2015), whilst normality in multivariate data were checked using quantile-quantile (Q-Q) plots (Bates *et al.* 2015; Wang *et al.* 2012).

Table 3.3 Functional traits of seal prey species used for trait-based analyses. Placement of species into each category was based on detailed species knowledge and corroboration from reference material (Collette & Nauen 1983; Froese & Pauly 2016; Hutchins & Swainston 1986; Kuiter 2002).

Functional Trait	Category	Description
Trophic	Trophic niche	Mesopredator, piscivore, omnivore, herbivore, cleaner and unknown
Spatial	Position of prey in the water column and in relation to the coast	*Benthic:* soft-sediment bottom dweller *Demersal:* associated with the soft-sediment benthos but positioned in the water column *Reef*: any benthic or demersal prey taxon found mostly/exclusively on rocky reefs *Coastal pelagic*: species mostly/exclusively associated with bays, estuaries and shallow coastal habitats; *Continental pelagic*: mid- and open-water species known to associate commonly with the continental shelf and slope; *Pelagic (or "true" pelagic)*: species not known to encounter any coastal or benthic structures and associate exclusively with open-water and oceanographic features; *Unknown*: of completely unknown spatial origin
Functional	Trophic interaction	Combination of trophic and spatial traits

Model fit was assessed by analysis of deviance, tested using log-likelihood ratios (sum-of-*LR*) and *P*-values calculated from 999 resampling iterations via PIT-trap resampling (Wang *et al.* 2012). For significant interactions between explanatory variables in the full model, the differences between levels of these variables were tested (Table 3.4). To then identify which response variables (i.e., species or functional traits) contributed most to the difference between levels, we performed post-hoc univariate tests with adjusted *P*-values fitted to each response variable (i.e., species or functional trait) (Wang *et al.* 2012). Response variables were ranked based on the test statistic and we calculated how many response variables were required to capture at least 50% of the deviance explained compared to the full model comprising all response variables. The deviance was calculated by taking the ratio of the percentage deviance explained by a subset of the response variables and the deviance explained by the full model containing all response variables (Guisan & Zimmermann 2000). Response variables (i.e., taxa) with the highest univariate test statistic, significant *P*-values, and capturing in aggregate at least 50% of the deviance explained by the full model therefore had the greatest effect size and were considered to have the strongest evidence for an effect of explanatory variables and thus likely to be contributing to differences between levels of the explanatory variables.

Additionally, differences in prey species richness were tested using analysis of variance (ANOVA) in the base package *stats* in *R* (R Core Team 2017). Trends in the data and model assumptions, including homogeneity of variances and normality of errors were checked graphically using boxplots, co-plots and quantile-quantile (Q-Q) plots. Model validity was assessed by plotting residuals against fitted values. The percentage frequency of occurrence (FO%) of prey items was used to graphically represent the data using *ggplot2* in *R* (Wickham 2009). Percentage frequency of occurrence of a given food item is defined as the number of samples in which that food item occurred, expressed as a proportion of the total number of samples that contained food (Amundsen *et al.* 1996; Davis *et al.* 2015). Thus, the total FO% of multiple diet items can exceed 100% due to the occurrence of multiple food items in samples.

3.4 Results

3.4.1 Overview of sequencing and broad trends

A total of 112 faecal samples passed our quality filtering (no human DNA, sufficient quantity and quality of prey DNA) and thus were included in further analyses (n_{AUFS} = 60, n_{LNFS} = 52; Table 3.1). One additional sample from LNFS from the JB summer time point also passed quality filtering, but could not be included in statistical analyses at it was the only sample found from that location and time (Table 3.1). The taxa identified in this sample are noted with the symbol "†" in Table 3.5 and Table B.1 (Appendix B). The sequencing runs produced in excess of 1.8 million DNA sequences of target taxa; of which 1.6 million remained in the dataset after quality filtering, with an average of over 14,200 target sequences per sample using up to four primer sets. Sequence data files are available online (Data Accessibility).

A total of 436 taxonomic assignments of fish, cephalopod, crustacean and bird taxa (AUFS n = 215, LNFS n = 221) met criteria for consideration in analyses as likely primary prey of AUFS and LNFS (Table 3.5 and X1). These represented a total of 115 individual prey taxa, 34 of which were common to both species, and a total of 76 and 73 prey taxa identified in AUFS and LNFS samples, respectively (Table 3.5 and X1). A further 48 taxonomic assignments were made of crustaceans (AUFS n = 21, LNFS n = 27), belonging to 25 genetically distinct taxa, however these taxa appeared in < 20% of samples, almost all were present in samples alongside possible mesopredators without any prior information on predation by fur seals on these taxa, they are considered likely to be secondary predation (Appendix B, Table B.2). Prior to removal from further analyses these taxa represented 10% of all taxonomic assignments made.

Fish were the most prevalent taxonomic group across time and location for AUFS, and for LNFS samples from Jervis Bay, whilst both fish and cephalopods were equally prevalent across time for LNFS at Montague Island (Figure 3.2). For AUFS samples, a total of 59 fish taxa occurred in 92–100% of samples, 16 cephalopod taxa occurred in 38–46%, one crustacean species occurred in 23.5% of samples in JB in the summer sampling, and no birds were detected (Figure 3.2). A further 13% of

AUFS samples contained 14 different crustacean taxa considered likely secondary predation (Table B.2). For LNFS samples, 54 fish taxa occurred in 64–100% of samples. We found 18 cephalopod taxa in LNFS samples, with cephalopods occurring in up to 33% of samples at JB in winter compared to 70–86% of samples at MI (Figure 3.2). Additionally, one bird species, the little penguin (*Eudyptula minor*) was identified in one LNFS sample from JB in the winter period. We found 14 different crustacean taxa likely to come from secondary predation in ca. 17% of LNFS samples (Table B.2).

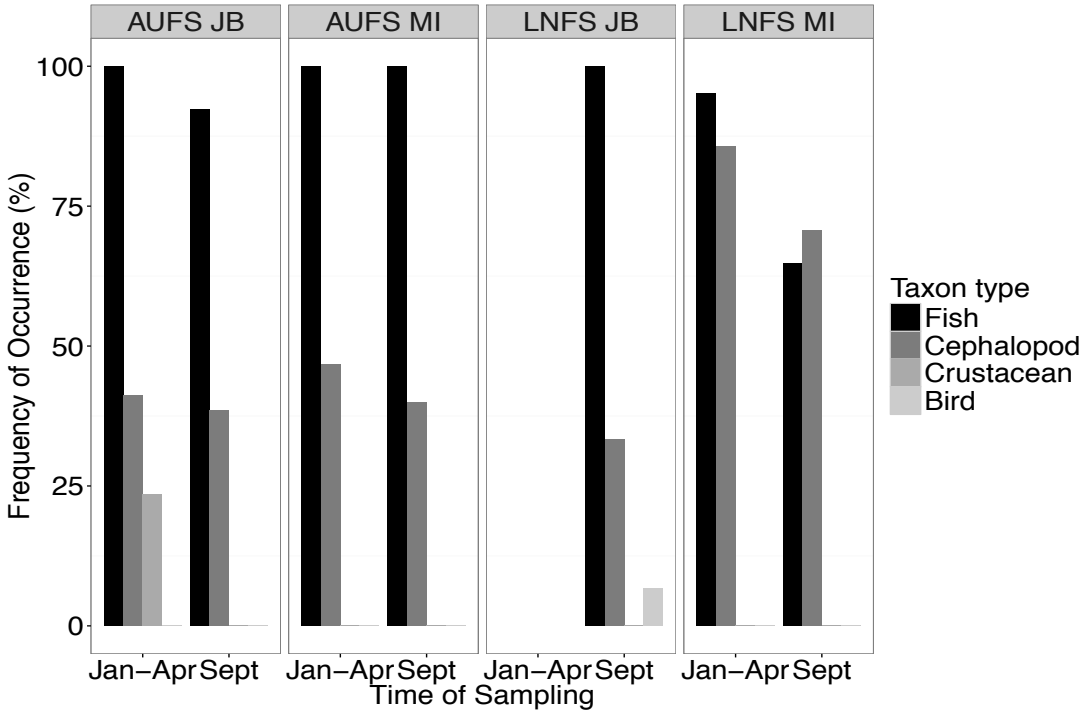


Figure 3.2 Prevalence of the four broad prey taxa for each seal species (AUFS and LNFS), location (MI and JB), and sampling time (Jan-Apr/summer* and Sept/winter 2014). Percentage frequency of occurrence (FO%) is expressed as a % of samples containing each main taxon across predator species, location, and time of sampling. No FO% data are available for LNFS at JB in the summer months.

3.4.2 Trophic, spatial and functional attributes of prey items

Mesopredators were the most common prey by trophic trait, found in 75–100% of samples of either seal species at any location or time (Figure 3.3). Analysis of diet composition in both fur seals by spatial traits showed that in samples from MI, benthic and demersal prey were more common in the summer compared to winter,

whilst coastal and continental pelagic prey were dominant in the winter samples (Figure 3.4, Appendix B, Table B.3). AUFS typically had greater FO% of benthic prey compared to LNFS at any time and location, whilst the most prevalent spatial traits in the diet of LNFS at MI were pelagics (Figure 3.4). Samples from JB were not significantly different based on prey spatial traits for all combinations of seal species and sampling time (Table B.3), and were characterised by primarily benthic, demersal and reef-associated prey taxa (Figure 3.4). This pattern was also observed for functional trait analyses (Table 3.4, Figure 3.5). Reef species and especially reef mesopredators were significantly more prevalent in JB samples, occurring in 23–35% of samples, while these prey traits were rare in MI samples for either species (FO% < 10% at MI) (Figure 3.5). As the functional trait includes both the trophic and spatial attributes of the prey taxa, encapsulating both trophic and spatial trait analyses, we present the results of the functional trait analyses in more detail (Figure 3.5).

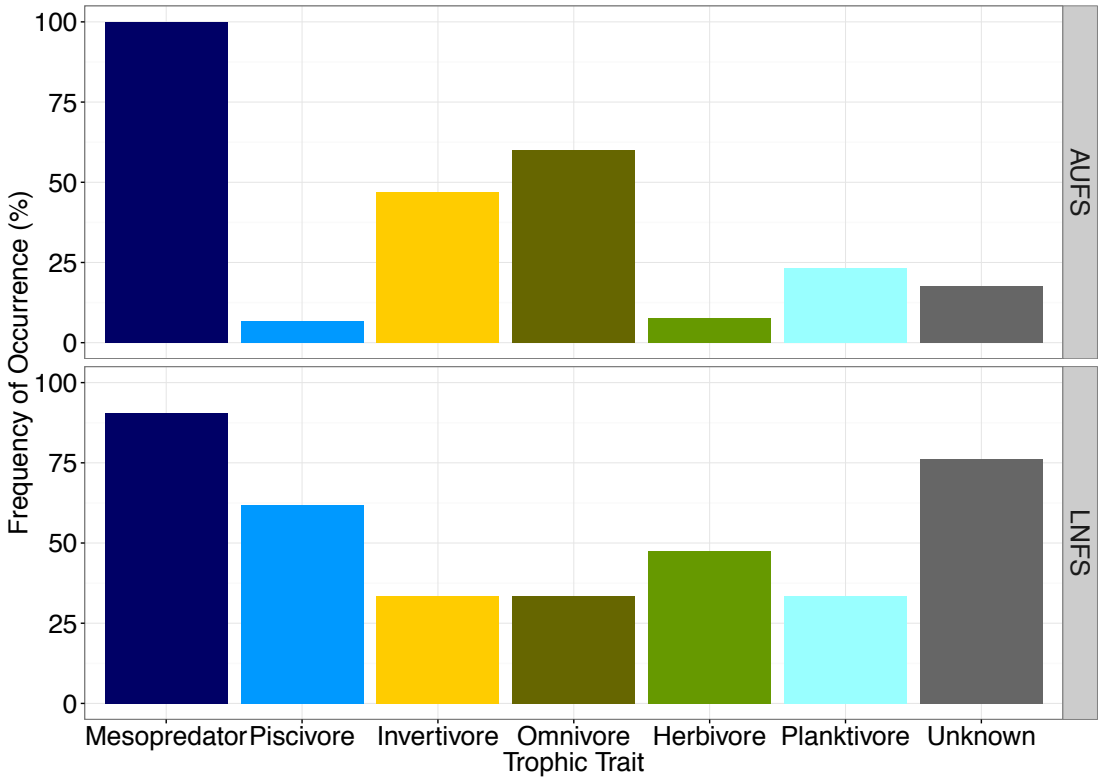


Figure 3.3 Summary of percentage frequency of occurrence (FO%) of trophic traits in the diets of sympatric eastern Australian fur seals, AUFS and LNFS.

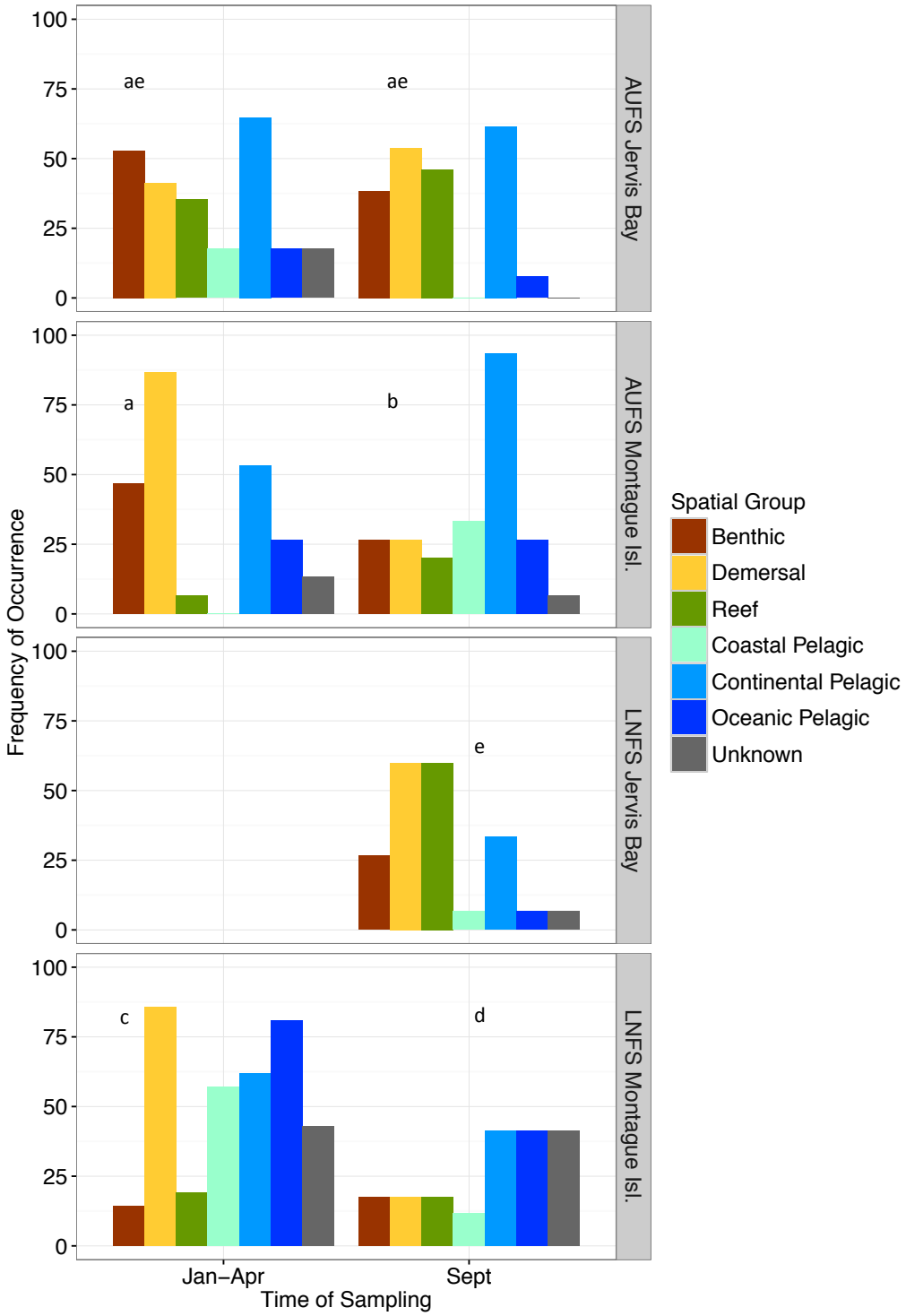


Figure 3.4 Percentage frequency of occurrence (FO) of spatial groups across seal species, location and sampling time. Different letters denote significant differences in the prey assemblage between pairwise treatment groups.

For analyses of the prey assemblage by functional traits, AUFS prey composition varied by sampling time and not location (Table 3.4, Figure 3.5), driven by a significant contribution of demersal omnivores in the summer samples compared

to winter samples from MI which had a higher FO% of coastal pelagic invertivores and continental pelagic mesopredators (Figure 3.5). Prey functional traits in AUFS diets at JB were not significantly different between sampling times. However, functional trait analyses for LNFS revealed that prey composition was significantly different for all combinations of location and sampling time (Table 3.4, Figure 3.5). LNFS samples from MI, contained significantly greater FO% of demersal mesopredators and omnivores, and coastal pelagic herbivores in summer samples compared to winter (Figure 3.5). LNFS samples from winter contained a greater FO% of pelagic mesopredators or prey of unknown trophic guild at MI, compared to greater FO% of demersal omnivores, reef planktivores and reef herbivores at JB (Figure 3.5).

3.4.3 Key prey species trends

Primary and secondary prey taxa are presented in separate tables (Table 3.5, B.1 and B.2). Species richness varied from 1 up to 13 taxa identified as potential primary prey in samples. For AUFS samples, species richness was broadly stable with an average of 3.6 (±0.4 SE) species per sample (Appendix B, Table B.4). For LNFS, richness per sample for LNFS was significantly greater in summer, with average richness of 5.7 (±0.5 SE) species compared to 3.2 (±0.4 SE) species in the winter samples, but there was no difference between winter samples from either location (Table B.4).

For AUFS and LNFS, 12 and 13 prey taxa, respectively, were encountered in over 20% of samples for a given location or sampling time (in bold in Table 3.5), and were considered common prey taxa. Virtually all combinations of the levels of explanatory variables (predator species, location and sampling time) were significantly different when the prey assemblage was analysed at the species level, with the exception of diet composition for AUFS from MI and JB sampled in winter, which were not significantly different even at the species level (Table 3.4 and 3.5). For AUFS from winter, seal diets consisted mainly of forage fish and continental pelagics, Australian sardine (*Sardinops sagax*) and jack mackerel (*Trachurus* sp.) (Tables 3.5). In summer, MI samples for AUFS had greater FO% of a taxon assigned to the family Monacanthidae (unknown Monacanthidae) and of *Octopus* sp. in summer (Tables 3.5). Species composition in AUFS diets from JB contained greater

FO% of less common and reef-associated taxa such as slipper lobster (*Crenarctus crenatus*), silver trevally (*Pseudocaranx georgianus*), a bream species (*Acanthopagrus* sp.), in the summer compared to winter samples; whilst JB samples from the winter had greater FO% of Australian sardine (*Sardinops sagax*) (Tables 3.5).

For LNFS, species composition within samples was significantly different for all combinations of location and time (Table 3.4 and 3.5). Differences between locations were due to greater FO% of the cephalopod red flying squid and king gar fish at MI, and at JB greater FO% of the reef-associated mado (*Atypichthys strigatus*), marblefish (*Aplodactylus* sp.), puller (*Chromis* sp.) and bastard trumpeter (*Latridopsis forsteri*) (Table 3.4 and 3.5). For MI samples, species composition in the diets of LNFS varied in time due to greater FO% of several cephalopod taxa with a peak in their prevalence in summer (Table 3.5).

Several previously rarely recorded or unrecorded taxa, such as mado, puller and silver sweep (*Scorpis lineolata*) (Table 3.5) were relatively common in samples from this study. They were all found together in at least one sample with no other taxa present, and as planktivores, it is improbable that they were consuming each other, and so they are likely to be primary prey items in eastern Australian fur seals. Several prey were only identified to genus or family level and each represented sequences from a single prey species, all of which were from taxa previously recorded as AUFS or LNFS prey (e.g., Monacanthidae, Macrouridae, Myctophidae, Sillangidae, Sepiidae) (Table 3.5 and B.1). Several taxa identified to sub-order, order or super-order include, respectively, unknown- Osmeriformes, Oegopsida and Decapodiformes (Table 3.5 and B.1), due to sequences having < 90% similarity to any existing sequences in Genbank. These taxa were included in analyses as they belong to taxonomic groups known to be consumed by fur seals, with the caveat that without better coverage of these groups in reference databases it is not possible to determine whether these taxa occur in samples due to primary or secondary predation.

Table 3.4 Analysis of deviance for multivariate generalised linear models (mvGLM) of species-level analyses and functional trait analyses of prey composition between fur seal species, locations and time points sampled, tested on four models. Where significant interactions occurred in the full model, reduced models tested the differences between levels of explanatory variables. Significance denoted by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. AUFS = Australian fur seal; LNFS = long-nosed fur seal; MI = Montague Island; JB = Jervis Bay.

	Response variables	**SPECIES**				**FUNCTIONAL**			
MODELS	*Factors*	*R.df*	*Df.diff*	*Dev*	*P-value*	*R.df*	*Df.diff*	*Dev*	*P-value*
(i) AUFS	*Intercept*	59				59			
	Time	58	1	106.53	0.051	58	1	34.43	0.044*
	Location	57	1	133.91	0.002**	57	1	32.34	0.065
	Time×Location	56	1	37.85	0.010**	56	1	22.32	0.084
AUFS in Summer	*Intercept*	31				-	-	-	-
	Location (Summer)	30	1	84.09	0.021*	-	-	-	-
AUFS in Winter	*Intercept*	27				-	-	-	-
	Location (Winter)	26	1	68.68	0.104	-	-	-	-
AUFS at MI	*Intercept*	29				-	-	-	-
	Time (MI)	28	1	92.51	0.003**	-	-	-	-
AUFS at JB	*Intercept*	29				-	-	-	-
	Time (JB)	28	1	81.59	0.021*	-	-	-	-

Table 3.4 Continued. AUFS = Australian fur seal; LNFS = long-nosed fur seal; MI = Montague Island; JB = Jervis Bay.

	Response variables	**SPECIES**				**FUNCTIONAL**			
MODELS	*Factors*	*R.df*	*Df.diff*	*Dev*	*P-value*	*R.df*	*Df.diff*	*Dev*	*P-value*
(ii) LNFS	*Intercept*	51				51			
	Group (Location+Time)	49	2	294.55	0.001**	49	2	125.93	0.001**
LNFS in Winter	*Intercept*	29				30			
	Location (Winter)	28	1	94.66	0.003**	29	1	44.48	0.014*
LNFS at MI	*Intercept*	35				35			
	Time (MI)	34	1	120.20	0.001**	34	1	47.52	0.008*
(iii) MI	*Intercept*	64				64			
	Time	63	1	188.00	0.001**	63	1	70.08	0.001**
	Seal sp.	62	1	228.93	0.001**	62	1	92.90	0.001**
	Seal sp.×Time	61	1	22.83	0.043*	61	1	15.02	0.296
MI in Summer	*Intercept*	35				-	-	-	-
	Seal sp. (Summer)	34	1	142.75	0.001**	-	-	-	-
MI in Winter	*Intercept*	27				-	-	-	-
	Seal sp. (Winter)	26	1	107.56	0.001**	-	-	-	-
(iv) JB	*Intercept*	45				45			
	Group (Seal sp.+Time)	43	2	204.78	0.003**	43	2	56	0.104

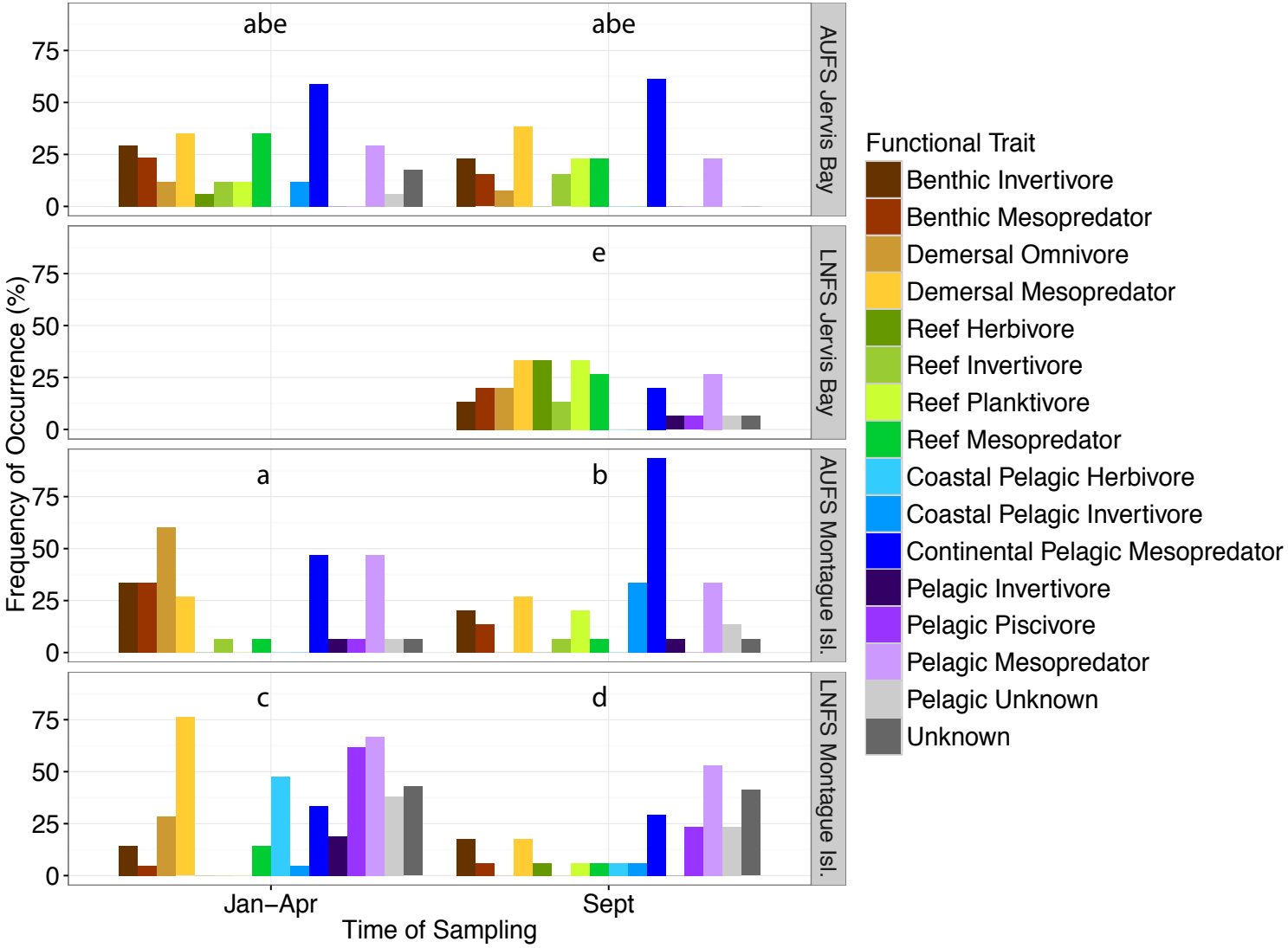


Figure 3.5 Percentage frequency of occurrence (FO%) of functional traits across predator species (AUFS and LNFS), location (MI and JB), and sampling time (Jan-Apr/summer* and Sept/winter 2014). Different letters denote significant differences in the prey assemblage between pairwise combinations of explanatory variables: seal species, location, and time. *No FO% data are available for LNFS at JB in the summer months.

Table 3.5 Taxonomic assignment and percentage frequency of occurrence (FO%) for samples of Australian (n = 60) and long-nosed (n = 53) fur seals for prey items occurring in ≥ 10% of samples. In bold, we highlight the species that occurred in 20% of samples for at least one given location or time sampled. Infrequent taxa occurring in < 10% of samples are presented in Table B.1 (Appendix B). *Trophic traits: PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Actinopterygii									
Congridae	*Gnathophis sp.* (conger eel)	PR, Benthic Predator	5.88	0.00	13.33	6.67	6.67	0.00	0.00
Belonidae	***Ablennes hians* (flat needlefish)**	**PI, Pelagic Piscivore**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**42.86**	**0.00**
Hemiramphidae	***Hyporhamphus melanochir* (southern sea garfish)**	**HE, Coastal Pelagic Herbivore**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**47.62**	**5.88**
Scomberesocidae	***Scomberesox saurus* (king gar)**	**PI, Pelagic Piscivore**	**0.00**	**0.00**	**6.67**	**0.00**	**6.67**	**19.05**	**23.53**
Berycidae	***Beryx decadactylus* (imperador)**	**PR, Demersal Predator**	**29.41**	**15.38**	**0.00**	**6.67**	**0.00**	**0.00**	**0.00**
Trachichthyidae	Unknown Trachichthyidae (roughies)	UN, Demersal Unknown	0.00	0.00	0.00	0.00	13.33	0.00	0.00
Coryphaenidae	*Coryphaena hippurus* (mahi mahi)	PR, Pelagic Predator	0.00	0.00	13.33	0.00	0.00	4.76	0.00
Clupeidae	***Sardinops sagax* (australian sardine)**	**IN, Coastal Pelagic Invertivore**	**11.76**	**0.00**	**0.00**	**26.67**	**0.00**	**4.76**	**5.88**
Macrouridae	Unknown Macrouridae (whiptails)	PR, Demersal Predator	0.00	0.00	0.00	13.33	0.00	0.00	0.00

Table 3.5 Taxonomic assignment table continued. *Trophic traits: PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Ophidiidae	***Genypterus blacodes* (ling, pink cusk-eel)**	**PR, Demersal Predator**	**0.00**	**0.00**	**0.00**	**20.00**	**0.00**	**0.00**	**0.00**
Aplodactylidae	***Aplodactylus* sp. (marblefishes)**	**HE, Reef Herbivore**	**0.00**	**0.00**	**0.00**	**0.00**	**26.67**	**0.00**	**0.00**
Carangidae	***Trachurus declivis* (common jack mackerel)**	**PR, Continental Pelagic Predator**	**23.53**	**30.77**	**13.33**	**66.67**	**0.00**	**4.76**	**11.76**
	Pseudocaranx georgianus (silver trevally)	PR, Reef Predator	11.76	0.00	0.00	0.00	13.33	0.00	0.00
Gempylidae	*Rexea sp.* (gemfish)	PR, Continental Pelagic Predator	0.00	15.38	0.00	0.00	0.00	0.00	0.00
	Thyrsites atun (barracouta)	PR, Demersal Predator	0.00	7.69	0.00	0.00	13.33	4.76	0.00
Kyphosidae	***Atypichthys strigatus* (mado)**	**PL, Reef Planktivore**	**11.76**	**7.69**	**0.00**	**6.67**	**26.67**	**0.00**	**0.00**
Latridae	*Latridopsis forsteri* (bastard trumpeter)	IN, Reef Invertivore	0.00	0.00	0.00	6.67	13.33	0.00	0.00
Nomeidae	*Cubiceps* sp. (drift fish)	PR, Demersal Predator	0.00	0.00	0.00	0.00	0.00	4.76	11.76
Pomacentridae	***Chromis* sp. (puller)**	**OM, Demersal Omnivore**	**0.00**	**0.00**	**0.00**	**0.00**	**20.00**	**0.00**	**0.00**
Pomatomidae	***Pomatus saltatrix* (bluefish/tailor)**	**PI, Pelagic Piscivore**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**23.81**	**0.00**

Table 3.5 Taxonomic assignment table continued. *Trophic traits: PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown. † Prey items found in the single LNFS sample from JB in Jan-Apr (Sillangidae).

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Scombridae	***Scomber australasicus* (spotted chub mackerel)**	**PR, Pelagic Predator**	**17.65**	**7.69**	**13.33**	**20.00**	**0.00**	**14.29**	**5.88**
Scorpididae	***Scorpis* sp. (sweep)**	**PL, Reef Planktivore**	**5.88**	**0.00**	**0.00**	**6.67**	**20.00**	**0.00**	**5.88**
Serranidae	*Caesioperca* sp. (butterfly/barber perch)	PL, Reef Planktivore	0.00	15.38	0.00	13.33	0.00	0.00	0.00
Sillaginidae †	*Sillago flindersi* (eastern school whiting)	IN, Benthic Invertivore	0.00	7.69	0.00	0.00	13.33	0.00	11.76
Sparidae	*Acanthopagrus* sp. (bream sp.)	PR, Reef Predator	11.76	0.00	0.00	0.00	0.00	0.00	0.00
Platycephalidae	***Neoplatycephalus richardsoni* (tiger flathead)**	**PR, Benthic Predator**	**17.65**	**7.69**	**20.00**	**6.67**	**6.67**	**4.76**	**5.88**
Monacanthidae	***Nelusetta ayraudi* (ocean jacket)**	**PR, Continental Pelagic Predator**	**47.06**	**38.46**	**40.00**	**60.00**	**20.00**	**28.57**	**11.76**
	Unknown Monacanthidae (leatherjackets)	**OM, Demersal Omnivore**	**5.88**	**0.00**	**60.00**	**0.00**	**0.00**	**9.52**	**0.00**
Tetraodontidae	*Lagocephalus* sp. (rabbitfishes)	IN, Pelagic Invertivore	0.00	0.00	0.00	0.00	0.00	14.29	0.00
Cephalopoda									
Loliginidae	***Sepioteuthis australis* (southern calamari squid)**	**PR, Demersal Predator**	**5.88**	**15.38**	**6.67**	**0.00**	**20.00**	**71.43**	**5.88**

Table 3.5 Taxonomic assignment table continued. *Trophic traits: PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Octopodidae	***Octopus maorum* (maori octopus)**	**IN, Benthic Invertivore**	**5.88**	**7.69**	**6.67**	**20.00**	**0.00**	**4.76**	**0.00**
	***Octopus* sp.**	**IN, Benthic Invertivore**	**0.00**	**7.69**	**26.67**	**6.67**	**6.67**	**9.52**	**0.00**
Enoploteuthidae	*Enoploteuthis galaxias* (galaxy squid)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	19.05	0.00
Ommastrephidae	***Nototodarus gouldi* (red arrow squid)**	**PR, Pelagic Predator**	**17.65**	**15.38**	**26.67**	**13.33**	**20.00**	**57.14**	**35.29**
	Nototodarus sp. (arrow squid)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	0.00	14.29	0.00
	***Ommastrephes bartramii* (red flying squid)**	**PR, Pelagic Predator**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**4.76**	**23.53**
	Todarodes filipovae (fillipova's squid)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	0.00	11.76
Sepiidae	*Sepia apama* (giant cuttlefish)	PR, Reef Predator	0.00	0.00	0.00	0.00	13.33	0.00	0.00
Oegopsida	Unknown Oegopsida (squid)	UN, Unknown	17.65	0.00	6.67	0.00	6.67	4.76	17.65
Decapodiformes	**Unknown Decapodiformes**	**UN, Unknown**	**5.88**	**0.00**	**0.00**	**0.00**	**0.00**	**42.86**	**35.29**
Malacostraca									
Scyllaridae	***Crenarctus crenatus* (slipper lobster)**	**IN, Benthic Invertivore**	**23.53**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

3.5 Discussion

The recent recolonisation of the coast of southeastern Australia (NSW) by Australian (AUFS) and long-nosed (LNFS) fur seals affords a unique opportunity to investigate trophic interactions in two sympatric, recolonising predators. Using taxonomically sensitive DNA metabarcoding methods to analyse diets, we identified a greater than expected diversity of prey items within the diets of both predator species and we provide baseline dietary information for two recolonising predators in the eastern Australian region. These methods enabled the identification of the ecological function of prey taxa and novel areas of differentiation and overlap in the diets of recolonising predators, affording greater characterisation of trophic interactions occurring within these temperate food webs.

Diet composition at the species level was different between predator species and locations, whilst there was considerable overlap in prey functional traits in the diet of both seal species at the range-edge haul-out site, with the most prevalent traits being benthic, demersal and reef-associated prey at this location (Figure 3.6). This result was unlike that of the diet composition of both seal species from the breeding colony, which exhibited a greater prevalence of prey from continental pelagic and true pelagic functional traits overall, and also a spike in benthic and demersal prey in the summer samples. At the breeding colony, diet composition also varied between predators (Figure 3.6), as expected from studies in these species from the centre of their geographic range. The hypothesis that diets would differ between seal species and seasons was thus supported at the breeding colony but not at the haul-out site. These data support the notion that there may be stronger trophic linkages for both seal species with coastal ecosystems at the haul-out site as compared to the more established breeding colony in eastern Australia.

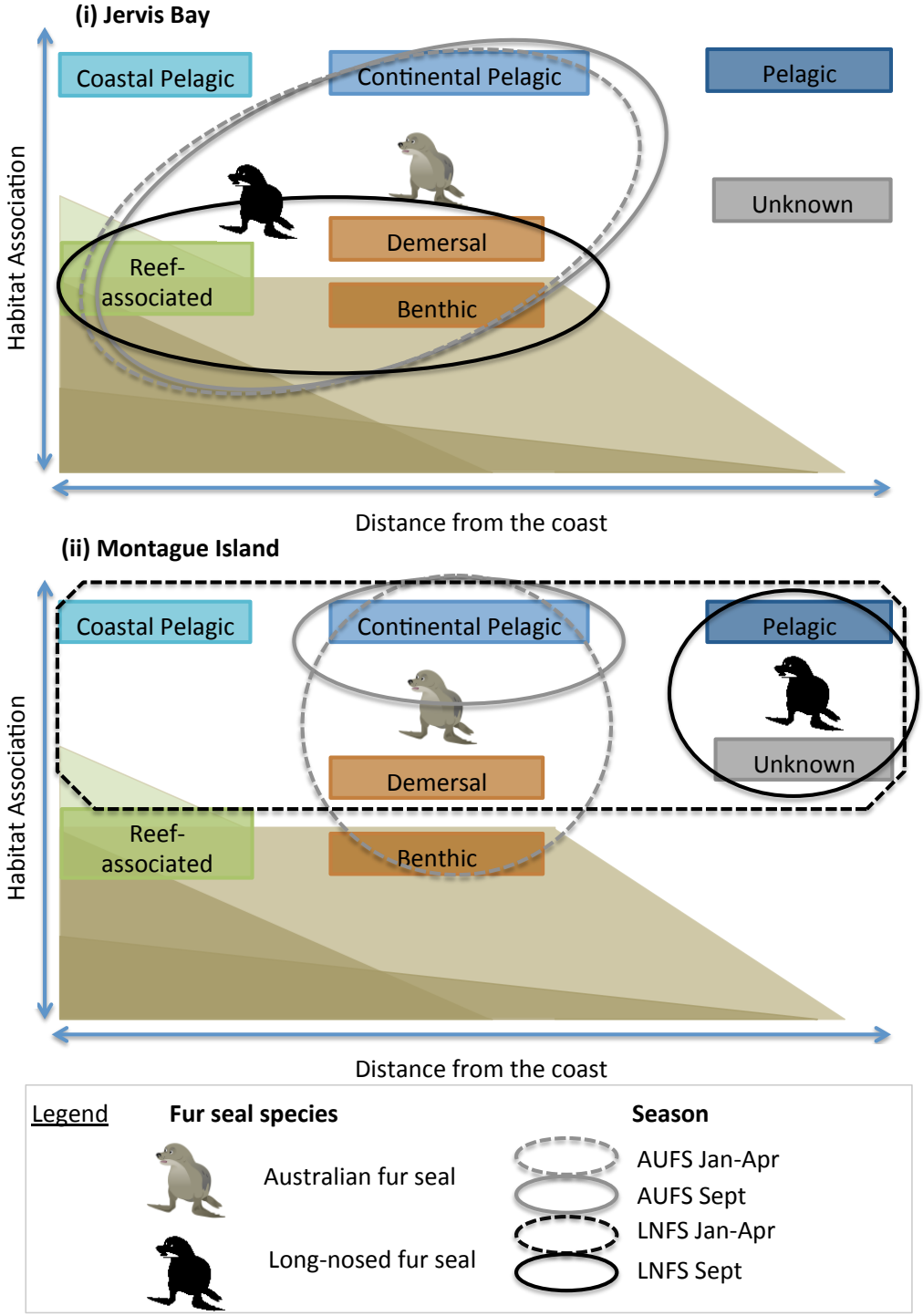


Figure 3.6 Conceptual diagram depicting changes in food web interactions for both Australian (AUFS) and long-nosed (LNFS) fur seals at (i) Jervis Bay and (ii) Montague Island, over time from summer (Jan-Apr, dashed polygon) to winter (Sept, solid polygon) samples. Only key prey traits are illustrated based on known spatial association with submerged habitats and coastal and continental shelf structures.

3.5.1 Ecological interactions of eastern Australian fur seals

Differences in diet composition at the species- and functional trait levels observed between predator species at the breeding colony (MI) were consistent with broad trends observed in the centre of their range (Deagle *et al.* 2009; Gales & Pemberton 1994; Harcourt *et al.* 2002; Kirkwood *et al.* 2008; Page *et al.* 2005). The main prey for AUFS were from benthic and demersal food webs, as well as from pelagic food webs over continental shelf waters from both the breeding colony (MI) and the haul-out site (JB); whilst for LNFS, the main prey for samples from the breeding colony (MI) were broadly pelagic (Figure 3.6). Temporal differences evident in the diet of both seal species at the breeding colony (MI) were also consistent with trends observed at other breeding colonies (Arnould *et al.* 2011; Harcourt *et al.* 2002; Page *et al.* 2005), with greater prevalence of benthic and demersal prey in the summer samples and pelagic prey in the winter samples (Figure 3.6). Summer is also the time of year when females are nursing young pups and are known to forage closer to breeding colonies, possibly contributing to these population-level trends in diets at MI (Harcourt *et al.* 2002; Kirkwood & Arnould 2011; Page *et al.* 2005; Page *et al.* 2006).

At the range-edge haul-out site (JB), however, the diets of both seal species were unexpectedly similar to each other and exhibited patterns atypical of other sites. The diet composition of LNFS samples from JB was more similar to AUFS samples from this location than to their own kind from the breeding colony (MI) (Figure 3.6), only several hundred kilometres away. Despite some differences in prey at the species level, our findings indicate that both fur seal species functionally overlap at this location, with prevalent trophic interactions with coastal ecosystems due to the dominance of benthic, demersal and especially reef-associated prey in their diets. Interestingly, the most common prey trait for AUFS at JB were still continental pelagic mesopredators, whilst pelagic prey were rare in LNFS from JB with the exception of *N. ayraudi*, contrary to what would be expected from studies from elsewhere for LNFS, mostly based at breeding colonies (Harcourt *et al.* 2002; Kirkwood & Arnould 2011; Page *et al.* 2005; Page *et al.* 2006), and contrary to what we observed in their diets at MI.

Reef fishes are a particular focus of coastal zone management due to their susceptibility to localised depletion from fishing and the need to mitigate anthropogenic effects through strategies including networks of marine protected areas (MPAs) (Kelaher *et al.* 2015). Although direct trophic effects of pinnipeds on reef communities are not well known, reef-associated prey are occasionally found in the diets of AUFS elsewhere in Australia (Deagle *et al.* 2009; Page *et al.* 2005). Fur seals are also suspected to affect reef fish assemblages at Montague Island, where their densities in eastern Australia are highest (Kelaher *et al.* 2015). Additionally, concerns about the trophic impacts of large predators on coastal reefs by local human communities and marine resource users are usually related to the densities of predators (i.e., the more seals the greater the concern) (Michelle Voyer, University of Technology Sydney, pers. comm.). The results of this study instead highlight the possibility that range edge and haul-out sites may experience greater trophic interactions between seals and coastal ecosystems.

Differences in predator diets may be influenced by site-specific differences in prey assemblages (Cherel & Hobson 2007; Deagle *et al.* 2009; Gales *et al.* 1993). Jervis Bay is a large coastal embayment, whereas Montague Island is a unique offshore island, 2˚ latitude further south, and may be more heavily influenced by oceanographic features that drive the distributions of highly mobile prey (Kelaher *et al.* 2015; Suthers *et al.* 2011). This influence may partially explain the greater prevalence of pelagic prey items in both fur seal species at Montague Island. However, both sites are positioned on a narrow continental shelf of ca. 20 km, proximal to a strong western boundary current that strongly affects prey distributions and availabilities, and both sites are associated with extensive networks of shallow and intermediate rocky reefs (Jordan *et al.* 2010).

It is more likely that the broad dietary patterns observed here are driven by differences in fur seal population demographics and densities between recolonised sites. The age cohorts and sex of fur seals differ between breeding colonies and haul-out sites, the latter consisting mainly of juvenile and sub-adult seals (Burleigh et al. 2008; R. Harcourt, Macquarie University, pers. comm.), differences that are known to influence foraging strategies of seals (Fowler *et al.* 2006; Lowther *et al.* 2013; Page *et al.* 2006). Juveniles have been found to make shorter, shallower and near-shore dives compared to adults in another otariid species, the Australian sea lion (Fowler *et al.*

2006; Lowther *et al.* 2013; Page *et al.* 2006). Additionally, density-mediated effects could be occurring at the breeding colony, observed in other recovering pinniped populations, such as northern fur seals (Kuhn *et al.* 2014), such that increasing population density, intra- and inter-specific competition between predator species, could lead to localised resource depletion at the breeding colony compared to a less established haul-out site over time. This raises the question of whether certain demographic and frontier cohorts of seals, particularly younger cohorts, may be more likely to forage in and impact shallower, near-shore reef communities before competition drives foraging effort further offshore, and importantly how long this effect might be observable. The ongoing NSW fur seal population recovery provides a unique opportunity to test these hypotheses for further research using a gradient of recolonising fur seal densities and demographics, as several more haul-out sites have become established since the commencement of this study.

The majority of prey taxa identified in this study were generalist 2nd or 3rd trophic-level mesopredators: invertivores, piscivores or generalist mesopredators. Prey included wide-ranging, generally schooling prey items that occur in a range of ecosystems. This observation confirms that both seal species are functionally 4th trophic-level generalist predators in the recently colonised east coast ecosystems and throughout their range (Goldsworthy *et al.* 2013). The direct trophic impact of these seals will therefore be felt primarily towards the middle of the food web, while indirect effects are expected for lower trophic levels through mesopredator release feedback mechanisms (Estes *et al.* 2016; Prugh *et al.* 2009). However, detailed information on prey diets, trophic linkages and dynamics are lacking and currently limit further interpretation of local food webs. Whole ecosystem trophodynamic modeling, such as performed for South Australia (Goldsworthy *et al.* 2013), is required to further evaluate at all trophic levels, the complex interactions between recolonising predators and eastern Australian ecosystems.

We also observed a previously known trophic linkage between long-nosed fur seals and little penguins. Predation of little penguins is known to occur in both fur seal species, but is more common in LNFS (Gales & Pemberton 1994; Page *et al.* 2005) and has been observed at Montague Island where little penguins nest (M. A. Coleman pers. obs.). A relatively high frequency of occurrence of their remains has been recorded in the scats and regurgitates of male LNFS in South Australia (~20% of

samples)round (Page *et al.* 2005) and in Victoria (up to 60% of samples) (R. McIntosh, Philip Island Nature Parks, pers. comm.). In contrast, we only found little penguin remains in a single scat in one season and at one location, the haul-out site at Jervis Bay (< 7% of samples) and none have yet been detected at Montague Island, a breeding colony. Given concerns about the potential impacts of recovering fur seal populations on little penguin populations elsewhere throughout their range, further monitoring of the degree of trophic interaction is warranted.

3.5.2 Recommendations for further work on DNA-based methods and predator diet analysis

High-taxonomic resolution was fundamental in identifying key trophic interactions of these recolonising predator species by enabling the identification of broader patterns in these predators' diets based on prey ecological traits. The number of prey species identified in the diets of either predator in this study, using DNA-based methods targeting four taxonomic prey groups, so far represents the highest number recorded in any study for either AUFS or LNFS, despite other studies typically employing greater sampling effort. Between 20 and 42 individual species are typically identified for either AUFS or LNFS in studies from Australia and New Zealand, with sampling efforts ranging from several hundred to over 1250 scats over multiple seasons, for 2–9 years of sampling effort (Fea *et al.* 1999; Gales & Pemberton 1994; Kirkwood *et al.* 2008; Page *et al.* 2005). A DNA-based study of AUFS diet from Bass Strait identified 54 bony fish, 4 cartilagenous fish, 4 cephalopods and one bird species in only one season (n = 90 scats) (Deagle *et al.* 2009), similar to the numbers found in the present study.

Differences in prey diversity can be influenced by study location, as a function of latitude and oceanographic parameters, and further studies directly comparing these methods across locations are warranted. However, geographic differences (Stuart-Smith *et al.* 2013) are not sufficient to explain the differences observed in prey diversity between DNA-based and morphological studies in these locations. DNA-based studies are known to be currently the most taxonomically sensitive method for diet analysis for marine predators (Berry *et al.* 2015; Casper *et al.* 2007; Deagle *et al.*

2009; Tollit *et al.* 2009), and aspects of predator diets may be overlooked by restricting diet investigations to morphological methods alone (Berry *et al.* 2015; Deagle *et al.* 2009). This method enabled this study to rapidly capture the breadth of these predator diets in novel locations.

At present, the use of multiple primers for DNA metabarcoding, to produce detailed taxonomic information on diet, incurs the loss of prey relative abundance information (Berry *et al.* 2015; Deagle *et al.* 2009), information that is crucial to assessing the relative importance of prey observed (**Chapter 2**). Biological and technical biases are known to affect sequence abundances and therefore sequence proportions recovered within samples and between primer sets (Deagle *et al.* 2006; Deagle *et al.* 2013; Thomas *et al.* 2014). Three recent studies have addressed these biases and offer solutions in the form of quantifying technical biases (Deagle *et al.* 2013), and in developing DNA correction factors either based on prey tissue composition or specific species (Thomas *et al.* 2016; Thomas *et al.* 2014). These developments will likely enable further breakthroughs to confidently using relative abundance or biomass information from DNA-based studies. However, further research and development of corrective factors across different predator species and ecosystems are needed before they can be more broadly applied. Additionally, whilst proportions of prey sequences observed for the same primer were found to be stable across sequencing runs from within the same laboratory group (Deagle & Tollit 2006), we caution that raw sequence abundances are contingent on accurate replication of library build conditions, will necessarily vary across laboratories, and are not comparable between primers. Due to the use of multiple DNA primer sets in this study to target multiple taxonomic groups, we conservatively analyse only presence data at high taxonomic resolution.

A second issue is that of secondary predation or the recovery of DNA not only from fur seal prey taxa but also from the taxa that the prey themselves consumed and that could, in theory, survive digestion twice (King *et al.* 2008; Sheppard & Harwood 2005). In practice, this has not, to our knowledge, been experimentally tested in any vertebrate predator for any method of diet analysis and secondary predation is a little understood issue common to all methods of diet analysis sampling predator tissues and faeces. Evaluating the extent to which trace amounts or greater quantities of material originating from secondary consumption is present in predator tissues would

require complex multi-trophic level captive feeding trials for each method of diet analysis. In the absence of such a study, present studies employ a weight of evidence approach outlined in the methods and rely on sound biological interpretation and prior knowledge of the system to increase confidence in prey identifications. This process is currently done manually and for most prey items detected it is relatively straightforward. Some of the lower taxonomic resolution taxa found here could in fact be secondary predation (e.g., unknown Decapodiformes), however, they could also represent species for which reference material is lacking in global genetic databases for entire taxonomic groups. Indeed poorly resolved taxonomy and a paucity of reference material are limiting factors in genetic analyses, particularly for certain taxonomic groups such as cephalopods and crustaceans (Berry *et al.* 2015). Our methods were careful to balance the risks of false negatives concerning legitimate prey items and also conservative towards false positives arising from contamination and potential secondary predation. Finally, through stringent quality control and manual curation of the prey database we are certain that these prey were ultimately consumed, and their relative importance will become clearer through longer-term research.

3.6 Conclusions

The results of this study provide a much-needed prey database for recolonising eastern Australian fur seal populations that can inform future work on their diet, trophic interactions and ecosystem trophodynamics. The differences observed in trophic linkages for predators at the haul-out site compared to the breeding site also highlight the need to further investigate different demographic and frontier cohorts of seals in recovering populations, which could result in different considerations for coastal management targeted to different cohorts of seals. We recommend continued research on seal diets in eastern Australia, a location at the frontier for the population and range recovery of two large-bodied predators, to provide valuable insights on the trophodynamics of similar predator recolonisations in temperate coastal ecosystems. Importantly, parallel sampling and analysis of the diets of mesopredators and lower trophic levels using complimentary multi-disciplinary and DNA-based methods will enable better resolution of trophic interactions across whole ecosystems.

Reconstruction of ecosystem-scale trophodynamics will be essential to managing the recovery of protected species and the marine resources they depend on.

3.7 Data Accessibility

Metabarcoding data (raw FASTQ files and filtered FASTA files) are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.2tk0q.

4 Trait-based analyses inform the relationship between fur seal aggregation sites and coastal fish community composition

4.1 Abstract

Despite large-scale changes in pinniped populations in the last century, relatively little is known about how these predators are affecting ecological communities. Sympatric predator populations, of Australian (*Arctocephalus pusillus doriferus*) and long-nosed (*Arctocephalus forsteri*) fur seals, are rebounding in eastern Australia following the cessation of sealing, and their diets have been shown to include nearshore coastal prey at their recolonisation frontier. Here, I used a trait-based approach to evaluate differences in fish assemblages at two recently recolonised sites harbouring fur seal aggregations (haul-out sites) compared to multiple local reference sites without seals. Trait-based analyses revealed that fish community composition differed between seal haul-out vs. reference sites, and that they differed in relation to fish functional traits, as well as the locations of the haul-out site. The prevalences of schooling fish and browsing herbivores were negatively correlated with haul-out sites, whilst the presence of mesopredators, as well as fish that formed pairs or small groups, were more common near haul-out sites. The total abundance and the proportional biomass of fish of different size classes were significantly different between one of the haul-out sites and reference sites. These results provide evidence for differences between reef fish communities adjacent to and away from fur seal aggregation sites. These differences did not, however, correspond to large ecological differences in reef community trophic structure or size structure, and differences were contingent on the location of the haul-out site sampled. At an early stage in the recovery trajectories of these fur seal populations, these results provide a baseline understanding for fish community composition in the context of natural recolonisation by large predators.

4.2 Introduction

Understanding how the recovery of predators affects prey communities at a functional level helps to identify critical trophic linkages and to predict flow-on effects within ecosystems. Large-scale changes to predator populations globally have led to a 'trophic downgrading' of our planet (reviewed by Ripple *et al.* 2014), whereby the disappearance of upper trophic levels can lead to cascading ecological effects. When predator populations return, however, unique opportunities arise to observe a potential reversal in the changes that may have resulted from their removal or local extinction. The recovery of predators typically results in prey limitation (Beschta & Ripple 2009; Estes *et al.* 2016; Ripple *et al.* 2014). However, it has been estimated that the ecological influences of over 90% of species of oceanic megafauna are largely unknown (reviewed by Estes *et al.* 2016).

Globally, pinnipeds have experienced large-scale population declines and alterations in geographic distributions, primarily due to harvesting (reviewed by Estes *et al.* 2016). Following protection and the cessation of harvesting, many seal species are also experiencing recovery (IUCN 2017; McCauley *et al.* 2015). Pinnipeds negatively impact prey populations (Boveng *et al*. 1998; Kelaher *et al*. 2015; Power & Gregoire 1978), but can also impact prey behaviour, as fish have been observed to be more cautious and reduce their time spent foraging in the presence of pinnipeds (Connell 2002; Shepherd *et al*. 2010). However, the functional role that pinnipeds may play in the their ecosystems remains poorly understood and may vary in different ecosystems (Bowen 1997; Estes 2009; Estes *et al.* 2016). Understanding the role of recovering populations of pinnipeds in structuring fish communities, whether significant or not, is required to ensure that marine management strategies account for interactions between these recovering predators and coastal ecosystems.

Cessation of harvesting and legal protection have promoted the recovery of two pinniped species on the coast of southeastern Australia, providing a unique opportunity to investigate relationships between these generalist-predators and complex prey communities at newly recolonised habitats. After more than a century of harvesting (Simon Goldsworthy, pers. comm., SARDI), Australian fur seals, *Arctocephalus pusillus doriferus* (hereafter AUFS), and long-nosed fur seals (formerly New Zealand fur seals), *A. forsterii* (LNFS), have recently re-established a

breeding colony and several non-breeding aggregation (hereafter 'haul-out') sites across 500 km of the coastline of southern New South Wales (NSW, eastern Australia) (McIntosh *et al.* 2014; Shaughnessy *et al.* 2001). Both fur seal species associate with the coast and both have broad diets spanning benthic to pelagic ecosystems, but typically exhibit resource partitioning (Arnould *et al.* 2011; Deagle *et al.* 2009; Harcourt *et al.* 2002; Page *et al.* 2005). Recent research on these recovering species from their northeastern range-edge revealed that the diets of both species converged in eastern Australian haul-out sites, with a particularly high prevalence of coastal reef-associated and benthic prey species (Hardy *et al.* 2017). It is not known how coastal fish communities may change in response to ongoing and natural predator recolonisation in eastern Australia, nor what the flow-on ecological effects might be on a complex marine ecosystem.

The use of a framework of analyses based on species' traits can improve our knowledge of the relationships between predators, their prey and their ecosystems as specific ecological and/or biological traits of the study species are used to inform statistical analyses (Luck *et al.* 2012; Spitz *et al.* 2014). A trait-based approach is also more likely to detect differences in ecological communities that would otherwise be missed when assessing overall community diversity indices, because biological and ecological changes are often trophically or functionally driven (Babcock *et al.* 2010; Coleman *et al.* 2015; Stuart-Smith *et al.* 2013). For example, the loss a predator that preys on herbivores will release those herbivores from predation pressure, and this will have ecological rammifications which are fundamentally linked to an animal's functional trait of being a herbivore rather than being due to that unique species.

Furthermore, trait-based analyses of ecological interactions may enable us to make sense of otherwise complex interacting webs of species, where we may have multiple species of herbivores and where their impact as a functional whole might be significant in an ecosystem when individual species may not have a significant effect. Species traits may therefore help to understand how complex assemblages of species respond to changing environmental conditions (Brown *et al.* 2014a; McGill *et al.* 2006). Thus, analyses of ecological communities can go beyond a taxonomic understanding towards a functional understanding of community ecology using trait-based analyses, however this is difficult to do in a coherent model using conventional multivariate statistics (Brown *et al.* 2014a; Spitz *et al.* 2014). The fourth-corner

solution developed by Brown *et al.* (2014a), and used in this study, enables researchers to combine and analyse in a single model: the relationships between species abundances or occurrences, to the environment in which they occur and their traits (Figure 4.1) (Brown *et al.* 2014a; Dray & Legendre 2008; Legendre *et al.* 1997; Spitz *et al.* 2014).

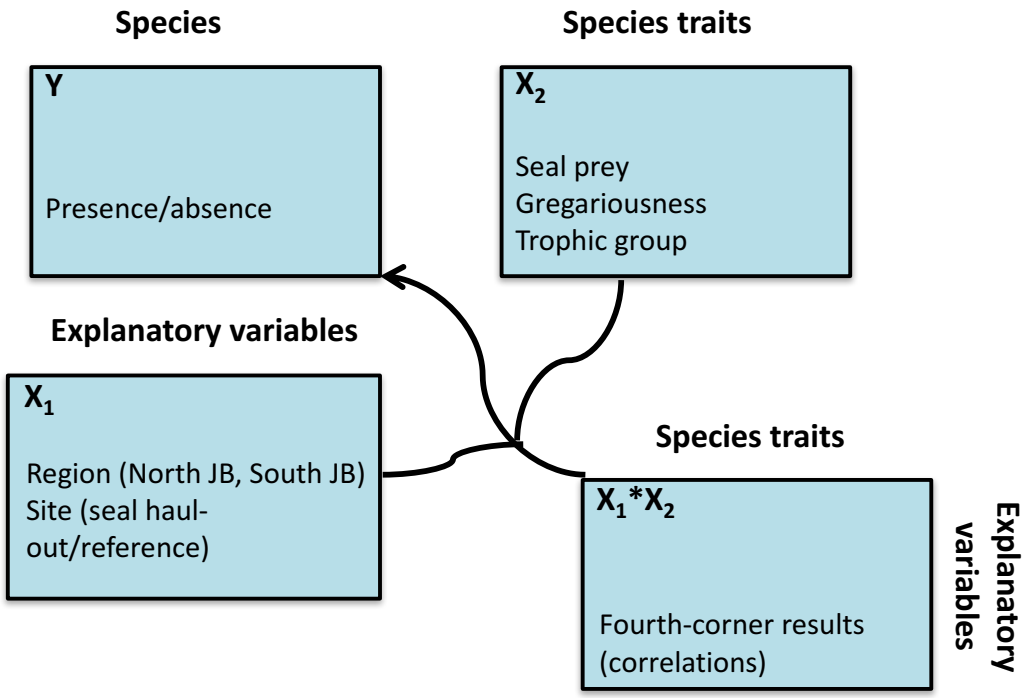


Figure 4.1 Graphical representation of the model-based solution to the fourth-corner problem (Brown *et al.* 2014b). The goal is to predict species occurrences (Y, 1st corner) as a function of predictor variables for the environment (X1, 2nd corner), species traits (X2, 3rd corner) and their interaction (X1*X2, 4th corner) (Brown *et al.* 2014a; Dray & Legendre 2008; Legendre *et al.* 1997; Spitz *et al.* 2014). Details for species traits are provided in Table 4.2. The matrix of coefficients for the interaction between X1 and X2 is the fourth-corner (Brown *et al*. 2014b) (Figure 4.5) and coefficients for the mains effects of traits and environmental variables are the second and third corner (Figure 4.6).

The objective of this study was to investigate localised differences in fish community composition, specifically relating to the ecological function of fish communities, in the context of recent fur seal recovery. Included in this study were two spatially distinct haul-out sites each paired with multiple reference sites that do not harbour fur seal aggregations, and these sites were used as proxies for locations harbouring frequent seal use and sites diffuse or little use by seals. Thus using an

asymmetrical design, I aimed to investigate localised differences in reef community metrics, including size structure of fish communities, and abundances and biomass of fish, between fur seal aggregation sites and reference sites. I also examine how fish functional traits may explain the relationship between fish community composition and locations of fur seal recovery in temperate reef ecosystems of eastern Australia (Table 4.1). For the latter, I use a fourth-corner model to identify trait-level differences in fish community composition, and also the strength of interaction between predator aggregation sites and particular functional groups within fish communities (Figure 4.1). It was expected that following the recent recovery of fur seals on temperate reefs I would observe trait-level differences, specifically reduced prevalences of mesopredators at haul-out sites, and differences in fish community size structure, whereby fish communities adjacent to haul-out sites would be composed of greater abundances and biomass of smaller fish compared to local reference sites.

4.3 Methods

4.3.1 Study sites and system

Jervis Bay, in eastern Australia, supports the two of the northernmost haul-out sites in use year-round by both Australian and long-nosed fur seals. This location represents the frontier for population and geographic range recovery in these species, at the time of study. Haul-out sites are non-breeding locations where pinnipeds come ashore to rest in between foraging trips (Burleigh *et al.* 2008). Whilst fur seal foraging strategies vary widely among species, individuals and locations, fur seals in NSW are known to forage locally as well as offshore (M. Salton, Macquarie University, pers. comm.), however data for the foraging distribution of the study population of seals ar currently unpublished. Additionally, previous work analysing the diets of fur seals from Jervis Bay found a large component of their diets to consist of coastal reef fish at this location (Hardy *et al.* 2017). The coast of Jervis Bay is lined with steeply sloping subtidal reefs reaching > 200 m in depth within kilometres of the coast (Jordan *et al.* 2010). Seal population densities in this frontier location are currently relatively low, and according to optimal foraging theory it is expected that predators

will exhaust resources locally prior to foraging further afield (Ashmole 1963; Birt *et al.* 1987; Orians & Pearson 1979). It is therefore likely that fur seals would begin foraging search patterns close to their point of departure, particularly haul-out sites.

The Jervis Bay haul-out sites are occupied by 30 to several hundred individual seals, with peak abundance in the winter and early spring (June-September) (Burleigh *et al.* 2008). Two haul-out sites were surveyed in Jervis Bay, NSW, Australia, these were: Drum & Drumsticks in North Jervis Bay (35° 2.799'S, 150° 50.552'E) and Steamer's Head in South Jervis Bay (35° 10.725'S, 150° 43.895'E) (sites labelled N-HO and S-HO in Figure 4.2). In South Jervis Bay, the haul-out site has been in regular use for ca. 15 years, whilst in North Jervis Bay, the haul-out has been in regular use for ca. 5–8 years at the time of the study, but with greater numbers of seals in the north compared to the south since the complete closure of the northern headlands to the public as part of the Department of Defense Beecroft Weapons Range in 2009 (Burleigh *et al.* 2008).

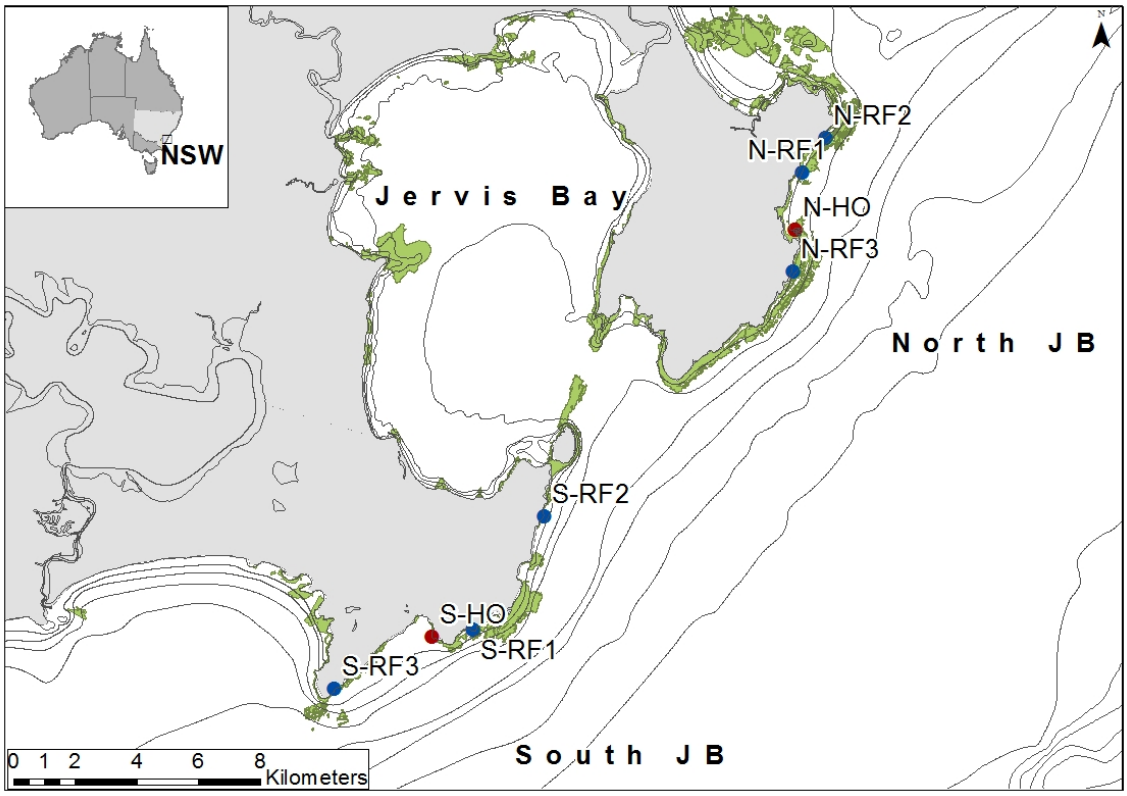


Figure 4.2 Locations of fur seal haul-out sites (N-HO & S-HO) and local reference sites (N-RF1 to -RF3, S-RF1 to -RF3) in North and South Jervis Bay, NSW, Australia. Illustration includes depth contours along the continental shelf in 20 m intervals from 0–240 m and shallow to intermediate reef habitat (up to 60 m, shaded in green) (OEH 2015).

Table 4.1 Summary of statistical analyses, models tested and datasets used for these analyses. Asterisks denote interactions performed under the full model. Abbreviations in parentheses are referred to in the results (Figure 4.3**,** Appendix C, Table C.2).

Question	Response variable	Statistical analyses
1) How did reef fish community parameters vary in relation to *region* and *site*? (Figure 4.3)	(a) Fish species richness (SPR)	GLM (Poisson)
	(b) Fish community Shannon-Wiener diversity index (H)	LM (normal)
	(c) Fish community evenness (E)	LM (normal)
	(d) Total abundance of prey fish species (Tabun_prey)	GLM (Poisson)
	(e) Total biomass of prey fish species (Tbiom_prey)	GLM (negbin)
2) Did the proportional abundances or biomass of fish within each size class vary according to *site type* within each *region*? (Figure 4.4)	Proportional abundance and biomass of fish size classes (2.5–62.5 cm)	Kolmogorov-Smirnov test
3) How do fish functional traits (X1) explain the differences in fish species prevalence (Y) between *region* and *site* (X2 = *region***site*) (Figures 4.5–4.8, Appendix C, Figures C.1 and C.2)	Fish species presence absence (Y)	Fourth-corner analyses

Data were collected from fur seal haul-out sites and reference sites that do not harbour seal aggregations. For each haul-out site, three local reference sites that are not used by fur seals for hauling-out were selected (Figure 4.2) using an asymmetrical design (Underwood 1994). Reference sites were focused on a localised radius of 2–10 km from the haul-out sites to test for localised differences between haul-out sites and reference sites, without confounding the study across a latitudinal gradient. Data on localized movements and geographical resource use by these populations of seals are unplushied (Marcus Salton, pers. comm., Macquarie University). The distance of 2 km from haul-out sites does not wholly exclude the presence of seals at reference sites, however as seals are not known to aggregate at any of the reference sites (Marcus Salton, pers. comm., Macquarie University) and thus any use of reference sites by fur seals assumed to be diffuse or transitory in this study. The sampling design for this study is therefore preliminary and changes could be recommended as further information becomes available for the potential foraging distribution of these predators. Reference sites were selected based on similar aspect (ENE to E facing for northern sites and S facing for southern sites) and similar habitat complexity (large boulder fields of generally > 25˚ slope) to their respective haul-out sites. Altogether, one haul-out site and three local reference sites were surveyed in each of two regions (North and South Jervis Bay) (Figure 4.2).

4.3.2 Data collection and processing

Surveys were conducted over a 14-day period in the austral spring (Nov–Dec) 2014, immediately following the peak in local seal abundances, to collect data at a time when the potential trophic pressure of these predators was expected to be the most acute and therefore more likely to be observed. Data on the diversity, abundances and sizes of fish were collected from shallow subtidal reefs at depths of 10–18 m by SCUBA divers using standard Reef Life Survey (RLS) visual census techniques described in Edgar and Stuart-Smith (2014) and in the RLS methods manual (https://reeflifesurvey.com/wp-content/uploads/2015/07/NEW-Methods-Manual_150815.pdf). Using RLS methods, data on the identity, abundance and size estimates for all fishes were collected along a 50×10 m transect (500 m^2 area). The majority of taxa were recorded to species-level, and unidentified taxa (<0.01% of

records) were classified at genus or family level. This survey method also records any large mobile and demersal invertebrates occurring on transects (i.e., cuttlefish), and they are included in analyses, however occurrences were rare. Overall, divers conducted 8 replicate transect surveys at each site (n = 8) using a random stratified design to sample evenly across the depth range, and data were collected for a total of 64 transects.

Fish species observed in this study were classified in relation to three traits; gregariousness, trophic group and seal prey status (Table 4.2). Traits for gregariousness and trophic group were assigned based on Ferrari *et al.* (2017) and Stuart-Smith *et al.* (2013) (Table 4.2, Appendix C, Table C.1). Species were categorized as seal prey using prior knowledge of the species, genera and families found in the diets of Australian and long-nosed fur seals from NSW (Hardy *et al.* 2017), Bass Strait (Deagle *et al.* 2009; Gales & Pemberton 1994; Kirkwood *et al.* 2008; Lake 1997) and New Zealand (Fea *et al.* 1999) (Table 4.2, Appendix C, Table C.1). Data on fish abundance and sizes were used to calculate fish biomass for each species and size class through the RLS data portal using known estimates relating fish size to weight for individual species. Additionally, the abundances and biomass data of all fish species were summed for each size class to conduct trait-based analyses.

Table 4.2 Functional traits of fish species used for trait-based modelling. Classification of fish traits for gregariousness and trophic group were sourced from a database developed by Stuart-Smith *et al.* (2013) and Ferrari *et al.* (2017), with the exception of the "seal prey" trait based on dietary information on fur seals for southeastern Australia and the local area (Hardy *et al.* 2017).

Functional Trait	Category	Type	Description
Trophic group	Trophic niche	Factor	Browsing herbivore, scraping herbivore, omnivore, benthic invertivore, cleaner, planktivore, mesopredator
Gregariousness	Behaviour	Ordered factor	Index from 1–3, representing solitary, paired to sometimes forming groups/small schools, always schools, respectively
Seal prey item	Trophic interaction	Binomial factor	Index of 0 and 1, representing 1 - a known or probable prey item at the family level, and 0 - not encountered in fur seal diet

4.3.3 Statistical models and analyses

Throughout the analyses summarised in Table 4.1, I assessed the significance of two categorical explanatory variables *region* (levels: North JB, South JB) and *site type* (levels: seal haul-out, reference) on various univariate and multivariate measures of assemblage structure (Table 4.1). In all cases, differences between *site types* were based on the comparison between the mean for all transects at each haul-out site and the mean for all transects at reference sites within both study *regions*.

Firstly, statistical differences between *regions* and *site types* were tested for the following univariate reef community metrics calculated per transect: (a) species richness calculated as the total number of unique fish and macro-invertebrate species observed; (b) the Shannon-Weiner diversity index; and (c) reef community evenness calculated by dividing the diversity index by species richness; (d) the total abundance and (e) the total biomass of fish species classified as seal prey (Table 4.1). Statistical analyses for these univariate metrics were performed in the packages *stats* and *MASS* (version 7.3-47) (Ripley *et al*. 2002) in *R* (version 3.4.1) (R Core Team 2017), for linear and generalised linear models respectively (Table 4.1). Secondly, variations in size structure of fish communities between *site type* were tested for each *region* using the Kolmogorov-Smirnov (KS) test (Lopes *et al.* 2007) and using data for the proportional abundances and biomass of fish within each size class (Table 4.1). To standardise for overall differences in abundance or biomass between replicate transects, the data for abundance and biomass of fish within each size class are expressed as a proportion of the total number or biomass of fish observed within each transect.

Thirdly, I aimed to investigate how fish functional traits may explain differences in fish assemblage composition in relation to *region* and *site type.* Fish functional traits relate to both their suitability as seal prey and also their relationship to reef ecosystems (Table 4.2). I used a fourth-corner model, a multivariate statistical technique, to predict the probability of occurrence of different fish species as a function of explanatory variables for the environment here *regions* and *site type* (X_1), species traits (X_2) and their interaction (X_1* X_2), where the matrix of coefficients for

the interaction between the species traits and explanatory variables is known as the fourth-corner solution (Figure 4.1) (Brown *et al.* 2014a). These coefficients for all trait-environment interactions are presented using a (GLM)-LASSO model (Brown *et al.* 2014a), where the strength and direction of the interactions are illustrated. Where significant interactions between environmental variables (*region, site type*) and traits are observed, the occurrences of fish species with these traits are interpreted as either negatively or positively correlated with certain environmental variables. The relative strength of the relationship between environmental variables and traits can also be compared between traits using this technique. The analytical routines for the fourth-corner analysis were performed using the package *mvabund* (version 3.12.3) (Wang *et al.* 2012) in *R* software (R Core Team 2017).

The model was fitted to data on fish species presence/absence using the function *traitglm* and using a LASSO penalty, specifying the fitting method as '*glm1path'*, a method of penalised likelihood that imposes a constraint on estimates of model parameters (Brown *et al.* 2014a; Hastie *et al.* 2009; Warton *et al.* 2015). This constraint effectively shrinks coefficients to zero when they are not statistically significant, providing a combined approach for model selection and parameter estimation to evaluate the magnitude and significance of an explanatory variable (Brown *et al.* 2014a; Hastie *et al.* 2009; Warton *et al.* 2015). Interaction plots were constructed to visualise the probabilistic relationship between environmental variables (*region, site type*) and the traits of fish. To determine species-level variations in the fish assemblage not explained by traits, a single predictive model was fitted for all species presence/absences at all *site types* and *regions* using a *traitglm*. This is the equivalent to fitting a multivariate species distribution model (SDM).

Broad trends, overdispersion and outliers in multivariate space were checked graphically by non-metric multi-dimensional scaling (nMDS) plots (Field *et al.* 1982) using the *vegan* package in *R* (Oksanen *et al.* 2015). An outlier in this dataset would consist for example of a replicate (transect) that contained a single or few species, especially a large numbers of these species, that were not observed in any other replicate. Such an outlier would have exceptionally large dissimilarity with other replicates that it would appear graphically as a point so distant that the rest of the assemblage would be tightly clustered. Model fit was assessed by plotting residuals against fitted values and plotting quantile-quantile (Q-Q) plots. The *ggplot2* package

in R was used to create all graphical illustrations (version 2.2.1) (Wickham 2009). The data on fish species assemblages were transformed to presence/absence to meet the assumptions of the fourth-corner model for trait-based analyses. The model was also intended to be tested on abundance and biomass estimates across the assemblage of reef species observed, however a tweedie distribution was required and this is not yet available for the *traitglm* function in *mvabund* (D. Warton, Stats Central, University of New South Wales, pers. comm.), and so was not done in the present study.

4.4 Results

4.4.1 Fish community summary metrics and fish community size structure at seal haul-out sites

Fish species richness was significantly higher at the haul-out site in North JB (average of 30 species/500 m^2, ± 2 SE) compared to reference sites and to the haul-out site in South JB, that were not significantly different from each other (average of 24–27 species/500 m^2, ± 1 SE) (Figure 4.3, Appendix C, Table C.2). The Shannon-Wiener diversity index was not statistically different between haul-out sites and reference sites within each region (H = 1.64 per 500 m^2 ± 0.04 SE) (Figure 4.3, Table C.2). There were no discernible differences in fish community evenness (E = 0.51 per 500 m^2 ± 0.01 SE) between the haul-out and reference sites or regions, and no interaction between these explanatory variables (Figure 4.3, Table C.2).

Total abundance of seal prey species was significantly lower at the haul-out site compared to reference sites in South JB (mean abundance: South HO = 176 ± 16 vs. South RF's = 330 per 500 m^2 ± 42) but not in North JB (Figure 4.3, Table C.2). For total biomass of seal prey species, biomass was lower at the South JB haul-out site compared to the reference sites, however this was not statistically significant in either North or South JB (Figure 4.3, Table C.2). Proportional abundances or biomass of fish were slightly higher for the smaller size classes (2.5–10 cm) at haul-out sites and higher in the larger size classes at reference sites (12.5–62.5 cm) (Figure 4.4).

However, this trend was only statistically significant for the proportional biomass of fish between the haul-out and reference sites in South JB (Figure 4.4).

4.4.2 Trait-based analyses of fish community response to seal haul-out sites

A total of 85 fish species and one mobile invertebrate were observed on surveys, and could be assigned traits (Table C.1). Of these fish species, 55% of surveyed species were benthic invertivores, 18% were browsing herbivores, 13% mesopredators, 9% were planktivorous species, 3% omnivores and 1% cleaner species (Appendix C, Table C.3). Cleaner species (n = 1) and omnivores (n = 3) were rare in this study, and were thus excluded from trait-based analyses, as inferences could not be made on their occurrences in this study. Regarding the gregariousness of fish species, 22% were schooling species, 25% occurred in pairs or small groups and 38% were solitary species (Table C.1). Thirdly, 74% of all recorded fish species were classified as potential seal prey according to previous diet work on southeast Australian fur seals, whilst 26% have not been recorded in fur seal diets (Table C.1).

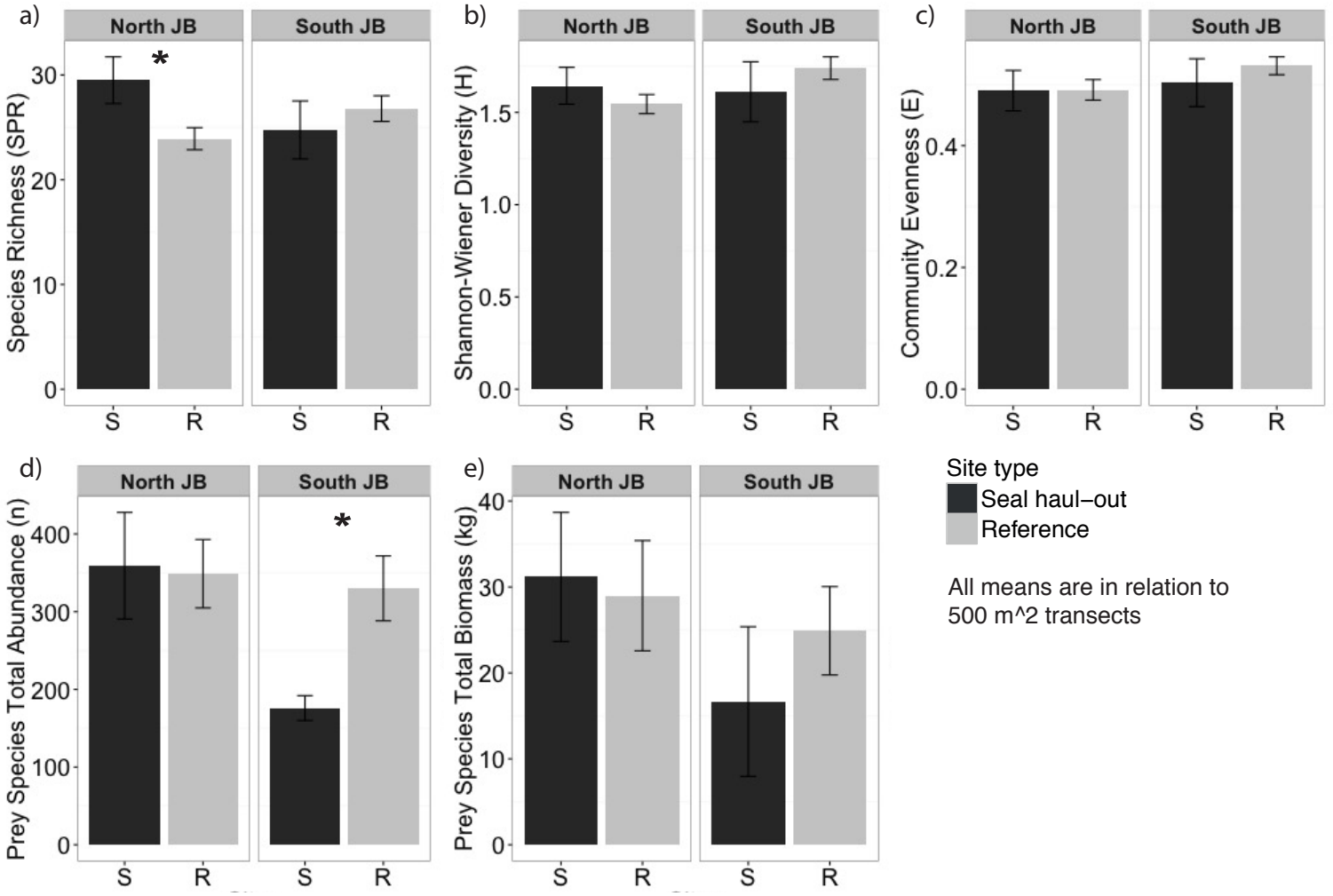


Figure 4.3
Comparisons for North and South JB between site types, fur seal haul-out sites and reference sites, of summary indices for reef fish communities: a) species richness across all fish species (SPR); b) Shannon-Wiener Diversity Index (H); c) community evenness (E); d) total abundance of seal prey species; and e) total biomass of seal prey species. All values are presented for an area of 500 m^2. Error bars represent standard errors and asterisks represent significant differences between *site types* and *regions*.

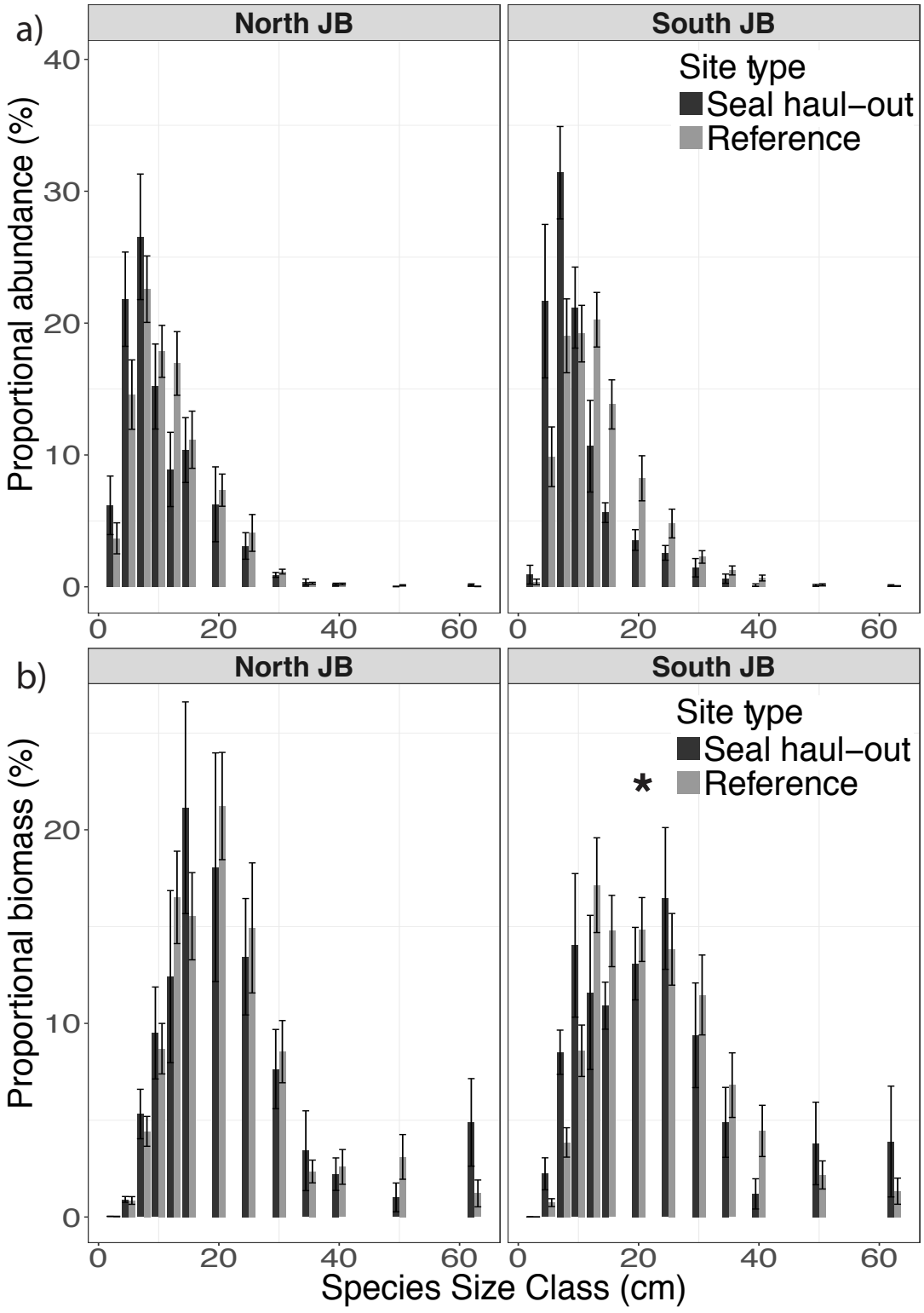


Figure 4.4 Fish size class distribution per 500 m^2 area by: a) abundance and b) biomass normalised for the total abundance and biomass within each site type. Proportional abundance and biomass are reported as percentages with standard error bars (KS-test for proportional abundance: $D_{\text{NorthJB}} = 0.1539, p = 0.9992$; $D_{\text{SouthJB}} = 0.2308, p = 0.8793$; for proportional biomass: $D_{\text{NorthJB}} = 0.1539, p = 0.9992$; $D_{\text{SouthJB}} = 0.6154, p = 0.0127$). Asterisks denote significant differences between *site types*.

The results of the fourth-corner model revealed that the probability of occurrence of fish species varied between seal haul out and reference sites (*site type*), between North JB and South JB (*region*), and between traits (Figure 4.5). Differences were also explained by interactions between environmental variables and certain fish functional traits (Figure 4.6). Coefficients for the model's main effects (Figure 4.5) revealed that the traits for seal prey (level "yes") and trophic groups (level "browsing herbivore") were important predictors of fish community assemblage, as were the variables for *region* and the haul-out vs. reference sites (*site type*). There was a greater probability of occurrence of seal prey species in general across all sites and regions simply because most species (~74%) were found to be known or likely seal prey items (Figure 4.5). Therefore known and likely seal prey species were common in both haul-out sites and reference sites, and prevalence as a metric for this trait was not sufficient to differentiate between *regions* and *site types* (Figure 4.6 and 4.7). The prevalence of browsing herbivores in general was also slightly higher compared to other trophic groups across all sites and regions (Figure 4.5 and 4.7). However, the results of the interaction between trophic groups and environmental variables showed that, despite a generally higher prevalence of browsing herbivores within the fish community, they were negatively correlated with haul-out sites (Figure 4.6 and 4.7). Schooling fish were also negatively correlated with haul-out sites, whilst mesopredators and fish species that formed pairs or small groups were positively correlated with haul-out sites (Figure 4.6 and 4.7).

Species-level variations between sites and region were observed (Appendix C, Figure C.1 and C.2), however the assemblages between site types and regions remained closely clustered even when traits were ignored (Figure 4.8). There was a negative correlation between haul-out sites and several schooling fish species, including *Pempheris affinis*, *P. analis* and *Trachurus novaezealandiae*, and also the browsing herbivore *Olithops cyanomelas* (Figure C.1 and C.2). Several species known to form pairs or small groups, including *Upeneichthys lineatus, Enoplosus armatus, Meuschenia trachylepis*, were positively correlated with haul-out sites. Additionally, the browsing herbivore, *Girella tricuspidata*, and two benthic invertivore species *Pagrus auratus* and *Hypoplectrodes maccullochi* were positively correlated with haul-out sites (Figure C.1 and C.2). Species prevalences are presented, whereby species are ranked by how common or uncommon they were in the study locations overall (Appendix C, Figure C.3 and C.4).

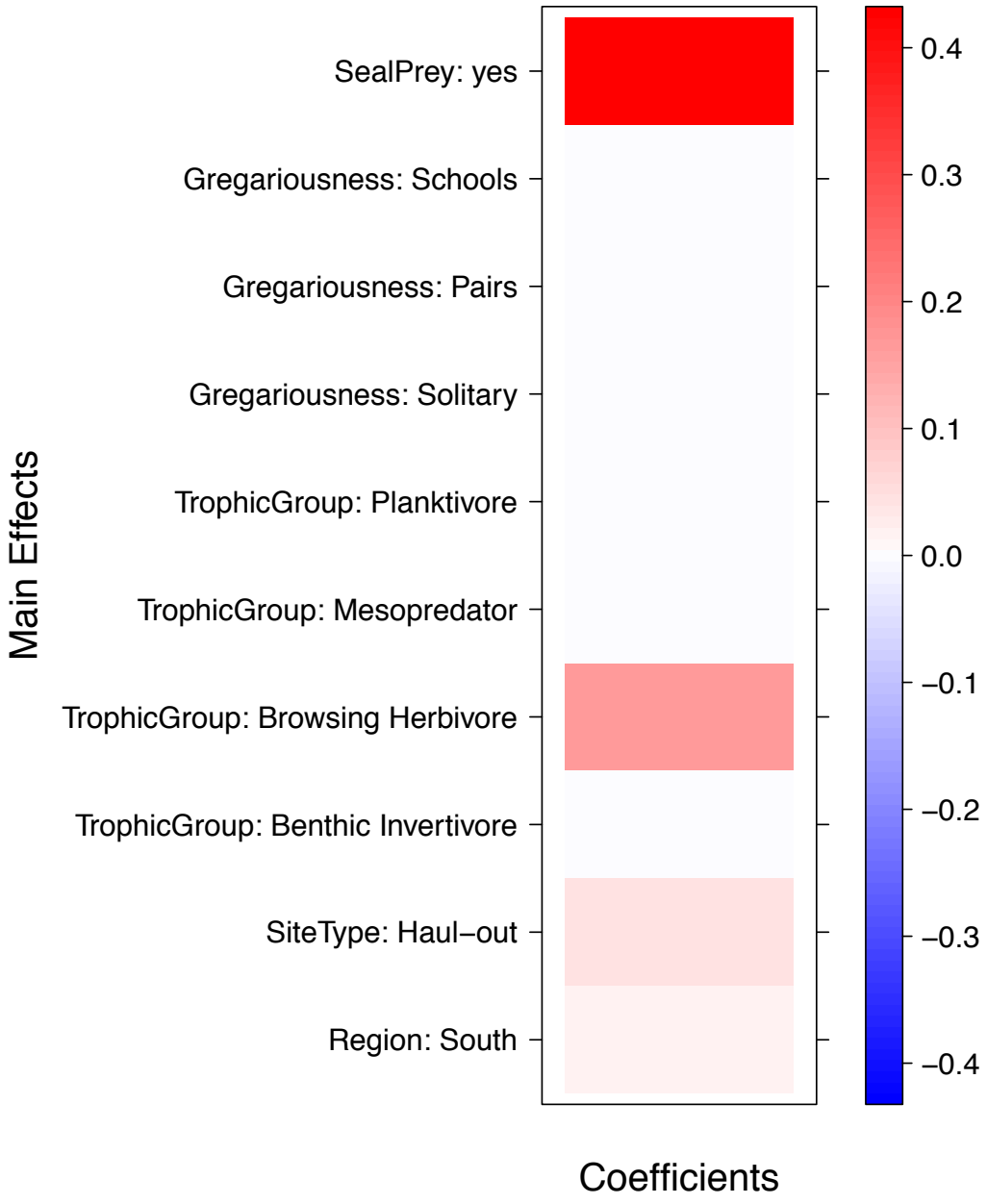


Figure 4.5 Standardised coefficients from the GLM-LASSO model for the relationship between the main effects terms and fish species assemblage (2nd and 3rd corners of the model). Colours denote statistical significance of traits, *region* and *site type* in explaining fish prevalences. Darker colours describe the strength of effect, whereby red indicates a positive relationship, whilst blue a negative relationship.

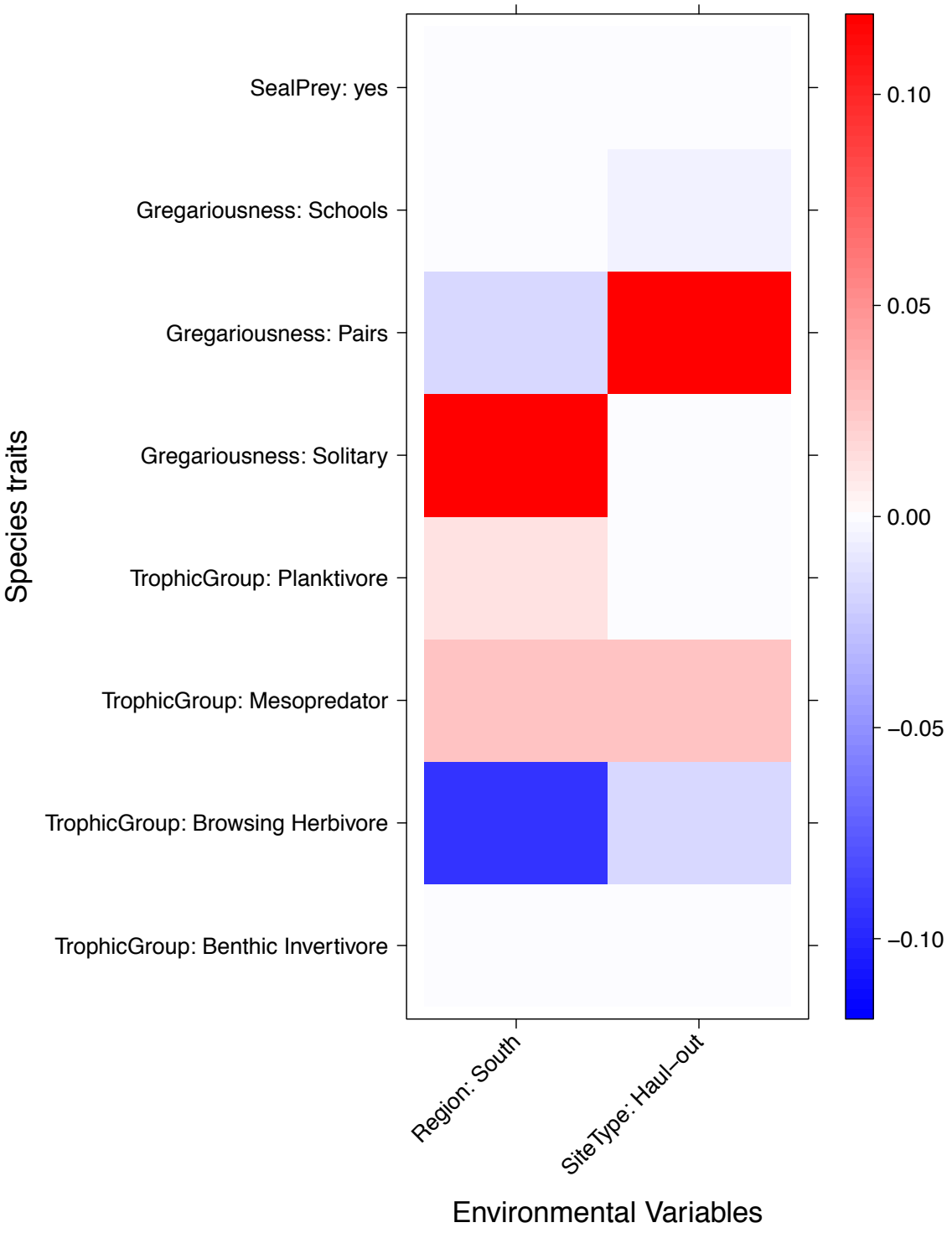


Figure 4.6 The fourth-corner modelling results displayed for standardised coefficients for all explanatory variable-trait interaction terms (X_1*X_2) from the GLM-LASSO model. Colours denote significant interactions between levels of each trait, the *region* sampled and *site type*, whereby darker colours describe the strength of effect. Red indicates a positive relationship, whilst blue a negative relationship.

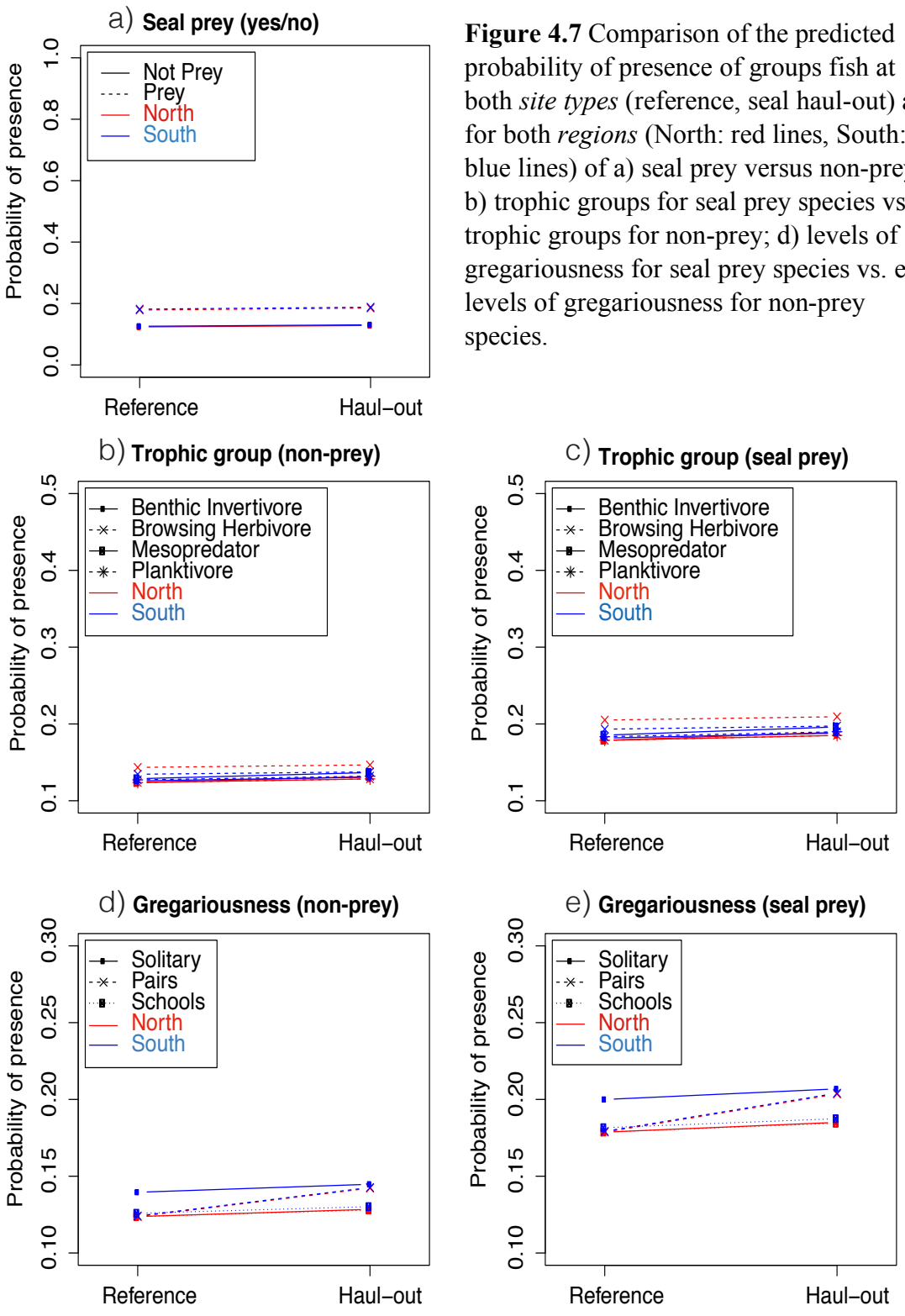


Figure 4.7 Comparison of the predicted probability of presence of groups fish at both *site types* (reference, seal haul-out) and for both *regions* (North: red lines, South: blue lines) of a) seal prey versus non-prey; b) trophic groups for seal prey species vs. c) trophic groups for non-prey; d) levels of gregariousness for seal prey species vs. e) levels of gregariousness for non-prey species.

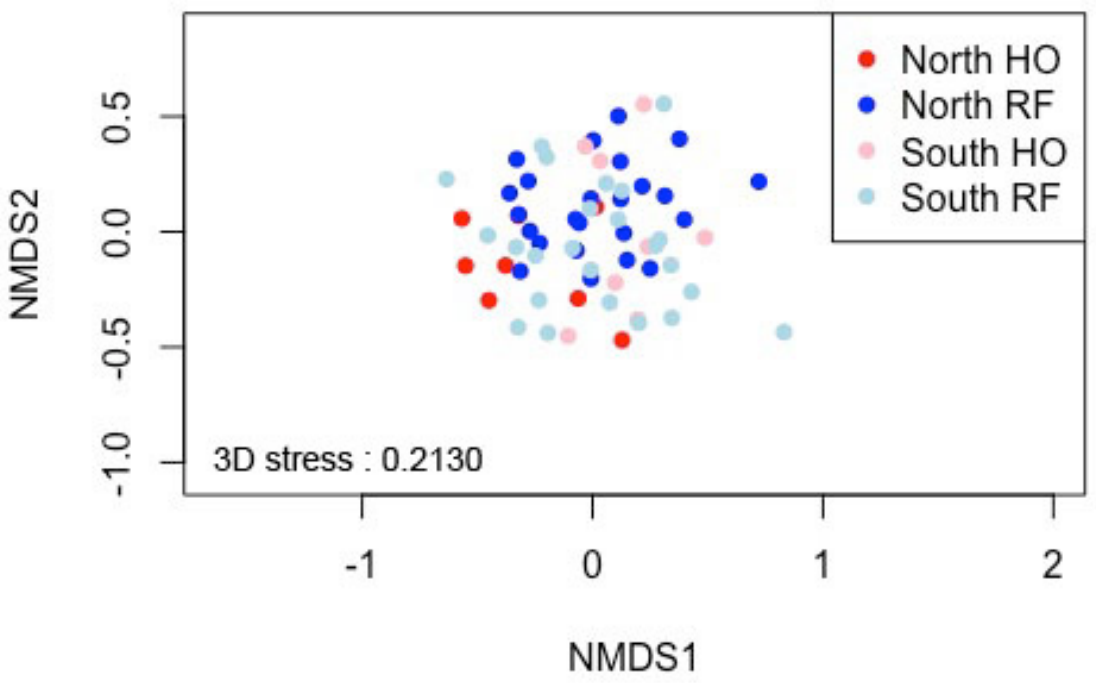


Figure 4.8 3D nMDS ordination for presence/absence data of fish species assemblages represented as centroids for each *region* (North, South) and *site type* (HO: seal haul-out, RF: references), illustrated in 2D.

4.5 Discussion

The potential functional effects of recovering fur seals along the coast of Australia, and elsewhere, are largle unknown. The early detection of differences in ecological communities following the recovery of large predators may help to understand trajectories of change in ecological communities. The fourth-corner modelling approach used here allowed for the exploration of functional and localised differences in reef fish community composition in the context of local fur seals recolonisation. This statistical technique provided a trait-based explanation to the interactions between fish species composition and locations of fur seal recovery. Correlations were identified between haul-out sites and certain fish grouped by traits, namely a lower occurrences of schooling fish and herbivorous fish at seal haul-out sites, however effect sizes were small. The total abundance and the proportional biomass of fish of different size classes were significantly different, namely with greater proportional abundance and biomass of smaller fish at one of the haul-out sites and associated reference sites, in South Jervis Bay. Some differences identified were

contingent on the location of fur seal recovery, which have different recolonisation histories.

This study identified correlations between reef community composition and locations of fur seal recolonisation, and this relationship could be explained by the traits of reef fish species. In contrast, there were no discernible differences in broader reef community indices, such as the Shannon-Wiener diversity index or reef community evenness, between seal haul-out sites and reference sites in this study. Although species richness was found to be higher at the northern haul-out site than nearby reference sites, the number of species observed and the values for other indices in this study were consistent with prior observations on temperate reefs in southern Australia, using underwater visual census methods (Coleman *et al.* 2015; Stuart-Smith *et al.* 2013). It is unlikely that the return of two native predator species will affect the beta-diversity and evenness of an ecosystem within only two decades, if at all, relative to other more significant ecological disturbances to ecosystems such as anthropogenic extractive activities (Babcock *et al.* 2010; Edgar & Barrett 1999), rather it is more likely that the prey community will be affected functionally (Coleman *et al.* 2015).

A lower probability of occurrence was observed for browsing herbivores at seal haul-out sites, whilst greater probability of occurrence was detected for mesopredators, regardless of whether or not these groups were also known to be seal prey items. Additionally, the probability of occurrence of seal prey items was not significantly different between locations of fur seal recolonisation. Mesopredators were expected to be rarer at haul-out sites because diet assessments of fur seals at this location showed they predominantly ate higher trophic-level fish (Hardy *et al.* 2017). The lack of a strong negative relationship between mesopredatory fishes adjacent to fur seal haul-out sites, may be due to the functional complexity of these ecosystems (Casey *et al.* 2017; Edwards *et al.* 2010), as this study surveyed 85 species of fish many of which are also generalist mesopredators, invertivores and omnivores. Prey diversity and trophic complexity, such as omnivory, intraguild predation and competition, are known to reduce the strength of cascading trophic effects (Bellwood *et al.* 2006; Polis *et al.* 2000). The diverse diet of fur seals and the complexity of Australian temperate ecosystems may result in similarly diverse and therefore weaker

trophic relationships between these recovering predators and reef ecosystems, as predicted by Paine (1969).

Species-level variations in mesopredators and benthic invertivores were not necessarily associated with haul-out sites in the same way. Several fish species that are important reef mesopredators and benthic invertivores were observed at sites in this study, and that also occur in the diets of fur seals, including *Pagrus auratus* (snapper) and *Achoerodus viridus* (blue groper), and other Labrids (Deagle *et al.* 2009; Hardy *et al.* 2017). The mesopredator, *Pagrus auratus*, and benthic invertivore, *Hypoplectrodes maccullochi* were actually more common at haul-out sites. Several species of the Pempheridae family, mostly schooling fish and benthic invertivores, were less common at haul-out sites. Whilst the diets of fur seals more commonly contained mesopredators than any other trophic group (**Chapter 3**), other trophic groups were also consumed and it is unlikely that fur seals select prey purely based on trophic groups.

Schooling fish were less common at haul-out sites, the prevalence of fish known to form small groups or pairs was higher at haul-out sites. Several species of schooling fish were found to be less common at haul-out sites compared to reference sites, *Pempheris affinis*, *P. analis* and *Trachurus novaezealandiae*, and these species are relatively common seal prey items (Hardy *et al.* 2017; McIntosh *et al.* 2006; Page *et al.* 2005). Empirically, a large number of seal prey identified throughout the geographic range of Australian and long-nosed fur seals are also known to be schooling fish. The lower prevalence of schooling fish at haul-out sites in this study could suggest localised avoidance of such sites by schooling fish, and thereby affecting fish community composition around fur seal haul-out sites.

The total abundance of fish classified as likely seal prey species was found to be significantly lower at the haul-out site from South Jervis Bay, and no differences between sites were detected in total biomass of fish classified as seal prey. Subtle differences were also observed in the size distribution of reef fish communities at haul-out sites compared to reference sites. The proportional abundances or biomass of the smaller fish size classes (2.5–10 cm) appeared to be greater at haul-out sites, whilst the proportional abundance or biomass of larger fish size classes (> 12.5 cm) appeared to be greater at the reference sites. This pattern was only statistically significant for proportional biomass of fish from South Jervis Bay. For the haul-out

site in South Jervis Bay, a lower total abundance of fish was detected in general in this region as well as a slight skew in the size structure of the fish community, whereby the proportional biomass of larger size classes of fish was greater at the South Jervis Bay reference sites compared to the haul-out site.

Overall, the effect sizes observed in this study were relatively small (typically $< \pm 5\%$) in terms of the actual differences in probability of occurrence in different functional groups of fish between the types of sites, as well as the differences in size structure of fish communities. It is an important finding to detect that there were few signs in the fish community of significant ecological changes at fur seal haul-out sites, and that those that were detected were at the haul-out site with the longest duration of fur seal recolonisation, in South Jervis Bay. This provides partial evidence to support further investigations into localized changes in reef fish communities adjacent to predator aggregation sites. However, several limitations of this study would need to be accounted for in future investigations of the hypotheses tested in this study.

Since this study was conducted, several new year-round haul-out sites have been established further north along the coast of NSW. As this is a study based on a natural experiment, a larger scale study incorporating at least a third, if not several more haul-out sites paired with appropriate reference sites is crucial to determining with confidence whether reef communities adjacent to haul-out sites differ from other reefs. Secondly, this kind of study needs at least several years of replication, as this study was conduced over just one season, the result scan only be taken as preliminary. Without many measurements of reef communities at locations prior to fur seal recolonisation, space for time substitution studies, such as this one, must exercise caution in identifying significant results and ideally need to identify a trajectory of change over time in reef communities that would be consistent with fur seal recolonisations and hypotheses of prey limitation.

Furthermore, the future and likely expansion of fur seal haul-out sites as well as likely niche partitioning that will occur with mature populations of seals will need to be taken into account in future studies. Due to the geographic uniqueness of both locations of fur seal aggregations in NSW at the time of study, being Montague Island and Jervis Bay, it was difficult to replicate across haul-out sites and also reference sites at Jervis Bay are likely to be too close to the haul-out sites if their usage by seals increases, which is probable. Fourth, the multivariate (multi-species) abundance and

biomass data in this study were overdispersed and had to be transformed to presence/absence in order to fit trait-based models for analyses. However, the effect of a predator would have to be very strong in order for these analyses to yield significant results and thus the results of this study are likely to be very conservative. I would therefore recommend greater replication over the depth gradient surveyed (10–18 m) to increase the power of the analyses when using abundance and biomass data. Additionally, the statistical package *mvabund* used in this study (R Core Team 2017; Wang *et al.* 2012), does not yet allow for the use of "quasi" distributions to account for overdispersed data, however this function will probably be available in the near-future and enable complex analyses of noisy ecological data.

Data on the precise geographic locations where the Jervis Bay fur seals forage are currently unpublished (Marcus Salton, pers. comm., Macquarie University). However, these data and continued monitoring of fur seal foraging activity in the area using animal-borne telemetry equipment are crucial to properly assessing what spaces and potentially even specific reefs these recovering predators may actually be using. Additionally, a recent diet study identified a frequent convergence of diets of both Australian and long-nosed fur seals on coastal reefs in animals from Jervis Bay (Hardy *et al.* 2017; **Chapter 3**). However, replication of the seasons sampled over years, using both high-taxonomic resolution methods of analysis (i.e., DNA metabarcoding) and also metods that integrate diet data over greater temporal scales (i.e., stable isotope analysis) would be useful to understand the relative importance of different ecosystems from the coast to the open oceanover time in these predators.

The natural recolonisation of eastern Australian temperate reefs by fur seals is relatively recent (≤ 15 years) (T. Lynch, CSIRO, Tasmania, pers. comm.; Burleigh *et al*. 2008). It may take decades for direct effects on food webs on temperate reefs to become apparent (Babcock *et al.* 2010; Barrett *et al.* 2007a), particularly with the recovery of a low density of two generalist predators. The strongest effects observed in this study were from the haul-out site associated with longest duration of use by fur seals, at South Jervis Bay (ca. 15 years) compared to the North (ca. 5–8 years, at time of the study in 2014). On this basis, I would recommend further investigation of reef assemblages from fish down to benthic assemblages in the study area. This is the subject of further investigation presented in a subsequent chapter in this thesis.

4.6 Conclusions

At an early stage in the recovery trajectories of two predator populations, these results shed light on poorly understood functional interactions between complex fish communities and locations of fur seal recovery. Using an asymmetrical experimental design and trait-based analyses, I identified a negative correlation between the prevalence of herbivorous fishes and locations of fur seal recovery, and a positive correlation between these locations and the prevalence of mesopredators. Additionally, schooling fish were less common at one haul-out site with a longer duration of use by fur seals. At this haul-out site, I also observed a lower total abundance of fish and reduced abundances of larger fish. However, the size and strength of any localised effects of recovering fur seals on reef fish community composition in southeastern Australia currently appeared to be minimal. These results provide information to support further research of the flow-on trophic effects of predator recovery on ecological communities, the subject of subsequent work in this thesis. Finally, ongoing monitoring of ecological communities is recommended to understand and conserve important ecological processes, in the context of recovering predator populations as well as anthropogenic pressure on coastal communities.

5 Investigating indirect effects of predator recolonisation on benthic reef communities

5.1 Abstract

Changes to high trophic level predator populations can alter ecological communities through trophic cascades. Two large predators, Australian (*Arctocephalus pusillus doriferus*) and long-nosed (*Arctocephalus forsteri*) fur seals, are recovering from near extinction on eastern Australian temperate reefs. Reef-based, higher trophic-level fish species are prevalent in the diets of these predators and negative impacts on fish abundances have been observed at recently recolonised sites in eastern Australia. However, very little is known about the potential for cascading, indirect effects on temperate reef food webs following recovery of their populations. This study aimed to quantify differences in benthic communities adjacent to newly recolonised fur seal haul-out sites compared to reference sites without seals. The overall trophic structure of benthic invertebrates was not significantly different between seal haul-out and reference sites. Abundances of the key herbivorous urchin, *C. rodgersii*, were lower adjacent to one of the seal haul-out sites compared to reference sites. The percentage cover of canopy-forming kelp and foliose macroalgae was lowest at haul-out sites compared to reference sites. Additionally, a negative correlation was observed between *C. rodgersii* abundances and the percentage cover of foliose macroalgae at haul-out sites. These results suggest that the abundances of *C. rodgersii* and the cover of foliose macroalgae are different at seal haul-out sites as compared to reference sites, providing early evidence for indirect effects of predator recovery on temperate reef ecosystems. However, longer term monitoring of eastern Australian temperate reef communities will be necessary to fully understand the community level implications of fur seal population recovery.

5.2 Introduction

Trophic cascades are triggered by changes to the abundances of strongly interacting predators (Paine 1969). In many examples, predators directly affect the abundances of their prey via consumption, as well as influencing their behaviour and other biological traits by virtue of being present (e.g. for wolves, fish and sharks) (Brown & Kotler 2004; Madin *et al.* 2011; Wirsing & Ripple 2010). Furthermore, impacts are known as "indirect effects" when the direct effects of predators on a food web successively progress through lower trophic levels (Pace *et al.* 1999), and this can lead to widespread ecological change.

The strength of trophic cascades is thought to be related to the intensity of interaction between species, and attenuated by the complexity of and functional redundancy within the food web (Polis & Holt 1992; Terborgh & Estes 2010). In three-tiered ecosystems, predator removal generally results in the release from predation pressure of herbivores, the subsequent degradation of vegetation and an ecosystem phase-shift. A kelp forest, for example, may be reduced to urchin barrens if sea otter populations decline in the northeastern Pacific (Estes & Palmisano 1974). The recovery of predators in these ecosystems typically results in the reversal of these ecosystem changes (Beschta & Ripple 2009; Estes & Palmisano 1974).

Much of our current understanding of trophic cascades involving large animals comes from inadvertent anthropogenic experiments involving the removal of predators and/or their natural or assisted reintroduction and recovery (Estes *et al.* 2016; Ripple *et al.* 2014). Our understanding of trophic cascades is particularly well documented under specific conditions involving strong interactions among diet specialists and in simpler three-tiered systems (Terborgh & Estes 2010). However, for marine environments where trophic networks consist of multiple and often-wide ranging, generalist predators and diverse prey assemblages, the trajectories of ecological change are often less clear and these ecosystems may not necessarily experience strong trophic structuring and therefore trophic cascades (Casey *et al*. 2017; Polis *et al*. 2000). The recent recovery of two generalist predator species, Australian (*Arctocephalus pusillus doriferus*) and long-nosed (*Arctocephalus forsteri*) fur seals, on temperate reefs of southern Australia presents a compelling natural experiment to examine how higher trophic processes influence these ecosystems.

Australian fur seals (hereafter AUFS), and long-nosed fur seals (formerly New Zealand fur seals) (hereafter LNFS), have recovered in numbers and geographic range since the cessation of commercial sealing in Australian waters (Goldsworthy *et al.* 2003; Kirkwood *et al.* 2010; McIntosh *et al.* 2014). These pinniped species are known to be 4th order predators on coastal and offshore ecosystems (Goldsworthy *et al.* 2013; Hardy *et al.* 2017), and also target reef associated prey species (Deagle *et al.* 2009; Hardy *et al.* 2017). Several fish species that are reef mesopredators and consume benthic invertebrates (invertivores) occur in the diets of fur seals, including *Pagrus auratus* (snapper) and *Achoerodus viridus*, and other Labrids (Deagle *et al.* 2009; Hardy *et al.* 2017). Negative correlations have been identified between fur seal prevalence and fish abundances at an established breeding colony in eastern Australia (Kelaher *et al*. 2015). Subtle differences in fish community composition and size distribution were detected adjacent to recently recolonised fur seal haul-out sites (**Chapter 4**). In **Chapter 4**, reduced total abundance of fish and reduced biomass of larger fish size classes were observed adjacent to one fur seal aggregation site, in particular.

Indirect and potentially cascading effects stemming from pinniped population recovery on lower trophic levels are likely to be even more cryptic or take more time to occur than direct effects (Babcock *et al.* 2010; Barrett *et al.* 2009). Yet, investigating these potential effects early in the recovery of fur seals may allow researchers to establish baseline information on reef community composition and assess possible trajectories of change in the future in relation to the likely expansion of fur seal aggregation sites over the course of their recovery, and could also enable an understanding of whether these predators can trigger phase shifts in marine ecosystems (Estes *et al.* 2016). Recent changes in abundances and distributions of important herbivores in Australia have triggered ecosystem phase-shifts on temperate reefs (Andrew 1991; Ling *et al.* 2009). In particular, the long-spined sea urchin (*Centrostephanus rodgersii*) has experienced both a range expansion due to both ocean warming and release from predation by overharvested lobster (Ling *et al.* 2009). Increases in abundances and sizes of *C. rodgersii* resulted in overgrazing, the reduction of kelp beds and phase-shifts to urchin barrens on affected temperate reefs in southern Australia (Kriegisch *et al.* 2016; Ling *et al.* 2009). These changes have highlighted the fragility of macro-algal canopies to urchin herbivory on Australian

temperate reefs. However, it is unknown whether the rapid recovery of 4th order predators, such as fur seals, may indirectly affect interactions between urchins and kelp forests, via the direct effects of seal predation on fish that eat urchins from recruitment to mature phases, for example, or other herbivorous invertebrates, or whether other functional groups may be affected.

Whilst, differences in fish communities were subtle and mainly identified at one haul-out site in **Chapter 4**, I identified a negative correlation between the prevalence of herbivorous fishes and seal haul-out sites, and an unexpected positive correlation between mesopredator prevalence and haul-out sites overall, leading to a testable hypothesis that benthic communities at haul-out sites may be characterized by higher cover of canopy-forming kelps and erect macroalgae (hereafter both are referred to as 'foliose macroalgae') compared to reefs without seals. However in preliminary observations, benthic reef communities adjacent to fur seal haul-out sites appeared to consist of urchin barrens (Brendan Kelaher, pers. comm., Southern Cross Univeristy). It remains possible that reef fish mesopredators and benthic invertivores may have reduced foraging efficiency on herbivorous invertebrates adjacent to fur seal haul-out sites. A greater abundance of herbivorous invertebrates, particularly of *C. rodgersii*, and lower prevalence of foliose macroalgae, would provide compelling evidence for lower levels of predation on benthic invertebrates adjacent to haul-out sites (i.e., "mesopredator release").

I first aimed to test for localised differences in the composition of sessile benthic communities between locations of fur seal recolonisation and reference sites, where the cover of foliose macroalgae, and the cover of encrusting algae were expected to be different between seal haul-out and reference sites (Table 5.1). I then investigated whether the trophic structure and the species assemblages of mobile benthic communities differed between locations of fur seal recolonisation and reference sites, in a pattern consistent with a mesopredator release scenario described above, or conversely that reflect the prevalences of mesopredators and herbivorous fishes observed in **Chapter 4**. Additionally, I tested for differences in the abundances of benthic invertivores and an urchin, *C. rodgersii*, at locations of fur seal recolonisation. Finally, I focused on the relationship between *C. rodgersii* and the cover of canopy-forming macroalgae and locations of fur seal recolonisation.

Table 5.1 Summary of hypotheses and statistical analyses

Question	Response variable	Statistical analyses
Sessile benthic community:		
1) How did sessile benthic community composition vary by *region* and *site type*?	Percent cover of sessile benthic organisms (benthic_%cover)	mvGLM (Poisson, link=*cloglog*)
Mobile benthic community: Benthic invertebrates & cryptic fish:		
2) How did benthic invertebrate and cryptic fish assemblages and trophic structure vary by *site type*, *region* and *macroalgae cover*?	Benthic invertebrate and cryptic fish species assemblage (presence/absence data)	Fourth-corner analyses using *traitglm* (binomial)
3) Did abundances of benthic invertivores vary according to *site type* and *region*?	Abundance of benthic invertivores	GLM (Poisson)
4) Did the abundance of the long-spined sea urchin, *C. rodgersii*, vary according to *site type*, *region* and *macroalgae cover*?	Abundance of *C. rodgersii*	GLM (negbin)

5.3 Methods

5.3.1 Study sites & system

Jervis Bay (JB), NSW, includes the newest haul-out sites for two recovering Australian and long-nosed fur seals in eastern Australia (Burleigh *et al.* 2008). This location represents the frontier for the natural recolonisation of the east Australian coast by these pinniped species. At this location in eastern Australia, I previously observed a high prevalence of reef prey species in the diets of both species of recolonising fur seals (**Chapter 3**; Hardy *et al*. 2017); as well as correlations between seal haul-out sites and fish community composition (**Chapter 4**).

Two haul-out sites were surveyed in Jervis Bay (JB), NSW, Australia: Drum & Drumsticks in North JB (35° 2.799'S, 150° 50.552'E) and Steamer's Head in South JB (35° 10.725'S, 150° 43.895'E) (sites labelled N-HO and S-HO in Figure 4.2, **Chapter 4**). The JB haul-out sites typically harbours 30 to several hundred individual seals, with peak abundances in the winter and early spring, at the time of writing (June-September) (Burleigh *et al*. 2008; N. Hardy, pers. obs.). The data for this study and those described in the previous chapter were collected during the same sampling expedition conducted in the austral spring (Nov-Dec) 2014, immediately following the peak in abundance of seals locally. The haul-out sites differed in the duration of time since recolonisation by fur seals. The South JB haul-out site has been in regular use for ca. 15 years, whilst the North JB haul-out site has been in regular use for ~5–8 years at the time of sampling. However, greater numbers of seals haul-out in North JB compared to South JB since the complete closure of the northern headlands to the public as part of the Department of Defence's Beecroft Weapons Range in 2009 (T. Lynch, pers. comm., CSIRO, Tasmania; Burleigh *et al*. 2008).

Data on invertebrate and benthic communities were collected from two types of sites: (i) fur seal haul-out sites, and (ii) reference sites that do not harbour seal aggregations. For both haul-out sites, three local reference sites were selected along the northern and southern coasts of JB. Altogether, this asymmetrical design (Underwood 1994) included four sites in each region of North and South JB (Figure 4.2, Chapter 4): one haul-out site and three reference sites for each region. The spatial sampling design is described in detail in **Chapter 4** (section 4.3.1). At each site, 8

replicate transect surveys were conducted using a random stratified design to sample evenly across the depth range (sampling methods are described below).

5.3.2 Data collection & processing

Data on invertebrate and benthic community assemblages were collected from shallow subtidal reefs at depths of 10–18 m by SCUBA divers using standard Reef Life Survey (RLS) visual census techniques described in Edgar and Stuart-Smith (2014) and in the RLS methods manual (http://reeflifesurvey.com/wp-content/uploads/2015/07/NEW-Methods-Manual_150815.pdf, accessed April 2014). Using RLS methods, divers collected data on the diversity and abundances of benthic mobile invertebrates (> 2.5 cm) and cryptic benthic fish species along a 50×2 m transect (100 m^2 area). For benthic mobile invertebrate and cryptic fish species, the majority of taxa were recorded to species level, but unidentified taxa (<0.01% of records) were classified usually at family level. Divers then took 25 photo-quadrats of 0.25 m^2 along the 50 m transects (6.25 m^2 area per transect) to collect data on the community composition of sessile benthic taxa.

Benthic mobile invertebrates and cryptic fish were assigned trophic group traits based on available information found in reference materials. A broad range of reference databases were consulted, such as FishBase (Froese & Pauly 2016), SeaLifeBase (Pauly & Palomares 2017), Atlas of Living Australia (ALA 2016), and the Australian Museum (2016) reference base (Appendix D, Table D.1). In addition, the total abundance of the urchin, *C. rodgersii*, was calculated for analysis.

Photo-quadrat images were uploaded into CoralNet (https://coralnet.ucsd.edu/), an online repository and resource for benthic image analysis that facilitates annotation of benthic survey images (Beijbom *et al*. 2015). CoralNet software randomly overlaid 25 points on each image and the taxon or substratum beneath each point was identified into one of 11 morpho-families according to the Collaborative and Automated Tools for Analysis of Marine Imagery classification scheme (CATAMI) version 1.2 (Appendix D, Table D.2) (Althaus *et al*. 2015). The percentage cover of each category in each quadrat was calculated as the

proportion of the number of points overlying that category. The percentage cover of each morpho-taxa was then averaged across all quadrats within each transect.

5.3.3 Statistical models and analyses

5.3.3.1 Benthic community composition

Localised differences in benthic community composition between *site type* and *regions* were tested using data on the percentage cover of 11 different morpho-families (Table D.2). Differences in benthic community assemblage composition were tested using multivariate generalised linear models (mvGLMs) fitted with the *manyglm* function in the *mvabund* package in *R* (R Core Team 2017; Wang *et al.* 2012), using a Poisson distribution and *cloglog* link for percentage data. An offset term was used to weight the total percentage of each morpho-family. Broad trends, overdispersion and outliers in multivariate space were checked graphically by non-metric multi-dimensional scaling (nMDS) plots (Field *et al.* 1982) using the *vegan* package in *R* (Oksanen *et al.* 2015). Normality in multivariate data were checked using quantile-quantile (Q-Q) plots, multivariate homoscedasticity was checked by plotting Dunny-Smith residuals against fitted linear predictor values (Bates *et al.* 2015; Wang *et al.* 2012).

Model fit was assessed by analysis of deviance, tested using log-likelihood ratios (sum-of-*LR*) and *p*-values calculated from 999 iterations via PIT-trap resampling (Wang *et al.* 2012). To then identify which response variables (i.e., species) expressed significant effects between *regions* and *site type*, post-hoc univariate tests were performed with adjusted *p*-values fitted to each response variable (Wang *et al.* 2012). Response variables were ranked based on the test statistic and I calculated how many response variables were required to capture at least 50% of the deviance explained compared to the full model comprising all response variables (as per section 3.3.4.2, **Chapter 3**). The deviance was calculated by taking the ratio of the percentage deviance explained by a subset of the response variables and the deviance explained by the full model containing all response variables (Guisan & Zimmermann 2000). Response variables with the highest univariate test statistic, significant *p*-values, and capturing in aggregate at least 50% of the deviance explained by the full

model therefore had the greatest effect size and were considered to have the strongest evidence for an effect of explanatory variables and thus likely to be contributing to differences between levels of the explanatory variables. The *ggplot2* package in R was used to create all graphical illustrations (version 2.2.1) (Wickham 2009).

5.3.3.2 Analyses of invertebrate assemblage and trophic structure

I then aimed to investigate whether invertebrate assemblages and the trophic structure of invertebrate communities differed between three environmental variables: (i) *site types*, haul-out sites and references, (ii) whether these differences were common to both *regions*, North and South JB, and (iii) whether patterns in the assemblage were influenced by the cover of foliose macroalgae found on the benthos. The relationship between the invertebrate species assemblage, the trophic traits of invertebrates, *site type*, *region* and the prevalence of *macroalgae*, was evaluated using a trait-based multivariate analysis technique, the fourth-corner model (Fig. 4.1) (Brown *et al.* 2014a).

I used a fourth-corner model to predict the probability of occurrence of different invertebrate species as a function of explanatory variables for the environment (here *regions*, *site type* and *macroalgae*; X_1), species traits (X_2) and their interaction (X_1* X_2), where the matrix of coefficients for the interaction between the species traits and explanatory variables is known as the fourth-corner solution (Figure 4.1) (Brown *et al.* 2014b). Analytical routines for the fourth-corner analysis, model fit, selection and assessment, are described in section 4.3.3 (**Chapter 4**), and were performed using the package *mvabund* (version 3.12.3) (Wang *et al.* 2012) in *R* software (version 3.4.1, R-Core Team 2017). The model was fitted to data on invertebrate species presence/absence using the function *traitglm* and using a LASSO penalty, specifying the fitting method as '*glm1path'* (Brown *et al.* 2014a; Hastie *et al.* 2009; Warton *et al.* 2015). The generalised linear model (GLM)-LASSO model effectively shrinks coefficients to zero when they are not statistically significant, and these are presented graphically to evaluate the magnitude and significance of an explanatory variable, and interactions between traits and explanatory variables (Brown *et al.* 2014b). Where significant interactions between environmental variables (*region, site type, macroalgae*) and invertebrate trophic traits are observed, I interpreted that the occurrences of invertebrate species with these traits are either

negatively or positively correlated with certain environmental variables. The relative strength of each trait and environmental variable could also be compared, as main effects for the models and predictors of the species assemblage.

A single predictive model was fitted to all species presence/absences at all *site types*, *regions* and values of *macroalgae* using a *traitglm* (described in section 4.3.3, **Chapter 4**). By excluding the trait input from the model, species-level variations in the invertebrate assemblage that were not explained by traits could then be determined. This is the equivalent to fitting a multivariate species distribution model. Broad trends, overdispersion and outliers in multivariate space were checked graphically by non-metric multi-dimensional scaling (nMDS) plots (Field *et al.* 1982) using the *vegan* package in *R* (Oksanen *et al.* 2015), see also section 4.3.3 (**Chapter 4**). Model fit was assessed by plotting residuals against fitted values and plotting quantile-quantile (Q-Q) plots.

Due to their ecological relevance, I also focused on trends in the abundances of benthic invertivores as a trophic group and of the ecologically important herbivore, *C. rodgersii*. Differences in benthic invertivore abundances were also tested between *regions* and *site type* using a GLM fitted with negative binomial distribution. The relationship between the total abundances of *C. rodgersii* and the explanatory variables of *regions*, *site type* and *macroalgae* was tested using a GLM fitted with negative binomial distribution, to account for overdispersion in the count data. These analytical routines were performed in the *MASS* package in *R* (version 7.3-47) (Ripley *et al*. 2002). The *graphics* package (R Core Team 2017) was used to plot residuals and assess model assumptions of homoscedasticity and normality. For both analyses of benthic invertivore and *C. rodgersii* abundances, the full models including all explanatory variables were simplified using backwards selection following the parsimony principle, and tested using *Chi-square* test on likelihood ratios, and using the Akaike Information Criterion (AIC) value comparison to determine the best fitting model of those tested (Buckland *et al*. 1997).

5.4 Results

5.4.1 Sessile benthic community composition

Benthic community composition varied between haul-out and reference sites, and these trends also varied according to the *region* sampled (Table 5.1, Figure 5.1a). Benthic community composition was similar across all reference sites in both North and South JB (Figure 5.1a and Figure 5.2). Community composition varied between haul-out sites and reference sites within *regions* (Figure 5.1a and 5.2). The reef community composition of both haul-out sites was also significantly different from each other (Figure 5.1a and 5.2). In South JB, the percentage cover of kelp was lower at the haul-out site compared to reference sites, whilst this was not significantly different between haul-out sites and reference sites at North JB (Figure 5.2). The percentage cover of encrusting algae and non-canopy forming macroalgae did not differ in South JB (Figure 5.2). In North JB, the percentage cover of encrusting algae was higher at the haul-out site compared to reference sites, whilst the percentage cover of non-canopy forming macroalgae was lower at the haul-out site compared to reference sites (Figure 5.2). Additionally, in South JB, the percentage cover of sponges was higher at the haul-out site compared to reference sites (Figure 5.2).

Table 5.2 Results of the multivariate generalised linear model (mvGLM) for the relationship between *regions* and *site type*, seal haul-outs and reference sites, and the benthic community composition (percentage cover of benthic morpho-taxa). These results are illustrated in Figure 5.2.

Metric	Model	Explanatory variables	Residual df	Df. Diff	Deviance	Pr(>Dev)
Benthic Community Composition (% cover)	Benthic ~ Region*Site type	(Intercept)	63			
		Region	62	1	45.52	0.001 ***
		Site type	61	1	21.65	0.034*
		Region:Site type	60	1	52.89	0.001 ***

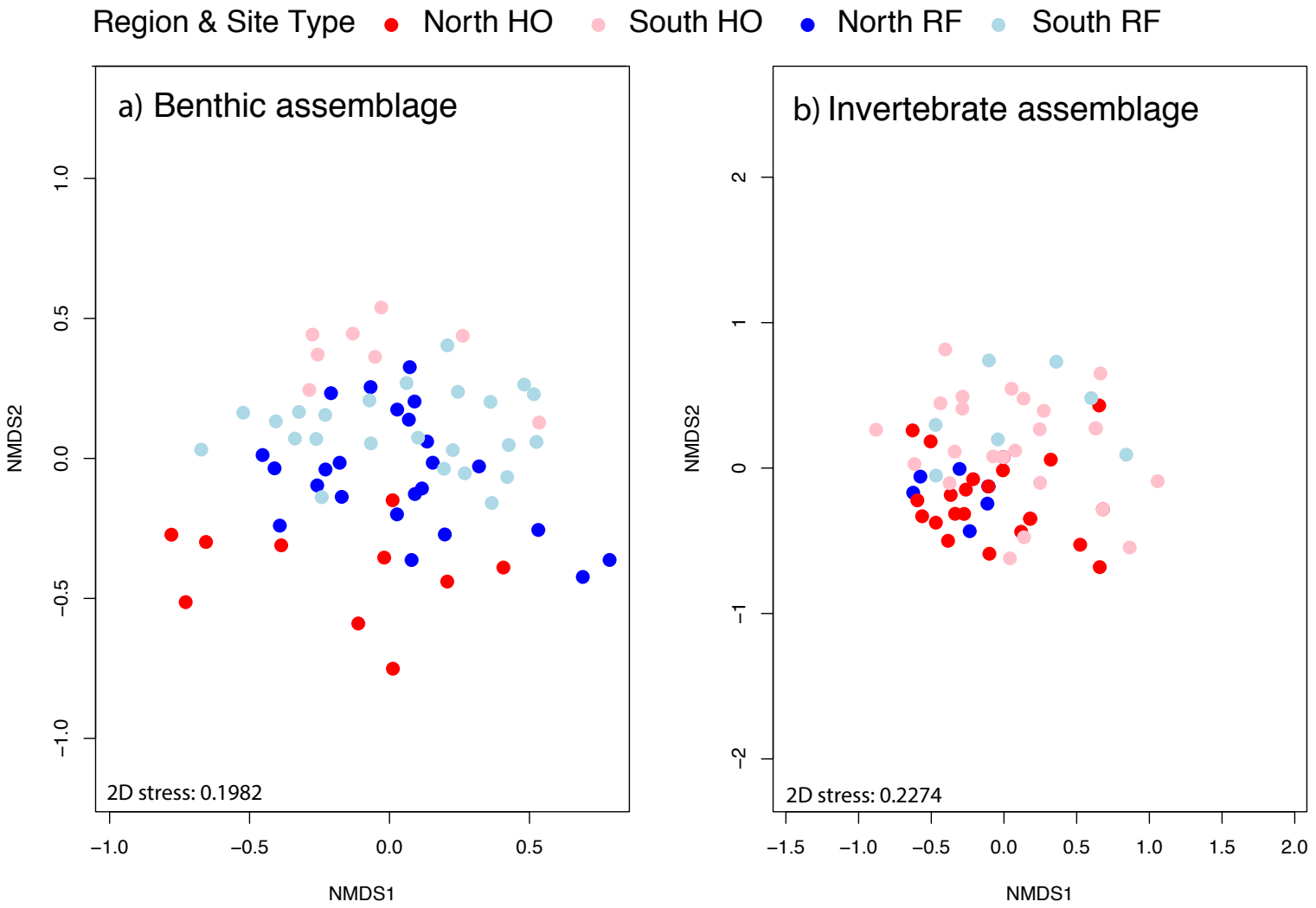


Figure 5.1 nMDS ordination for: a) benthic community composition (% cover of benthic morpho-taxa), and b) invertebrate species assemblages (presence/absence data), represented as centroids for each *region* (North, South) and *site type* (HO: seal haul-out, RF: references).

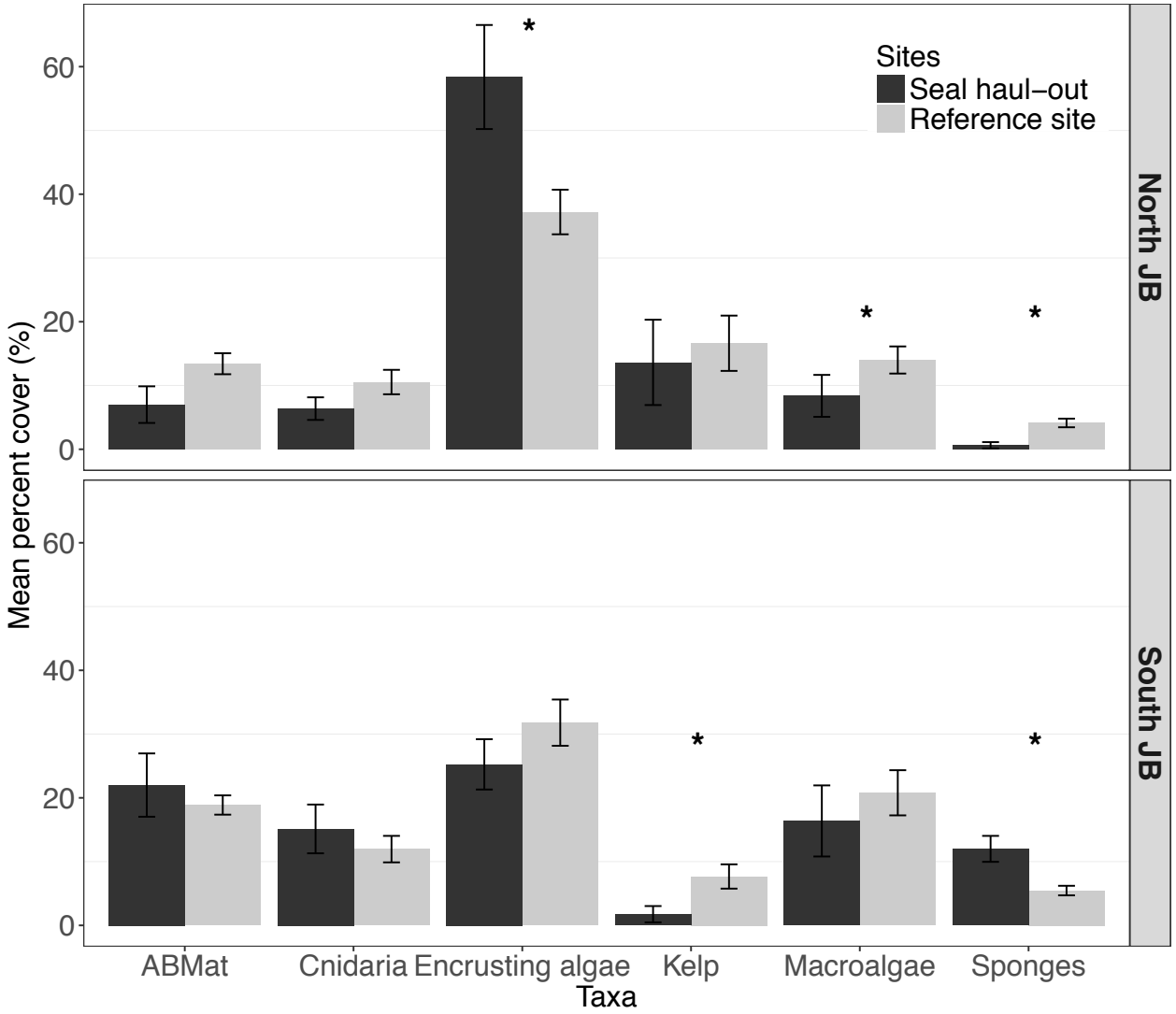


Figure 5.2 Percentage cover of benthic morpho-families across seal haul-out sites and reference sites in north and south JB. Error bars represent standard errors. Asterisks denote morpho-families that contributed significantly to the differences between *regions and site type*. ABMat = algal and biotic matrix (Appendix D, Table D.2).

5.4.2 Macro-invertebrate and cryptic fish community

A total of 36 species were observed on benthic surveys, including 11 cryptic fish species and 25 macro-invertebrates (> 2.5 cm). Of these macro-invertebrate and cryptic fish species, 22% were mesopredators, 31% were benthic invertivores consuming mobile species, whilst 19% were benthic invertivores consuming sessile invertebrates, 19% were herbivores and 8% were detritivores or filter feeders (Table D.1). Detritivorous and filter-feeding species were numerically rare in this study, and were thus excluded from trait-based analyses, as inferences could not be made on their occurrences (Table D.1). The most common species in the mobile benthic assemblage were: the teleosts and mesopredators, *Acanthistius ocellatus* and *Scorpaena cardinalis*; a teleost and benthic invertivore, *Hypoplectrodes maccullochi*; a gastropod and benthic invertivore, *Cabestana spengleri*; and two herbivores, the echinoderm *C. rodgersii*, and gastropod *Astralium tentoriformis* (Figure D.1, Table D.1).

The results of the fourth-corner analysis revealed that the trophic structure of benthic macro-invertebrate and cryptic fish species was not affected by *site type* (haul-out sites compared to references), or the percentage cover of *macroalgae* (Figure 5.3). There was an interaction between the trophic structure of the invertebrate assemblage and *region*, where herbivores were less common in South JB generally (Figure 5.3). For invertebrate species occurrences, the percentage cover of foliose *macroalgae* and the *region* sampled were more important predictors than *site type*. Overall, the percentage cover of foliose macroalgae was negatively correlated with species probabilities of occurrence (Figure 5.4). It must be noted that this finding was not due to methodological issues of detecting invertebrates amongst foliose macroalgae because invertebrate prevalence data are based off visual searches by divers, involving under-canopy surveying wherever macroalgae were large enough to hide invertebrates, and these data are independent from the photo-quadrat image analyses for sessile benthic communities. This result may, however, reflect a simply large abundance of certain invertebrates, such as urchins and tent shells, on hard reef substrates.

Species-level variations between *site type,* cover of *macroalgae* and *regions* were observed (Figure 5.5). The benthic invertivore, *Chironemus marmoratus* (fish

species), was significantly less common at haul-out sites. The mesopredator, *A. ocellatus* (fish), and benthic invertivore, *Dicathais orbita* (gastropod), were significantly more common at haul-out sites (Figure 5.5). The percentage cover of *macroalgae* was negatively correlated with the probability of occurrence of several common rocky subtidal species: two mesopredators, *A. ocellatus* and *Gymnothorax prasinus* (fish species), the benthic sessiles invertivore, *Fromia polypora* (seastar), the benthic invertivore, *H. maccullochi* (fish), and the herbivore, *A. tentoriformis* (gastropod) (Figure 5.5). The probability of occurrence of the benthic invertivores *Cabestana spp.* (gastropods) and *C. marmoratus* (fish), and the herbivore, *Heliocidaris tuberculata* (sea urchin), were positively correlated with the percentage cover of *macroalgae* (Figure 5.5). These patterns simply reflect the micro-habitat preferences of these reef species.

Overall, abundances of benthic invertivores were not significantly different between haul-out sites and reference sites (Figure 5.6a). The urchin, *C. rodgersii*, was ubiquitous across study sites analysed and thus differences in the probability of occurrence of *C. rodgersii* according to explanatory variables were not detected (Figure 5.5). On average, the abundance of *C. rodgersii* was lowest at the haul-out site in South JB and this was significally different to reference sites, but a difference was not observed in North JB (Figure 5.6b). The abundances of *C. rodgersii* were also negatively correlated with the percentage cover of *macroalgae* at haul-out sites in both *regions* (Figure 5.7, Table 5.3), whilst this relationship was not significant at reference sites. This relationship was observed at locations where both *C. rodgersii* abundance and the cover of foliose macroalgae were low (Figure 5.7).

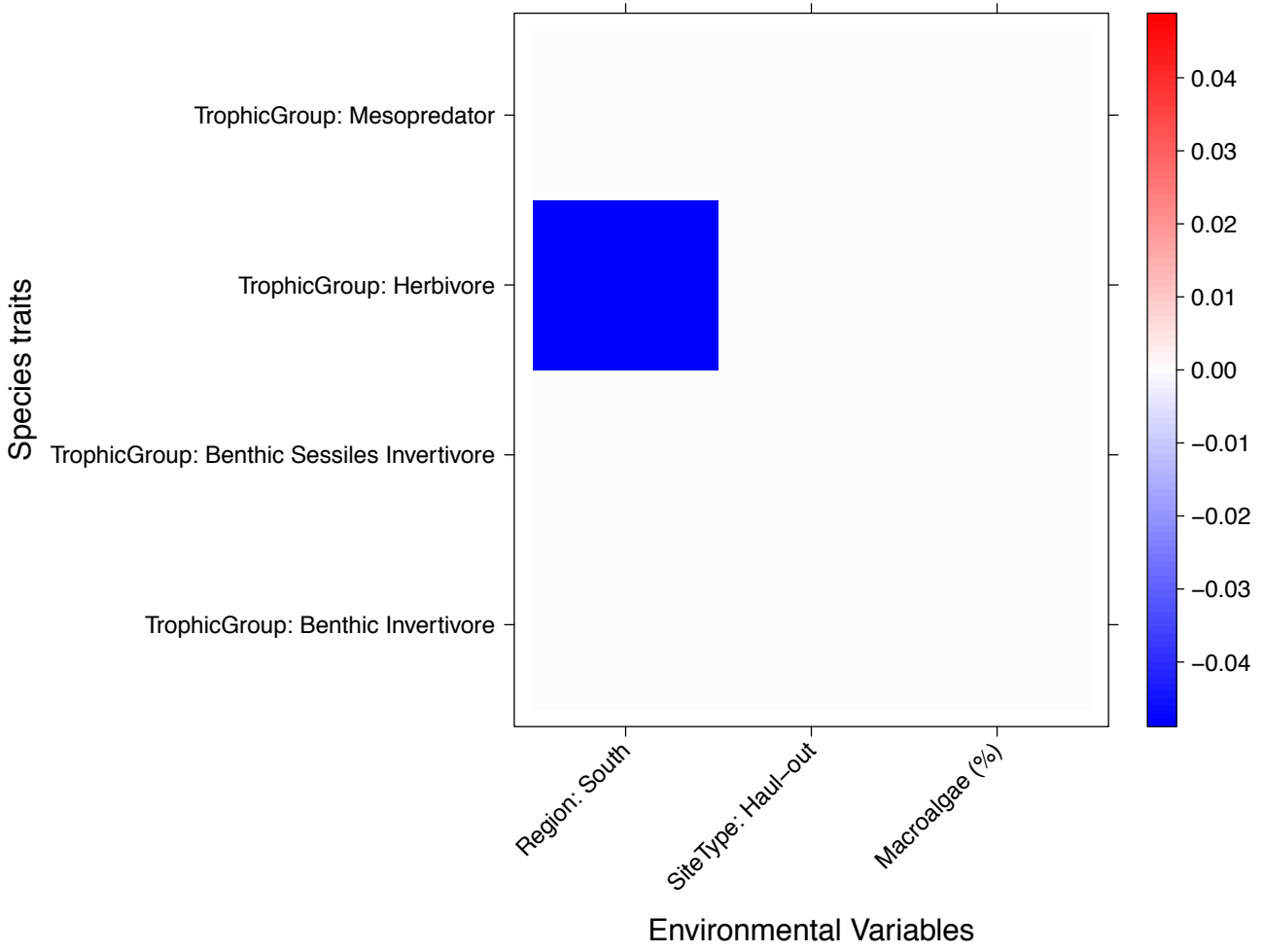


Figure 5.3 The fourth-corner modelling results displayed for standardised coefficients for all explanatory variable-trait interaction terms (X_1*X_2) from the GLM-LASSO model. Colours indicate significant interactions between levels of each trait and the environmental variables, *site type*, *region* sampled and percentage cover of *macroalgae*. Darker colours describe the strength of effect, whilst red indicates a positive relationship, and blue a negative relationship. Coefficients have been shrunk to zero (white) for non-significant terms in the GLM-LASSO model.

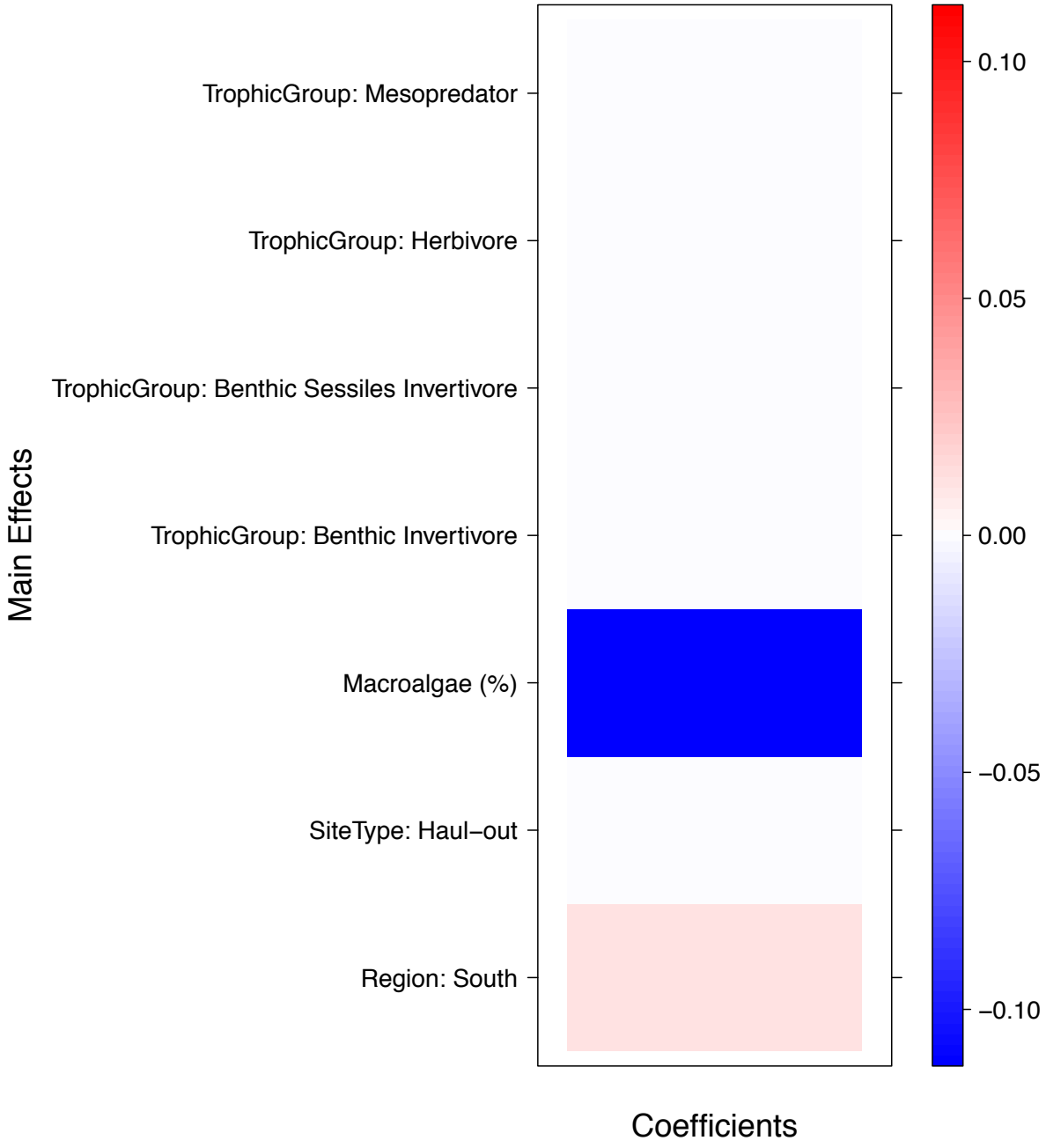


Figure 5.4 Standardised coefficients from the GLM-LASSO model for the relationship between the main effects terms and invertebrate species assemblage.

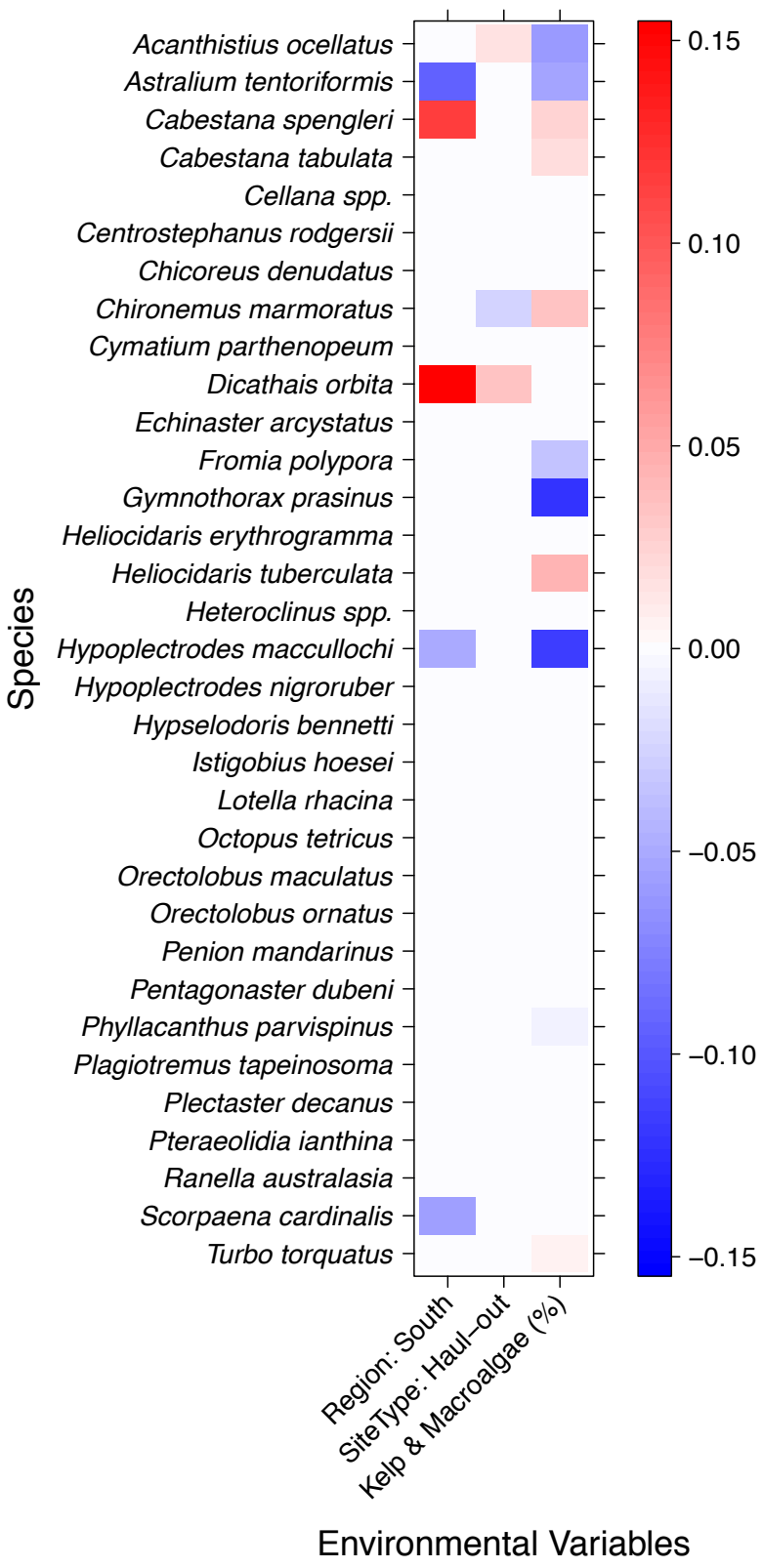


Figure 5.5 Results of the fourth-corner model fitted for the relationship between species and environmental variables, without traits. Standardised coefficients for all species and environmental variable interaction from the GLM-LASSO model.

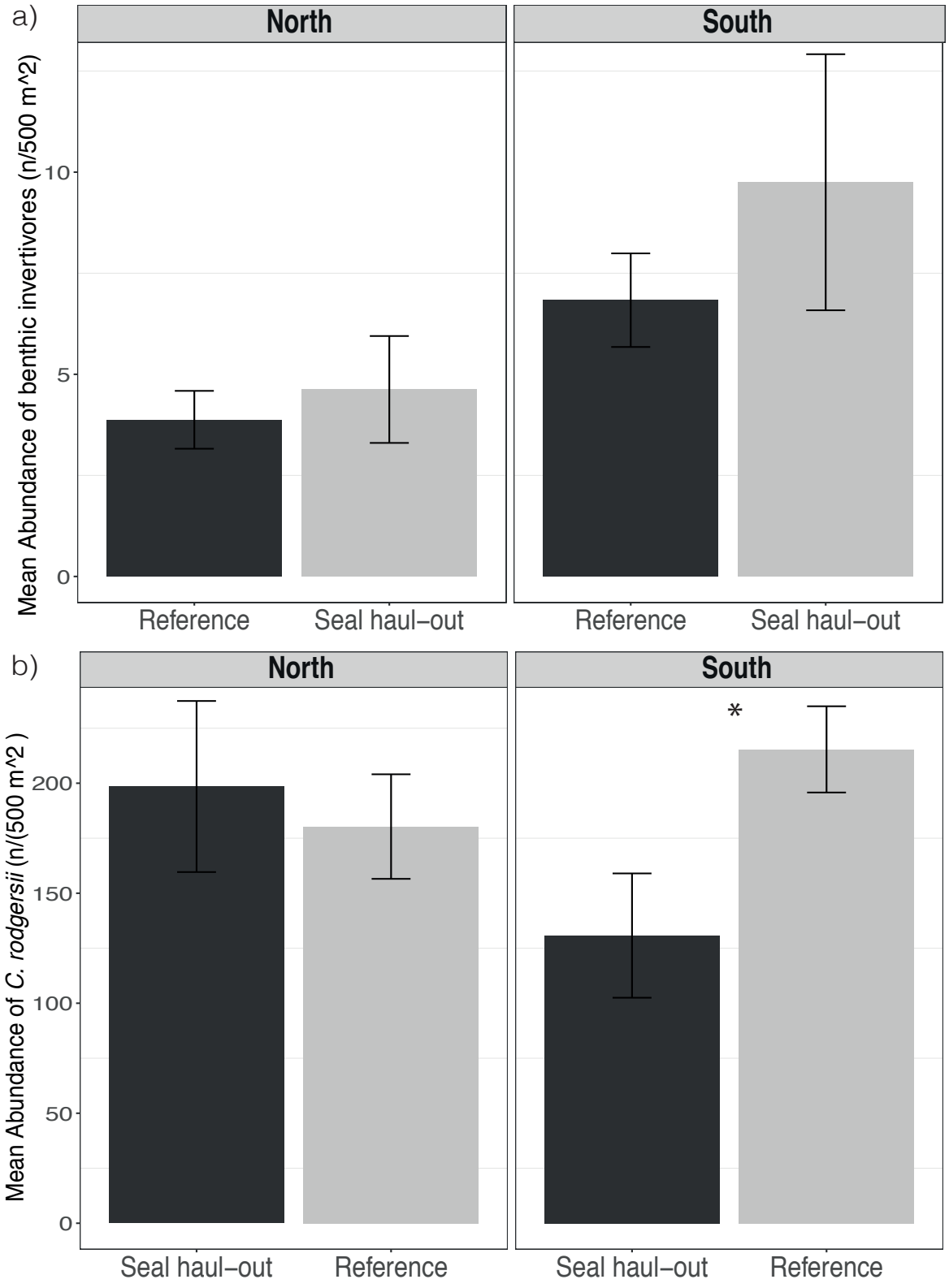


Figure 5.6 The comparison between a) mean abundances of benthic invertivores and b) mean abundances of the long-spined sea urchin at different *site types* and *regions*, error bars represent standard errors and asterisks represent significant differences between *site types* and *regions*.

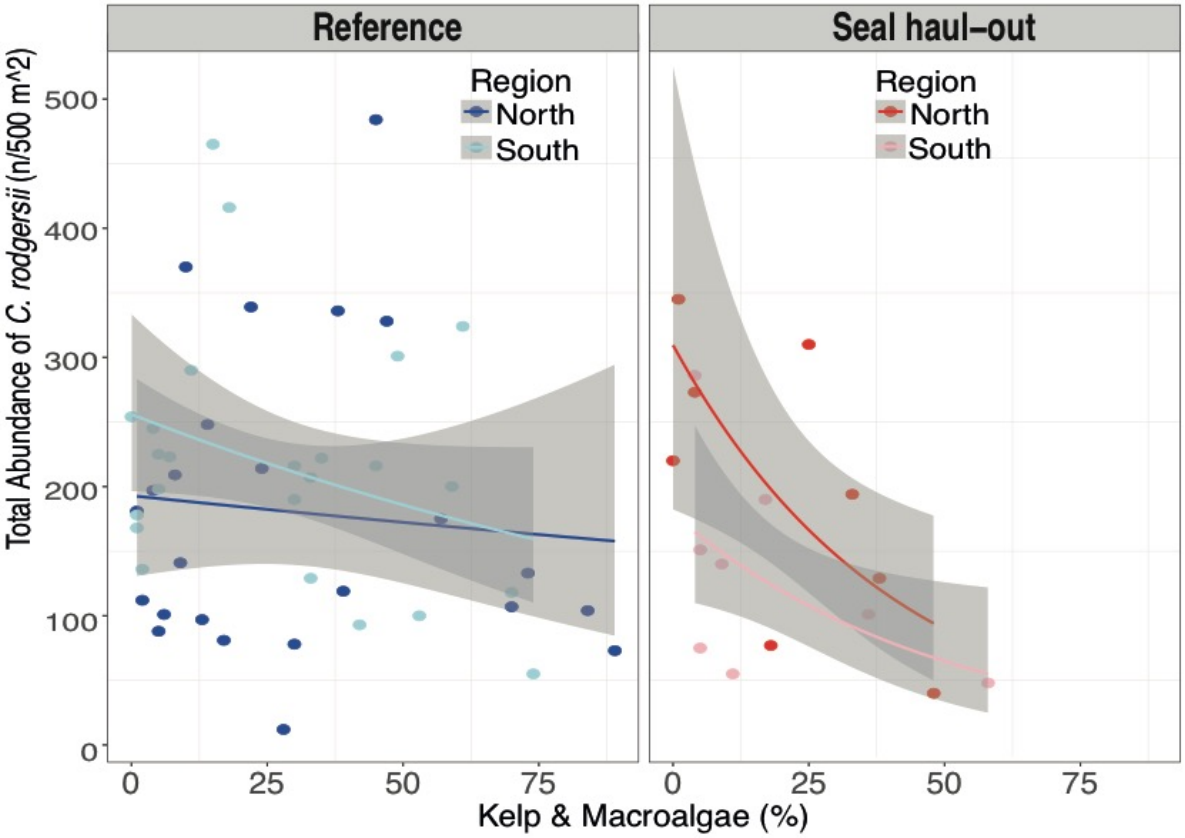


Figure 5.7 The relationship between total abundance of the long-spined sea urchins (*Centrostephanus rodgersii*) and the percentage cover of kelp and macroalgae, between *site types*, seal haul-out (HO) and reference (RF) site, and between *regions*, North and South JB, fitted lines and confidence intervals (95%) are for a generalised linear model with negative binomial distribution.

Table 5.3 Results of the generalised linear model for the relationship between *regions* and *site type*, seal haul-outs and reference sites, and the abundances of the long-spined sea urchin (*Centrostephanus rodgersii*), using a negative binomial distribution.

Metric/Model	Explanatory variables	Estimate	Std. Error	z-value	Pr (>|z|)
Total Abundance *C. rodgersii* T_abun ~ Region + Site Type + Macroalgae + Site Type*Macroalgae + Region*Site Type	(Intercept)	5.322237	0.142866	37.253	<2e-16 ***
	Region (South)	0.160697	0.152870	1.051	0.2932
	SiteType (Seal haul-out)	0.361431	0.283791	-1.274	0.2028
	Macroalgae	-0.004183	0.003057	-1.368	0.1712
	SiteType (Seal haul-out):Macroalgae	0.018130	0.008221	-2.205	0.0274 *
	Region (South):SiteType (Seal haul-out)	0.620930	0.307205	-2.021	0.0433 *

5.5 Discussion

This study aimed to identify differences in coastal reef benthic communities, that would be consistent with potential indirect effects stemming from predator recovery, adjacent to locations that have been colonised by two high-order predator species, Australian and long-nosed fur seals, on temperate reefs of eastern Australia. The trophic structure, or prevalences of different trophic groups, of mobile benthic communities did not significantly differ between fur seal haul-out and reference sites. Species-level variation in mobile benthic communities were observed in relation to fur seal haul-out sites, but were best predicted by the percentage cover of canopy-forming and foliose macroalgae, consistent with micro-habitat preferences in these species. Additionally, abundances of the ecologically important herbivore, *C. rodgersii*, were lower adjacent to one of the seal haul-out sites compared to reference sites. The percentage cover of canopy-forming and foliose macroalgae were also found to be lowest at haul-out sites compared to reference sites. Whilst patterns in *C. rodgersii* abundance and sessile benthic community composition were generally similar at all reference sites, patterns at haul-out sites were contingent on the region sampled (southern versus northern Jervis Bay).

In the previous study investigating temperate reef fish communities adjacent to locations of fur seal recolonisation (**Chapter 4**), a negative correlation was observed between the prevalences of herbivores and fur seal haul-out sites, whilst a positive relationship was observed for mesopredators. The patterns observed in the previous chapter are thus not entirely consistent with those of reduced macro-algal cover and also lower urchin abundances at haul-out sites observed in the present study, unless the lower abundances and prevalences of herbivores at haul-out sites actually corresponds with their being larger and also more efficient foragers.

At both haul-out sites, there was a negative relationship between *C. rodgersii* abundance and the percentage cover of foliose macroalgae, in contrast to reference sites where this relationship was weak. This relationship could be consistent with localised indirect effects on marine benthic communities adjacent to fur seal haul-out sites, where lower cover of foliose macroalgae may occur despite sea urchin abundances being lower at haul-out sites, if the size or biomass of urchins adjacent to seal haul-out sites is greater (e.g., size specific predation by sea otters on urchins)

(Estes & Steinberg 1988). Differences in sizes of urchins at haul-out sites compared to reference sites were not examined and warrant further investigation.

Predation pressure from fur seals on fish mesopredators and invertivores could also affect the size structure of urchins (Ling *et al.* 2009). Differences in reef fish community size structure were effectively detected in southern Jervis Bay, whereby larger fish size classes were more abundant at reference sites, but not in northern Jervis Bay (**Chapter 4**). Additionally, the presence of seals at haul-out sites could promote differences in the foraging behaviour of fish mesopredators and invertivores such as reduced foraging time or effort (Connell 2002). Reductions in foraging effort have been observed in fish species in the presence of fur seals on temperate reefs (Connell 2002), and on coral reefs for herbivorous fish in response to greater mesopredator abundances (Madin *et al.* 2011). A release for urchins from predation pressure of larger teleost predators or changes in behaviour by teleost predators could be occurring at least in southern Jervis Bay and could partially explain the relationship between sea urchins and macroalgae observed at both haul-out sites. Surveys of the size structure of sea urchins at these locations could elucidate if this were the case. Additionally, surveys targeting the biomass and ideally foraging behavior of teleost urchin predators such as *P. auratus* and *A. viridis* adjacent to fur seal aggregation sites may help to resolve these trophic questions.

A significant sea urchin predator group, nocturnal lobsters, were not surveyed in this study (Ling *et al.* 2009; Redd *et al.* 2008), requiring specialised observational techniques. Lobsters are known to control the abundances of *C. rodgersii* and promote the recovery of kelp forests from overgrazing on Australian temperate reefs, Tasmania (Ling *et al.* 2009). The larger lobsters are also the most effective predators, particularly predating on the larger urchins (Ling *et al.* 2009). Crustaceans are only occasionally consumed by fur seals (Deagle *et al.* 2009; Hardy *et al.* 2017; Page *et al.* 2005), thus predation pressure on lobsters is not expected in relation to recovering fur seals. However, lobsters populations are heavily impacted by overfishing and therefore positively affected by protection measures, including marine protected area designation over time (Ling *et al.* 2009; Montgomery & Liggins 2013). Marine protected areas have been designated at fur seal colonies and haul-out sites, among other sites, along the coast of NSW (Coleman *et al.* 2015; Kelaher *et al.* 2015), including in Jervis Bay (Coleman *et al.* 2015). It is therefore likely that marine park

protection and conversely the extraction of urchin predations by recreational and commercial fisheries would influence urchin and algal abundances (Ling *et al.* 2009), more so than the recovery of large generalist predators.

In the previous study (**Chapter** 4), negative correlations were observed for the total abundance of fish species known to be seal prey, and for the size structure of fish communities adjacent to the haul-out site compared to references, in southern JB. This is also the location at which the lowest abundances of sea urchins and foliose macroalgae were observed in the present study. Whilst effect sizes were small, differences in benthic reef community composition were stronger at the seal haul-out (South JB) with the longest history of seal recolonisation and use, ca. 15 years. Differences were subtle or not detected at the seal haul-out site with ca. 5–8 years of use (North JB). Direct ecological changes to target species abundances and biomass are known to take between 5–20 years to manifest, whilst indirect, community-level changes can take even longer to manifest at lower trophic levels and in primary producers (Babcock *et al.* 2010; Barrett *et al.* 2009). These observations correspond to an early stage in the recovery of fur seals on temperate reefs in eastern Australia. The identification of more significant differences in both reef fish and benthic communities adjacent to the haul-out site with a greater duration of use by seals, is compelling and warrants further investigation over a longer time scale, in order to verify any trajectories of change that would be consistent with predator-mediated effects to lower trophic levels following ongoing recovery of fur seals in eastern Australia.

The present study suffers from the same limitations on inference as the previous study (**Chapter 4**). In section 4.5, I offer several recommendations for further investigation of the hypotheses tested in these studies. Recommendations to address the limitations of this study include: (i) including additional fur seal haul-out sites to the north and south of the present study area, as well as (ii) a gradient of reference sites sampling according to distance from fur seal haul-out sites and using a multiple regression statistical approach to analyse the data; and (iii) increasing the sampling effort in both the number of replicate surveys and the temporal scale of the project in order to assess any trajectories of ecological change over time in relation to both fur seal recovery and the recovery of ecological communities under marine protection measures.

Finally, with a high diversity of reef species and of trophic relationships on Australian reefs (Casey *et al.* 2017; **Chapter 4**), a significant knowledge gap exists considering our understanding of relationsips between intermediate (i.e., mesopredators, invertivores, omnivores) levels of food webs. It is likely that any effects on middle trophic level components of these ecosystems could be mitigated by trophic complexity or take time to manifest.

5.6 Conclusions

Using trophically and functionally driven metrics, this and the previous chapter may be detecting subtle ecological shifts early in the recovery of large predators on Australian temperate reefs. I observed a lower abundance of an important reef herbivore, the long-spined sea urchin (*C. rodgersii*) but also a lower prevalence of canopy-forming and foliose macroalgae at the fur seal aggregation site with the longest duration of use. These data are preliminary and it could take decades of monitoring for any conclusive ecological effects to become apparent, particularly at a community-level. Additionally, the abundances or biomass of important and strongly interacting species may need to pass above or below certain thresholds before phase shifts actually occur. Finally, the recovery of large predators on the coast of eastern Australia is not occurring in isolation from other factors affecting reef ecosystems. Human extractive activities, such as commercial and recreational fishing of temperate reef fish and invertebrates, as well as spatial measures for their protection influence the structure and composition of temperate reef ecosystems. Combined long-term monitoring of coastal ecosystems adjacent to locations of fur seal recolonisation and marine protected areas is therefore recommended to develop a sound understanding of their relative importance on Australian temperate reefs, with particular focus on changes in in populations of urchin predators, especially lobsters, as well as urchin populations and macroalgal communities.

6 Synthesis

In this thesis, I primarily aimed to investigate the ecological consequences of the recovery of two important coastal predators, Australian and long-nosed fur seals, on complex, temperate ecosystems in southeastern Australia. I have completed this project having produced many answers, but far more questions have arisen from this work than I was able to answer in that time. Here, I synthesise what I have achieved in this investigation of recovering predators and complex ecosystems. I highlight many exciting questions that have arisen from this work and I provide recommendations for monitoring predator recovery and important ecological interactions in this and other ecosystems.

6.1 Summary of research and findings

The recovery of two large predators on temperate and coastal ecosystems in southeastern Australia raised a number of interesting questions for science. Australian and long-nosed fur seals are large, warm-bodied predators, with females weighing up to 120 kg and males up to 350 kg (Arnould & Warneke 2002), and are rapidly recovering from previous overharvesting to well over 100,000 individuals for each species in Australian waters (Kirkwood & Arnould 2011; Shaughnessy *et al.* 2015). Kirkwood and Arnould (2011) posited that Australian fur seals, and to this I add long-nosed fur seals, are likely to represent a significant resident biomass of high trophic-level predators in coastal waters of southeastern Australia. The majority of the work on the foraging ecology and diets of these two species comes from the centre of their geographic range in Bass Strait and South Australia.

How then would they interact with coastal ecosystems in eastern Australia? A coastline characterized by significant rocky reef systems and the narrowest continental shelf anywhere in Australia (Jordan *et al.* 2010). And, would the recovery of large, warm-bodied predators result in cascading effects on prey and coastal communities in eastern Australia? Firstly, evidence for top-down forcing between pinnipeds and their prey existed, but was often circumstantial (Estes *et al.* 2016). Further, little was known about the east coast fur seals, apart from reports documenting their recovery and very rough population estimates in

NSW (Burleigh *et al.* 2008; Shaughnessy *et al.* 2001). The first task would be to investigate the diets of these newly recovering populations of fur seals in eastern Australia.

Prior information on trophic interactions between fur seals and east coast ecosystems was lacking, which also promised to be a complicated task involving two sympatric generalist predators known to forage in biodiverse coastal and pelagic ecosystems. DNA-based methods for predator diet analysis offered to produce taxonomically sensitive information on predator diets, to produce data across a range of potentially foraged ecosystems of which we lacked prior knowledge, and importantly offered a minimally invasive technique to study the diets of these protected species. In **Chapter 2**, I undertook a systematic review of the recent applications of genetic techniques for predator diet analysis to assess recommendations and limitations in the use of genetic techniques for investigating ecological interactions. Genetic techniques have emerged as an excellent tool for the exploration of trophic interactions, particularly in complex ecosystems, for generalist predators and in ecosystems for which we have little prior knowledge. DNA metabarcoding, or the mass amplification, sequencing and identification of prey DNA from multiple taxa, was particularly appropriate for the aims of this thesis.

In **Chapter 3**, I therefore undertook a detailed analysis of the diets of both recolonising predator species using DNA metabarcoding techniques. The diets of both species were characterized from the frontier of their geographic recolonisation of eastern Australia, including from an established breeding colony and from a new but permanent haul-out site in NSW, across multiple seasons. This study produced an unprecedented diversity of prey taxa, identifying over 70 potential prey species for each fur seal species, and the results were published (Hardy *et al.* 2017). Patterns in the diets of both fur seal species at a newly established breeding colony in southeastern Australia were similar to that of breeding colonies in the centre of their geographic range, in southern Australia. Namely, the likely provenance of prey included a broad range of ecosystems in the diets of fur seals from a breeding colony, including coastal benthic and demersal prey as well as pelagic prey. The breadth of their diet appeared to narrow in the winter when fur seal diets primarily contained coastal and offshore pelagic prey. However, these patterns were not observed at a non-breeding haul-out site at the species' geographic range edge. The diets of both seal species at their range edge contained a high prevalence of coastal and particularly benthic and reef-associated prey items (Figure 6.1). The convergence of diets, and thus ecological interactions,

of two predator species at their range edge correlates with known differences in seal population densities and demographics at sites that are newly recolonised by predators.

Detailed information on the diets of Australian and long-nosed fur seals thus helps to provide answers to the first question that I asked in this thesis: how these predators interacted with temperate ecosystems of eastern Australia. The first hypothesis of this thesis was also accepted: in that important consumptive interactions linking fur seals to temperate reef ecosystems were identified. According to optimal foraging theory and in central place foragers, such as fur seals, it is commonly expected that predators will exhaust resources locally prior to foraging further afield (Ashmole 1963; Birt *et al.* 1987; Orians & Pearson 1979). The localized depletion of resources and subsequent increase in foraging effort has been observed in a natural recolonisation event by northern fur seals in the Aleutian Islands (Kuhn *et al.* 2014). This led to predictions that I would observe limiting effects on prey (mesopredator) populations, the release of secondary consumers from pressure by mesopredators, and thereby increasing herbivory on temperate reefs of eastern Australia, a pattern observed in other temperate systems (Estes *et al*. 2016; Ripple *et al*. 2016).

Chapters 4 and 5 investigated both direct trophic effects on reef fish communities and potential indirect effects on benthic reef communities following the natural recolonisation of discrete locations by fur seals on temperate southeast Australian reefs. As it is not possible to go back in time and survey reefs prior to the recolonisation of fur seals in eastern Australia, and prior data is lacking, these investigations were conducted at two spatially distinct fur seal haul-out sites, each paired with multiple reference sites that do not harbour fur seal aggregations. I aimed to quantify localized differences in reef fish community composition, in the size and trophic structure of fish communities, between fur seal haul-out sites and reference sites. I then aimed to identify differences between seal haul-out sites and references sites in benthic macro-invertebrate and cryptic fish communities, as well as sessile benthic communities. I hypothesized that these differences would be consistent with increased pressure from fur seals on larger fish and higher-trophic level prey species, and a subsequent release from predation of lower-trophic level components of reef ecosystems, for example by increasing reef herbviores and decreasing macroalgal cover adjacent to fur seal aggregation sites.

The results of **Chapters 4** and **5** provided evidence for differences between reef fish and benthic communities adjacent to fur seal aggregation sites compared to local reference sites along the coast (Figure 6.1). Interestingly, through multivariate trait-based analyses of

reef fish assemblages, I identified that schooling fish and herbivorous fish were less prevalent at fur seal haul-out sites compared to reference sites. However, mesopredators occurences were greater at haul-out sites compared to reference sites and invertivorous fish occurrences were similar at both types of sites. At the haul-out site with a greater history of use by fur seals (ca. 15 years), I also observed a lower total abundance of fish compared to reference sites, and a lower biomass of larger-sized fish. At the same site, the abundances of an ecologically important herbivore, *Centrostephanus rodgersii*, were also unexpectedly lower compared to reference sites. Despite lower average urchin abundances, fur seal haul-out sites had lower average cover of canopy-forming kelps and foliose macroalgae compared to reference sites. These results lend only partial support to hypotheses of direct effects of fur recovering fur seals on reef prey and little support for indirect effects on benthic reef communities (Figure 6.1). These differences did not correspond to strong ecological differences in reef community trophic structure or size structure, and differences were contingent on the location of the haul-out site sampled (Figure 6.1). Additionally, the complexity of Australian temperate reef food webs, including the effects of other mesopredators and the diversity of the fur seals' prey base, likely dampen the potential for trophic effects linking fur seals to benthic community composition (Figure 6.1).

At an early stage in the recovery of two high-order predator species, this thesis provides important baseline information: (i) on the diets of sympatric predators and important trophic interactions in complex temperate ecosystems, (ii) on evidence of subtle direct effects on prey communities, and weak indirect effects on benthic coastal ecosystems following the recovery of fur seals. This information contributes to addressing a critical knowledge gap regarding trophic effects linking pinnipeds, their prey and lower levels of their food webs. This work offers important baseline information and novel insights with which to continue monitoring ecological interactions in recovering fur seals and assess future trajectories of change on Australian temperate reefs.

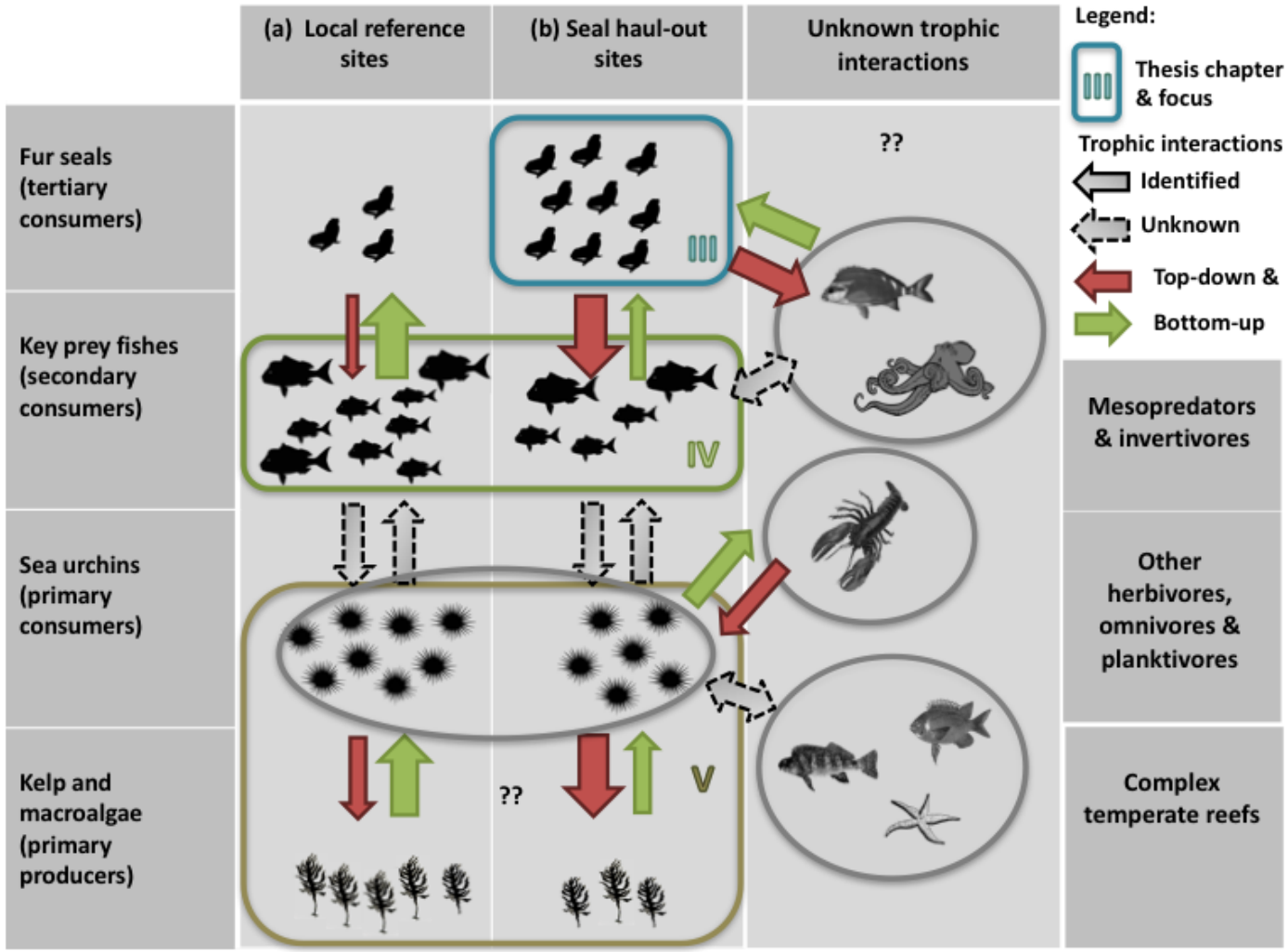


Figure 6.1 Conceptual diagram of the findings and structure of the three data chapters of this thesis. Illustrated are simplified communities of predators, secondary and primary consumers, and primary producers. Trophic interactions pictured include those identified in this thesis or from the literature, and unknown interactions representing key knowledge gaps. Evidence for trophic effects were found linking fur seals to secondary consumer communities, however prey are diverse and trophic interactions beyond the seal's prey are not clear. Lower average abundances of urchins were observed at haul-out sites, but also lower cover of foliose macroalgae. Australian temperate ecosystems are complex, containing diverse guilds of mesopredators, invertebrate consumers, omnivores, planktivores and herbivores.

6.2 Significance for Australian temperate ecosystems and wider implications

This thesis highlights the need to further investigate frontier predator populations rather than considering them peripheral, at the detriment of understanding local food web dynamics and conserving ecological processes. The diets, foraging ecology and ecological interactions of frontier predator populations can vary greatly from mature and established populations, and therefore warrant further research effort.

6.2.1 On the diets and foraging ecology of frontier predator populations

The findings from genetic analyses of the diets of Australian and long-nosed fur seals (Hardy *et al.* 2017; **Chapter 3**) corroborate our knowledge that these species are generalist consumers of fish and cephalopods ranging from shallow coastal to epipelagic ecosystems (Harcourt *et al.* 2002; Kirkwood *et al.* 2008; Page *et al.* 2005). The results were broadly consistent with dietary analyses in these pinniped species at other locations in Australia and using other methods. However, differences in the prevalence of shallow coastal, and particularly reef-based prey, were observed in the diets of fur seals at haul-out sites compared to breeding colonies, and these differences were consistently observed in both species and across two seasons that represented nearly 5 months of sampling in a year (Hardy *et al.* 2017). From the results of this thesis and from the centre of their geographic range, we would expect fur seals from breeding colonies to largely rely on productive benthic and epipelagic prey resources from the continental shelf, slope and offshore waters in eastern Australia. Fur seals from haul-out sites appeared to include more coastal and reef-associated prey than fur seals from the breeding colony in NSW (Hardy *et al.* 2017). The latter finding has been partially supported by preliminary data from a telemetry study on fur seals from the same locations studied in this thesis (M. Salton, Macquarie University, pers. comm.).

The differences observed in the diets of fur seals across a recolonisation frontier correlate with the demographics and densities of seals at breeding colonies compared to haul-out sites. Haul-out sites contain a relatively high proportion of juvenile and subadult seals (Burleigh *et al.* 2008) compared to breeding colonies

dominated by adult breeding animals. Juveniles are known to make shorter, shallower and near-shore dives in other pinniped species (Fowler *et al.* 2006; Lowther *et al.* 2013; Page *et al.* 2006). Additionally, seals are known to forage for longer and further in established breeding colonies that are at or above carrying capacity, and that have locally depleted prey resources (Kuhn *et al.* 2014). The effects of demographics and densities on foraging effort are also known in other animals, such as in seabirds (Ashmole 1963; Birt *et al.* 1987; Orians & Pearson 1979). Eastern Australian temperate ecosystems are experiencing the rapid recovery of two sympatric fur seal species whose diets were found to overlap in particular at the frontier for recolonisation. This raises multiple questions, including: for how long could this overlap occur? Did this happen at Montague Island prior to population growth and potential local resource depletion? It would be difficult to know the answer to the latter question, however, will fur seal diets overlap and will both species frequently consume reef-based prey at new haul-out sites as these species continue to recover northward on the coast of eastern Australia? And, do similar patterns occur at other frontiers of recolonisation in these predators in southwestern Australia?

The answers to the questions posed above can provide insights for Australian temperate ecosystems, and also for ecosystems experiencing recovering predator populations globally. The first step to answering these questions is increasing our sampling effort both in space and time to investigate generalities in these patterns at the local scale of eastern Australia, the scale of Australian pinnipeds, and of course on a global scale in other recovering pinniped populations. The study presented in **Chapter 3** provided valuable insights from only two seasons' worth of sampling in two sympatric predators at the main locations at which they occur in eastern Australia. Since the commencement of this PhD project, both fur seal species have been observed to establish new permanent haul-out sites a further 150 km north of Jervis Bay, my northernmost study site (G. Ross, Office of Environment and Heritage, NSW Government, pers. comm.). I would recommend conducting further dietary analysis including at least two more locations on the eastern coast of Australia, including one breeding colony Gabo Island, Victoria, and the Five Islands Nature Reserve, NSW, as a second haul-out site. Including these sites would allow for replication of the type of study site (breeding colony vs. haul-out site) within the temperate east coast bioregion of Australia. Additionally, I would then recommend

conducting a larger geographic study of the overall dietary patterns of both seal species at haul-out sites compared to breeding colonies across their geographic range in Australia (Figure 6.2) and elsewhere to investigate broader dietary patterns that may be occurring in established compared to peripheral or frontier populations of central place foragers.

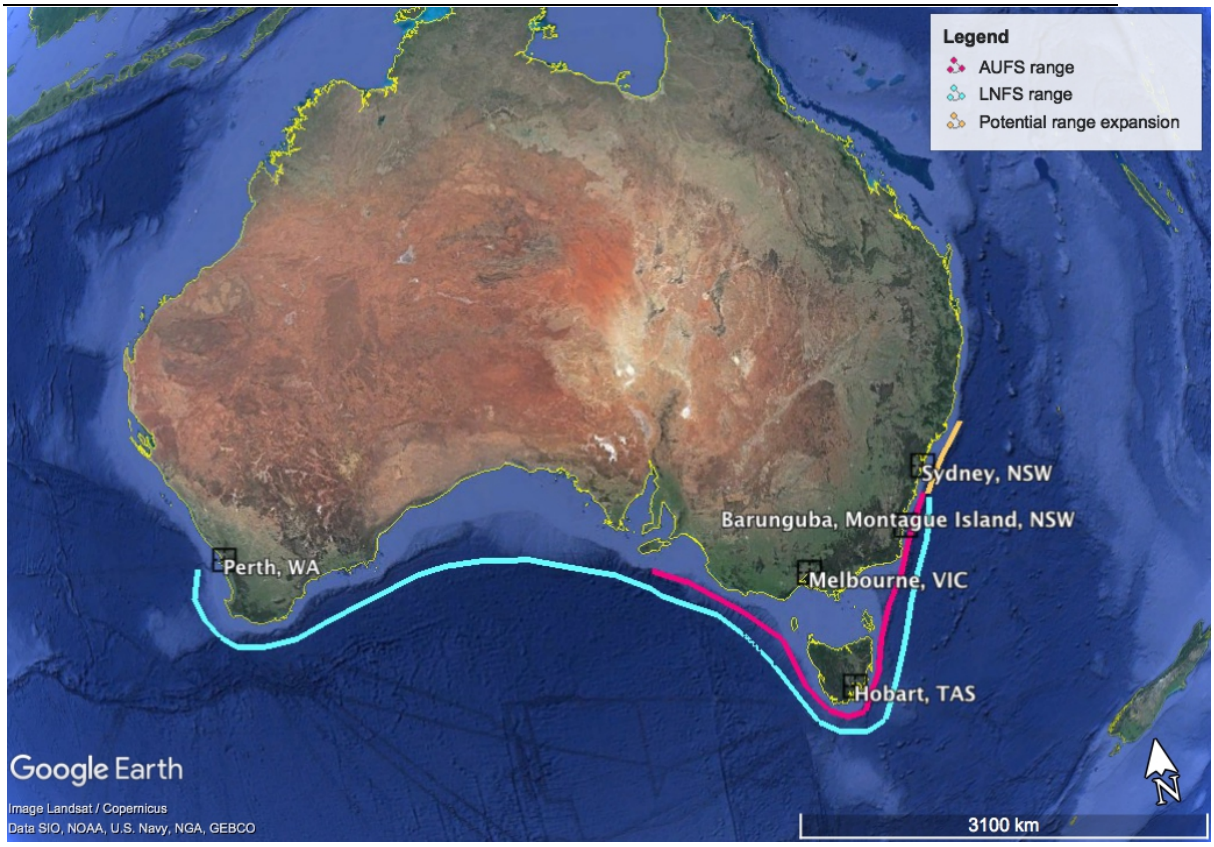


Figure 6.2 Geographic range of Australian fur seals (AUFS, in pink), of long-nosed fur seals (LNFS in blue), highlighting areas of overlap in range. Bass Strait, however is dominated by large Australian fur seal colonies (McIntosh et al. 2014), whilst long-nosed fur seals are more common in South Australia (Shaughnessy et al. 2014). Their potential northward range expansion in eastern Australian (in orange) is based on historic anecdotes of their former geographic prior to sealing activities in the 19th and 20th centuries.

The next step in answering these questions is what methods to use? All methods of analysis of predator diets and foraging ecology provide valuable information and are complementary. Genetic methods of diet analysis enable scientists to investigate the breadth of a predator's diet, and repeated over space and time, these techniques can provide detailed information of diets with enough taxonomic information to infer the likely provenance of most species (Hardy *et al.*

2017). Genetic methods and morphological analyses of diets can provide reliable semi-quantitative information on the relative importance of diet items (Thomas *et al.* 2016). However, other molecular methods such as the analyses of stable isotope and fatty acid signatures provide information on trophic interactions at longer time-scales and can give a better idea of the importance of these trophic interactions over time. Additionally, animal-borne telemetry provides researchers with detailed 3D spatial information on foraging ecology, critical to understanding where predators were likely to have foraged (Knox *et al.* 2017; Kuhn *et al.* 2010; Page *et al.* 2006). This information can inform us of important ecological processes that support large predator populations (Carroll *et al.* 2016). All of these methods provide important information and also have serious limitations (Bowen & Iverson 2013). Strategies that combined methods can provide the most accurate information on a predator's true diet (Chiaradia *et al*. 2014; **Chapter 2**). I would therefore recommend designing studies that integrate multiple methods for the analyses of these and other predators to more deeply understand their diets and broader patterns in their foraging ecology.

6.2.2 On the top-down effects of pinnipeds

The recovery of large, warmed-bodied predators in temperate marine ecosystems, and in particular where these predators are known to frequently consume shallow coastal and reef-based prey, is eventually expected to cause some level of resource depletion (Borer *et al.* 2005). Some evidence of predator effects on reef fish communities were detected (**Chapter 4**), however the evidence did not point to overwhelmingly strong trophic interaction between fur seals and particular functional groups of fish on eastern Australia temperate reefs. Nor did these subtle effects appear to strongly affect major functional groups of mobile and sessile benthic invertebrates and macroalgae on these temperate reefs.

The absence of strong visible direct or indirect effects of fur seals at the spatial and temporal scale that was sampled, does not necessarily mean there is no effect. The question lies in the temporal and spatial scale that was and needs to be sampled. These are wide-ranging animals and it is possible that their effects will occur at a range of spatial scales. But they are also central-place foragers with discrete aggregation sites for resting and/or breeding. There was an absence of any knowledge

on cascading trophic effects of pinnipeds recovering on Australian temperate reefs and there is a relatively small population of fur seals in NSW, Australia. However, central-place foragers first deplete prey resources close to their location of aggregation (Ashmole's Halo); and there is evidence in other systems for wide-ranging predators to have localised effects on prey communities (Estes 2009; Estes *et al.* 1998). Large, warm-bodied and wide-ranging mammalian predators are known for their ability to efficiently forage on and deplete localized prey resources (Bowen 1997; Estes 2009; Estes *et al.* 1998). Newly established fur seal haul-out sites are unique in that fur seal densities are still low and localised effects are more likely, before predator densities increase above a critical threshold and predation pressure expands potentially from a reef scale to a coastal scale. The first step was therefore to start searching for localized, 'halo' effects (Ashmole 1963) on nearshore coastal reefs. This was also a scale that was achievable by divers and has only really been done involving one other marine mammal (Estes & Palmisano 1974), and which produced detailed information on important trophic processes.

The strongest evidence for visible direct and indirect effects of recovering fur seals on Australian temperate reefs stemming from this study came from the fur seal aggregation site with the longest duration of use (ca. 15 years) (Burleigh *et al.* 2008), whilst the weaker evidence came from the aggregation site with a shorter duration of use (~5–8 years, at the time of study). Decadal-scale observations of marine reserves have indicated that direct effects on target species were detectable within 5–7 years of protection (Babcock *et al.* 2010). If establishment of marine reserves and reduction in human predation pressure on temperate reefs is relatable to a local increase in large, warm-bodied predators also on temperate reefs, then the durations of use of the seal haul-out sites in this study of ca. 8–15 years are within the expected, aforementioned, time frame for observing direct effects on seal prey communities (Babcock *et al.* 2010; Barrett *et al.* 2009; Barrett *et al.* 2007a). However, the complete protection of sites and thus immediate spatial closure of recreational and commercial fishing activities in sanctuary zones is likely to produce far more and visible recovery in reef communities from the effects of those activities, than would the slow and piece-meal recovery of generalist predators to coastal ecosystems. Additionally, these predators are wide-ranging and predation pressure is likely to be more spatially diffuse than a marine reserve. Thus, I offer that any measurable predator-mediated effects of fur

seals on temperate reefs would occur far more slowly than those observed from marine reserve protection. Therefore, the observations in this thesis of potential and weak trophic effects linked to fur seals on temperate east Australian reefs could be significant within the time-frame of their recovery (~8–15 years) and continued monitoring is recommended.

A study of natural recolonisation events by northern fur seals in the North Pacific identified changes in fur seal foraging behaviour and local resource depletion within 31 years of recolonisation at a breeding colony and that they estimate was reaching carrying capacity in that time (Kuhn *et al.* 2014), and animal tracking was carried out over a period of 15 years to determine this. Two prominent studies also compared predator foraging behaviour inferred from telemetry data with the known (Kuhn *et al.* 2015) or likely distribution of prey (Carroll *et al.* 2016). A greater sampling effort in time and space, and combined with temporally-relevant information on predator diets and prey resource distributions (Kuhn *et al.* 2015) is strongly recommended for Australian pinnipeds, to determine patterns in their foraging ecology, their evolving relationships with various ecosystems, in particular coastal ecosystems.

Eastern Australian temperate and tropical reefs are also complex ecosystems (Carey *et al.* 2017; **Chapters 3** and **4**). Dietary analyses of Australian and long-nosed fur seals revealed over 70 prey species each across multiple seasons and ecosystems. Reef-based surveys in my eastern Australian temperate study sites included over 80 species including a diverse number of mesopredators, benthic invertivores, omnivores and planktivores. In a global meta-analysis of consumer effects on benthic communities, Edwards *et al.* (2010) found that prey species richness was the most significant predictor for, and was negatively correlated with, the strength of consumer effects on benthic communities in models of studies including up to 40 prey species. The level of ecological complexity found in the study system investigated in this thesis is therefore comparatively large on a global scale. Prey diversity, and related trophic complexity, including omnivory, intraguild predation and competition, are known to significantly dampen cascading trophic effects (Bellwood *et al.* 2006; Polis *et al.* 2000). The ecological complexity in Australian temperate reefs could therefore dampen any potential consumer effects of recovering, large and warm-bodied

predators, or these effects may take longer still to manifest. Only further research investigating these questions can provide the answers.

6.2.3 On what to do next?

As the population of fur seals in southeastern Australia continues to expand, it is increasingly important to understand the resources that this large biomass of coastal predators rely on and determine potential cascading effects of predation particularly where these resources may be limiting. The second half of this thesis ultimately focused on coastal reef ecosystems due to their importance for coastal communities (Figure 6.3). However, **Chapter 3** also identified a diversity of trophic interactions involving benthic to pelagic habitats from the coast and into deeper waters. The most pressing research questions surrounding the recovery of sympatric fur seal species, and large predators on coastal eastern Australia, involve looking more broadly again to understand the resources that fur seals exploit across the breadth of ecosystems that support them (Figure 6.3). Specifically, I recommend that the next step in this research is to undertake ecological trophodynamic modelling, accounting for the energy budgets of recovering Australian and long-nosed fur seal populations in eastern Australia and identifying the relative importance of different ecosystems to growing fur seal popultions. Biomass-informed modelling could also be compared to other areas with seal populations, such as for the Great Australian Bight (Goldsworthy *et al.* 2013).

In eastern Australia, fur seals are recovering on a coastline that also supports Australia's densest human populations and where predators may increasingly be competing for marine resources with humans. This competition, perceived or real, certainly generates heated debate among key stakeholders and has significant ethical and conservation repercussions, not least the calls for culling of a marine mammal (Shaughnessy *et al.* 2015). The preliminary findings in this thesis indicate that fur seals are not significantly changing ecological communities near their aggregation sites. However, I recommend further research including ecological biomass modelling and more extensive ecosystem surveys temporally and spatially, sampling across the breadth of their geographic range, to positively identify potential areas of competition and conflict with humans.

There is merit in trying to understand how these recovering predators may interact with temperate reefs in the context of also designating and managing marine protected areas for the benefit of fish species targeted by fishing activities (Boncoeur *et al.* 2002; Kelaher *et al.* 2015). Economic and ecological modelling in another system involving seals and fish stocks recommended increasing the size of no-take marine reserves in order to account for seal predation on fish stocks (Boncoeur *et al.* 2002). Continued monitoring of fur seal diets using multiple methods of analyses, and including modelling and quantification of ecological interactions, would help to map the exchange of energy between predators and coastal ecosystems and thus determine the significance of coastal ecosystems to fur seals. I would additionally recommend using animal tracking methods to identify specific foraging patterns of fur seals in eastern Australian, and for example this could potentially identify foraging "hotspots". If coastal reef ecosystems are confirmed to be important for certain populations of fur seals, then measures to account for this could include designing larger marine parks in areas of high fur seal foraging activity.

There is also merit in seeking to understand the productive offshore and continental shelf resources that support these large predators and investigate areas of conflict for resources with fishing activities. Additionally, southeastern Australian ecosystems are part of a global warming hotspot, where range expansion in reef species is already occurring (Figueira & Booth 2010; Ling *et al.* 2009) and where ecosystem changes have been observed (Vergés *et al.* 2014). It is unknown how the return of large predators to eastern Australian ecosystems and environmental changes may interact. There are obvious implications of further research to the conservation and management of Australian species and of ecological processes on Australian temperate reefs, such as understanding ecological stable states or potential phase-shifts following large-scale changes to predator abundances. This information can help to identify when significant ecological changes occur and may require interventions, such as additional spatial protections for reef communities if they show signs of predator-mediated effects.

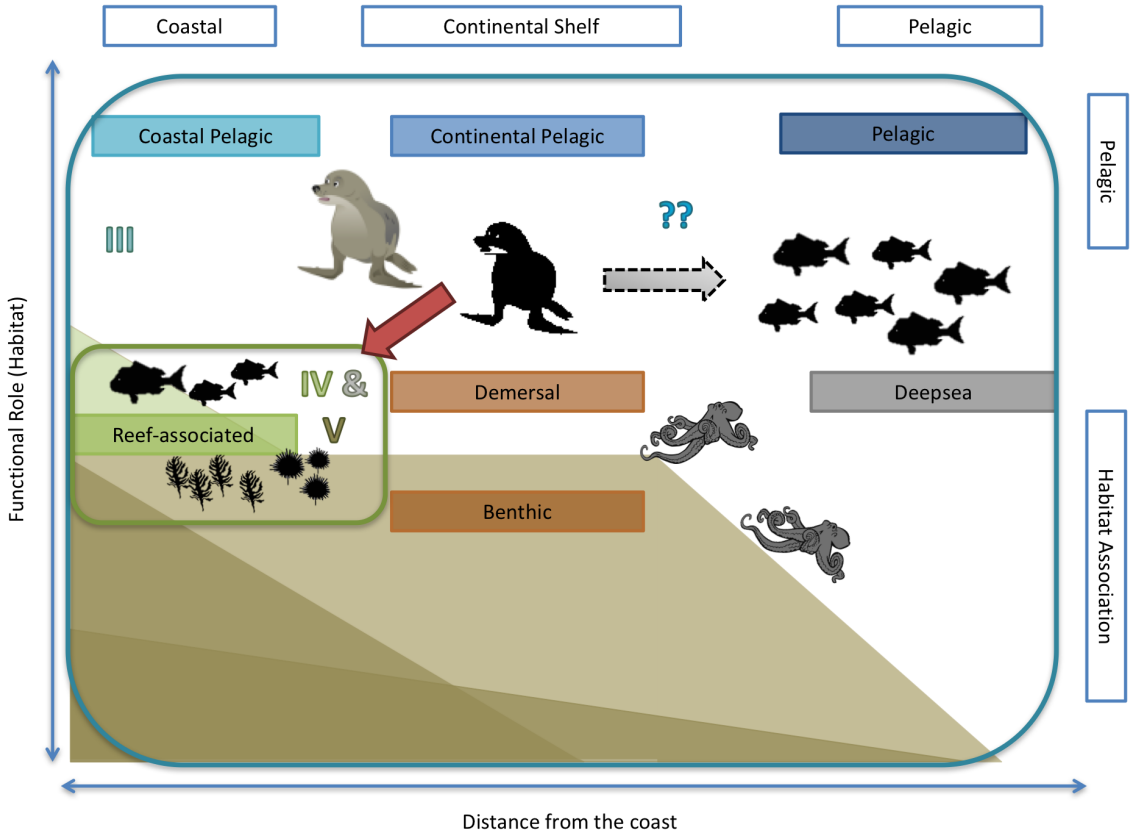


Figure 6.3 Diagram illustrating the ecological focus of this thesis spanning the breadth of fur seal diets in eastern Australia (Chapter 3) to focusing on trophic interactions between recovering fur seals and reef communities (Chapters 4 & 5). Ecosystems (boxes) in which fur seals forage are represented, where simplified trophic interactions are expected (arrows). This diagram highlights how much we don't know about ecological interactions linking these generalist predators to a broader range of ecosystems in which they are known to forage.

The southeastern coast of Australia is a unique location in which to test ecological hypotheses regarding predator recovery (Figure 6.2). Here, populations of two large, warm-bodied and generalist predators are recovering and exploiting complex coastal and pelagic ecosystems. The eastern Australian range of fur seals spans 32˚ to 37˚S in latitude across a coastline that is broadly characterised by a narrow continental shelf, extensive temperate rocky reefs, and heavily influenced by one prominent oceanographic feature in particular, the East Australian Current (Suthers *et al.* 2011). Elsewhere in their geographic range, large differences in oceanographic features and characteristics occur from Bass Strait to the Great Australian Bight in Southern Australia. These differences offer diverse opportunities to investigate predator ecology across natural latitudinal, oceanographic and

biogeographical gradients (Figure 6.2). For future studies investigating potential consumer effects of fur seals, I recommend sampling across a larger array of fur seal breeding and haul-site in order to test for trophic effects across varying densities of fur seals, and spatial gradients using a multiple regression statistical design rather than categorical as was done in this study.

For those embarking on an investigation of ecological interactions between predators and ecosystems, this thesis offers a useful exploratory strategy with which to start: using diet analysis for accurate food web characterization, combined with community trait-based modelling to identify potential consumer effects of predation. This strategy is particularly useful where prior knowledge on the diets of generalist predators and on important ecological interactions is lacking. Where these interactions have been identified, as has been done for eastern Australian temperate reefs presented in this thesis, the next step is to undertake more targeted investigations of key ecological interactions to understand their relative importance and dynamics. Scientists can use the information presented in exploratory food web investigations to inform further targeted analysis of specific species of conservation or management interest and to quantify these interactions in space and time. The third step is then to use detailed and quantitative information to build ecosystem-scale trophodynamic models to understand interactions and exchanges of energy within and across ecosystems (Goldsworthy *et al.* 2013). Australian and long-nosed fur seals interact with a broad range of temperate ecosystems from the coast to offshore ecosystems, and a trophic mass-balance model for these interactions would help to understand and attribute the potential impacts of recovering predators, of fishing activities and of ecological change in these ecosystems.

6.3 Conclusions

This thesis first provides a synthesis of genetic tools applied to the analysis of predator diets and trophic ecology, and it is my hope that this synthesis will prove a useful guide for ecologists aiming to apply genetic techniques in a broad range of ecosystems and predator taxa. My third chapter, now published, offered the first analyses of the diets of recovering and sympatric fur seal species in eastern Australia, and yielded information on novel trophic interactions. Through this work, I identified

important knowledge gaps and further avenues of investigation in frontier predator populations, and that led to further investigations of trophic effects from fur seals on coastal reef ecosystems. This thesis provides valuable information from an early time in the recovery trajectory of two predator species and I expect it will be very interesting to revisit this study throughout the recovery of these species and their continued range expansion. Simply getting in and having a look is not a common tactic in pinniped research generally, for many good reasons of course, primarily because we lack their diving capabilities. For a coastal predator, however, timely surveys of the reefs that they could impact is especially important to provide a baseline assessment of reef communities whilst predator densities are still low. Without the approach applied in this thesis, we would have no local information on temperate reefs adjacent to fur seal aggregation sites with which to compare potential trajectories of change, and we would not later know whether any localised prey depletion occurred at all or whether a 'halo' effect broadened spatially, if one is later detected. Although small in scale, this is the most significant offering to scientific knowledge that I make in the second half of my thesis. This thesis provides an investigative framework with which to undertake ecological monitoring in this study system and others, and also provides suggestions for multiple stages of ecological monitoring relevant to researchers and managers across a broad range of ecosystems.

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Appendix A

Supplementary material from **Chapter 2**. Research papers used in the systematic review of genetic analyses of predator diets and trophic interactions.

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Appendix B

Supplementary material from **Chapter 3**.

Table B.1 Taxonomic assignment and frequency of occurrence for samples of Australian (n = 60) and long-nosed (n = 53) fur seals for incidental prey taxa occurring in <10% of samples (Primer sets: Fish16S and S_Cephalopoda, Crust16S and Bird12S). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Actinopterygii									
Congridae	*Gnathophis* sp. (conger eel)	PR, Benthic Predator	0.00	0.00	6.67	0.00	6.67	0.00	0.00
Aulopidae	*Latropiscis purpurissatus* (sergeant baker)	PR, Benthic Predator	0.00	7.69	0.00	0.00	6.67	0.00	0.00
Belonidae	Unknown Belonidae (needlefishes)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	9.52	0.00
Hemiramphidae	*Hyporhamphus regularis* (river garfish)	OM, Coastal Pelagic Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Clupeidae	*Etrumeus teres* (maray)	IN, Coastal Pelagic Invertivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Sardinella gibbosa (gold-belly sardinella)	OM, Coastal Pelagic Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Engraulidae	*Engraulis australis* (australian anchovy)	IN, Coastal Pelagic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Moridae	*Lotella rhacina* (rock cod, beardie)	PI, Reef Piscivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Pseudophycis barbata (bearded rock cod)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00

Table B.1 Taxonomic assignment table continued (Actinopterygii). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown. † Prey item found in the single LNFS sample from JB in Jan-Apr (*Myxus elongatus*).

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Mugilidae	*Aldrichetta forsteri* (yelloweye mullet)	OM, Demersal Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Liza argentea (goldspot mullet)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	9.52	0.00
	Mugil cephalus (sea mullet)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	9.52	0.00
†	*Myxus elongatus* (sand mullet)	OM, Demersal Omnivore	5.88	0.00	0.00	0.00	0.00	4.76	0.00
	Unknown Mugilidae (mullets)	OM, Demersal Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Myctophidae	*Lampadena* sp. (lanternfishes)	IN, Pelagic Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Myctophum sp. (spotted lanternfish)	IN, Pelagic Invertivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
	Hygophum hanseni (hansen's lanternfish)	PL, Pelagic Planktivore	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Ophidiidae	*Genypterus* sp. (cusk-eels)	PR, Demersal Predator	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Bramidae	*Brama brama* (atlantic pomfret)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Carangidae	*Seriola lalandi* (yellowtail kingfish)	PR, Coastal Pelagic Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Centrolophidae	*Seriolella brama* (blue warehou)	PR, Demersal Predator	0.00	0.00	6.67	0.00	0.00	0.00	0.00

Table B.1 Taxonomic assignment table continued (Actinopterygii). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Cheilodactylidae	*Nemadactylus douglasii* (grey morwong)	PR, Reef Predator	5.88	7.69	0.00	6.67	6.67	0.00	0.00
	Unknown Cheilodactylidae (morwongs)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Nemadactylus macropterus (jackass morwong)	PR, Reef Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Chironemidae	*Chironemus marmoratus* (eastern kelpfish)	PR, Reef Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Dinolestidae	*Dinolestes lewini* (longfin pike)	PR, Reef Predator	5.88	0.00	0.00	0.00	6.67	4.76	0.00
Emmelichthyidae	*Emmelichthys nitidus* (redbait)	PR, Continental Pelagic Predator	0.00	7.69	0.00	6.67	0.00	0.00	0.00
Gempylidae	*Nealotus tripes* (black snake mackerel)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Girellidae	*Girella* sp. (greenfish)	OM, Demersal Omnivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Girella tricuspidata (luderick)	HE, Demersal Herbivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Kyphosidae	*Kyphosus sydneyanus* (silver drummer)	HE, Reef Herbivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Kyphosus vaigiensis (brassy drummer)	OM, Reef Omnivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Unknown Kyphosidae (drummers)	OM, Reef Omnivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00

Table B.1 Taxonomic assignment table continued (Actinopterygii). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Labridae	*Achoerodus viridis* (eastern blue groper)	IN, Reef Invertivore	5.88	0.00	0.00	6.67	0.00	0.00	0.00
	Austrolabrus maculatus (blackspotted wrasse)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Bodianus sp. (pigfish)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Mullidae	*Upeneichthys* sp. (goatfish)	IN, Benthic Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Odacidae	*Odax cyanomelas* (herring cale)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pempheridae	*Parapriacanthus* sp. (sweepers)	IN, Reef Invertivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pentacerotidae	Unknown Pentacerotidae (armourheads)	PR, Demersal Predator	0.00	0.00	0.00	0.00	6.67	0.00	0.00
Pinguipedidae	*Parapercis allporti* (barred grubfish)	IN, Benthic Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Polyprionidae	*Polyprion oxygeneios* (hapuku)	PR, Pelagic Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00
Sphyraenidae	*Sphyraena* sp. (pikes)	PR, Reef Predator	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Tetragonuridae	*Tetragonurus atlanticus* (bigeye squaretail)	IN, Pelagic Invertivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Trichiuridae	*Lepidopus caudatus* (silver scabbardfish)	PR, Continental Pelagic Predator	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Scorpaenidae	*Scorpaena* sp. (scorpionfishes)	PR, Reef Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Scorpaenodes scaber (pygmy scorpionfish)	PR, Reef Predator	0.00	7.69	0.00	0.00	0.00	0.00	0.00

Table B.1 Taxonomic assignment table continued (Actinopterygii). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Sebastidae	*Helicolenus* sp. (ocean perch)	PR, Demersal Predator	5.88	0.00	6.67	6.67	0.00	0.00	0.00
Triglidae	*Chelidonichthys* sp. (gurnard)	PR, Benthic Predator	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Lepidotrigla argus (eye gurnard)	IN, Benthic Invertivore	5.88	0.00	6.67	0.00	0.00	0.00	0.00
	Lepidotrigla papilio (spiny gurnard)	IN, Benthic Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	5.88
Diodontidae	*Allomycterus pilatus* (australian burrfish)	IN, Reef Invertivore	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Monacanthidae	*Cantherhines dumerilii* (barred leatherjacket)	OM, Reef Omnivore	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Meuschenia australis (brownstriped leatherjacket)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	6.67	0.00	0.00
	Meuschenia scaber (velvet leatherjacket)	IN, Reef Invertivore	0.00	7.69	0.00	0.00	0.00	0.00	0.00
	Eubalichthys mosaicus (mosaic leatherjacket)	HE, Reef Herbivore	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Ostraciidae	*Anoplocapros inermis* (eastern smooth boxfish)	IN, Reef Invertivore	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Tetraodontidae	*Arothron firmamentum* (starry toad)	IN, Demersal Invertivore	0.00	0.00	6.67	0.00	0.00	4.76	0.00
Osmeriformes	Unknown Osmeriformes (argentines)	UN, Unknown	0.00	0.00	0.00	6.67	0.00	0.00	0.00

Table B.1 Taxonomic assignment table continued (Cephalopoda and Aves). * PR = predator; PI = piscivore; PL = planktivore; IN = invertivore; OM = omnivore; UN = unknown.

			Australian fur seal				Long-nosed fur seal		
			JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Trophic* & Functional Trait	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Cephalopoda									
Argonautidae	*Argonauta nodosa* (knobby argonaut)	IN, Pelagic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Octopodidae	*Octopus tetricus* (Common Sydney Octopus)	IN, Benthic Invertivore	0.00	7.69	6.67	0.00	0.00	4.76	0.00
	Unknown Octopodidae (octopus)	IN, Benthic Invertivore	0.00	0.00	0.00	6.67	0.00	0.00	0.00
	Octopus sp. #2 (*O. berrima* or *O. pallidus*)	IN, Benthic Invertivore	0.00	7.69	0.00	6.67	0.00	4.76	0.00
Ocythoidae	*Ocythoe tuberculata* (tuberculate pelagic octopus)	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	9.52	0.00
Cranchiidae	*Leachia* sp.	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Taonius sp. (glass squid)	UN, Pelagic Unknown	0.00	0.00	0.00	6.67	6.67	0.00	0.00
Enoploteuthidae	*Abralia* sp. (midwater squid)	UN, Pelagic Unknown	5.88	0.00	0.00	0.00	0.00	0.00	0.00
	Abraliopsis sp.	UN, Pelagic Unknown	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Ommastrephidae	*Eucleuteuthis* sp. (luminous flying squid)	UN, Pelagic Unknown	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Ornithoteuthis volatilis (flying squid)	UN, Pelagic Unknown	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Pyroteuthidae	*Pterygioteuthis microlampas*	UN, Pelagic Unknown	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Sepiidae	Unknown Sepiidae (cuttlefish)	PR, Reef Predator	5.88	7.69	6.67	0.00	6.67	0.00	5.88
Aves									
Spheniscidae	*Eudyptula minor* (little penguin)	PR, Pelagic Predator	0.00	0.00	0.00	0.00	6.67	0.00	0.00

Table B.2 Taxonomic assignment table for species present in Australian and long-nosed fur seal scats likely due to secondary predation (Malacostraca, primer set: Crust16S).

		Australian fur seal				Long-nosed fur seal		
		JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Malacostraca								
Acanthephyridae	*Notostomus* sp. (shrimp)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Callianassidae	*Biffarius* sp. (ghost shrimp)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Diogenidae	Unknown Diogenidae (hermit crabs)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Euphausiidae	*Euphausia recurva* (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Nyctiphanes australis (euphausiid)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Unknown Euphausiidae (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
Pandalidae	*Chlorotocus crassicornis* (green shrimp)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
Penaeidae	*Melicertus plebejus* (eastern king prawn)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Metapenaeus sp. (school prawn)	0.00	0.00	0.00	13.33	0.00	0.00	0.00
Polybiidae	*Ovalipes* sp. (sand crab)	0.00	0.00	6.67	0.00	6.67	0.00	23.53
	Unknown Polybiidae (swimmer crabs)	0.00	0.00	20.00	0.00	0.00	0.00	0.00
Portunidae	*Portunus sanguinolentus* (blue-spot swimming crab)	0.00	0.00	6.67	0.00	0.00	19.05	0.00
	Thalamita admete (swimming crab)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Thalamita sima (four-lobed swimming crab)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
Raninidae	*Ranina ranina* (spanner crab)	11.76	0.00	0.00	0.00	0.00	0.00	0.00
Scyllaridae	*Galearctus rapanus* (slipper lobster)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Sergestidae	Unknown Sergestidae (sergestid shrimps)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Squillidae	*Busquilla plantei* (stomatopod crustacea)	0.00	0.00	0.00	0.00	0.00	4.76	0.00
	Oratosquillina sp. (mantis shrimp)	0.00	0.00	0.00	0.00	0.00	9.52	0.00

Table B.2 Continued taxonomic assignment table for like secondary predation species (Malacostraca, primer set: Crust16S).

		Australian fur seal				Long-nosed fur seal		
		JB		MI		JB	MI	
Class/Family	Genus species (Common Name)	Jan-Apr	Sept	Jan-Apr	Sept	Sept	Jan-Apr	Sept
Squillidae	Unknown Squillidae (mantis shrimps)	5.88	0.00	0.00	0.00	0.00	0.00	0.00
Xanthidae	Unknown Xanthidae (rubble crabs)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Unknown Decapod	11.76	0.00	13.33	0.00	13.33	4.76	17.65
	Unknown Dendrobranchiata (shrimps)	0.00	0.00	6.67	0.00	0.00	0.00	0.00
	Unknown Euphausiacea (krill)	0.00	0.00	0.00	0.00	0.00	0.00	5.88
	Unknown Stomatopoda (mantis shrimps)	0.00	0.00	0.00	0.00	0.00	4.76	0.00

Table B.3 Analysis of deviance table for multivariate generalised linear models (mvGLM) for trophic and spatial group analyses of prey composition between fur seal species, at different locations and time points sampled, tested on four models. Where significant interactions occurred in the full model, reduced models tested the differences between levels of explanatory variables. Significance denoted by: *$p <$ 0.05, **$p < 0.01$, ***$p < 0.001$.

	Response variables		**SPATIAL**		
MODELS	*Factors*	*R.df*	*Df.diff*	*Dev*	*P-value*
(i) AUFS	*Intercept*	*59*			
	Time	58	1	12.37	0.185
	Location	57	1	11.38	0.245
	Time×Location	56	1	25.03	0.011*
AUFS in Summer	*Intercept*	31			
	Location (Summer)	30	1	33.37	0.006**
AUFS in Winter	*Intercept*	27			
	Location (Winter)	26	1	26.49	0.034*
AUFS at MI	*Intercept*	29			
	Time (MI)	28	1	36.44	0.001**
AUFS at JB	*Intercept*	29			
	Time (JB)	28	1	12.09	0.492
(ii) LNFS	*Intercept*	51			
	Group (Location+Time)	49	2	83.46	0.001**
LNFS in Winter	*Intercept*	30			
	Location (Winter)	29	1	31.55	0.009**
LNFS at MI	*Intercept*	35			
	Time (MI)	34	1	34.67	0.006**
(iii) MI	*Intercept*	65			
	Time	64	1	33.98	0.001**
	Seal sp.	63	1	36.68	0.002**
	Seal sp.×Time	62	1	24.47	0.008**
MI in Summer	*Intercept*	35			
	Seal sp. (Summer)	34	1	40.17	0.002**
MI in Winter	*Intercept*	29			
	Seal sp. (Winter)	28	1	20.98	0.031*
(iv) JB	*Intercept*	45			
	Group (Seal sp.+Time)	43	2	15.74	0.457

Table B.4 Analysis of variance table for species richness between fur seal species, locations and time points sampled, tested on four models. Significance denoted by: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

MODELS	Response variables / *Factors*	*Df*	*Sum Sq*	*Mean Sq*	*F value*	*P-value*
		SPECIES RICHNESS				
(i) AUFS	*Time*	1	14.8	14.8	0.208	0.65
	Location	1	2.8	2.82	1.095	0.3
	Time×Location	1	0.3	0.28	0.021	0.886
	Residuals	56	757	13.517		
(ii) LNFS	*Group (Location+Time)*	2	80.02	40.01	6.887	0.002**
	Residuals	49	284.66	5.81		
(iii) MI	*Time*	1	25	25.03	4.155	0.081
	Seal sp.	1	19	19.01	3.155	0.046
	Seal sp.×Time	1	37.5	37.51	6.227	0.015*
	Residuals	62	373.5	6.02		
(iv) JB	*Group (Seal sp.+Time)*	2	1.8	0.87	0.106	0.9
	Residuals	43	355.7	8.27		

Appendix C

Supplementary material from **Chapter 4**.

Table C.1 Matrix for the 85 fish species and one mobile macro-invertebrate, and their traits for gregariousness, trophic group and categorisation in terms of seal predation. Traits for gregariousness and trophic group were assigned based on information provided by Stuart-Smith et al. (2014) . Categorisation of species as seal prey was conducted using prior knowledge of the diets of Australian and long-nosed fur seals from NSW from Hardy et al. (2016) and from Bass Strait (Gales & Pemberton 1994; Kirkwood et al. 2008; Deagle et al. 2009; Lake 1999) and New Zealand (Fea et al. 2002).

Species	Family	Gregariousness	Seal Prey	Trophic Group
Prionurus microlepidotus	Acanthuridae	schools	no	browsing herbivore
Aplodactylus lophodon	Aplodactylidae	pairs/group	yes	browsing herbivore
Arripis trutta	Arripidae	schools	no	mesopredator
Aulopus purpurissatus	Aulopodidae	solitary	no	mesopredator
Plagiotremus laudandus	Blennidae	solitary	no	cleaner
Brachaelurus waddi	Brachaeluridae	solitary	no	mesopredator
Pseudocaranx georgianus	Carangidae	schools	yes	benthic invertivore
Seriola lalandi	Carangidae	schools	yes	mesopredator
Trachurus novaezelandiae	Carangidae	schools	yes	planktivore
Chelmonops truncatus	Chaetodontidae	solitary	no	benthic invertivore
Amphichaetodon howensis	Chaetodontidae	solitary	no	benthic invertivore

Table C.1 Matrix for the 85 fish species and one mobile macro-invertebrate, and their traits for gregariousness, trophic group and categorisation in terms of seal predation.

Species	Family	Gregariousness	Seal Prey	Trophic Group
Cheilodactylus fuscus	Cheilodactylidae	pairs/group	yes	benthic invertivore
Cheilodactylus spectabilis	Cheilodactylidae	solitary	yes	benthic invertivore
Nemadactylus douglasii	Cheilodactylidae	solitary	yes	benthic invertivore
Chironemus marmoratus	Chironemidae	solitary	yes	benthic invertivore
Cirrhitichthys aprinus	Cirrhitidae	solitary	no	benthic invertivore
Dasyatis thetidis	Dasyatidae	solitary	no	benthic invertivore
Dinolestes lewini	Dinolestidae	schools	yes	mesopredator
Enoplosus armatus	Enoplosidae	pairs/group	no	benthic invertivore
Heterodontus portusjacksoni	Heterodontidae	solitary	no	benthic invertivore
Atypichthys strigatus	Kyphosidae	schools	yes	planktivore
Girella elevata	Kyphosidae	schools	yes	browsing herbivore
Girella tricuspidata	Kyphosidae	schools	yes	browsing herbivore
Kyphosus bigibbus	Kyphosidae	schools	yes	browsing herbivore
Kyphosus sydneyanus	Kyphosidae	schools	yes	browsing herbivore
Microcanthus strigatus	Kyphosidae	schools	yes	planktivore
Scorpis lineolata	Kyphosidae	schools	yes	planktivore
Tilodon sexfasciatus	Kyphosidae	pairs/group	yes	benthic invertivore
Achoerodus viridis	Labridae	solitary	yes	benthic invertivore
Austrolabrus maculatus	Labridae	solitary	yes	benthic invertivore
Coris aurilineata	Labridae	solitary	yes	benthic invertivore

Table C.1 Matrix for the 85 fish species and one mobile macro-invertebrate, and their traits for gregariousness, trophic group and categorisation in terms of seal predation.

Species	Family	Gregariousness	Seal Prey	Trophic Group
Coris picta	Labridae	pairs/group	yes	benthic invertivore
Coris sandeyeri	Labridae	pairs/group	yes	benthic invertivore
Eupetrichthys angustipes	Labridae	solitary	yes	benthic invertivore
Labrid spp.	Labridae	solitary	yes	benthic invertivore
Notolabrus fucicola	Labridae	pairs/group	yes	benthic invertivore
Notolabrus gymnogenis	Labridae	pairs/group	yes	benthic invertivore
Notolabrus tetricus	Labridae	pairs/group	yes	benthic invertivore
Ophthalmolepis lineolata	Labridae	solitary	yes	benthic invertivore
Pictilabrus laticlavius	Labridae	solitary	yes	benthic invertivore
Pseudolabrus luculentus	Labridae	pairs/group	yes	benthic invertivore
Suezichthys arquatus	Labridae	solitary	yes	benthic invertivore
Latridopsis forsteri	Latridae	schools	yes	benthic invertivore
Sepioteuthis australis	Loliginidae	schools	yes	benthic invertivore
Acanthaluteres vittiger	Monacanthidae	pairs/group	yes	browsing herbivore
Eubalichthys bucephalus	Monacanthidae	pairs/group	yes	browsing herbivore
Eubalichthys mosaicus	Monacanthidae	solitary	yes	browsing herbivore
Meuschenia flavolineata	Monacanthidae	pairs/group	yes	browsing herbivore
Meuschenia freycineti	Monacanthidae	pairs/group	yes	browsing herbivore
Meuschenia trachylepis	Monacanthidae	pairs/group	yes	browsing herbivore
Scobinichthys granulatus	Monacanthidae	solitary	yes	browsing herbivore

Table C.1 Matrix for the 85 fish species and one mobile macro-invertebrate, and their traits for gregariousness, trophic group and categorisation in terms of seal predation.

Species	Family	Gregariousness	Seal Prey	Trophic Group
Schuettea scalaripinnis	Monodactylidae	schools	no	planktivore
Lotella rhacina	Moridae	solitary	yes	mesopredator
Parupeneus spilurus	Mullidae	pairs/group	yes	benthic invertivore
Upeneichthys lineatus	Mullidae	pairs/group	yes	benthic invertivore
Upeneichthys vlamingii	Mullidae	pairs/group	yes	benthic invertivore
Gymnothorax prasinus	Muraenidae	solitary	no	benthic invertivore
Olisthops cyanomelas	Odacidae	solitary	yes	browsing herbivore
Carcharias taurus	Odontaspididae	pairs/group	no	mesopredator
Orectolobus maculatus	Orectolobidae	solitary	no	mesopredator
Anoplocapros inermis	Ostraciidae	solitary	yes	benthic invertivore
Pempheris affinis	Pempheridae	schools	yes	benthic invertivore
Pempheris analis	Pempheridae	schools	yes	benthic invertivore
Pempheris compressa	Pempheridae	schools	yes	benthic invertivore
Pempheris multiradiata	Pempheridae	schools	yes	benthic invertivore
Paraplesiops bleekeri	Plesiopidae	solitary	no	benthic invertivore
Trachinops taeniatus	Plesiopidae	schools	no	benthic invertivore
Chromis hypsilepis	Pomacentridae	schools	yes	planktivore
Mecaenichthys immaculatus	Pomacentridae	pairs/group	yes	planktivore
Parma microlepis	Pomacentridae	solitary	yes	browsing herbivore
Parma unifasciata	Pomacentridae	solitary	yes	browsing herbivore

Table C.1 Matrix for the 85 fish species and one mobile macro-invertebrate, and their traits for gregariousness, trophic group and categorisation in terms of seal predation.

Species	Family	Gregariousness	Seal Prey	Trophic Group
Pomacentrid spp.	Pomacentridae	NA	yes	omnivore
Pomacentrus coelestis	Pomacentridae	pairs/group	yes	omnivore
Trygonorrhina fasciata	Rhinobatidae	solitary	no	benthic invertivore
Scorpaena cardinalis	Scorpaenidae	solitary	yes	benthic invertivore
Sepia spp.	Sepiidae	pairs/group	yes	benthic invertivore
Acanthistius ocellatus	Serranidae	solitary	yes	mesopredator
Hypoplectrodes maccullochi	Serranidae	solitary	yes	benthic invertivore
Hypoplectrodes nigroruber	Serranidae	solitary	yes	benthic invertivore
Acanthopagrus australis	Sparidae	pairs/group	yes	benthic invertivore
Pagrus auratus	Sparidae	pairs/group	yes	benthic invertivore
Rhabdosargus sarba	Sparidae	pairs/group	yes	benthic invertivore
Synodus variegatus	Synodontidae	solitary	no	mesopredator
Canthigaster callisterna	Tetraodontidae	solitary	no	omnivore
Torquigener squamicauda	Tetraodontidae	solitary	no	benthic invertivore
Trygonoptera spp.	Urolophidae	solitary	no	benthic invertivore

Table C.2 Results of linear and generalised linear models for species richness, species diversity Shanon-Wiener index and community evenness. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Metric	Model	Explanatory variables	Estimate	Std. Error	z-value	Pr (>|z|)
Species Richness (SPR)	SPR ~ Region*Site type	(Intercept)	23.917	1.222	19.567	<2e-16
		RegionSouth	2.875	1.729	1.663	0.0480*
		SealHO	5.583	2.445	2.284	0.0066**
		RegionSouth:SealHO	-7.625	3.457	-2.206	0.0100*
Shanon-Wiener Diversity Index (H)	H ~ Region*Site type	(Intercept)	1.5451	0.0623	24.7920	<2e-16***
		RegionSouth	0.1947	0.0881	2.2090	0.031*
		SealHO	0.0994	0.1246	0.7970	0.4280
		RegionSouth:SealHO	-0.2274	0.1763	-1.2900	0.2020
Evenness (E)	E ~ Region*Site type	(Intercept)	0.4917	0.0172	28.6460	<2e-16***
		RegionSouth	0.0395	0.0243	1.6280	0.1090
		SealHO	-0.0014	0.0343	-0.0400	0.9690
		RegionSouth:SealHO	-0.0264	0.0485	-0.5440	0.5890
Total Abundance Prey Fish (T_Abun)	T_Abun ~ Region*Site type	(Intercept)	5.8550	0.1140	51.3670	<2e-16***
		RegionSouth	-0.0557	0.1612	-0.3460	0.7296
		SealHO	0.0291	0.2279	0.1280	0.8985
		RegionSouth:SealHO	-0.6578	0.3229	-2.0370	0.0417 *
Total Biomass Prey Fish (T_Biom)	T_Biom ~ Region*Site type	(Intercept)	10.0730	0.1528	65.9130	<2e-16***
		RegionSouth	-0.1376	0.2161	-0.6370	0.5240
		SealHO	0.2744	0.3008	0.9120	0.3620
		RegionSouth:SealHO	-0.4880	0.4254	-1.1470	0.2510

Table C.3 Summary statistics in relation to trophic groups observed at Jervis Bay study sites.

Trophic group	Species richness	%Species richness	Total Abundance	%T abundance	Total Biomass (kg)	%sT biomass
Higher carnivore	11	13%	787	2.70%	191.7	16.00%
Benthic invertivore	48	55%	10418	35.80%	290.5	24.20%
Browsing herbivore	16	18%	1592	5.50%	164.9	13.80%
Omnivore	3	3%	37	0.10%	1.4	0.10%
Planktivore	8	9%	16240	55.90%	550.7	45.90%
Cleaner	1	1%	1	0%	0.0	0%

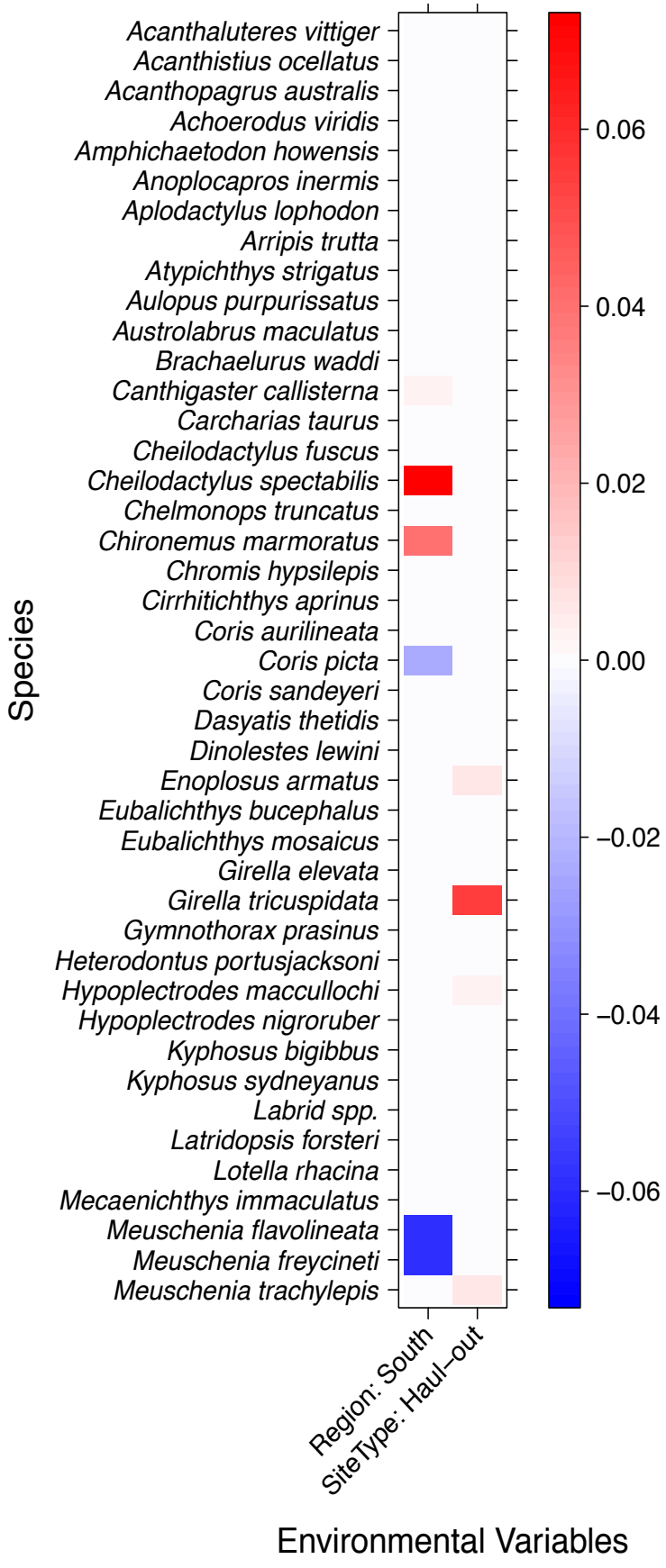

Figure C.1 Standardised coefficients for all species and environmental variable interaction from the GLM-LASSO model, fitted without traits (part 1). Colours denote significant interactions between species and the *region* and *site type*, whereby darker colours describe the strength of effect. Red indicates a positive relationship, whilst blue a negative relationship. The first half of the species assemblage is displayed here, the second half are in the following graph (Figure C.2).

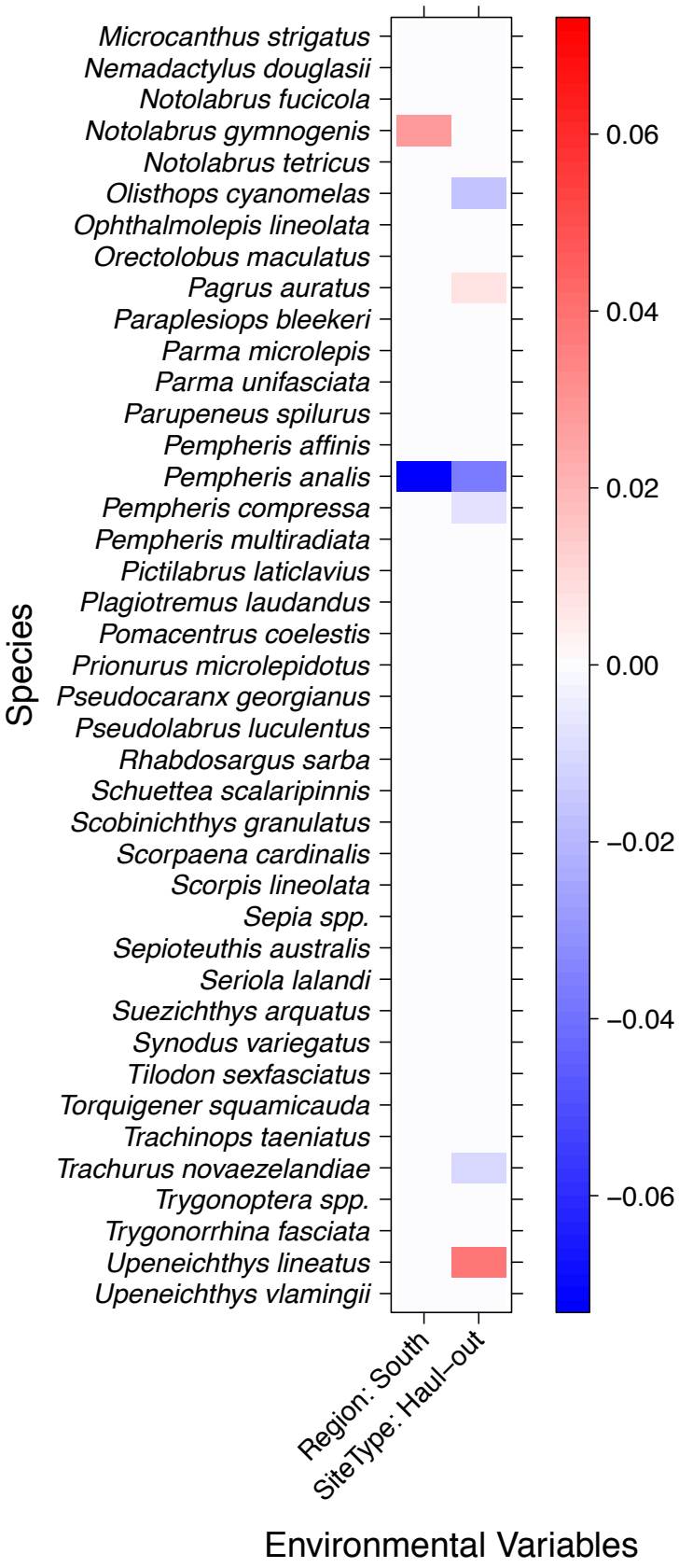

Figure C.2. Results of the fourth-corner model fitted for the relationship between species and environmental variables, without traits (part 2).

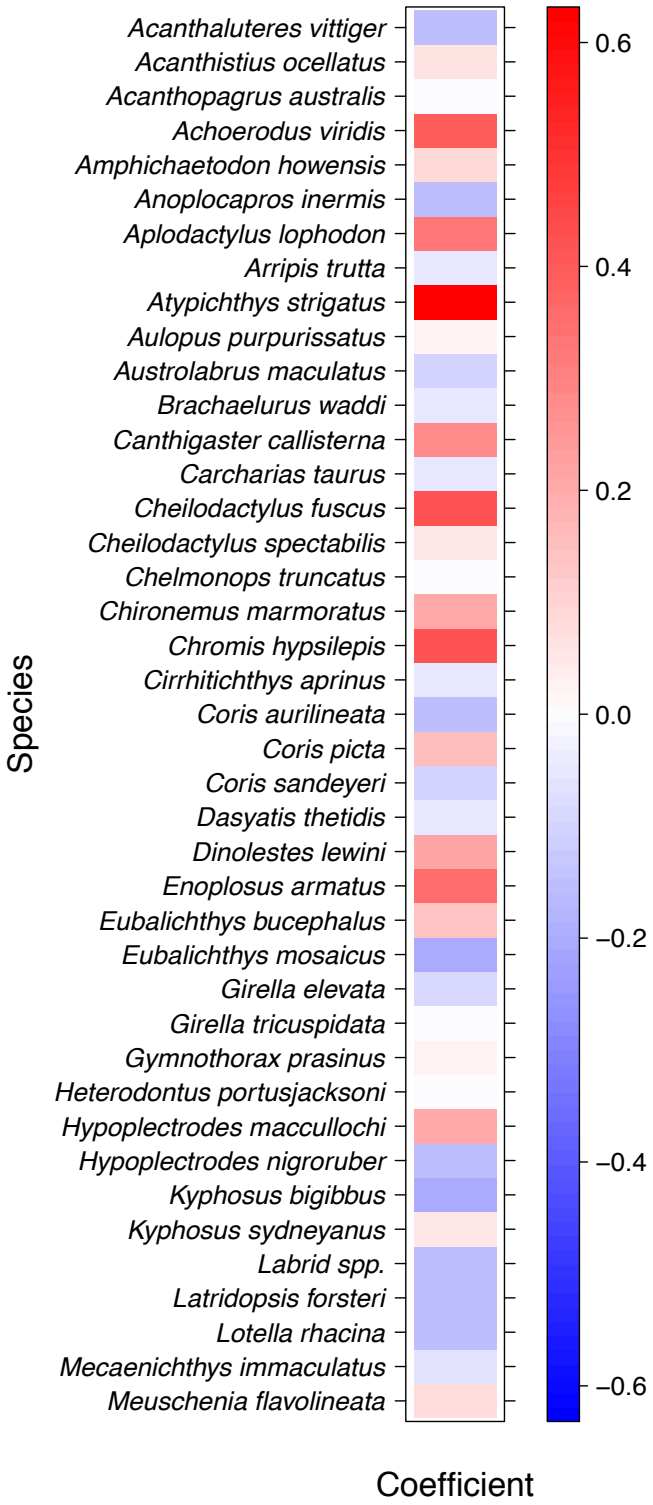

Figure C.3 Standardised coefficients for individual species prevalence in the fourth-corner model, most common or uncommon species (Part 1).

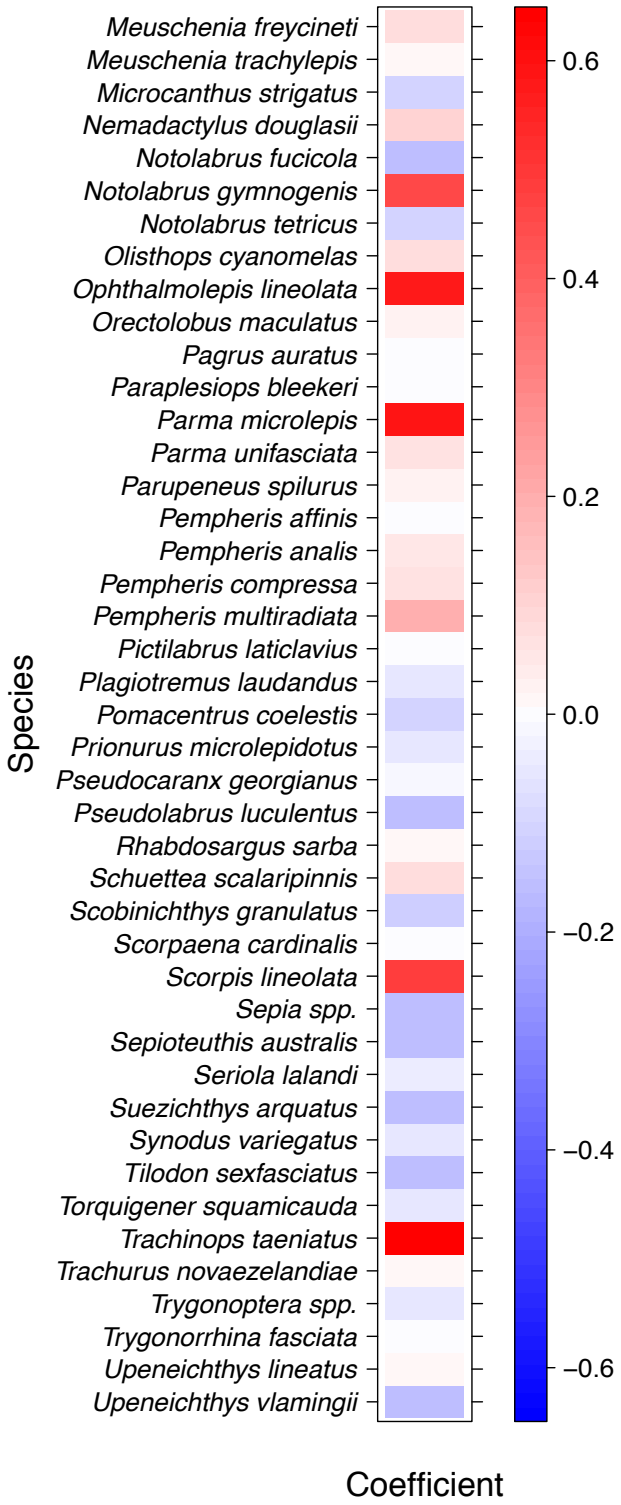

Figure C.4 Standardised coefficients for individual species prevalence in the fourth-corner model, most common or uncommon species (Part 2).

Appendix D

Supplementary material from **Chapter 5**.

Table D.1 Matrix for the 36 species benthic mobile invertebrates and cryptic fish, and their trophic group trait used for trait-based multivariate analyses. † Denotes detritivorous and filter-feeding species (n = 3), rare in this study, and thus excluded from trait-based analyses.

Species	Trophic Group
Acanthistius ocellatus	mesopredator
Asterodiscides truncates †	detritivore filter feeder
Astralium tentoriformis	herbivore
Cabestana spengleri	benthic invertivore
Cabestana tabulata	benthic invertivore
Cellana sp.	herbivore
Centrostephanus rodgersii	herbivore
Chicoreus denudatus	benthic invertivore
Chironemus marmoratus	benthic invertivore
Comanthus trichoptera †	detritivore filter feeder
Cymatium parthenopeum	benthic invertivore
Dicathais orbita	benthic invertivore
Echinaster arcystatus	benthic sessiles invertivore
Fromia polypora	benthic sessiles invertivore
Gymnothorax prasinus	mesopredator
Heliocidaris erythrogramma	herbivore
Heliocidaris tuberculata	herbivore
Heteroclinus spp.	benthic invertivore
Hypoplectrodes maccullochi	benthic invertivore
Hypoplectrodes nigroruber	mesopredator
Hypselodoris bennetti	benthic sessiles invertivore
Istigobius hoesei	benthic invertivore
Lotella rhacina	mesopredator
Octopus tetricus	benthic invertivore
Orectolobus maculatus	mesopredator
Orectolobus ornatus	mesopredator
Penion mandarinus	benthic invertivore
Pentagonaster dubeni	benthic sessiles invertivore
Phyllacanthus parvispinus	herbivore
Plagiotremus tapeinosoma	mesopredator
Plectaster decanus	benthic sessiles invertivore
Pteraeolidia ianthina	benthic sessiles invertivore
Ranella australasia	benthic sessiles invertivore
Scorpaena cardinalis	mesopredator
Turbo torquatus	herbivore
Unidentified hermit crab †	detritivore filter feeder

Table D.2 Categories of epibenthic biota identified using the standardised classification scheme (CATAMI v1.2). Broad morpho-taxa were used for statistical analyses of benthic community composition.

Broad morpho-taxa	Categories
Algal & Biotic Matrix	Algae matrix
Algal & Biotic Matrix	Biotic Matrix
Ascidians	Ascidians: stalked
Ascidians	Ascidians: stalked: solitary
Ascidians	Ascidians: stalked: colonial
Ascidians	Ascidians: unstalked
Ascidians	Ascidians: unstalked: colonial
Ascidians	Ascidians: unstalked: solitary
Bryzoa	Bryozoa: hard
Bryzoa	Bryozoa: hard: branching
Bryzoa	Bryozoa: hard: encrusting
Bryzoa	Bryozoa: hard: fenestrate
Bryzoa	Bryozoa: soft
Bryzoa	Bryozoa: soft: foliaceous
Cnidaria	Cnidaria: anemones
Cnidaria	Cnidaria: anemone: solitary
Cnidaria	Cnidaria: anemone: colonial
Cnidaria	Corals
Cnidaria	Corals: black/octocoral (3D)
Cnidaria	Corals: black/octocoral (3D): fleshy: arborescent
Cnidaria	Corals: black/octocoral (3D): non-fleshy: bushy
Cnidaria	Corals: black/octocoral: encrusting
Cnidaria	Corals: stony corals: encrusting
Crustacea	Crustacea: barnacles
Echinoderms	Echinoderms: feather stars: unstalked crinoids
Echinoderms	Echinoderms: sea urchin
Hydroids	Hydroids
Encrusting macro-algae	Macroalgae (encrusting)
Encrusting macro-algae	Macroalgae: encrusting: calcareous
Encrusting macro-algae	Macroalgae: encrusting: brown
Encrusting macro-algae	Macroalgae: encrusting: green
Encrusting macro-algae	Macroalgae: encrusting: red
Macro-algae	Macroalgae: articulated calcareous: red
Macro-algae	Macroalgae: erect coarse branching
Macro-algae	Macroalgae: erect coarse branching brown
Macro-algae	Macroalgae: erect coarse branching green
Macro-algae	Macroalgae: erect coarse branching red
Macro-algae	Macroalgae: erect fine branching
Macro-algae	Macroalgae: erect fine branching brown

Table D.2 Categories of epibenthic biota identified using the standardised classification scheme (CATAMI v1.2). Broad morpho-taxa were used for statistical analyses; "substrate" and "unclear" were not included in analyses.

Broad morpho-taxa	Categories
Macro-algae	Macroalgae: erect fine branching green
Macro-algae	Macroalgae: erect fine branching red
Macro-algae	Macroalgae: filamentous/filiform
Macro-algae	Macroalgae: filamentous/filiform: brown
Macro-algae	Macroalgae: filamentous/filiform: green
Macro-algae	Macroalgae: filamentous/filiform: red
Macro-algae	Macroalgae: globose/saccate
Macro-algae	Macroalgae: globose/saccate: brown
Macro-algae	Macroalgae: globose/saccate: green
Macro-algae	Macroalgae: laminate
Macro-algae	Macroalgae: laminate: brown
Macro-algae	Macroalgae: laminate: green
Macro-algae	Macroalgae: laminate: red
Macro-algae	Macroalgae: large canopy-forming
Macro-algae	Macroalgae: large canopy-forming: brown
Macro-algae	Macroalgae: large canopy-forming: Ecklonia
Macro-algae	Macroalgae: sheet-like/membraneous
Macro-algae	Macroalgae: sheet-like/membraneous: brown
Macro-algae	Macroalgae: sheet-like/membraneous: green
Macro-algae	Macroalgae: sheet-like/membraneous: red
Sponges	Sponges: erect
Sponges	Sponges: erect forms
Sponges	Sponges: erect forms: laminar
Sponges	Sponges: erect forms: branching
Sponges	Sponges: erect forms: simple
Sponges	Sponges: hollow forms
Sponges	Sponges: hollow forms: cups and alikes
Sponges	Sponges: massive forms
Sponges	Sponges: massive forms: simple
Sponges	Sponges: massive forms: cryptic
Sponges	Sponges: crusts
Substrate	Substrate: consolidated
Substrate	Substrate: consolidated (hard): boulders
Substrate	Substrate: consolidated (hard): rock
Substrate	Substrate: unconsolidated
Substrate	Substrate: unconsolidated: pebble/gravel
Substrate	Substrate: unconsolidated: sand/mud (<2mm)
Unclear	Transect hardware
Unclear	Unclear
Unclear	Water
Unclear	Shadow

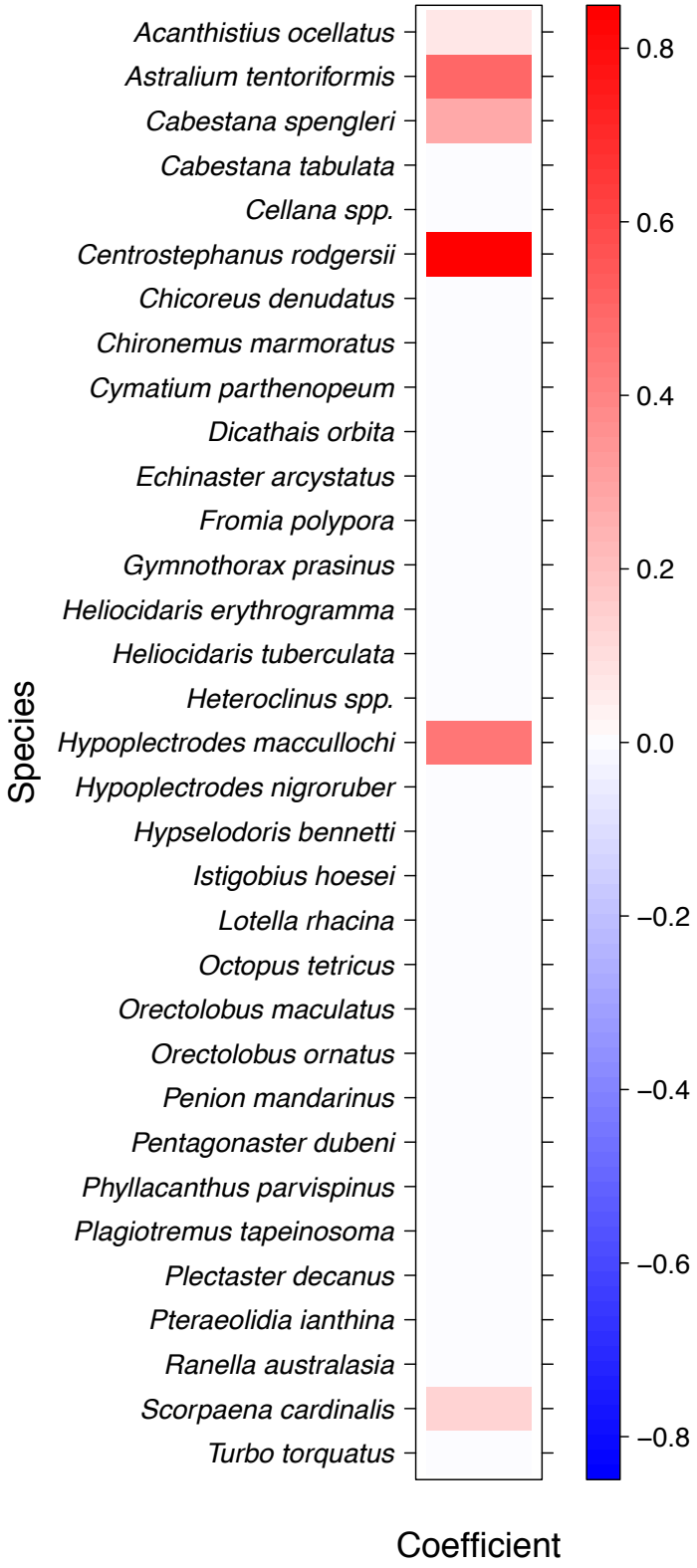

Figure D.1 Coefficients for individual species, illustrating species with the greatest influence on the assemblage. Colours denote species with significant effects on the overall assemblage. Red indicates a common species, and blue an uncommon species; whilst darker colours describe the strength of effect.