The effect of habitat degradation on parasitism of coral reef fishes

Thesis submitted by:

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in November 2021

for the degree of Doctor of Philosophy in Marine Biology

in the Centre of Excellence for Coral Reef Studies,

James Cook University, Townsville, Queensland, Australia

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Coral Reef Studies

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‘*Unless someone like you cares a whole awful lot, nothing is going to get better, it’s not.*’

The Lorax, Dr Seuss

Acknowledgements:

I wish to sincerely and whole-heartedly thank my supervisors for their mentorship, kindness and wisdom. Professor Andrew Hoey, thank you for pushing me to be my best and to be a better scientist. Thank you for your unwavering support and commitment, the many laughs and thank you for checking not only on the status of my research, but on the person behind it. Dr Kate Hutson, thank you for the hours spent in the lab and for the wonderful lab community and support network you created. Thank you for making draft edits an absolute joy, for Gorf (the charismatic green tree frog) and thank you for being there.

Thank you to the staff at the ARC Centre of Excellence (CoE) for Coral Reef Studies, for the financial, administrative and academic support. Thank you for introducing me to some of the world's leading ecologists and providing many invaluable opportunities. Thank you to Vivian Doherty and Janet Swanson for the seemingly endless supply of Fantales, hiking tips and support. Thank you to Alana Grech from the CoE and Jodie Wilson from the Graduate Research School for being in our (the student's) corner. Thank you also to staff at the Great Barrier Reef Marine Park Authority, the Centre for Sustainable Tropical Fisheries and Aquaculture; to staff at JCU's Boating and Diving and IT departments and the JCU Ethics Committee for all your assistance. This thesis would not have been possible without the spectacular support from staff at Orpheus Island Research Station, particularly Marta Espinheira, Bryn Jones and Jimmy - thank you for helping my fieldwork to run smoothly and for making it some of my best moments and fondest of memories. I am extremely grateful for the technical assistance, dedication and time given to me by Ben Lawes, Simon Wever and Andrew Thompson, from the Marine and Aquaculture Facilities Unit at James Cook University. Enormous thanks to the Holsworth Wildlife Research Endowment for funding my research.

I am extremely grateful to Rob Adlard, Diane Barton, Ian Beveridge, Geoff Boxshall, Neil Bruce, Evan Goulden, Kathryn Hall, Delane Kritsky, Marlies Monnens and Shokoofeh Shamsi for their time and assistance with parasite identifications. I also wish to thank Thomas Cribb, Scott

Cutmore, Berilin Duong, Russell Yong and the Marine Parasitology Laboratory, UQ for taking me under your wing and taking the time to help and teach me.

I am indebted to my volunteers, thank you for your time, your skill, (your remarkable cooking) and for being fantastic humans to be stranded on an island / in a lab with for weeks/months on end. Each of you made those experiences infinitely better, run smoother and more joyful by being there. Thank you also to the Marine Parasitology Lab at James Cook University for being a constant source of light, support and coffee. In particular, thanks to David Vaughan for all of his lab assistance and endless wisdom, and to Pauline – for your friendship, encouragement and glowing positivity. Thank you to Jonathan Barton, Saskia Juriaans, Alexia Landry, Sophie Stephenson and Kevin Bairos-Novak for your advice and assistance. Thank you to Dr Jenni Donelson for your help in all matters of life and science, for being a wonderful colleague, friend and mentor. Thank you to our 'planting' group for the encouragement and GIFs.

Thank you to Katie Sambrook, Tyto and Ruby, my absolute lifelines. Words cannot express my gratitude to you, how much you've helped me and how enjoyable you have made even the toughest of times - thank you for the delirium. Thank you to Thea for lending me your home, letting me be a third unofficial flatmate and for being my very own cheerleading squad. Thank you to my family and friends for making one of the most challenging experiences among the most wonderful. Thank you to my Townsville friends/family (Jacinta Bonaventura, Chloe Boote, David Caligari, Grace Cole, Kelly Hannan, Tracy MacKeracher, Eva McClure, Laura Richardson, Katie Sievers, Robert Strite, Anna Loizou, Liza Robbins and Izzy-Bee) for making Townsville a home, for the adventures, for your friendship, support and for being some of the most fantastic, inspiring people I know.

Special thanks to J.K. Rowling, Stephen Fry, Karen and Georgia for getting me through long hours and late nights in the lab.

For Ray, Sarah and Tyto.

Statement of the Contribution of Others

The research presented in this thesis includes collaborative work with my supervisors, Professor Andrew S. Hoey and Dr Kate S. Hutson, who provided intellectual guidance, editorial assistance, financial support, and technical assistance.

A JCU Postgraduate Research Scholarship (JCUPRS) covered tuition fees at James Cook University and 3.5 years of stipend. Financial support was provided by the Holsworth Wildlife Research Endowment (R2 2017; R2 2018; K. Motson) and the ARC Centre of Excellence for Coral Reef Studies (2019; K. Motson). Additional project expenses were covered by the ARC Centre of Excellence (CoE) for Coral Reef Studies research allocation awarded to Prof. Andrew Hoey and a James Cook University Development Grant: *Parasite cultivation techniques: in vitro and in vivo culture methods for ecological and applied aquatic parasitology research*. I was financially supported to attend two interstate symposia funded by the ARC Centre of Excellence for Coral Reef Studies and one interstate conference funded by the Australian Coral Reef Society. James Cook University provided laboratory space at the Townsville campus, Orpheus Island Research Station and funding for technicians at the Marine and Aquaculture Research Facilities Unit. The Marine Parasitology Laboratory, University of Queensland provided laboratory space and resources.

Olivia Bell, Chloë Boote, Jenni Calcraft, Jonathan Chew, Brayden Cockerell, Mila Grinblat, Kelly Hannan, Aero Leplastrier, Alejandro Usobiaga Fabian, Philip Jones, Eva McClure, Mike Mixalitsis, Pat Monarca, Pauline Narvaez, Ryan Ramasamy, Cassy Thompson, Hannaleena Väisänen, David Vaughan provided field and lab assistance. Rob Adlard, Diane Barton, Ian Beveridge, Geoff Boxshall, Neil Bruce, Thomas Cribb, Scott Cutmore, Berilin Duong, Evan Goulden, Kathryn Hall, Delane Kritsky, Marlies Monnens, Shokoofeh Shamsi and Russell Yong provided assistance with parasite identifications.

This research was conducted under JCU ethics approvals A1989 and A2449.

Abstract

Parasites are a ubiquitous, but often overlooked, component of the world's ecosystems. Parasites can affect the health, behaviour, and survival of individuals they infect, host populations, community composition and the functioning of entire ecosystems, and so contribute to ecosystem health and function. Despite the potential importance of parasites in the functioning of ecosystems, our understanding of parasite communities on coral reefs, and how these communities are influenced by habitat condition, is limited. The objective of this thesis was to investigate the effect of coral reef substrata, representing a gradient of reef health (coral, macroalgae and rubble), on the parasite communities and host-parasite interactions of herbivorous coral reef fishes on inshore reefs of the Great Barrier Reef.

To date, most studies of parasites of coral reef fishes have focused on particular taxa, or interactions between cleaning species and parasites. Very few studies have quantified the entire parasite communities of coral reef fish. Therefore, in **Chapter 2** I quantified and compared the abundance, taxonomic richness, and composition of parasite communities among three co-occurring herbivorous coral reef fishes (*Siganus doliatus*, *Pomacentrus wardi* and *Pomacentrus adelus*) from an inshore reef on the Great Barrier Reef (GBR). The parasite communities of *P. wardi* and *P. adelus* were broadly similar and characterised by pennellid copepods, derogenid and lecithasterid digeneans, and were distinct from those of *S. doliatus* that were characterised by a higher abundance of attractotrematid and gyliauchenid digeneans. Overall, *S. doliatus* had a higher abundance of parasites and was infected with a higher parasite taxon richness than *P. adelus*, likely due to its mobility and use of multiple habitats. Interestingly, *P. wardi* (10 cm maximum total length, TL) was infected with a similar number of ectoparasites as *S. doliatus*, a significantly larger species (25 cm maximum TL), and a significantly greater abundance of ectoparasites than its conspecific, *P. adelus* (8 cm maximum TL), suggesting that body size was not a primary driver of ectoparasite abundance. Rather differences in parasite communities among the three species are likely related to differences in diet, behaviour and mobility. These

findings highlight the importance of a holistic approach to understanding a species' parasite community, using multiple infection and community metrics, and incorporating both parasite and host ecology.

In forest, stream and saltmarsh ecosystems, changes in the composition of habitat forming taxa, due to habitat degradation, have been found to alter the community composition, richness, prevalence and abundance of parasites. In **Chapter 3** I investigate how different coral reef habitats, representing a gradient of coral reef health, influenced the parasite community and infection parameters of the site-attached herbivorous coral reef damselfish, *Pomacentrus wardi*. A minimum of 30 *P. wardi* were collected from each of three habitats on three inshore reefs of the GBR and their parasite communities quantified. *Pomacentrus wardi* from macroalgae habitats had lower prevalence of ectoparasitic infection (i.e., proportion of fish with ectoparasites) than those collected from rubble habitats, with an intermediate prevalence of infection in coral habitats. Yet, there were no consistent differences in the abundance of ectoparasites among habitats. For the endoparasite community, abundance differed among habitats, with higher abundances of endoparasites infecting *P. wardi* in coral relative to macroalgae and rubble habitats, whereas there were no differences in the prevalence or richness of parasites among habitats. These difference in the composition and abundance of parasites infecting *P. wardi* among habitats may reflect differences in the densities of intermediate invertebrate hosts, habitat requirements and life cycles of individual parasites, predation of parasites, or exposure to parasites among habitats. This study finds a significant effect of different coral reef habitats on both ecto- and endo-parasitism of *P. wardi*.

The difference in ectoparasite communities among habitats observed in Chapter 3 may relate to a range of factors, such as cleaning interactions and host-specific behaviours, such as the avoidance of areas in which parasites are prevalent. Therefore, in **Chapter 4** I used enclosure cages to isolate the effects of benthic habitats on ectoparasite transmission and colonisation of *S. doliatus* over specific coral reef substrata. *Siganus doliatus* were cleaned of external parasites

and placed in individual enclosure cages over each of three habitat types (coral, macroalgae and rubble; n = 10 per habitat) for 72 hours. After 72 hours the fish were collected and the external parasite communities quantified. *Siganus doliatus* were infected with a greater abundance of ectoparasites when caged over rubble compared to coral and macroalgae habitats, with this pattern driven by the abundance of gnathiid isopods. There were no detectable differences in the taxonomic composition or richness of parasite communities among the three habitats. Gnathiid isopods may be strongly affiliated with rubble habitats due to the reduced abundance of predators (both corals and cleaner fish) in these habitats. Transitions from coral- to macroalgal-dominance are viewed as one of the greatest threats to the functioning of coral reef ecosystems and results from the present study suggest that transitions to rubble may have the greatest effect on parasite transmission and infection, namely by gnathiid isopods.

Of the few studies investigating the connections between parasites and coral reef substrata, the majority have focused upon the abundance and habitat preferences of gnathiid isopods. In **Chapter 5**, I examine how different coral reef substrata (live coral, macroalgae and coral rubble) influence the development, hatching, and infection success of a common ectoparasite of coral reef fish, *Neobenedenia girellae* (Monogenea: Capsalidae). This study found that water conditioned with coral and macroalgae substrata significantly reduced the hatching success of *N. girellae* relative to control seawater, likely due to the chemical and/or microbial activity of these substrata. Infection of adult *N. girellae* was comparable among coral reef substrata, implying that once *N. girellae* larvae have contacted the host, the environment becomes less prohibitive. If the results for *N. girellae* here are representative of other ectoparasite taxa (in particular other capsalid monogeneans), then any differences in parasite communities among habitats may relate to factors other than hatching and infection success.

The research presented in this thesis is the first to categorise the metazoan parasite communities of *S. doliatus*, *P. wardi* and *P. adelus,* finding several novel host records as well as two potentially novel parasite species. This work is also the first to investigate how entire parasite communities

and parasite life histories may respond to changes in the benthic composition of coral reefs. My findings emphasise that alongside increased degradation of coral reefs, and predicted ongoing declines in live coral cover, we may observe shifts in parasitism, and potentially the health and function, of coral reef fishes. Habitat degradation, however, not only causes the benthic composition of coral reefs to change, but also causes them to fragment, creating discrete 'patches' of reef habitats. It is, therefore, imperative that future research not only examines the effect of benthic composition on the parasite communities of coral reef fishes, but also the effect of seascape composition and configuration on the transmission and infection of parasite communities, and the effects of these changes on the health and fitness of individuals, populations and assemblages of reef fishes.

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Chapter 1: General Introduction

1.1 The role of parasites in ecosystem function

Parasites are a key, but often overlooked, component in the functioning of the world's ecosystems (Hudson et al., 2006; Poulin, 1999; Thomas et al., 1999). Through effects to individual health and behaviours, parasites can modify species interactions and competitive and predatory outcomes (Hatcher et al., 2006; Hudson et al., 1992; Poulin, 1999; Tompkins et al., 2000). Parasites can also modify host populations through altering the disease susceptibility (Hudson et al., 1997; Thomas et al., 2005), reproductive output (Lafferty & Kuris, 2009), phenotype (Mouritsen, 2002) and local abundance of a species (Tompkins et al., 2002) and in turn, modify the community composition of an ecosystem and transfer of energy within it (Dunne et al., 2013; Mouritsen & Haun, 2008; Mouritsen & Poulin, 2005; Preston et al., 2016). In instances where parasite hosts are ecosystem engineers (e.g., marine benthic systems; Dairain et al., 2019; Mouritsen & Haun, 2008), or predators/competitors of ecosystem engineers, (e.g., herbivores of kelp, Hagen, 1992; and plant communities, Arneberg et al., 1996; Phoenix & Press, 2005; Pywell et al., 2004), they can alter the structure and complexity of the local environment through their effects on the abundance and/or phenotype of habitat-forming organisms (Mouritsen & Haun, 2008; Thomas et al., 1999). For example, in soft-bottom communities, trematode infection of the cockle, *Austrovenus stutchburyi*, causes swelling of the cockle's foot, preventing burrowing (Mouritsen & Poulin, 2005). Increased cockle parasitism thereby increases the abundance of surfaced cockles and structure for epifaunal attachment, as well as changing sediment particle composition, causing increases in epi- and in-faunal biodiversity.

1.2 Parasite life histories and habitat degradation

While parasites can influence the structure and complexity of local environments, the environment also plays an integral role in the dynamics and outcomes of host-parasite interactions. The effect of the environment on host-parasite interactions may manifest through

changes in host (i.e., intermediate and final hosts) and parasite population densities (Lafferty & Kuris, 2005; Marcogliese, 2005) and survivorship (Lafferty, 1997), the abundance of parasite predators (Artim & Sikkel, 2013) and the creation/removal of host and parasite habitats (Patz et al., 2000). Since the industrial revolution, increasing human population pressures, urban expansion, greenhouse gas emissions, land-use change and demand for natural resources have resulted in a rapidly changing climate and the reduction and degradation of ecosystems and habitats worldwide (IPCC, 2018; Millennium Ecosystem Assessment, 2005). Habitat degradation has changed the composition of multiple ecosystems through the loss of habitat forming species – with forest degradation and deforestation resulting in the loss of 'primary' forest to alternate land uses (Curtis et al., 2018); desertification altering drylands into arid dust plains (Burrell et al., 2020) and permafrost peatlands degrading to flooded arctic wetlands (Swindles et al., 2015). These changes in foundation species have influenced the communities and infection dynamics of parasites in multiple ecosystems (Chapman et al., 2015; Gillespie & Chapman, 2008; Huspeni & Lafferty, 2004), with repercussions to the physiology and life history traits of parasites, their intermediate hosts and vectors (Patz et al., 2000; Afrane et al., 2006; Yasuoka & Levins, 2007; Burkett-Cadena & Vittor, 2018).

1.3 Parasite benthic associations and habitat degradation

Habitat degradation and the loss of habitat-forming species may affect the availability of the intermediate hosts of parasites. For example, corals from the genus *Porites* are intermediate hosts to the digenean, *Podocotyloides stenometra,* which is transmitted to corallivorous fish, such as *Chaetodon multicinctus*, through consumption of infected polyps (Aeby et al. 2002). Through the increased frequency and severity of climatic and anthropogenic disturbances, such as coral bleaching, reductions in the abundance of *Porites* on degraded reefs has been shown to reduce the transmission and abundance of *P. stenometra*. For some parasites, specific substrata can act as intermediate hosts, facilitating their transmission to and infection of the next host in their life cycle. For example, in coastal Mississippi, two species of trematode encyst on algae and other

substrata that form the diet of local mullet (Overstreet, 2005). These habitat associations thus facilitate trematode transmission. As a result of Hurricane Katrina in 2005, these substrata were lost and the associated trematode species were absent for several years (Overstreet, 2007). Through removing a substratum key to their transmission, the trematode species were unable to infect their mullet host and therefore complete their life cycle, causing a temporary loss of these parasites from the system. Habitat degradation and its influence on intermediate host and habitat availability, can therefore alter the local prevalence, abundance and infection intensity of parasites.

1.4 Parasites and the declining condition of coral reefs

Coral reefs are among the world's most vulnerable ecosystems to climatic change and local anthropogenic stressors. The cumulative impact of these stressors, in particular the increasing frequency and severity of coral bleaching (Hughes, Kerry, et al., 2017), have caused the structure, condition and composition of coral reefs to change, with some reefs shifting from coral- to macroalgal- or rubble-dominance (Adam et al., 2021; Contreras-Silva et al., 2020; Vieira, 2020). Considerable research effort has been made to understand the effects of coral loss and reef degradation upon reef fish communities, with significant reductions in the abundance, biodiversity and fitness of coral reef fishes observed (Pratchett et al., 2018; Richardson et al., 2018; Thompson et al., 2019; Wilson et al., 2019). However, very few studies have investigated the effects of benthic composition on the life history, abundance, richness, transmission or community composition of coral reef parasites.

On coral reefs, initial research into the relationship between benthic composition and parasite abundance and distribution have been investigated in host-generalist ectoparasites, in particular, *Gnathia* spp. (Artim et al., 2020; Artim & Sikkel, 2013; Narvaez et al., 2021; Paula et al., 2021; Santos & Sikkel, 2019). The abundance of gnathiid isopods is significantly affected by the benthic composition of coral reefs, with reduced emergence in areas of high density, live coral cover (Artim et al., 2020; Artim & Sikkel, 2013; Paula et al., 2021; Santos & Sikkel, 2019). Specifically,

gnathiid isopods show a preference for rubble, sponge and dead coral substrata, avoiding live coral, as coral polyps can feed heterotrophically on the free-living life stages (Artim & Sikkel, 2013; Paula et al., 2021). If the continued effects of anthropogenic stressors and climatic change cause an increase in the abundance and extent of rubble-dominated habitats, we may therefore see a concomitant increase in the prevalence and abundance of gnathiid isopods (Artim et al., 2020). As for other species of coral reef parasites, qualitative connections have been made between high abundances of monogenean and crustacean parasite infections and reduced coral cover/degraded habitat (Sikkel et al., 2000, 2009). However, the relationship between these parasites and coral cover has never been tested experimentally or directly quantified. While these studies have shown the potential effects of habitat degradation on coral reef parasites, they have been taxonomically limited, sampling a single parasite species or group. Coral reef fishes however are infected by multiple parasite species (metazoan and protozoan; Justine, 2010; Rohde, 1976a). Therefore, through examining only a subset of the parasite community, we understand only a portion of how parasite communities, parasitism and consequently the health and function of host individuals and populations may be affected by habitat degradation.

Whilst the effect of coral cover upon the abundance of parasites other than *Gnathia* spp. is yet to be quantified, a laboratory study by Hutson et al., (2012) investigated the effect of several tropical marine algal species on the embryonation and hatching success of the monogenean ectoparasite, *Neobenedenia girellae*. Polar extracts from *Ulva* sp. and *Asparagopsis taxiformis* resulted in delayed egg embryonation, hatching and reduced hatching success in *N. girellae*. Some algal species may therefore have negative effects on the development, hatching success and life history traits of parasites in the wild. On coral reefs, *Sargassum* is a genus of brown algae commonly found in degraded habitats, however the effect of *Sargassum* on parasite life histories is unknown. Moreover, the relationship between different coral reef substrata and the life cycles, survivorship, habitat associations and infection dynamics of other coral reef parasites has not been investigated.

1.5 Aims and thesis outline

Given the importance of parasites for ecosystem function and the likelihood of continued changes in coral reef ecosystems with climatic change, understanding the interactions of parasites and habitat condition in a coral reef context is key to understanding the system-wide implications of coral reef degradation. The objective of this thesis was to determine the effect of habitat degradation on parasitism of herbivorous reef fishes on inshore reefs of the Great Barrier Reef. This thesis addresses this objective using four aims:

1. To characterise the metazoan parasite communities and infection parameters of three herbivorous coral reef fishes.
2. To quantify the effect of different coral reef habitats, representing a gradient of coral reef condition (i.e., live coral, macroalgae and rubble) on the parasite community composition and infection dynamics of a common herbivorous coral reef fish.
3. To investigate the effect of different coral reef substrata (i.e., live coral, macroalgae and rubble) on the transmission and colonisation of coral reef ectoparasites on a common herbivorous coral reef fish.
4. Determine how different coral reef substrata, representing a gradient of coral reef health (i.e., live branching coral, macroalgae and coral rubble), influence the development, hatching and infection success of a common coral reef ectoparasite, *Neobenedenia girellae.*

Each of these aims is addressed in a separate chapter. **Chapter 2** quantifies and compares the parasite community composition, abundance, richness and prevalence of ecto- and endo-parasites infecting three common and co-occurring herbivorous coral reef fishes, *Siganus doliatus, Pomacentrus wardi* and *Pomacentrus adelus*, from an inshore reef of the Great Barrier Reef. The results of this chapter provide a baseline of parasite communities that inform the subsequent chapters. Using parasite community and infection data from Chapter 2, **Chapter 3** quantifies and compares the parasite community composition, abundance, richness and prevalence of ecto- and endo-parasites infecting a common, herbivorous coral reef fish, *Pomacentrus wardi*, among

habitat types representing a gradient of coral reef health (live coral, macroalgae and rubble). **Chapter 4** builds directly on Chapter 3 and aims to identify the parasite species that infect a common coral reef fish, the barred rabbitfish, *Siganus doliatus*, from different coral reef substrata (i.e., live coral, macroalgae and rubble) and quantify and compare how infection parameters (parasite abundance and richness) vary among them. This field experiment used enclosure cages to minimise external sources of parasite infection and removal; to prevent movement among substrata; and to minimise host-host transmission. Having explored the effect of coral reef habitats, substrata and benthic composition upon coral reef parasite communities and infection parameters, in **Chapter 5** I sought to identify the interactions between coral substrata and the different life stages of a common coral reef ectoparasite, *Neobenedenia girellae*. Chapter 5 investigates the effects of live coral, macroalgae and rubble substrata upon the development and hatching success of *N. girellae* embryos; the infection success of *N. girellae* larvae; and the maturation, time to egg production and survivorship of *N. girellae* adults.

Together, these four original research chapters advance our understanding of the effects of coral reef degradation on parasitism of coral reef fishes and how parasite species and communities are affected by different coral reef substrata. Importantly, I identify the aspects of the ecology of the parasites, and three host species used, that may be key in determining their parasite communities and the response of these communities to habitat degradation. Understanding how parasite communities and parasitism of coral reef fishes may shift with declining coral reef condition will allow us to further our understanding of the implications of coral reef degradation upon the diversity and function of coral reef ecosystems.

Chapter 2: Variation in the parasite communities of three co-occurring herbivorous coral reef fishes

2.1 Introduction

Parasites are ubiquitous, abundant and diverse components of natural ecosystems, and are increasingly recognised for their roles in ecosystem functioning (Marcogliese, 2004; Mouritsen & Poulin, 2005; Preston et al., 2016). Within an ecosystem, parasites play important roles in trophic interactions and energy transfer, with parasitism, in its broadest sense, hypothesised to be the most common means of food acquisition among organisms (Dunne et al., 2013; Lafferty, Allesina, et al., 2008; Lafferty et al., 2006; Price, 1977). In estuarine ecosystems for example, parasites have been found to contribute more to overall ecosystem biomass than predatory birds and fishes, due to their high productivity and food conversion ratios (Kuris et al., 2008). Parasites are also highly diverse, with approximately 40% of all known species estimated to be parasitic at some stage within their life cycle (Dobson et al., 2008; Rohde, 1984). Moreover, the host specificity of most parasites means that the biodiversity of the parasite community often reflects the biodiversity of the ecosystem itself (Cribb, Bray, Barker, et al., 1994; Hudson et al., 2006; Marcogliese, 2004). Despite the diversity of parasites and their potential importance in ecosystem function, parasites are often overlooked within ecological studies. Including parasites and host-parasite interactions within ecological research will improve our understanding of the function, health and resilience of these ecosystems. This is particularly important for those ecosystems vulnerable to growing anthropogenic and climatic stressors, such as coral reefs.

Coral reefs are one of the world's most biodiverse ecosystems and are estimated to support a diverse community of parasites; up to ten-fold greater than the number of coral reef fish species they support (Cribb, Bray, Barker, et al., 1994; Rohde, 1976b). Despite considerable research focus on the taxonomy of some families of marine parasites (Bray & Cribb, 1998; Kritsky et al., 2007), and on the interactions between cleaner organisms (i.e., cleaner wrasse and shrimps) and

parasitic gnathiid isopods (Grutter et al., 2019; Sikkel et al., 2006), relatively few studies have described the parasite communities of coral reef fishes in their entirety or how these communities vary among fish species (Duong et al., 2019; Muñoz et al., 2007; Vignon & Sasal, 2010). Quantifying the parasite communities of coral reef fishes and establishing their 'baseline' composition will not only increase our understanding of coral reef biodiversity but provide insights into the mechanisms structuring their parasite communities. Moreover, establishing community baselines will allow us to identify how changes in reef condition and disturbances such as coral bleaching, cyclones and terrestrial run-off can influence parasite communities and, for those parasites that utilise multiple host species, be used as a means of monitoring system recovery (Overstreet, 2007).

Of the few studies that have quantified the parasite communities of adult coral reef fishes, most have focused on piscivorous (Vignon & Sasal, 2010: Lutjanidae 3 spp., Serranidae 5 spp.), invertivorous (Muñoz et al., 2007: Labridae 14 spp.) and omnivorous species (Lo et al., 1998: Pomacentridae 4 spp.; Sun et al., 2012: *Pomacentrus amboinensis*). Few studies have quantified the parasite communities of herbivorous reef fish, with exceptions being *Siganus rivulatus* (Red Sea; Dzikowski et al., 2003a), *Acanthurus nigricans,* and the detritivorous *Ctenochaetus marginatus* (Line Islands Archipelago; Wood et al., 2015). Ingested material is a major source of infection by internal (or endo-) parasites, and as such differences in diet and feeding ecology have been related to differences in parasite communities across a range of taxa (Aponte et al., 2014; King et al., 2008; Vitone et al., 2004). Herbivorous coral reef fishes help to maintain a healthy balance between coral and macroalgal assemblages on coral reefs. The consumption of macroalgal biomass by herbivores reduces competition for benthic space and facilitates coral recruitment, growth and survivorship (Burkepile & Hay, 2008; Hughes et al., 2007). There is, however, considerable variation in diet and feeding ecology among herbivorous fishes (e.g., Hoey et al. 2013; Rasher et al. 2013), and these factors are likely to influence their parasite communities.

The aim of this study was to quantify and compare the parasite communities of three common and co-occurring herbivorous coral reef fishes from inshore reefs of the GBR: the barred rabbitfish, *Siganus doliatus* (Guérin-Méneville, 1829-38), a gregarious and mobile algal cropping species; and two territorial, algal farming damselfish: Ward's damsel, *Pomacentrus wardi* (Whitley, 1927), and the obscure damsel, *Pomacentrus adelus* (Allen, 1991). Given the similar diet, ecology, body size and phylogeny of the two damselfishes (*P. wardi* and *P. adelus*) it is reasonable to hypothesise that they would host similar parasite abundances, taxonomic richness and parasite communities distinct from that of the rabbitfish, *S. doliatus*.

2.2 Methods

2.2.1 Fish collection

This research was conducted under JCU ethics approval A2449 and GBRMPA permit G13/35909.1. Three common, co-occurring herbivorous coral reef fishes: *S. doliatus, P. adelus* and *P. wardi* were selected for this study, as they are abundant on inshore reefs of the Great Barrier Reef (Emslie et al., 2012; Hoey et al., 2013). *Siganus doliatus* is a relatively large (up to 25 cm total length, TL; Kuiter & Tonozuka, 2001) species that typically forms conspecific pairs, or less commonly, larger conspecific or mixed species schools (Woodland 1990). *Siganus doliatus* has a relatively large home range (1.53 ± 0.13 SE ha; Brandl & Bellwood, 2013) that likely encompasses a range of habitat types (e.g., coral, macroalgae, rubble, sand). *Pomacentrus adelus* and *P. wardi* are two common species of herbivorous, farming (or 'territorial') damselfishes on the GBR (maximum TL: *P. adelus*: 8 cm; *P. wardi*: 10 cm; Allen et al., 2015). Both species are solitary, highly site-attached with small territory sizes (approx. 0.6 – 2.0 m^2; Ceccarelli et al., 2005, 2006) and typically only form pairs to breed (Breder & Rosen, 1966). While previous studies have investigated the prevalence and composition of specific parasite taxa for *S. doliatus* (Kritsky et al., 2007; Nolan & Cribb, 2006) and *P. wardi* (Bray et al., 1993; Gunter & Adlard, 2008) no studies have documented the entire ecto- and endo-parasite communities of *S. doliatus* or *P. wardi*, and there are no parasite species recorded for *P. adelus*.

Thirty individuals of each species were collected from reef crest and outer reef flat (1-3 m depth) of Pioneer Bay, Orpheus Island within the central GBR in July and August 2017. Orpheus Island is a high continental island approximately 16 km from the Queensland coast that has extensive fringing reef development on the western (leeward) margin (Figure 2-1). The sample size was selected given 30 individuals achieves approximately 95% confidence of recovering parasite taxa with a prevalence of >10% (Post & Millest, 1991). Individuals were collected using a weak clove oil solution, barrier nets, and hand nets and immediately placed in an individual sealed aquarium-grade bag, provided with supplemental oxygen and transported to Orpheus Island Research Station (OIRS). At OIRS each fish was transferred into an aquarium filled with filtered, UV-sterilised seawater and supplemental aeration. Given differences in body size (*S. doliatus* mean TL = 22.4 ± 0.3 cm SE; *P. wardi* mean TL = 7.3 ± 0.2 cm SE; *P. adelus* mean TL = 6.9 ± 0.2 cm SE), the pomacentrids were placed in individual 10 L aquaria and *S. doliatus* were placed in individual 15 L aquaria. Static, as opposed to flow-through, systems were used to ensure that any dislodged parasites were retained within each aquarium. Water exchanges were conducted every 24 hours whereby approximately 80% of the water from each aquarium was siphoned, filtered through a 63 μm sieve to capture dislodged parasites, and replaced with fresh, filtered UV-sterilised seawater. Any parasites captured on the sieve were preserved in a 70% ethanol solution for subsequent identification. Fish were fed twice daily *ad libitum*; the two pomacentrid species were fed commercial pellets (NRD 5/8 pellets, INVE Aquaculture Nutrition) and *S. doliatus* fed commercially supplied, dried *Pyropia* sp. (i.e., nori). Fish were held in aquaria for a maximum of six days before being transported to James Cook University for necropsy. Prior to transport, fish were not fed for 24 hours to minimise nitrogenous waste during transport, placed in separate, sealed aquarium-grade bags filled with fresh, filtered UV sterilised seawater and filled with supplemental oxygen. Aquarium water was filtered, and any captured parasites preserved as described above.

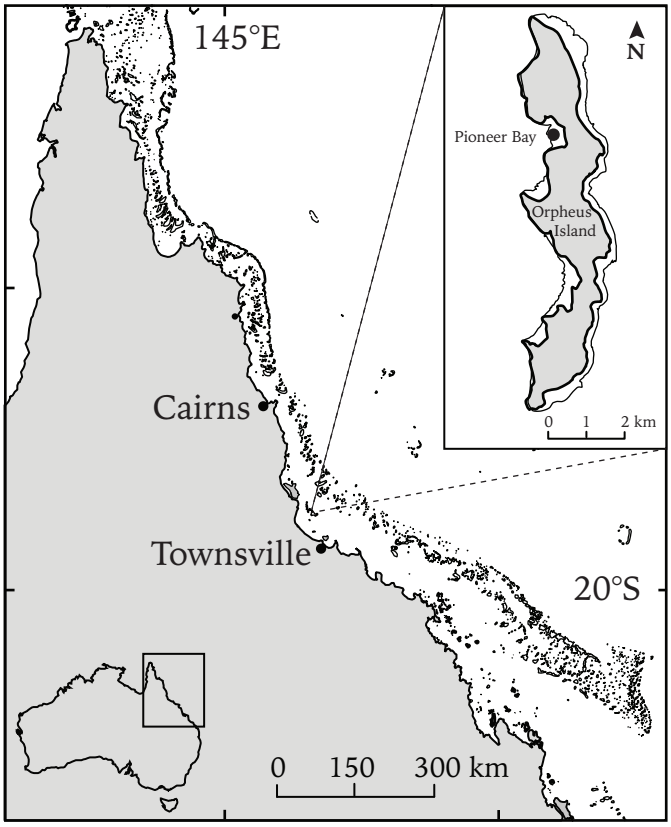

Figure 2-1: Map of the Great Barrier Reef; inset: Orpheus Island within the central Great Barrier Reef; the black circle indicates the fish collection location at Pioneer Bay.

2.2.2 Fish dissection

Fish were euthanised in a 0.15% solution of 2-Phenoxyethanol and subsequently measured (total length and wet weight), photographed, and the entire body surface, including inside the oral cavity and buccal folds, inspected for ectoparasites under a dissection microscope (range 6.7 to 45 x magnification as required). Gills were then removed and placed in filtered seawater for inspection under the dissecting microscope at 6.7 to 45x magnification. Following gill removal, each fish was placed in individual freshwater baths for 5 mins. The contents of each bath were then filtered through a 63 µm sieve to collect any dislodged ectoparasites. Parasitological analyses of visceral organs (i.e., heart, liver, spleen, gall bladder, white muscle, brain, stomach and intestines) were conducted following Hutson et al. (2007) and Cribb & Bray (2010). For both pomacentrid species, a sample of white muscle tissue (from around the visceral cavity) and the

entire heart, liver, gall, spleen and brain were squashed onto slides, forming a tissue layer approximately one cell thick. Slides were examined for parasites under a compound microscope at 200x magnification (400x magnification for the gall bladder). Due to the larger organs of *S. doliatus*, a sample of the white muscle tissue (from around the visceral cavity), heart, liver and spleen was removed consistently from the same area, squashed onto a slide and examined as above. The remainder of the organs were dissected and inspected for parasites under a dissecting microscope at 6.7 to 45x magnification. The abundance of all parasites was quantified, the exceptions being ceratomyxid myxozoans (f. Ceratomyxidae) and ancyrocephalid monogeneans (f. Ancyrocephalidae). The extremely high abundances of these taxa in the gall bladder and on the gills, respectively, made accurate estimates of abundance unfeasible and hence only presence/absence data was recorded. All parasites found during dissections were preserved in 70% ethanol. Due to logistical and ethical considerations, thirteen *S. doliatus* were euthanised in a 0.15% solution of 2-Phenoxyethanol and immediately frozen for dissection later. At the time of dissection, the frozen *S. doliatus* were defrosted overnight in a refrigerator and dissected the following morning following the protocol described above (see Appendix A: Methods).

Parasites were identified to the lowest taxonomic ranking using morphological characters with the assistance of taxonomic keys and/or soliciting taxonomic expertise on parasite groups.

2.2.3 Statistical analysis

Statistical analyses were conducted using family-level assignments of the parasite taxa to provide objective comparisons among the three fish species, unless otherwise indicated (Locke et al., 2011; Poulin & Leung, 2010).

Due to non-normality of parasite abundance data and the high number of zeros within the dataset, PERMANOVA was used to test for differences in parasite community composition (infection intensity of each parasite family) among the three fish species (fixed categorical variable with three levels). PERMANOVA was conducted using the '*vegan'* package in R (Oksanen et al. 2020) and post-hoc comparisons among species were conducted using the '*RVAideMemoire*'

package (Hervé, 2020). Model validation was confirmed using stress values and stress plots. Differences in the composition of the parasite communities among fish species were visualized using non-metric multidimensional scaling (nMDS). This technique produces an ordination of community composition data for each fish, based on the Bray-Curtis dissimilarity matrix of parasite infection intensity data (i.e., excluding uninfected fish; see A. O. Bush et al. (1997)) using the '*vegan*' package in R (Oksanen et al. 2020). Infection intensity data was square root transformed and Wisconsin double standardised to reduce the influence of extreme values. As individual fish without parasitic infection were removed from the analysis, nMDS was used to compare the communities of 25x *S. doliatus,* 24x *P. wardi* and 13x *P. adelus*.

For abundance and richness data, candidate models were created using Poisson and negative binomial distributions, as these error distributions are typically used for count data. To inform the appropriate error structure for each model, assumptions of the error distributions were examined for candidate models using residual plots, Chi square goodness-of-fit tests and dispersion (the ratio of the variance to the mean). Residual diagnostics, zero-inflation and overdispersion were also tested for each model using the '*DHARMa*' package. The error distribution that best satisfied model assumptions and candidate model with the best model fit (lowest AICc values within 2 units) was selected for the analysis.

To compare the abundance of total parasites and endoparasites among the three fish species (*S. doliatus*, *P. wardi* and *P. adelus*) a generalised linear mixed effects models (GLMM) was used, with fish species as a fixed factor. An observation-level random effect was included to model extra variation present in the data. As abundance data for ectoparasites and the two most common parasite taxa independently (graffillid turbellarians and pennellid copepods) were not over-dispersed, abundances were compared among the three fish species using generalised linear models (GLM), with fish species included as a fixed factor. Due to collinearity of fish species and total length, total length was not included as a factor within the analyses. The only exception to this was the abundance of pennellid copepods that infected the two pomacentrid species only,

as species and total length were not collinear for the two pomacentrid species. Therefore, the natural log of total length was included as an offset in the model to account for any potential effect of body size on pennellid abundance. Total parasite and endoparasite abundance were therefore modelled against a Poisson distribution, and the abundance of ectoparasites, pennellid copepods and graffillid turbellarians were modelled against a negative binomial distribution. A single *S. doliatus* was removed from the total and endoparasite abundance analysis, as the abundance of gyliauchenid digeneans in its gastro-intestinal tract (n = 1,916 gyliauchenids) was an order of magnitude greater than the average abundance of all thirty *S. doliatus* investigated (mean = 110.8 gyliauchenids per fish ± 64 SE – including heavily infected individual).

The number of parasite families (i.e., taxon richness) found to infect the three fish species was modelled as a function of the covariate 'Species' (categorical with three levels; n = 30 individuals per fish species). Parasite richness data (i.e., the number of parasite families infecting each fish) was significantly under-dispersed ('*DHARMa*' nonparametric dispersion test; $P < 0.05$). Therefore, standard errors for the Poisson distribution are over-estimated (Harris et al., 2012). The overall prevalence of parasitic infection (i.e., proportion of hosts infected), as well as prevalence of ecto- and endo-parasitic infection was compared among species (n = 30 individuals per species) using generalised linear models (GLM). Presence/absence data was modelled against a binomial distribution, using fish species as a fixed factor, to determine parasite prevalence.

Tukey's post-hoc analyses comparing parasite abundances, taxonomic richness and prevalence among fish species were conducted using the '*emmeans*' package. All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018). The parasite taxa for which only presence/absence data was recorded (i.e., ceratomyxid myxozoans and ancyrocephalid monogeneans) were included in the analyses of parasite richness and prevalence but excluded from the analysis of abundance and community composition. Nematodes and encysted and larval parasitic worms were removed from species richness and community composition analyses but

were included within analyses of prevalence and total abundance, as these specimens could not be identified to family (see Appendix A: Table S2.1).

2.3 Results

A total of 3,978 metazoan parasites were recorded from 17 families (7 families of ectoparasites and 10 families of endoparasites) across the 90 fish examined (Table 2-1). From these 90 fish (including the heavily infected individual with 1,916 gyliauchenids), gyliauchenid digeneans (ex. *S. doliatus* total = 3,324 individuals) accounted for 84% of all parasites recorded. Encysted and larval parasitic worms were also highly abundant, accounting for 8% of all parasites recorded (total = 335 individuals), however these could not be could not be identified to family with any certainty. The abundance of parasites ranged from 0 to 1,947 parasites per fish, with a mean abundance (calculated from raw data) of 123.8, 6.6 and 2.2 parasites per *S. doliatus, P. wardi* and *P. adelus*, respectively. Ceratomyxid myxozoans and encysted and larval parasitic worms were the most prevalent parasites recorded, with encysted and larval worms found in 63% of all fish examined and ceratomyxid myxozoans present in 48% of all fish examined. Overall parasite prevalence was high, with 100% of *S. doliatus* (n = 30), 97% of *P. wardi* (n = 29) and 83% of *P. adelus* (n = 25) examined having parasite infections.

Table 2-1: Summary of all known host-parasite records for *Siganus doliatus*, *Pomacentrus wardi* and *Pomacentrus adelus* from the Indo-Pacific Region. Host records identified within the present study are highlighted in bold. Novel host records are indicated by (*); Known parasite microhabitats that were not specified in the original study are indicated by ($^{+}$). Records from the present study that may be the same species as those identified in previous studies are indicated by (•). Locations on the Great Barrier Reef (GBR) are abbreviated as follows: 'HI' = Heron Island, 'LI' = Lizard Island, 'CB' = Capricorn Bunker, 'PI' = Palm Island Group, 'GI' = Green Island; locations outside of the GBR are abbreviated as follows: 'NC' = New Caledonia, 'N' = Noumea, 'P' = Palau.

Host sp.	**Group/Class/Family**	**Taxon**	**Record**	**Microhabitat**	**Location**
Siganus doliatus					
Ectoparasites:					
	Malacostraca				
	Corallanidae	***Argathona* cf. *macronema****	Current study	Body surface	PI
	Cymothoidae	*Anilocra* sp.	Grutter, 1994	Body surface^{+}	GBR
	Gnathiidae	***Gnathia* spp.** •	Grutter, 1994	Body surface / Gills^{+}	LI; HI
			Current study	Body surface	PI
		Gnathia falcipines •	C. M. Jones et al., 2007	Body surface / Gills^{+}	LI
	Hexanauplia				
	Bomolochidae	*Acanthocolax / Orbitacolax* sp. nov.	Grutter, 1994	Body surface^{+}	LI; HI
	Caligidae	*Lepeophtheirus* sp.	Grutter, 1994	Body surface / Gills^{+}	LI; HI

		Caligus sp. •	Grutter, 1994	Body surface / Gills^{+}	LI; HI
		***Caligus* cf. *uniartus**•**	Current study	Body surface	PI
	Trematoda				
	Transversotrematidae	*Transversotrema licinum*	Grutter, 1994	Body surface^{+}	LI
	Monogenea				
	Ancyrocephalidae	*Glyphidohaptor sigani*	Kritsky et al., 2007	Gills	HI
		Pseudohaliotrema sphincteroporus°	P. D. Olson & Littlewood, 2002	Gills	GI
		***Pseudohaliotrema* sp. 1•**	Current study	Gills	PI
		***Pseudohaliotrema* sp. 2•**	Current study	Gills	PI
		Tetrancistrum* sp.	Current study	Gills	PI
	Capsalidae	**Capsalidae n. sp.*•**	Current study*	Body surface	PI
		'Benedeninae' •	Grutter, 1994	Body surface / Gills^{+}	HI
	Neophora				
	Piscinquilinidae	*Ichthyophaga* sp.	Lockyer et al., 2003	Body surface / Gills^{+}	GI
	Piscinquilinidae *or* Graffillidae	*Ichthyophaga* sp. *or* *Paravortex* sp.	Grutter, 1994	Body surface / Gills^{+}	LI; HI
	Graffillidae	***Paravortex* sp.**	Current study	Body surface / Gills	PI
Endoparasites:					
	Chromadorea				
	Raphidascarididae	***Hysterothylacium* sp.***	Current study*	Heart, Stomach, Intestines	PI

	Raphidascarididae sp.*	Current study*	Intestines	PI
Trematoda				
Bucephalidae	**Bucephalidae sp.**	Current study*	Stomach, Intestine	PI
Gyliauchenidae	*Flagellotrema reburrus*	Hall & Cribb, 2008	Intestine	HI
	Gyliauchen zancli	Hughes-Stamm et al., 1999	Intestine	HI
		Current study	Intestine	PI
	"Medousogyliauchen" cydippe	Hall, 2004	Intestine	HI
		Current study	Stomach, Intestine	PI
	Ptychogyliauchen thetidis	Hall & Cribb, 2004	Intestine	LI; HI
		Current study	Intestine	PI
	Ptychogyliauchen thistilbardi	Hall & Cribb, 2004	Intestine	N; NC
Lecithasteridae	*Hysterolecithoides frontilatus* syn. *H. epinepheli*	Bray & Cribb, 2000	Stomach/Intestine	LI; NC
	Machidatrema leonae	Bray & Cribb, 2000	Stomach	HI
	Thulinia microrchis	Bray et al., 1993	Stomach	HI
Microscaphidiidae	***Hexangium* cf. *sigani****	Current study*	Intestine	PI
Aporocotylidae	*Phthinomita hallae*	Nolan & Cribb, 2006	Heart	HI
	Phthinomita jonesi	Nolan & Cribb, 2006	Heart	LI
	Phthinomita sasali	Nolan & Cribb, 2006	Heart	P; GBR
Atractotrematidae	***Atractotrematrema sigani****	Current study*	Stomach, Intestine	PI
Enoplea				

Capillariidae	Capillariidae sp.	Moravec, 2001	Digestive Tract	NC
Myxozoa				
Ceratomyxidae	***Ceratomyxa* sp. 1***	Current study*	Gall	PI
	Ceratomyxa* sp. 2	Current study*	Gall	PI
	Ceratomyxa* sp. 3	Current study*	Gall	PI
Pomacentrus wardi				
Ectoparasites:				
Malacostraca				
Gnathiidae	***Gnathia* sp.***	Current study*	Body surface	PI
Hexanauplia				
Pennellidae	**Pennellidae sp.***	Current study*	Body surface	PI
Monogenea				
Ancyrocephalidae	gen. sp.	Rohde & Hobbs, 1988	Gills	CB / LI
	Pseudohaliotrema* sp. 1	Current study*	Gills	PI
Neoophora				
Graffillidae	***Paravortex* sp.***	Current study	Body surface / Gills	PI
Endoparasites:				
Trematoda				
Derogenidae	**Derogenidae sp.***	Current study*	Intestine	PI
Lecithasteridae	*Hysterolecitha nahaensis* •	Barker et al., 1994	N/A	HI
	Lecithaster stellatus •	Bray et al., 1993	Intestine	GBR

		Lecithasteridae sp. •	Current study*	Intestine	PI
	Lepocreadiidae	*Lepotrema monile*	Bray & Cribb, 1998	N/A	HI
	Bivesiculidae	***Bivesicula claviformis****	Current study*	Intestine	PI
	Cryptogonimidae	*Mitotrema anthostomatum*	Cribb et al., 1996	N/A	HI
	Faustulidae	**Faustulidae n. sp.***	Current study*	Intestine	PI
	Heterophyidae	*Galactosomum bearupi*	Beuret et al., 2000	Brain	HI
	Chromadorea				
	Camallanidae	*Spirocamallanus* sp.	Lester & Sewell, 1989	Intestine	HI
	Myxozoa				
	Ceratomyxidae	*Ceratomyxa sewelli*	Gunter & Adlard, 2008	Gall	LI
		Ceratomyxa moseri	Gunter & Adlard, 2008	Gall	LI
		***Ceratomyxa* sp. 4 •**	Current study	Gall	PI
		***Ceratomyxa* sp. 5 •**	Current study	Gall	PI
Pomacentrus adelus					
Ectoparasites:					
	Hexanauplia				
	Pennellidae	**Pennellidae sp.***	Current study	Body surface	PI
	Monogenea				
	Ancyrocephalidae	***Pseudohaliotrema* sp. 1***	Current study*	Gills	PI
	Neoophora				
	Graffillidae	***Paravortex* sp.***	Current study	Body surface / Gills	PI

Endoparasites:					
	Trematoda				
	Derogenidae	**Derogenidae sp.***	Current study	Intestine	PI
	Lecithasteridae	**Lecithasteridae sp.***	Current study	Intestine	PI
		Hysterolecitha* sp.	Current study	Stomach	PI
	Faustulidae	**Faustulidae sp. nov.***	Current study	Intestine	PI
	Chromadorea				
	Raphidascarididae	***Hysterothylacium* sp.***	Current study*	Intestine	PI
		Raphidascarididae sp.	Current study*	Stomach	PI
	Myxozoa				
	Ceratomyxidae	***Ceratomyxa* sp. 6***	Current study	Gall	PI

2.3.1 *Parasite community composition*

There was a clear separation of the parasite community of *S. doliatus* from those of the two pomacentrid species (PERMANOVA: $F_{2,61} = 8.31$, $P < 0.01$; Figure 2-2), with a high degree of overlap in the parasite communities of *P. adelus* and *P. wardi* (PERMANOVA: $F_{2,61} = 8.31$, $P = 0.23$). The parasite community of *S. doliatus* was characterised by relatively high abundance of caligid copepods, corallanid isopods and microscaphid, attractotrematid and gyliauchenid digeneans, while those of *P. adelus* and *P. wardi* were characterised by pennellid copepods and bivesiculid, derogenid and lecithasterid digeneans.

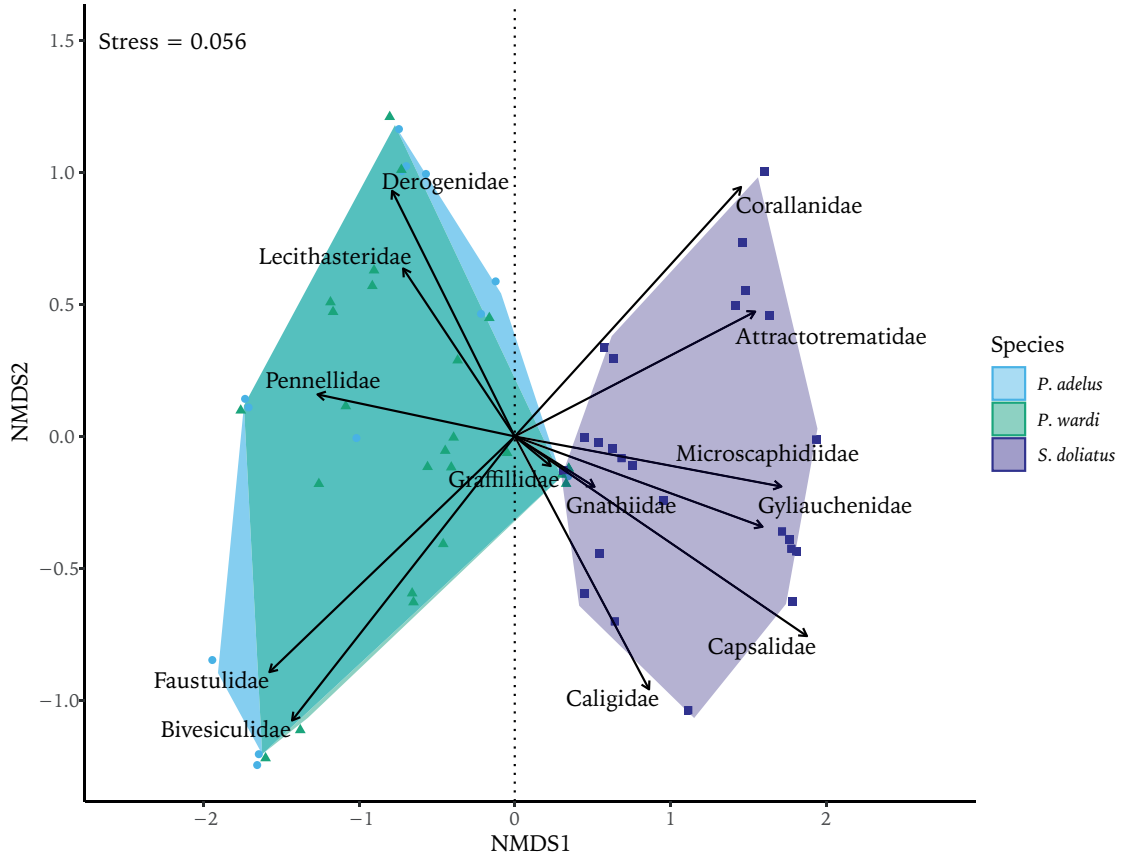

Figure 2-2: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the overall parasite communities of *Siganus doliatus* (n = 25), *Pomacentrus wardi* (n = 24) and *Pomacentrus adelus* (n = 13) from Pioneer Bay, Orpheus Island, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed infection intensity data (i.e., only infected hosts). Each point represents individual fish. Polygons

represent each fish species. Vectors represent the partial regression coefficients of the original variables (parasite species) with the two dimensions. Vector length is proportional to the degree of correlation between the parasite family and the ordination. See Appendix A: Fig. S2.1 and S2.2 for additional ordinations.

2.3.2 Parasite abundance and taxon richness

Abundance of parasites was highly variable among and within species, ranging from 0 – 8 parasites per fish in *P. adelus*, 0 – 22 in *P. wardi,* and 0 – 1,947 parasites per fish (0 – 333 parasites per fish excluding the outlier) in *S. doliatus*. Total parasite abundance (excluding ceratomyxid myxozoans, ancyrocephalid monogeneans, and the outlier) varied significantly among the three fish species and was greatest in *S. doliatus* (adjusted mean = 20.7 ± 4.8 SE parasites per fish), lowest in *P. adelus* (adjusted mean = 1.4 ± 0.4 SE parasites per fish; $P < 0.01$), and intermediate in *P. wardi* (adjusted mean = 4.8 ± 1.1 SE parasites per fish; $P < 0.01$; Figure 2-3a).

The taxon richness of parasite communities also varied among species with *S. doliatus* and *P. wardi* infected by a significantly greater number of parasite families (*S. doliatus* adjusted mean = 3.2 ± 0.3 SE families per fish, $P < 0.01$; *P. wardi* adjusted mean = 2.3 ± 0.3 SE; $P < 0.01$) than *P. adelus* (adjusted mean = 0.93 ± 0.2 SE families per fish; Figure 2-3b).

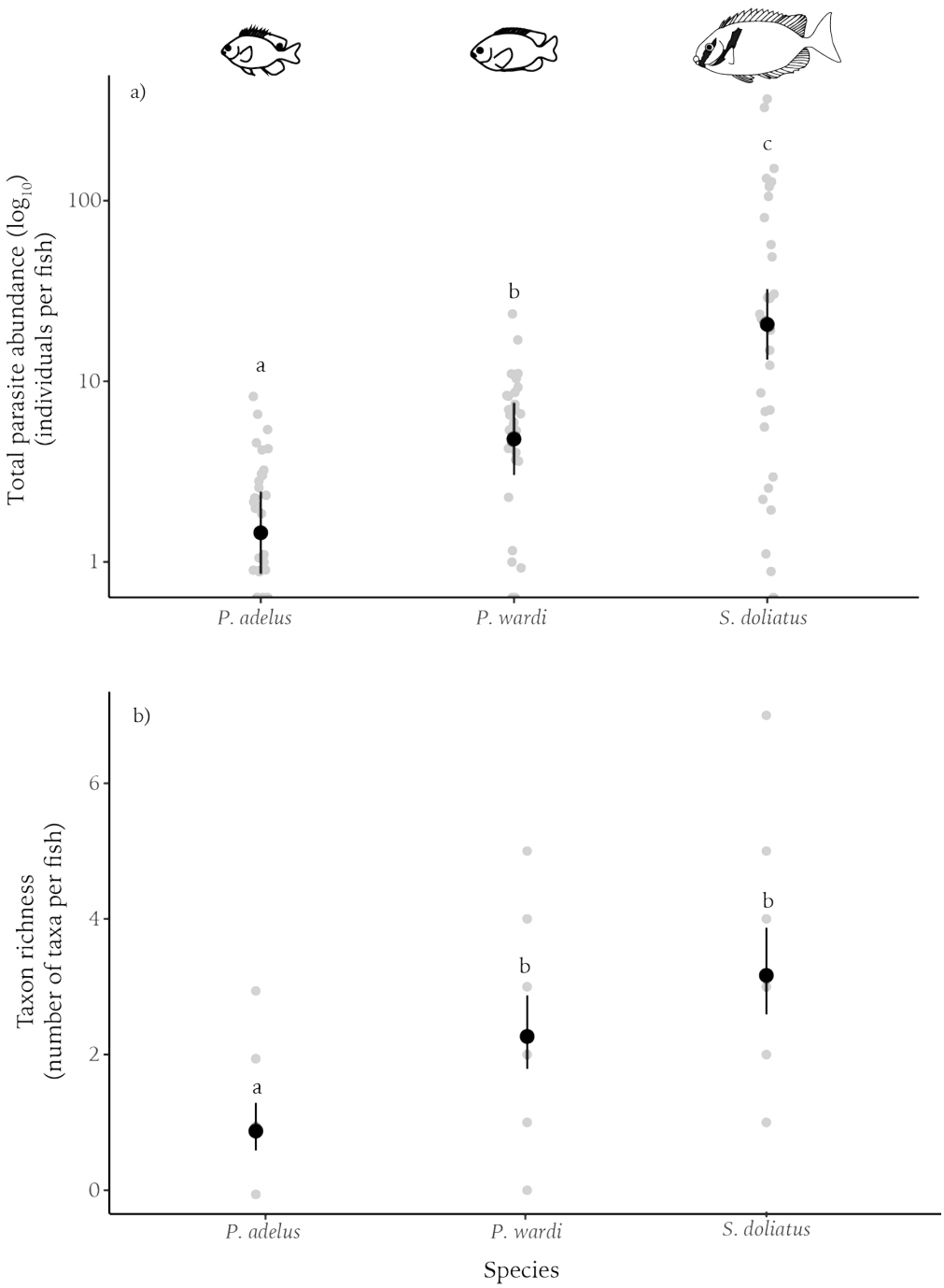

Figure 2-3: Differences in (a) total parasite abundance and (b) taxon richness (number of parasite families) among three co-occurring herbivorous fishes, *Pomacentrus adelus*, *Pomacentrus wardi* and *Siganus doliatus*, from Pioneer Bay, Orpheus Island, central Great Barrier Reef. Lines represent 95% confidence intervals; black points represent adjusted means; grey points represent raw data. Letters represent significant differences between species (Tukey's HSD; $P < 0.05$).

2.3.3 Ecto- and endo-parasite abundance

The abundance of both ecto- and endo-parasites differed significantly among species. Ectoparasite abundance was lower on *P. adelus* (adjusted mean = 0.2 ± 0.1 SE ectoparasites per fish) than the other two species, with *P. wardi* (adjusted mean = 2.0 ± 0.5 SE ectoparasites per fish; $P < 0.01$) and *S. doliatus* (adjusted mean = 1.3 ± 0.3 SE ectoparasites per fish; $P < 0.01$) having similar abundance of ectoparasites ($P = 0.4$; Figure 2-4a). In contrast, the abundance of endoparasites was significantly greater in *S. doliatus* (adjusted mean = 17.3 ± 4.4 SE endoparasites per fish, $P < 0.01$) than the two pomacentrid species, with *P. wardi* and *P. adelus* having similar abundances of endoparasites (adjusted mean = 2.2 ± 0.6 SE endoparasites per fish and 1.1 ± 0.3 SE, respectively; $P = 0.22$; Figure 2-4b).

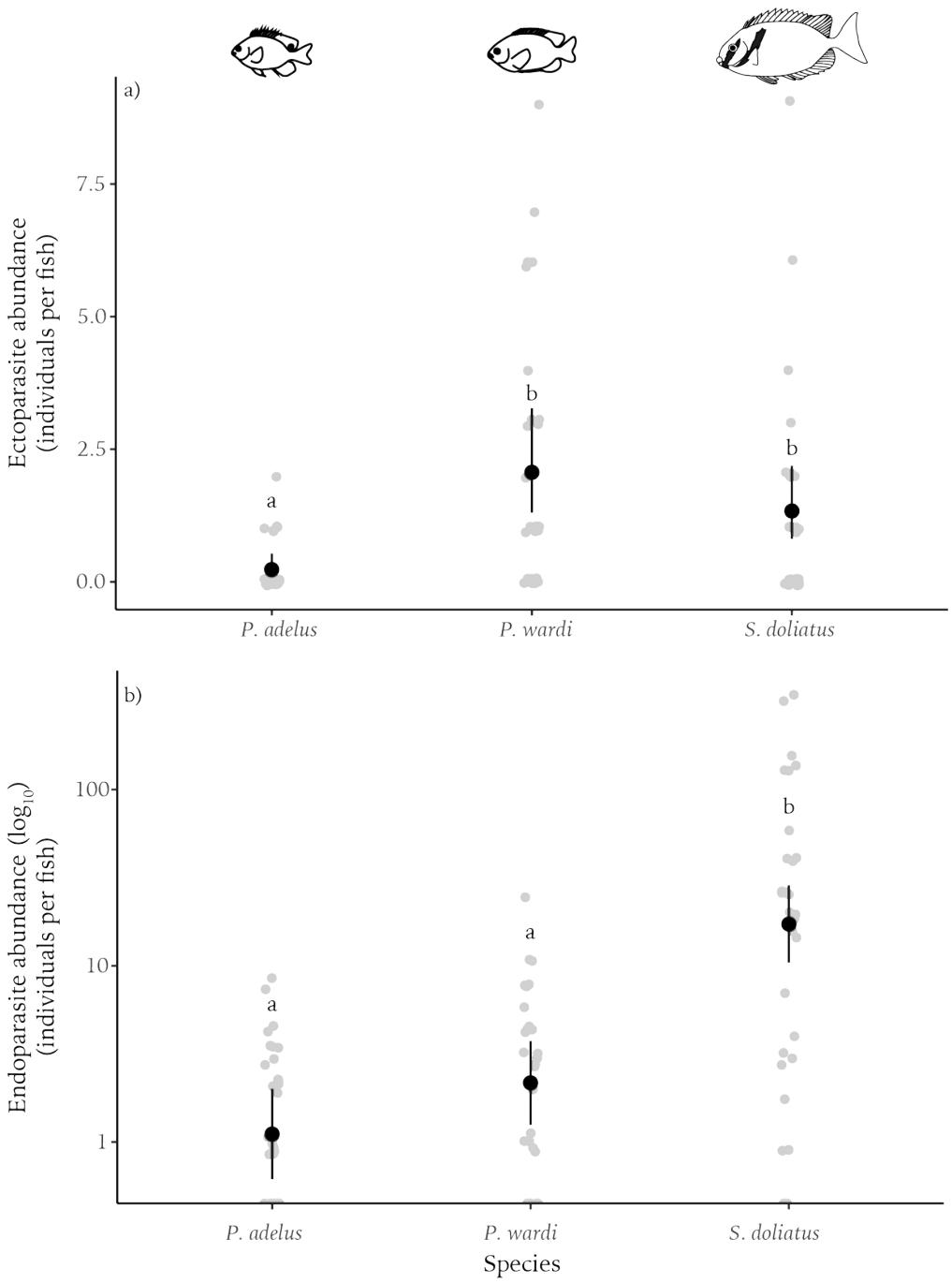

Figure 2-4: Differences in the abundance of a) ectoparasites and b) endoparasites infecting three co-occurring herbivorous fishes, *Pomacentrus adelus*, *Pomacentrus wardi* and *Siganus doliatus*, from Pioneer Bay, Orpheus Island, central Great Barrier Reef. Lines represent 95% confidence intervals; black points represent adjusted means; grey points represent raw data. Letters indicate significant differences between species (Tukey's post-hoc test, $P < 0.05$).

2.3.4 Overall prevalence

Due to the high number of fish infected with parasites (*S. doliatus*: n = 30, *P. wardi*: n = 29, *P. adelus*: n = 25), no significant difference in parasite prevalence was detected among species (see Appendix A: Table S2.2). However, the proportion of hosts infected with ectoparasites was significantly greater in *S. doliatus* (adjusted mean = 0.7 ± 0.1 SE) and *P. wardi* (adjusted mean = 0.7 ± 0.1 SE) relative to *P. adelus* (adjusted mean = 0.3 ± 0.1 SE; $P < 0.01$). No significant difference in endoparasite prevalence was observed among the three fish species (see Appendix A: Table S2.3). Whilst encysted and larval parasitic worms were unidentifiable to family, a high proportion of all three fish species were infected by encysted and larval parasitic worms (*S. doliatus*: 70%, *P. wardi*: 60%, *P. adelus*: 60%).

2.3.5 Abundance of common parasite taxa

Graffillid turbellarians and pennellid copepods were the most abundant parasite taxa, although the latter was only recorded from the two pomacentrid species. The abundance of both graffillid turbellarians and pennellid copepods varied among fish species. *Pomacentrus adelus* had the lowest abundance of graffillid turbellarians (adjusted mean = 0.1 ± 0.1 SE graffillid turbellarians per fish) relative to *P. wardi* (adjusted mean = 0.9 ± 0.3 SE; $P < 0.01$) and *S. doliatus* (adjusted mean = 0.7 ± 0.2 SE; $P < 0.05$; Figure 2-5a). Similarly, the abundance of pennellid copepods was significantly lower in *P. adelus* (adjusted mean = 0.1 ± 0.1 SE pennellid copepods per fish) than *P. wardi* (adjusted mean = 1.2 ± 0.3 SE, $P < 0.01$; Figure 2-5b).

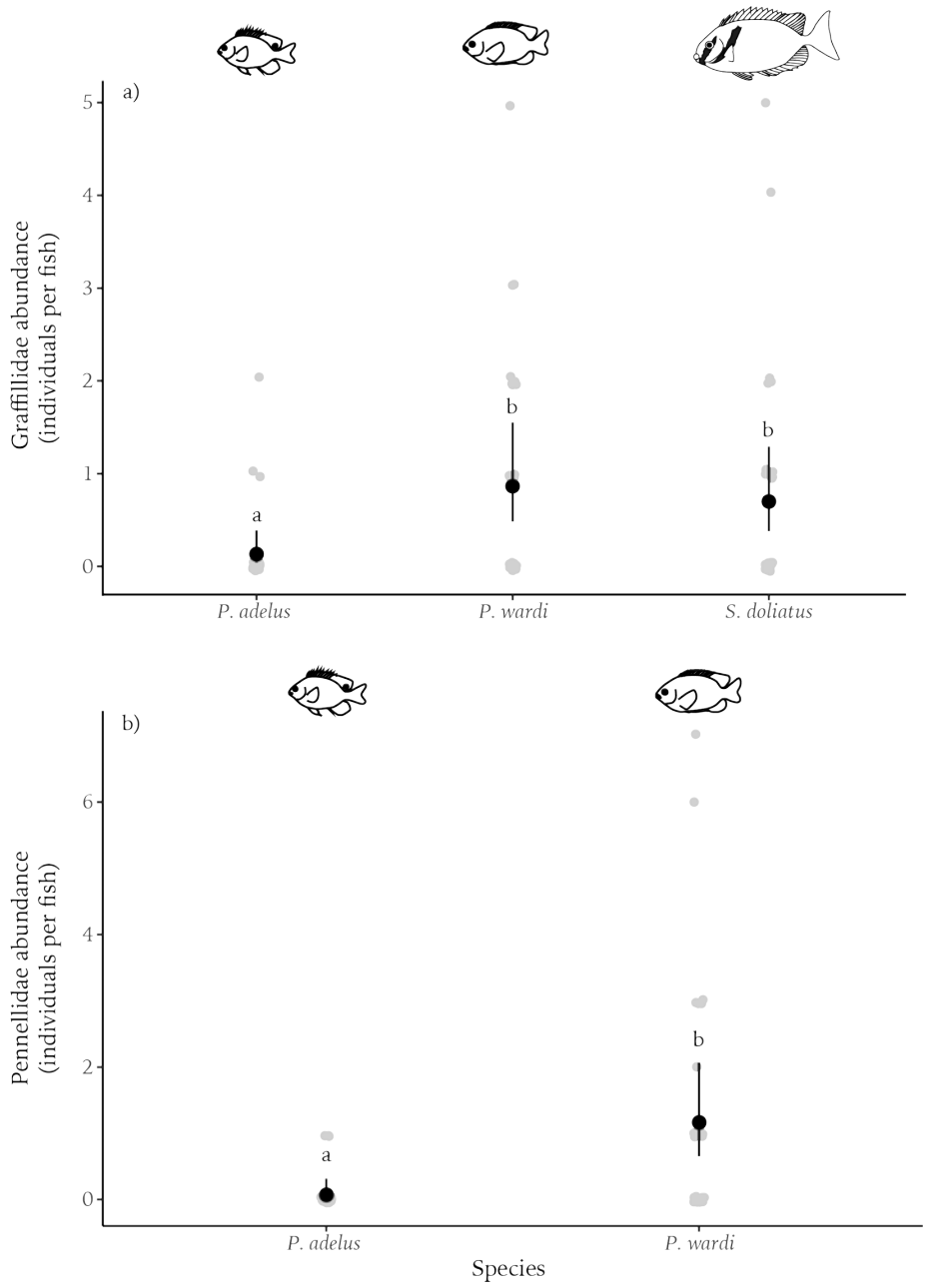

Figure 2-5: Differences in the abundance of a) graffillid turbellarians (Graffillidae) and b) pennellid copepods (Pennellidae) infecting three species of co-occurring herbivorous fish, *Pomacentrus adelus*, *Pomacentrus wardi* and *Siganus doliatus*, from Pioneer Bay, Orpheus Island, central Great Barrier Reef. No pennellid copepods were recorded from *S. doliatus*. Lines represent 95% confidence intervals; black points represent adjusted means; grey points represent raw data. Letters represent significant differences between species (Tukey's HSD; $P < 0.05$).

2.3.6 Host-parasite records and novel taxa

Two probable, novel species were also recorded in this study, an ectoparasitic capsalid monogenean, Capsalidae n. sp., found on the skin of *S. doliatus* and an endoparasitic digenean, Faustulidae n. sp., in the intestines of both *P. wardi* and *P. adelus.* Novel records of six parasite families and at least sixteen species were recorded for *S. doliatus,* as well as new records of six families and at least six species were documented for *P. wardi*. The parasite community of *P. adelus*, including eight families and at least ten species is documented for the first time (Figure 2-6).

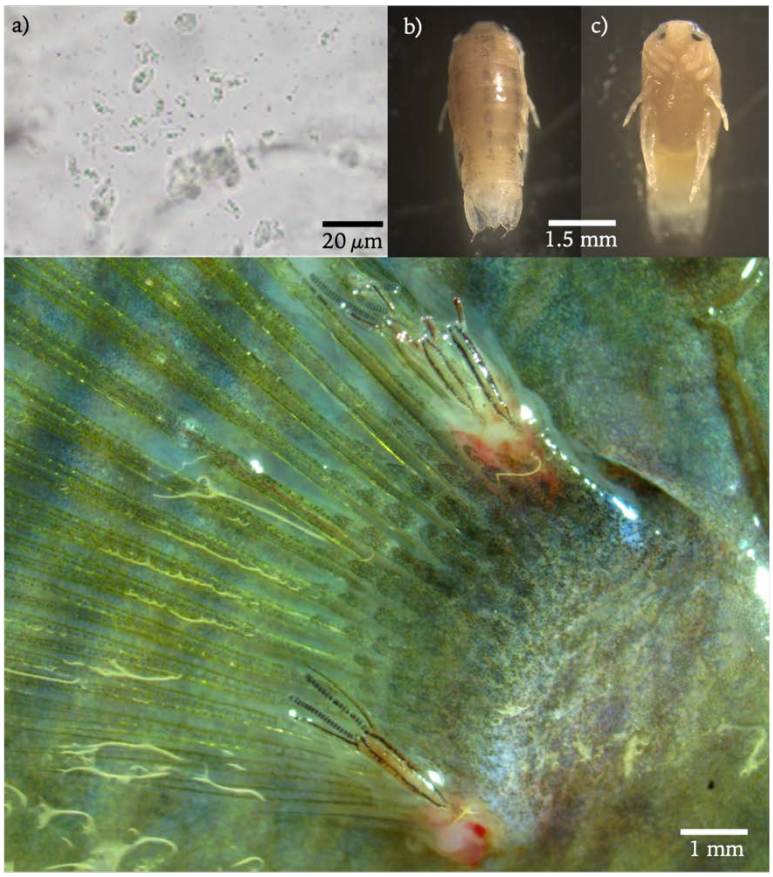

Figure 2-6: Photographs of parasites recovered from *Siganus doliatus*, *Pomacentrus wardi* and *Pomacentrus adelus* from Pioneer Bay, Orpheus Island, central Great Barrier Reef. a) *Ceratomyxa* spp. ex *Pomacentrus adelus* gall bladder; a species of Corallanidae ex *Siganus doliatus* external surfaces, b) dorsal view, c) ventral view; d) copepods from the family Pennellidae ex *Pomacentrus wardi*.

2.4 Discussion

Characterising the parasite communities of coral reef fishes is important to our understanding of coral reef biodiversity, ecology and inter-specific interactions. This study found significant differences in the abundance, richness and composition of parasites infecting three co-occurring herbivorous fishes (*S. doliatus*, *P. wardi* and *P. adelus*) from an inshore reef in the central GBR. Consistent with the initial hypotheses, the parasite communities of the two territorial pomacentrids were broadly similar, but distinct from those of the larger-bodied and more mobile rabbitfish, *S. doliatus*. Further, the larger-bodied *S. doliatus* (22.4 ± 0.3 cm SE), was infected with the greatest abundance and richness of parasite taxa relative to the smaller bodied *P. wardi* and *P. adelus* (mean TL: 7.3 ± 0.2 cm SE and 6.9 ± 0.2 cm SE cm, respectively). These patterns were largely driven by differences in the abundance and richness of endoparasites among the three fish species. Despite the similarity in their parasite community composition, there were differences in the abundance and richness of parasites infecting the two pomacentrid species. In general, *P. wardi* had a greater abundance and richness of parasites overall and a greater abundance of and prevalence of infection by ectoparasites than *P. adelus*. Differences in the parasite communities of *S. doliatus* and the two pomacentrid species, and between the two pomacentrids, may be related to differences in their diet, phylogeny, behaviour and/or body size.

2.4.1 Diet

The observed differences in the endoparasite communities of *S. doliatus* and the two pomacentrids may be related to differences in their diet and/or feeding ecology (Campbell et al., 1980; Rohde, 2005). *Pomacentrus wardi* and *P. adelus* are territorial, or farming, damselfishes that cultivate and feed predominantly on several species of algae, together with detritus and invertebrates within their territories (Ceccarelli, 2007; Kramer et al., 2013). Three of the major/most abundant endoparasites infecting both *P. adelus* and *P. wardi* (i.e., the bivesiculid *Bivesicula claviformis*, derogenid and lecithasterid digeneans) occurs through the consumption of either a first intermediate gastropod or second intermediate crustacean host infected with the

parasite larvae (cercariae or metacercariae respectively; Cribb, Bray, & Barker, 1994; Cribb et al., 1998; Køie & Gibson, 1991; Rohde, 2005). While invertebrates only represent a very small proportion of the material ingested by *P. wardi* and *P. adelus*, it seems likely that their direct or incidental consumption may be a likely source of infection for these parasites. In contrast, *S. doliatus* typically feeds on a broader range of larger corticated and foliose macroalgae (e.g., *Hypnea* spp., *Gracilaria* spp., *Padina* spp.; Fox et al., 2009; Hoey et al., 2013). The dominant endoparasites infecting *S. doliatus* in the present study (Atractotrematidae, Gyliauchenidae and Microscaphidiidae) also use a molluscan first intermediate host, from which cercariae (larval digeneans) emerge and encyst on aquatic vegetation and have been shown to infect *S. doliatus* through its consumption of specific algal taxa (Al-Jahdali & Hassanine, 2012; Hassanine et al., 2016; Huston et al., 2018). Although further research is required to ascertain the infection pathways of these endoparasites, and whether these, or similar parasite assemblages, are shared by other fish species with similar feeding ecologies, it seems possible that differences in diet may have contributed to the observed differences in endoparasite communities of these three species.

2.4.2 Host-parasite interactions

The greater abundance of endoparasites in *S. doliatus* was largely attributed to gyliauchenid digeneans (f. Gyliauchenidae) that were found to infect 73% of the *S. doliatus* individuals examined, with up to 1,916 specimens found to infect a single fish. Gyliauchenids are digenetic trematodes found exclusively in herbivorous coral reef fishes (Hall & Cribb, 2005). Within the Indo-West Pacific, the rabbitfishes (f. Siganidae) are host to up to 52% of described gyliauchenid species (Hall, 2004). Gyliauchenid digeneans feed on host gut contents and may be particularly prevalent and in high abundance in the digestive tract of herbivorous fishes with a fermentative gut (Clements & Choat, 1995; Hall & Cribb, 2005). They have been hypothesised to benefit the host by assisting in the digestion of macroalgae (Hughes-Stamm et al., 1999; M. K. Jones et al., 2000). Further, host mortality rate associated with gyliauchenid infections is thought to be negligible, with few known incidences of pathogenesis, as these worms are mobile and create

little site-specific damage (Rohde, 2005). The relationship between gyliauchenid digeneans and *S. doliatus* may therefore be more mutualistic than parasitic, allowing a single *S. doliatus* to harbour significant numbers with little or no adverse effects. In contrast, most digenean families, such as those found to infect *P. adelus* and *P. wardi* (Bivesiculidae, Derogenidae, Lecithasteridae and Faustulidae), are generally considered more damaging to the host, feeding on mucus, epithelial cells and sometimes blood, often leading to significant pathogenesis (Rohde, 2005).

2.4.3 Body size and parasitism

The abundance and richness of ectoparasites infecting coral reef fish and other animal taxa have previously been linked to differences in body size, with larger bodied animals providing increased surface area, greater resource volume and a potentially greater number of niches (Dáttilo et al., 2020; Lo et al., 1998; Muñoz et al., 2007; Poulin, 1995). Of the three fish species studied, *S. doliatus* is the largest and was infected with the greatest abundance of parasites overall, largely driven by its high abundance of endoparasites (discussed above). However, no difference in ectoparasite abundance was observed between *P. wardi* and *S. doliatus* despite substantial differences in body size and mobility. Moreover, *P. wardi* (mean TL = 7.3 cm) was infected by a significantly greater abundance and richness of ectoparasites than *P. adelus* (mean TL = 6.9 cm), despite a minimal difference in mean body size (i.e., 0.4 cm) between them. It appears unlikely that such a small difference in body size would contribute to the variation in ectoparasite infection between these two pomacentrid species. Similarly, Caro et al., (1997) found differences in the parasite richness of confamilial fish species (f. Mugilidae and Sparidae) of similar size and ecology. The differences in parasitism of *P. wardi* and *P. adelus* may therefore be due to differences in their ecology, demography (e.g., age; Lo et al., 1998), immunity and infection history (Sol et al., 2003), differences in host and parasite behaviours (Bush & Clayton, 2018), such as differences in diurnal activity (Strohm et al., 2001), and host densities (Arneberg, 2002; Arneberg et al., 1998). Differences in farmed algal communities may also affect the abundance and richness of parasites infecting *P. wardi* relative to *P. adelus.* The algae within the

territories of *P. wardi* are typically dominated by *Polysiphonia*, *Lobophora* and *Jania*, whereas territories of *Pomacentrus adelus* are typically dominated by *Polysiphonia* and *Galaxaura,* although the composition can vary geographically (Ceccarelli, 2007). These differences in farmed algal communities may provide alternate attachment structures for parasite species, different habitats for parasite intermediate hosts and free-living stages, and thus potentially influence the abundance and richness of parasites infecting these two species.

2.4.4 Geographic variation in parasite communities

This study is the first to characterise the metazoan parasite communities of *S. doliatus*, *P. wardi* and *P. adelus*. Comparisons to existing host-parasite records for *S. doliatus* and *P. wardi* within the broader GBR (see Table 2-1), and for *S. doliatus* and siganid species in other regions (*Siganus sutor* in Kenya: Martens & Moens, 1995; *Siganus argenteus, Siganus luridus and Siganus rivulatus* from the Red Sea Diamant & Paperna, 1986; Dzikowski et al., 2003a; Hassanine & Al-Jahdali, 2007), provide insights into the potential influence of host-parasite co-evolution and geography to the parasite communities of these fish species. For example, abundant parasite taxa infecting *P. wardi* (pennellid copepods and derogenid digeneans) and *S. doliatus* (*Pseudohaliotrema* sp.) at Orpheus Island have not been recorded to infect these species on mid-shelf reefs of the GBR (i.e., Lizard or Heron Island; see Table 2-1). Conversely, parasites recorded to infect *P. wardi* (*Spirocamallanus* sp., Lester & Sewell, 1989; *Lepotrema* sp., Bray & Cribb, 1998) and *S. doliatus* (*Phthinomita* sp., Nolan & Cribb, 2006; *Lepeophtheirus* sp., Grutter, 1994) at other GBR locations were not recorded to infect these species within the present study at Orpheus Island. These apparent differences in the parasite communities of *S. doliatus* and *P. wardi* add to a growing body of research documenting geographic variation in the parasite communities of reef fish within the GBR (Cribb et al., 2014; Grutter, 1994; Trieu et al., 2015). Conversely, some parasite taxa appear to be common across greater geographic distances. For example, several families and parasite genera appear to be conserved across *S. doliatus* within the Pacific Ocean (e.g., *Gyliauchen* sp. at Heron, Lizard and Orpheus Island, Noumea and New

Caledonia, Hall, 2004; Hall & Cribb, 2004, 2008; Hughes-Stamm et al., 1999; *Phthinomita* sp. at Heron Island, Lizard Island and Palau, Nolan & Cribb, 2006; Hughes-Stamm). Moreover, these parasites are also conserved in congeneric species, with *Siganus argenteus, Siganus rivulatus* and *Siganus luridus* from the Red Sea reported to be infected with *Ceratomyxa* sp., gyliauchenid digeneans and *Gnathia piscivora* (Diamant & Paperna, 1986). Similarly, *Siganus sutor* from the Indian Ocean shares infections of *Tetrancistrum* sp., *Pseudohaliotrema* sp., *Caligus* sp., *Gnathia* sp., *Hexangium sigani* and gyliauchenid digeneans with *S. doliatus* from the GBR (Martens & Moens, 1995). These parasite species that are shared among congeneric hosts may potentially be more resilient to disturbances and habitat loss. However, with coral reefs experiencing increasingly frequent climatic and anthropogenic stressors, the potential loss and fragmentation of these habitats may reduce populations of coral reef parasites, particularly those species with smaller distributions, found in specific regions of the GBR.

2.4.5 Summary

This study was the first to document the parasite communities of *S. doliatus, P. wardi* and *P. adelus*, expanding on our understanding of parasite species' associations of these fishes. In doing so it has identified two potentially new species, provided several novel host-parasite records and the first parasite records for *P. adelus.* Baseline data on the parasite communities of these three common, herbivorous fish species can facilitate future comparisons to understand how parasite communities vary with environmental degradation and change. This is particularly relevant for coral reefs given the current and predicted future disturbances to which they are exposed (Hughes, Barnes, et al., 2017; Vercelloni et al., 2020). Whilst phylogeny and geography are major determinants of fish parasite communities, differences in parasitism and parasite communities observed among the three species are also likely related to differences in their diet, mobility and habitat use. Further comparisons of the parasite communities of multiple reef fishes from within and across functional groups, fish families and locations are required to evaluate the role and

interplay of these ecological, geographic and phylogenetic variables in determining parasite community composition and parasitism in coral reef fishes.

Chapter 3: Benthic habitat composition affects parasite communities of a common coral reef fish, *Pomacentrus wardi*

3.1 Introduction

Interactions among and within species (e.g., competition, predation, pollination, seed-dispersal, symbiosis, reproduction and parasitism) play a vital role in the structure and function of ecosystems (Nagelkerken & Munday, 2016; Valiente-Banuet et al., 2015). The nature and strength of these interactions are affected by the composition and abundance of individual species, and the availability, diversity and complexity of habitats (Gosnell et al., 2012; Reynolds et al., 2018). Changes in global climate and increased anthropogenic pressures are affecting the health and structure of habitats across the world's ecosystems (Alvarez-Filip et al., 2009; Wang et al., 2008). As a result, climate change and anthropogenic stressors are becoming the dominant drivers of the composition and structure of ecosystems (Casatti et al., 2006; Graham et al., 2006). For example, habitat degradation on coral reefs has led to increased rates of predation of reef fishes when associated with dead, bleached and algal-covered coral colonies relative to those associated with healthy coral colonies (Coker et al., 2009). Changes in species interactions are likely to have flow-on effects to populations, community composition, and ultimately the functioning of the entire ecosystem. Host-parasite interactions are among the most prevalent inter-specific interactions across a range of ecosystems. Therefore, understanding how parasite communities vary in response to habitat degradation is increasingly important due to ongoing climatic change and local anthropogenic stressors.

The degradation of habitats and consequent changes in the composition of habitat-forming taxa have been shown to alter parasite communities and host-parasite interactions in terrestrial and aquatic systems (Chapman et al., 2015; Gillespie & Chapman, 2008; Huspeni & Lafferty, 2004). Through changing the availability of suitable habitats for the proliferation of parasite life stages (dry forest; Kiene et al., 2021) and affecting host exposure to parasites (Behie et al., 2014), habitat

degradation, especially changes in the composition of habitat-forming taxa, can affect the abundance of hosts, parasites and/or vectors (rainforest; Tchoumbou et al., 2020). Shifts in the composition of habitat-forming species can also increase host stress and their susceptibility to infection (streams; Ramírez-Hernández et al., 2019), and may affect the ability of hosts to avoid parasite infections through changes in the chemical environment (coral reefs; Narvaez et al., 2021). Whilst there has been a considerable body of work on the effects of habitat change and degradation on host-parasite interactions and parasite communities across a range of freshwater and terrestrial ecosystems, relatively few have examined such relationships in coral reef ecosystems (for exceptions see Artim et al., 2020; Artim & Sikkel, 2013; Dzikowski et al., 2003a; Narvaez et al., 2021; Paula et al., 2021; Santos & Sikkel, 2019).

Of the few studies that have investigated the relationship between habitat degradation and parasitism on coral reefs, the majority have focused on a single taxon, gnathiid isopods (Artim et al., 2020; Artim & Sikkel, 2013; Narvaez et al., 2021; Santos & Sikkel, 2019). These studies found that predation-risk was likely responsible for the increased abundance and emergence of gnathiid isopods in rubble habitats, with coral polyps capable of heterotrophically consuming gnathiid isopod larvae (Artim & Sikkel, 2013; Paula et al., 2021). With the prevalence of rubble habitats likely to increase in the future due to disturbance, gnathiid isopods are expected to increase in abundance (Artim et al., 2020; Santos & Sikkel, 2019). Changes in the chemical landscape of degraded coral reefs has also been linked to the number of gnathiid isopod infections, with higher abundances of gnathiid isopods infecting juvenile coral reef fish in water conditioned with dead coral, relative to those held in water conditioned with live coral (Narvaez et al., 2021).

In addition to gnathiid isopods, coral reef fishes typically harbour species-rich and diverse communities of ecto- and endo-parasites that will likely be affected by the degradation of coral reefs. However, to date, no study has investigated the effect of coral reef habitats and substrata on the composition of the entire parasite community of a coral reef fish. The aim of this study

was to compare parasite communities infecting a common coral reef fish, *Pomacentrus wardi*, among three habitat types representing a gradient of coral reef health (live coral, macroalgae and rubble).

3.2 Methods

3.2.1 Study site

This study was conducted between January and March 2018 at three inshore islands (Orpheus, Pelorus and Fantome Islands) within the Palm Island Group, central Great Barrier Reef (GBR), Australia (Figure 3-1). These continental islands are situated approximately 15–22 km east from the mainland coast and have well-developed fringing reefs on their western, or leeward, margins. Three sites were selected on the reef crest and outer reef flat (2–5 m depth) of each island (i.e., nine sites in total) with one site on each island representing each of three distinct benthic compositions: coral, macroalgae and rubble habitats. Each site was approximately 100m x 10m (1,000m^2) in areas of contiguous reef and consisted of relatively homogenous habitat types. Coral habitats were characterised by relatively high cover of branching *Porites* (i.e., *P. cylindrica*) and massive *Porites*; macroalgae habitats were characterised by high cover of macroalgae, predominantly the corticated red macroalgae *Hypnea* spp. and *Laurencia* spp., and rubble habitats by a lack of live coral and macroalgae, and high rubble cover (see Appendix B: Methods and Results).

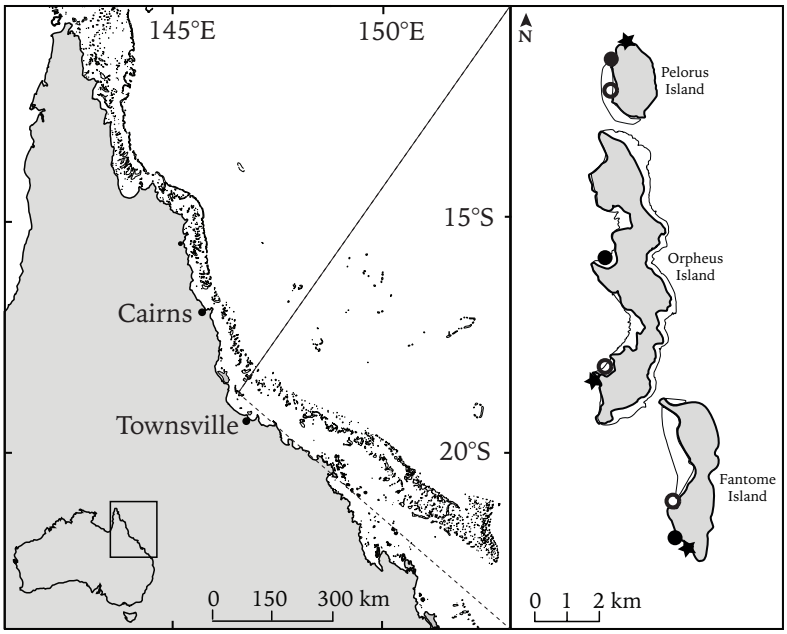

Figure 3-1: Map of the Great Barrier Reef; inset: Pelorus, Orpheus and Fantome Islands in the central Great Barrier Reef, Australia, showing the location of the nine study sites. Filled circles represent coral sites, open circles represent macroalgae sites and stars represent rubble sites.

3.2.2 Study species

Ward's damsel, *Pomacentrus wardi* (Allen 1990) was selected as the study species as it is abundant on inshore reefs of the GBR where it occurs in a range of habitats (Ceccarelli, 2007). *Pomacentrus wardi* is a small-bodied (max total length, TL 10.0 cm; Allen et al., 2015), herbivorous, farming (or 'territorial') damselfish that maintains relatively small territories (1–2 m^2: Breder & Rosen, 1966; Ceccarelli et al., 2006) dominated by *Polysiphonia*, *Lobophora* and *Jania* algal species (Ceccarelli, 2007). Being highly site attached means the parasites of individual *P. wardi* are likely to have originated from the habitat in which individuals are found.

3.2.3 Fish collection

This research was conducted under JCU ethics approval A2449 and fish were collected in accordance with section 20 of the Great Barrier Reef Marine Park Regulations 2018. A minimum of thirty *P. wardi* individuals were collected from each of the nine sites (mean total length = 6.4

cm; range = 4.3–9.9 cm) using a dilute clove oil solution, barrier nets and hand nets. Once captured, each fish was immediately placed in an individual, sealed aquarium-grade bag, provided with supplemental oxygen and transported to Orpheus Island Research Station. Each fish was then transferred into an individual 10 L static aquarium filled with filtered, UV-sterilised seawater and supplemental aeration. The bags in which the individual fish were held and transported were rinsed with filtered, UV-sterilised seawater, the contents filtered through a 63 µm mesh, and any parasites captured were preserved in 70% ethanol solution. Fish were maintained in individual aquaria for a maximum of seven days prior to dissection.

3.2.4 Site surveys

To account for any potential effects of local fish assemblages as sources of parasites and/or transmission of the parasites to *P. wardi*, four replicate 4 x 10 m belt transects were conducted at each site, with adjacent transects separated by approximately 10 m. The transect tape was laid simultaneously as the diver recorded all diurnally active, non-cryptic fish within a 4 m wide belt. Each fish species within the belt was identified to species or genus (see Appendix B: Methods and Results).

3.2.5 Fish dissections

Collected *P. wardi* were euthanised in a 0.15% solution of 2-Phenoxyethanol and then placed in individual freshwater baths for five minutes to dislodge any ectoparasites. The contents of each bath were filtered through a 63 µm sieve and any parasites captured on the sieve were preserved in a 70% ethanol solution. The total length and wet weight of each fish was then measured and the entire body surface, including inside the oral cavity and buccal folds, was inspected under a dissection microscope at 6.7 to 45x magnification for any ectoparasites that were still attached. Gills and opercula were removed, placed in filtered seawater and inspected under a dissecting microscope at 6.7 to 45x magnification. Parasitological analyses of visceral organs (i.e., heart, liver, spleen, gall bladder, brain, stomach and intestines and white muscle tissue) were conducted as per standard methods (Cribb & Bray, 2010; Hutson et al., 2007). Briefly, a sample of white

muscle tissue from around the visceral cavity, the entire heart, liver, spleen, gall bladder and brain were squashed onto slides to form a tissue layer approximately one cell thick. Slides were examined for parasites under a compound microscope at 200x magnification (400x magnification for the gall bladder). Due to the extremely high number of ancyrocephalid monogeneans (f. Ancyrocephalidae) infecting the gills, and the small size and high abundance of ceratomyxid myxozoans (f. Ceratomyxidae) infecting the gall bladder, their presence was recorded but abundance was not quantified. Parasites were removed from the stomach and intestines using a gut wash in which the organs were opened, sectioned and shaken vigorously in a physiological saline solution to dislodge any parasites. The tissues were then examined under a dissecting microscope at 6.7 to 45x magnification, and the saline solution allowed to settle and separate. After several minutes, approximately three quarters of the supernatant was discarded from the solution and the settled contents inspected for parasites under the dissecting microscope (Cribb & Bray, 2010). The eyes were also removed, dissected and visually inspected for parasites. All specimens recovered during dissections were preserved in 70% ethanol.

Due to logistical considerations, 159 of the 304 *P. wardi* were euthanised as described above and the entire fish preserved in 70% ethanol solution for later dissection and quantification of endoparasites. To ensure that internal organs and their parasites were preserved, the opercula were removed prior to preservation, exposing the gills, and an incision made along the ventral surface from the anus to the heart, exposing the internal organs to the ethanol solution. Prior to dissection of the preserved fish, the ethanol solution was filtered through a 63 µm sieve, and any dislodged parasites were collected. Parasites were identified to the lowest taxonomic rank using morphological characters with the assistance of taxonomic keys and/or soliciting specialist expertise (see Appendix B: Methods).

3.2.6 Statistical analyses

Statistical analyses were conducted using family-level assignments of parasite taxa. The only exceptions to this were tetraphyllidean metacestodes (15 individuals) and hemiruid digeneans (3

individuals) for which families were indeterminable based on the larvae detected. Of the nematode specimens recovered, only some were amenable to family-level identification (the remaining were identified to phyla) and so were excluded from taxon richness and community composition analyses, as were encysted larvae, as these likely form a complex of parasite taxa. Ancyrocephalidae (monogenean gill parasites) and Ceratomyxidae (myxozoans from the gall bladder) were excluded from abundance and community composition analyses due to abundance data not being collected for these taxa.

Prior to analyses, collinearity of independent variables was checked using variance inflation. The abundance of potential hosts (i.e., all fish), pomacentrids, as well as dissection method (fresh or after preservation) were included as predictor variables within candidate models, with both additive (Habitat + Island) and interactive (Habitat * Island) terms tested. To account for differences in body size among *P. wardi* individuals, the natural log of total length was included as an offset within all models (see Appendix B: Table S3.1). Akaike's Information Criterion corrected for small sample size (AICc) was used to determine the best candidate model, and the simplest model with the lowest AICc (within two units) was selected (See Appendix B: Table S3.2). All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018).

The abundance, prevalence (i.e., proportion of hosts infected) and richness of parasites (following A. O. Bush et al., 1997) were compared among habitats (categorical with three levels) and islands (categorical with three levels) using generalised linear and generalised linear mixed models, with separate models for ecto- and endo- parasites. Candidate models for richness and abundance data were created using both Poisson and negative binomial distributions, as these are typically used for count data. To inform the appropriate error structure for each model, residual diagnostics, over-dispersion and zero-inflation were tested using the '*DHARMa*' package to determine model fit and assumptions of the error distributions were examined using residual plots, Chi square

goodness-of-fit tests and dispersion (the ratio of the variance to the mean). The error distribution with the best model fit and satisfaction of model assumptions was selected for the analysis.

The abundance of ecto- and endo- parasites were modelled against a negative binomial distribution, the prevalence of ecto- and endo- parasite infection against a binomial distribution and taxon richness against a Poisson distribution. Tukey's post-hoc analyses were conducted using the '*emmeans'* package. The taxonomic richness and abundance of fish assemblages were compared among habitats and islands using a generalised linear model and generalised linear mixed effects model respectively, fitted against a Poisson distribution (typical for count data). For fish abundance, an observation-level random effect was used to control for over-dispersion.

The composition of the ecto- and endo-parasite communities, and local fish assemblages were compared among islands and habitats using PERMANOVA, with dissimilarities in parasite and fish communities among islands and habitats visualised using a non-metric multidimensional scaling (nMDS). The nMDS was based on Wisconsin double standardisation of count data for fish and parasite taxa and a zero-adjusted Bray-Curtis dissimilarity matrix using the '*vegan'* package in R. For parasite community analyses, any *P. wardi* without any ecto- or endo- parasitic infection were removed prior to analysis (ectoparasite community: n = 62 infected fish; endo-parasite community: n = 198 infected fish). Due to low abundance and prevalence of some parasite taxa, a 'dummy' species was added to each dataset, with equal abundance (n = 1) for each fish. Adding a 'dummy' species allows two individuals that may not share any parasite taxa, to have a single 'species' in common, with the same abundance (Clarke et al., 2006). Pairwise comparisons were conducted using the '*RVAideMemoire'* package (Hervé, 2020).

3.3 Results

Overall, 4,066 parasites from 17 families were recovered from the 304 *P. wardi* examined (mean abundance = 13.4 ± 0.8 SE parasites per individual; range = 0 – 81 parasites per individual). A total of 3,189 of the parasites recovered were in encysted form and so unidentifiable to family. The vast majority of *P. wardi* collected were infected by parasites, with only 22 of the 304

individuals examined (7.2%) showing no signs of any parasite infection. Copepods from the families Pennellidae (n = 114) and Caligidae (n = 108) were the two most abundant ectoparasite families recorded, representing 47% and 44% of total ectoparasite abundance, respectively. The three most abundant endoparasite families recorded were the digenean families Derogenidae (n = 192), Bivesiculidae (n = 163) and Lecithasteridae (n = 157) constituting approximately 10%, 9% and 8.5% of total endoparasite abundance, respectively (Figure 3-2).

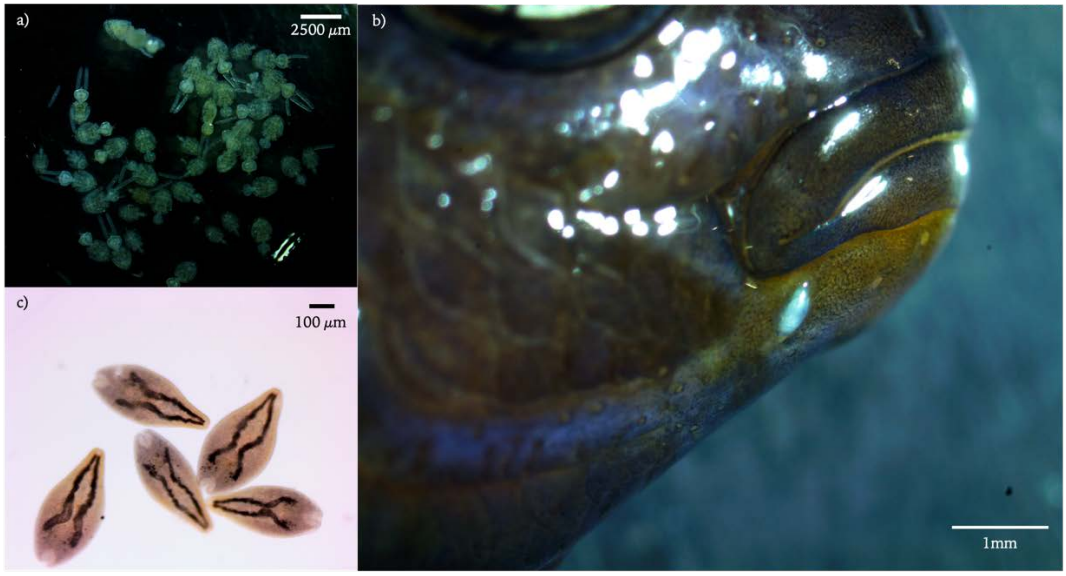

Figure 3-2: Photographs of common parasites ex *Pomacentrus wardi* from the Palm Island Group, Central Great Barrier Reef: a) caligid copepods from external surfaces; b) a turbellarian trematode infecting the lower jaw; c) juvenile *Bivesicula claviformis* from the intestines.

3.3.1 Ectoparasites

Variation in ectoparasite abundance was best explained by a negative binomial GLM featuring an interaction between habitat and island as the only independent variables (Appendix B: Table S3.2). Ectoparasite abundance did not differ among habitats on Fantome or Orpheus Island but was greatest at the macroalgae site at Pelorus Island (adjusted mean = 5.6 ± 2.4 SE parasites per host; $P < 0.05$) relative to the coral (adjusted mean = 0.2 ± 0.1 SE) and rubble sites (adjusted mean = 0.5 ± 0.3 SE). The lowest mean abundance of ectoparasites was observed at the

macroalgae site at Fantome Island (adjusted mean = 0.06 ± 0.05 SE parasites per host; Figure 3-3a).

Variation in the prevalence of ectoparasite infection and taxonomic richness were best explained by the model including habitat as the only independent variable. The proportion of *P. wardi* individuals infected with ectoparasites was lower in macroalgae (adjusted mean = 0.14 ± 0.04 SE) than rubble habitats (adjusted mean = 0.31 ± 0.05 SE) and intermediate in coral habitats (adjusted mean = 0.24 ± 0.04 SE; Figure 3-3b). Taxonomic richness of ectoparasites tended to be greatest on *P. wardi* collected from rubble (adjusted mean = 0.35 ± 0.06 SE parasite taxa per host), lowest on those from macroalgae (adjust mean = 0.18 ± 0.05 SE parasite taxa per host) and intermediate on those from coral habitats (adjusted mean = 0.25 ± 0.04 SE parasite taxa per host; Figure 3-3c), however this result was not significant.

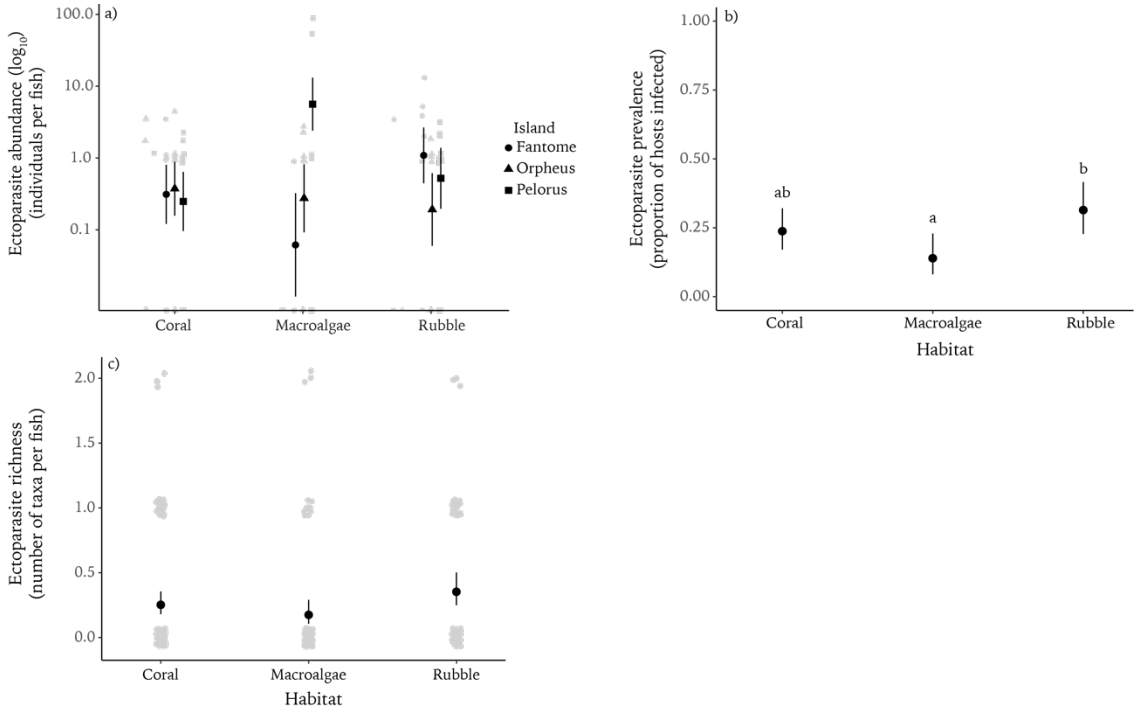

Figure 3-3: Differences in the a) abundance, b) prevalence, and c) taxonomic richness of ectoparasites infecting *Pomacentrus wardi* among coral, macroalgae and rubble habitats at Fantome, Orpheus and Pelorus Island of the central Great Barrier Reef. Lines represent 95%

confidence intervals; black circles represent adjusted means; grey points represent raw data. Letters represent significant differences between groups.

The ectoparasite community of *P. wardi* differed significantly among habitats (PERMANOVA: $F_{2,61} = 4.66$, $P < 0.01$; Figure 3-4). Ectoparasite communities of *P. wardi* within macroalgae habitats were characterised by a higher relative abundance of pennellid and caligid copepods and differed from those in coral and rubble habitats that were characterised by a higher abundance of graffillid turbellarians.

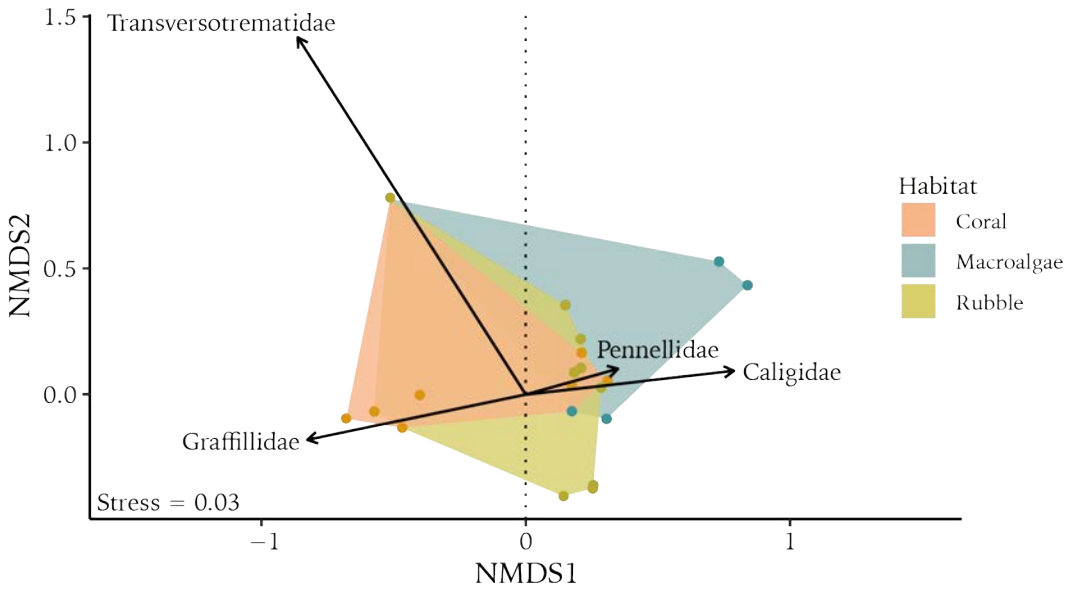

Figure 3-4: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the ectoparasite community composition of nine sites categorised as coral, macroalgae or rubble habitats, at Fantome, Orpheus and Pelorus Islands within the Palm Island Group, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed species abundance data. Each point represents a single infected *Pomacentrus wardi* (n = 62 infected fish). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (parasite taxa) with two dimensions. Vector length is proportional to the degree of correlation between the species and the ordination.

3.3.2 Endoparasites

Variation in endoparasite abundance was best explained by an additive negative binomial GLM of habitat, island, pomacentrid abundance and dissection method. Endoparasite abundance was greatest in coral (adjusted mean = 8.7 ± 1.1 SE parasites per host) intermediate in rubble (adjusted mean = 4.5 ± 0.4 SE parasites per host; $P < 0.01$) and lowest in macroalgae habitats (adjusted mean = 3.0 ± 0.6 SE parasites per host; $P < 0.01$; Figure 3-5a). Endoparasite abundances were also significantly greater at Fantome (adjusted mean = 7.4 ± 0.6 SE parasites per host) relative to Orpheus (adjusted mean = 5.1 ± 0.5 SE parasites per host; $P < 0.01$) and Pelorus Island (adjusted mean = 3.1 ± 0.3 SE parasites per host; $P < 0.01$; Figure 3-5b).

The candidate model that best explained the variation in the prevalence of endoparasite infection was an additive binomial GLM that included habitat, island and dissection method. Whilst the prevalence of endoparasitic infection in rubble habitats (adjusted mean = 0.95 ± 0.02 SE) was marginally greater than in macroalgal habitats (adjusted mean = 0.86 ± 0.04 SE), no significant difference in the prevalence of endoparasite infection among habitats was observed ($P = 0.054$; Figure 3-5c). However, the proportion of hosts infected at Pelorus Island (adjusted mean = 0.85 ± 0.04 SE; $P < 0.05$) was significantly lower than at Fantome Island (adjusted mean = 0.90 ± 0.02 SE; $P < 0.05$; Figure 3-5d). Variation in the taxonomic richness of endoparasites was best explained by the null model (i.e., logged total length as the only fixed factor) and indicated there were no differences among habitats or islands.

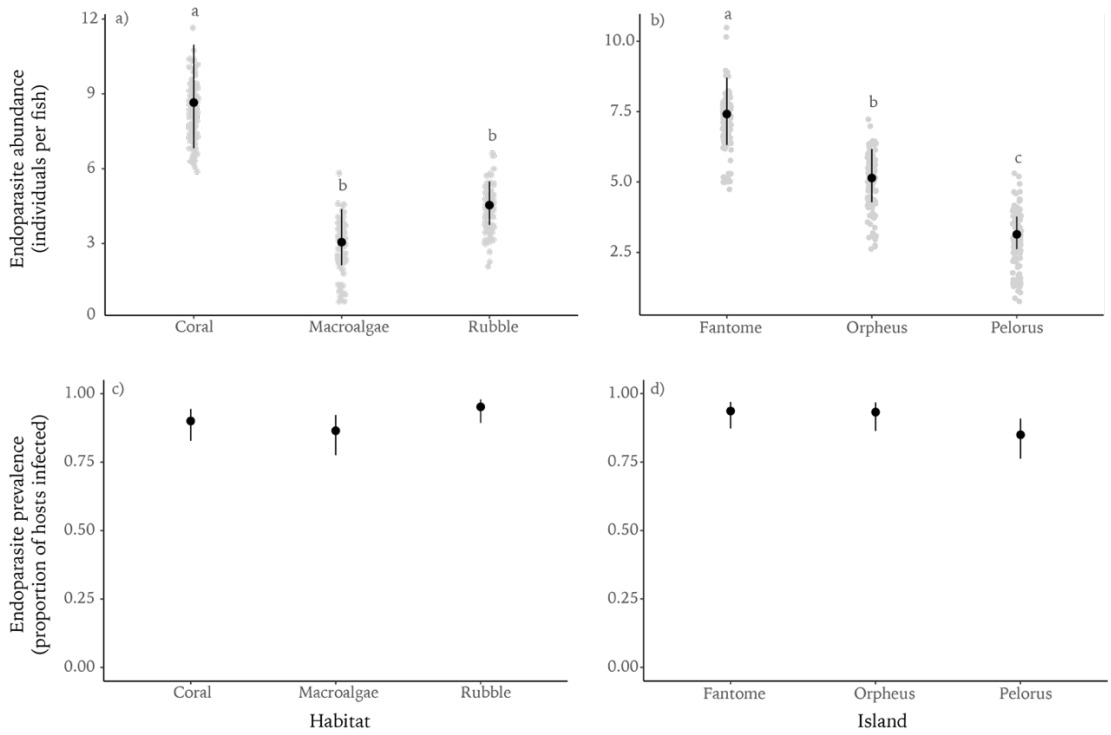

Figure 3-5: Differences in the a, b) abundance and c, d) prevalence of endoparasites infecting *Pomacentrus wardi* among coral, macroalgae and rubble habitats and Fantome, Orpheus and Pelorus island of the central Great Barrier Reef. Lines represent 95% confidence intervals; black circles represent adjusted means; grey circles represent model residuals. Letters represent significant differences between groups.

For the composition of the endoparasite community, there was a significant interaction between habitat and island (PERMANOVA: $F_{4,197} = 2.14$, $P < 0.05$; Figure 3-6). Coral sites at Fantome and Pelorus were typified by bivesiculid and lecithasterid digeneans. Rubble sites across all islands, as well as macroalgae sites at Fantome and Pelorus Island were typified by derogenid digeneans, whereas the macroalgae site at Orpheus Island was typified by bivesiculid digeneans.

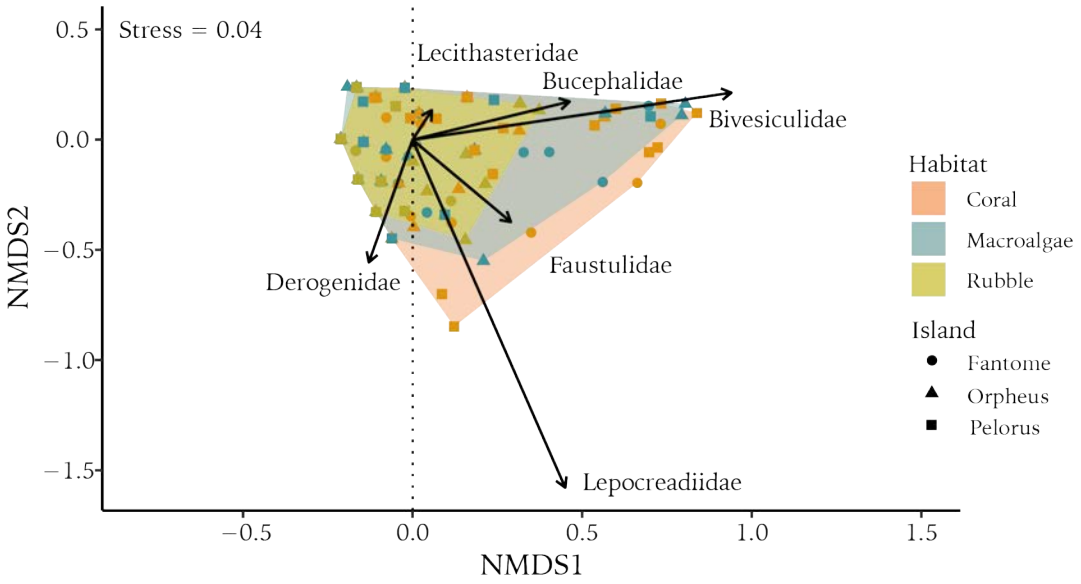

Figure 3-6: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the endoparasite community composition of nine sites categorised as coral, macroalgae or rubble habitats, at Fantome, Orpheus and Pelorus Islands within the Palm Island Group, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed species abundance data. Each point represents a single infected *Pomacentrus wardi* (n = 198 infected fish). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (parasite taxa) with two dimensions. Vector length is proportional to the degree of correlation between the species and the ordination. See Appendix B: Fig. S3.4 for additional ordinations.

3.4 Discussion

Understanding how changes in benthic composition may affect parasite communities on coral reefs, and the level of infection experienced by coral reef fishes, is crucial to our broader understanding of how habitat degradation may affect coral reef communities, biodiversity and ecosystem function into the future. This study found differences in ectoparasite community composition and prevalence, and in the abundance of endoparasites infecting *P. wardi* among coral reef habitats. A higher proportion of *P. wardi* were infected with ectoparasites in rubble (42%) relative to macroalgal habitats (15%). Habitat had no effect upon endoparasite community

composition or prevalence, but significantly affected endoparasite abundance, with *P. wardi* from coral habitats having higher abundances of endoparasites to those living in macroalgae and rubble habitats. The lower infection in macroalgal and rubble habitats may reflect a reduction in the abundance of intermediate invertebrate hosts in these habitats. Habitat condition appears to play an important, but poorly understood role in host-parasite interactions on coral reefs. Whilst there is evidence of the effects of degraded, rubble habitats on the abundance of the ectoparasite, *Gnathia* spp., this study provides evidence of an effect of habitat degradation upon the ecto- and endo-parasite community of *P. wardi*.

3.4.1 Endoparasite abundance

Differences in the abundance of endoparasites infecting *P. wardi* among habitats in this study may reflect the habitat associations of their intermediate invertebrate hosts. Whilst the invertebrate community was not surveyed in the present study, more than half of coral-associated invertebrates have an obligate association with live coral (Stella et al., 2011). Species of the Bivesiculidae, Derogenidae and Lecithasteridae, the most abundant endoparasite families infecting *P. wardi*, are transmitted via an intermediate gastropod or crustacean invertebrate host (Cribb, Bray, & Barker, 1994; Cribb et al., 1998; Køie & Gibson, 1991; Rohde, 2005). Therefore, the potential increase in intermediate host density and proximity in coral-dominated habitats, likely exposes *P. wardi* to a higher probability of encountering parasite transmissive stages, resulting in the greater abundance of endoparasites infecting *P. wardi* in coral-, relative to macroalgae- and rubble-dominated habitats (Arneberg et al., 1998; Behie et al., 2014).

The importance of habitat degradation in determining intermediate host abundance, and thereby parasitism, has been highlighted in other systems. For example, in marine coastal systems, the loss of polychaete and bivalve intermediate hosts due to sediment disruption following Hurricane Katrina caused numerous trematode parasite species to be absent within inshore systems for several years (Overstreet, 2007). In rainforests, habitat degradation has led to shifts in habitat-forming taxa to primary successional species. Ant intermediate hosts that associate with these

successional species consequently increased in abundance, increasing the abundance of endoparasites infecting black howler monkeys, the final host (Behie et al., 2014). Whilst under much debate, the reduced abundance of endoparasites in macroalgae and rubble relative to coral habitats supports other evidence that, in degraded habitats, heteroxenous parasites (i.e., parasites requiring multiple hosts to reach sexual maturity) are less likely to complete their life cycle, and so may be negatively affected by habitat degradation, relative to monoxenous (i.e., directly transmitted) parasites (Diamant et al., 1999; Dzikowski et al., 2003a, 2003b; Kiene et al., 2021; Pérez-del Olmo et al., 2007). The trends in ectoparasite abundance observed in the present study may therefore represent reduced habitat health in the macroalgae and rubble sites sampled.

Several studies have found the abundance of heteroxenous parasites infecting reef fish to reduce, and the abundance of monoxenous parasites to increase, in response to increases in fishing pressure (Wood et al., 2014; 2015; Wood & Lafferty, 2015). Whilst these studies investigate the effects of a different stressor to those in this thesis, they yield comparable results and as such provide evidence for the role of parasite life cycles in predicting their response to disturbance and environmental stressors (Dzikowsky et al., 2003; Olmo et al. 2007). One hypothesis for these observations is that heteroxenous parasites may be negatively affected by environmental disturbance through reductions in the abundance or removal of one of their hosts necessary to complete their life cycle (Dzikowsky et al, 2003). Alternatively, monoxenous parasites may be more resilient to changes in their environment as they are typically ectoparasitic and so are exposed to the external environment and its fluctuations (Olmo et al. 2007). Further investigation is required to determine the generality of these findings and the underlying mechanism/s.

3.4.2 Endoparasite community composition and prevalence

Despite the abundances of individual endoparasitic taxa varying among habitats, the endoparasite community and prevalence of infection were similar among coral, macroalgae and rubble habitats. This may be because *P. wardi* is a farming damselfish, cultivating its algal territory, the species within it and thereby controlling its algal food source. Therefore, if *P. wardi* cultivates

the same algal species in coral-, macroalgae- and rubble-dominated habitats, endoparasitic infection by the same endoparasitic species is likely, through consumption of the same intermediate invertebrate hosts that associate with these algal species. In the Caribbean, algal gardens of the territorial dusky damselfish, *Stegastes adustus*, contain more species of algae present in rubble territories compared to territories in areas of high live coral cover (di Santo et al., 2020). However, the variation in algal composition of *P. wardi* territories among coral reef habitats has yet to be investigated.

3.4.3 Prevalence of ectoparasitic infection

The increased proportion of *P. wardi* infected with ectoparasites in rubble-, relative to macroalgal-dominated habitats, may be due to differences in the structural complexity of rubble and macroalgal habitats and the availability of shelter and habitat for *P. wardi*. Whilst rubble habitats provide structural complexity and diversity at small scales, sufficient for macroparasites (Kramer et al., 2014), macroalgae habitats provide complex habitats for larger coral reef species, such as juvenile reef fishes and adult pomacentrids to use as shelter (e.g., Tang et al., 2020). Sheltering is a common behavioural strategy adopted by aquatic species to evade parasitism (Behringer et al., 2018). Whilst no studies to date have tested whether coral reef fishes avoid or use specific habitats in order to reduce their risk of ectoparasitism, there is evidence to suggest that shelter availability may affect ectoparasitism of coral reef fishes (Sikkel et al., 2006). Moreover, through visual signs and their infection history, fish are capable of learning which environments are associated with parasitism and subsequently avoid these infection sources (e.g., rainbow trout from a lake system, Karvonen et al., 2004b; Klemme & Karvonen, 2016). For example, in coastal marine systems, juvenile sticklebacks have been found to avoid ectoparasitic infection. When swimming closer to the benthos and in vegetated habitats, juvenile sticklebacks experienced higher levels of ectoparasitism. However, in the presence of parasites, juveniles swam at the surface, resulting in their reduced infection by ectoparasites (Poulin & FitzGerald, 1989a). Positive phototaxis exhibited in some monogenean ectoparasites, means that hosts in

increased shade have reduced infection intensities (Shirakashi et al., 2013). Therefore, the increased shade and shelter offered by macroalgae habitats may have reduced the proportion of *P. wardi* infected relative to rubble habitats.

For coral reef fishes, their environment is perceived through visual, chemical and olfactory cues (Derby & Sorensen, 2008). In degraded habitats, changes in olfactory and chemical cues of these habitats can have negative effects on predator avoidance (McCormick & Allan, 2017); learning and the social transmission of alarm cues (Chivers et al., 2016); and can alter the efficacy of alarm cues (McCormick et al., 2017). In terms of parasitism, changes in olfactory and chemical cues may prevent juvenile fish from avoiding gnathiid isopod infections when maintained in water that has been in contact with dead relative to live coral (Narvaez et al., 2021). There is therefore an olfactory component to parasitism and parasite avoidance, with fish capable of detecting alarm cues from conspecifics infected with parasites (Poulin et al., 1999). For *P. wardi,* its olfactory capacity or the detectability of its alarm cues may be compromised in degraded habitats, with *P. wardi* slow to recognise chemical alarm cues when held in water from degraded relative to healthy coral (McCormick & Allan, 2017). Due to the capacity for chemical cues from degraded corals to disrupt olfaction and behaviour in *P. wardi*, rubble habitats in the present study may potentially have masked alarm cues from conspecifics or otherwise prevented *P. wardi* from identifying and therefore avoiding ectoparasitism, potentially increasing the prevalence of ectoparasitism observed in *P. wardi* in rubble habitats. However, parasites often use olfaction and chemical cues to locate their hosts (Mordue & Birkett, 2009; Sikkel et al., 2011). It is therefore possible that olfactory and chemical cues from live coral substrata may also affect the behaviour of gnathiid isopods, due to their higher predation risk in live coral habitats (Artim & Sikkel, 2013; Paula et al., 2021). To the best of my knowledge, the effect of coral reef habitats upon olfaction and chemical detection of coral reef parasites has not been tested.

3.4.4 Summary

In the present study, significant differences in parasitism and parasite communities were observed among coral reef habitats that represent a gradient of habitat condition. Trends in endoparasite abundance reflected the notion that, the abundance of heteroxenous, trophically transmitted parasites represents a healthier system in which multiple hosts are present. However, whilst differences in parasite prevalence, abundance and community composition were observed among habitats, it is unknown whether the differences observed are sufficient to alter the pathological effects of parasitism to *P. wardi* under ongoing habitat degradation into the future. The territorial damselfish, *P. wardi*, was particularly useful as a model species within the present study, as it cultivates algae within its territories and thereby its food source. This behaviour may inhibit the detection of shifts in its endoparasite community composition among habitats. However, shifts in the prevalence and abundance of endoparasites infecting *P. wardi* may provide more insight into shifts in habitat condition and host-parasite-habitat relationships. However, potential differences in the diet, stomach contents and algal species farmed by *P. wardi* among habitats must be confirmed.

The effects of habitat on parasite communities and infection dynamics are multifaceted. This study highlights the need to understand the conditions and thresholds under which changes in benthic composition will increase or decrease parasitism, and the parasite traits that determine this response, a task initiated by Artim et al. (2020). This study highlights the interrelationships between host, parasite and the physical environment and the multifactorial, less visible consequences of habitat degradation on coral reefs.

Chapter 4: Reef habitat affects ectoparasite colonisation of a coral reef fish

4.1 Introduction

Parasites are a diverse and abundant component of terrestrial and aquatic ecosystems, with a recent assessment of global biodiversity estimating up to 75% of the 0.2 to 5.7 billion species on Earth may be parasitic (Larsen et al., 2017). Parasites play key roles in structuring ecological communities and shaping ecosystem functions. For example, parasites can influence ecological communities by altering the behaviour (Barber et al., 2000), fecundity (Lafferty & Kuris, 2009) and survival (Finley & Forrester, 2003) of hosts, thereby moderating population and community dynamics and energy transfers (Anderson & May, 1978), and the topology and connectivity of trophic webs (Dunne et al., 2013). Parasites can also affect the incidence of disease through acting as a vector (Hudson et al., 1997) and through the infection and removal of immune-compromised and diseased individuals from populations (Thomas et al., 2005). Over longer temporal scales, parasites can also drive host evolution of parasite avoidance behaviours (Brunner & Eizaguirre, 2016; Poulin & FitzGerald, 1989b). Given the potential importance of parasites in influencing the structure and functioning of ecosystems, understanding how parasite communities respond to changes in habitat configuration merits greater attention (Granath, 2015; Lõhmus & Björklund, 2015; Marcogliese, 2001).

The combined effects of local anthropogenic activity and climatic change are causing many of the world's ecosystems to shift from structurally complex, high diversity systems to structurally simple, low diversity systems (McPherson et al., 2021; Scheffer et al., 1993; Staver et al., 2011). On coral reefs, climate change and local anthropogenic activities (e.g., overfishing, landuse change and water pollution) have led to reductions in the abundance and shifts in the composition of hard corals (Hughes, Kerry, et al., 2018), and increases in the cover of other benthic substrata, such as macroalgae and rubble (Cheal et al., 2010; Hughes, 1994; Mumby, 2009; Norström et

al., 2009). Such shifts in the benthic composition of coral reefs have been shown to have a substantial effect on reef-associated taxa, with coral reef fish and macro-invertebrate communities experiencing declines in richness and abundance as well as shifts in species composition following the loss of live corals (Bellwood et al., 2006; Pratchett et al., 2011; Wilson et al., 2006). Despite the ubiquity of parasites and their potential importance in ecosystem functioning, few studies have investigated the effects of habitat degradation on the structure of parasite communities. The only exception to this is the finding that gnathiid isopods may increase in abundance as coral cover declines (Artim et al., 2020). Other than the effect of habitat degradation (i.e., shifts in both benthic and fish assemblages) on the parasite community of *P. wardi* (see Chapter 3), no prior studies appear to have investigated the effect of benthic composition on the transmission of coral reef parasites and colonisation of coral reef fishes.

The aim of this study was to investigate the effect of different coral reef habitats (i.e., live coral, macroalgae and rubble) on the transmission and colonisation of coral reef ectoparasites on a common coral reef fish, the barred rabbitfish, *Siganus doliatus* (Guérin-Méneville 1829-1838) using in-situ experiments. Complex reef habitats typically harbour a greater diversity of coral reef taxa due to a high diversity of niche microhabitats (Gratwicke & Speight, 2005; Stella et al., 2011; Wilson et al., 2006). As many species of scleractinian coral can feed heterotrophically on macrofauna, such as gnathiid isopods (Artim & Sikkel, 2013; Paula et al., 2021), reductions in coral cover (i.e., potential predators) and structural complexity are hypothesised to result in decreased ectoparasite abundance and richness from coral (i.e., complex) to macroalgae and rubble (i.e., less complex) habitats.

4.2 Methods

4.2.1 Study sites

This study was conducted during austral summer on fringing reefs on the leeward side of three islands (Orpheus, Pelorus and Fantome Islands) in the Palm Island Group, central Great Barrier Reef, Australia (Figure 4-1). These islands are situated approximately 15-22 km from the

mainland coast and have extensive fringing reef development on their leeward (western) margins. Three reef crest sites (2-5 m depth) were selected on each island (i.e., nine sites in total) with one site on each island representing each of three distinct benthic compositions: coral, macroalgal and rubble habitats. Each site was approximately 100m x 10m (1,000m^2) in areas of contiguous reef and consisted of relatively homogenous habitat types. Coral habitats were characterised by relatively high cover of branching *Porites* (i.e., *P. cylindrica*), massive *Porites*, and branching and corymbose *Acropora*; macroalgae habitats were characterised by high cover of the red corticated macroalgae *Hypnea* spp. and *Laurencia* spp., and rubble habitats by a lack of live coral and macroalgae, and >75% rubble cover.

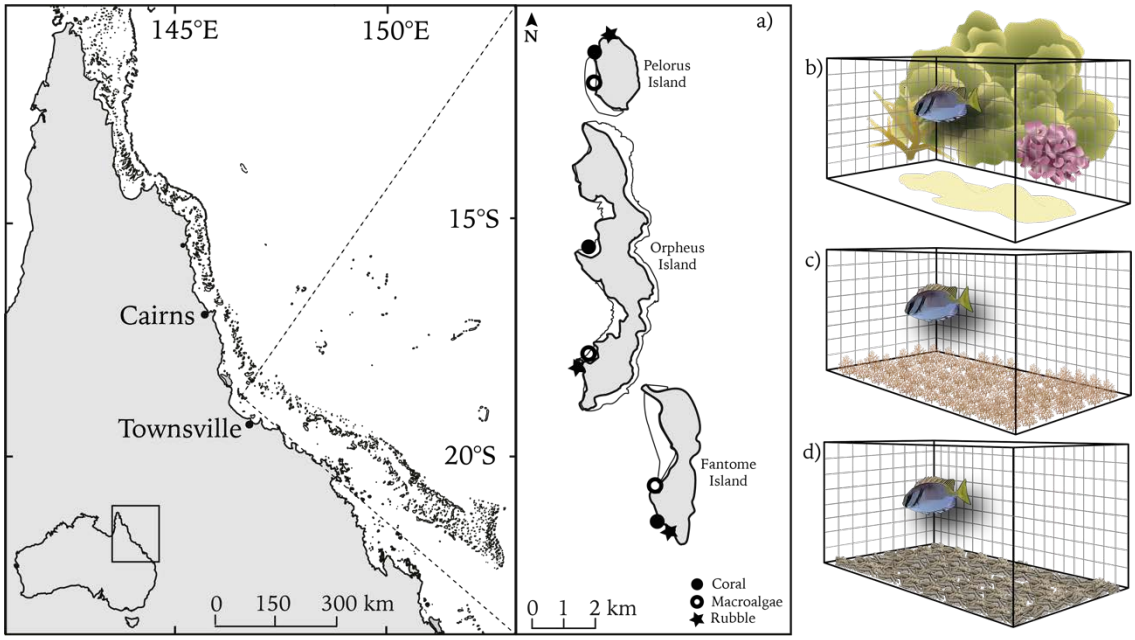

Figure 4-1: a) Map of the Great Barrier Reef; inset: the nine habitat sites (coral n = 3, macroalgae n = 3, rubble n = 3) at Pelorus, Orpheus and Fantome Island within the Palm Island Group, central Great Barrier Reef. b-d) Diagrams of cage design, containing an individual *Siganus doliatus*, deployed at a b) coral (filled circle), c) macroalgae (open circle), d) rubble (filled star) site. Substrata graphics obtained from the Integration and Application Network (ian.umces.edu/media-library).

4.2.2 Study species

The barred rabbitfish, *Siganus doliatus,* was selected as the model species as it is abundant on inshore reefs of the Great Barrier Reef (Cheal et al., 2012; Hoey et al., 2013), occurs within each of the three habitats (i.e., coral, rubble and macroalgae habitats (Cvitanovic & Hoey, 2010; Tang et al., 2020), and has been reported to be infected by a diversity of ectoparasite taxa (>16 putative ectoparasite species; see Appendix C: Table S4.1).

4.2.3 Fish collection and ectoparasite removal

This research was conducted under JCU ethics approval A2449 and GBRMPA permit G18/40033.1. Seventy-eight adult *S. doliatus* (mean total length = 198.8 ± 2.7 mm SE) were collected from shallow (2-5 m depth) fringing reefs on the leeward side of the three islands using hand nets and a large barrier net (Pelorus n = 22 *S. doliatus* captured; Orpheus n = 29; Fantome n = 27). Once captured, fish were immediately transported to Orpheus Island Research Station and their ectoparasites removed by placing each fish in dechlorinated freshwater for 4 mins (Chambers & Ernst, 2005; Sikkel et al., 2004). The solution contained a mild concentration of AQUI-S aquatic anaesthetic to reduce stress and minimise self-induced harm to the fish (0.5 mL per 5 L dechlorinated freshwater; Grutter, 1995; Harms, 1996). The body surface of each fish was then visually inspected under a dissection microscope (range 6.7-45x magnification as required) for approximately 1 min and any remaining attached ectoparasites were removed using forceps. Following parasite removal, fish were transferred to individual 110 L aquaria and supplied with flow-through, filtered, UV-sterilised seawater, and supplemental aeration. A 10L plastic hide was provided for shelter in each aquarium. Fish were held for 3 to 7 days prior to being used in the field experiment and fed *Ulva ohnoi* (defrosted from frozen to prevent contamination) daily, *ad libitum*.

4.2.4 Fish caging

Individual *S. doliatus* were randomly allocated to one of the three habitat types (coral, macroalgae, rubble) within their island of collection. At each of the nine sites, six to ten fish were placed inside individual cages and deployed to the reef between 0830h and 1030h, with all fish within a site deployed at the same time. Cages were used to ensure fish remained directly above/adjacent to the target substratum and that interactions with other fishes and cleaner organisms were limited, whilst allowing infection by ectoparasites from the surrounding environment and benthos. Cages (50 x 45 x 30cm; 6.5mm square mesh) were fully enclosed, constructed of galvanised steel mesh, and were held in position with weights attached to the base. Cages were positioned directly over macroalgae and rubble substrata in the respective habitats and were placed immediately adjacent to coral colonies to prevent damage to live coral in the coral habitats. Cages and fish were left undisturbed at each site for three days.

Cages and fish were retrieved between 0630h – 0830h after three days on the reef. Fish were collected as close to dawn as practical to ensure the capture of mobile ectoparasites, such as gnathiid isopods, that are more abundant on fish at this time (Côté & Molloy, 2003; Grutter & Hendrikz, 1999; Sikkel et al., 2006). Each cage, with an individual *S. doliatus* inside, was carefully transferred into a 70 L plastic aquarium underwater and slowly brought to the surface by divers on SCUBA. Fish were transferred into individually labelled aquarium-grade plastic bags with aeration and transported to Orpheus Island Research Station within 1 hr of collection. Each fish and the contents of the corresponding bag were transferred to individual 20 L holding aquaria for 2 – 3 hours, containing static UV-sterilised, filtered seawater with supplemental aeration. Ectoparasites were removed from the fish as described previously and the saltwater from the aquarium filtered through a 63 μm mesh, which was then thoroughly rinsed into a Petri dish, to capture any dislodged parasites. All ectoparasites collected were preserved in 70% ethanol for later identification. Following parasite removal, fish were measured (total length to

the nearest 0.1 cm), weighed (nearest 0.1 g) and held in 2,000 L aquaria for a recovery period of up to 3 days prior to release back to their site of capture.

4.2.5 Parasite identification

All ectoparasites removed from experimental fish were identified by examination of diagnostic morphological features from whole-mounted or cleared, preserved material (see Chapter 2). Distinctive features were used to classify parasites to species. As gnathiid isopods are only parasitic in their larval stage and species identification is determined from adult males that reside in the substratum (Monod, 1926), all gnathiids collected were grouped as '*Gnathia* spp.', as per (Grutter, 1994, 1995).

4.2.6 Statistical analyses

All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018).

The species richness and abundance of ectoparasites, and the abundance of the two most prevalent putative ectoparasite species (*Pseudohaliotrema* sp.1 and *Gnathia* spp.) infecting *S. doliatus* were compared among habitats (categorical with three levels) and islands (categorical with three levels) using generalised linear models or generalised linear mixed models (Appendix C: Table S4.2). Candidate models for richness and abundance data were created using both Poisson and negative binomial distributions, as these are typically used for count data. To inform the appropriate error structure for each model, residual diagnostics, over-dispersion and zero-inflation were tested using the '*DHARMa*' package to determine model fit and assumptions of the error distributions were examined using residual plots, Chi square goodness-of-fit tests and dispersion (the ratio of the variance to the mean). The error distribution with the best model fit and satisfaction of model assumptions was selected for the analysis.

Total ectoparasite and *Gnathia* spp. abundance were compared among habitats and islands using a generalised linear mixed effects model with Poisson distribution using the '*lme4*' package, with an observational-level random effect included to account for overdispersion (Harrison, 2014).

Ectoparasite richness and *Pseudohaliotrema* sp.1 abundance were compared among habitats and islands using a generalised linear model with negative binomial distribution using the '*stats*' package. The natural log of total length was included as an offset within each model to account for any differences in fish body size on rates of infection. Tukey's post-hoc analyses were conducted using the '*emmeans*' package.

Ectoparasite community composition was compared among islands and habitats using a PERMANOVA and visualised using a non-metric multidimensional scaling (nMDS). The PERMANOVA and nMDS were based on Wisconsin double standardisation of putative ectoparasite species count data and a Bray-Curtis dissimilarity matrix using the '*vegan'* package in R. Any *S. doliatus* without any ectoparasitic infection were removed prior to analysis. Due to low abundance and prevalence of ectoparasites obtained from *S. doliatus*, a 'dummy' ectoparasite species was added to the dataset with equal abundance (n = 1) for each individual *S. doliatus*. The 'dummy' species meant that two individuals without any shared parasite taxa have a single 'species' with the same abundance in common, creating a dissimilarity of zero (Clarke et al., 2006). SIMPER analysis, from the package *'vegan*', was used to determine the most influential species driving differences in the ectoparasite community among habitats and islands.

4.3 Results

After three days on the reef, a total of 128 ectoparasites from six putative species, representing four families, were recorded from the 78 caged *S. doliatus* (mean abundance = 1.6 ± 0.5 SE parasites per individual; range = 0 – 33 parasites per individual). Gnathiid isopods (*Gnathia* spp.; n = 67) and the ancyrocephalid, *Pseudohaliotrema* sp.1, (n = 39) accounted for 52% and 30% of all parasites recorded, respectively (Figure 4-2). Parasite prevalence (i.e., the proportion of *S. doliatus* infected with parasites) ranged from 0% at the Pelorus coral site to 78% (seven out of nine fish) at the Orpheus rubble site (see Appendix C: Table S4.3).

Figure 4-2: Photograph of gnathiid isopods ex *Siganus doliatus* caged at the Pelorus Island rubble site, Palm Island Group, Central Great Barrier Reef.

4.3.1 Ectoparasite abundance

Ectoparasite abundance on *S. doliatus* differed among habitats, with significantly greater number of parasites recorded from fish caged in rubble habitats (adjusted mean = 1.2 ± 0.4 SE parasites per fish) compared to those in coral habitats (adjusted mean = 0.5 ± 0.2 SE parasites per fish; $P < 0.05$). Ectoparasite abundance was intermediate on *S. doliatus* caged in macroalgal habitats (adjusted mean = 0.7 ± 0.2 SE parasites per fish) and did not differ from those in coral ($P = 0.67$) or rubble habitats ($P = 0.41$; Figure 4-3a). The abundance of ectoparasites infecting caged *S. doliatus* did not differ between islands ($P = 0.17$; Figure 4-3b).

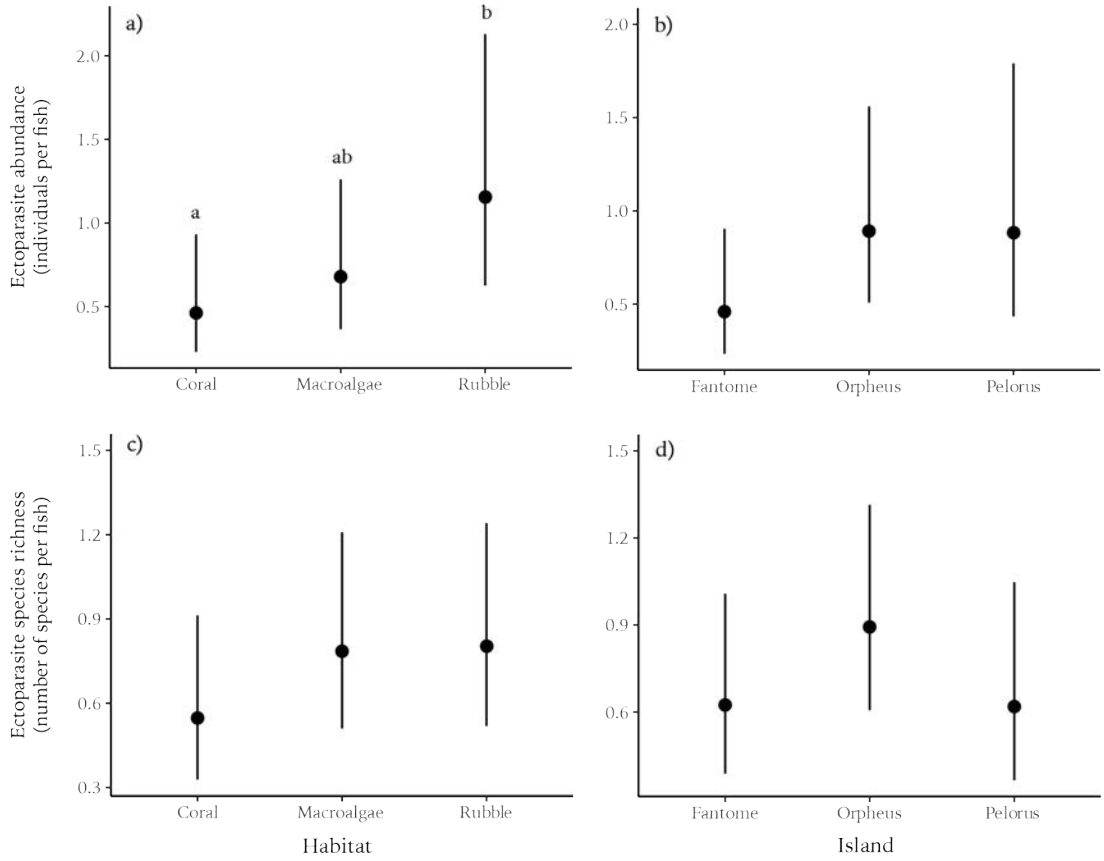

Figure 4-3: Differences in the a, b) abundance and c, d) species richness of ectoparasites infecting caged *Siganus doliatus* (n = 78) among coral, macroalgae and rubble habitats and Fantome, Orpheus and Pelorus island of the central Great Barrier Reef. Lines represent 95% confidence intervals; black circles represent adjusted means. Letters represent significant differences between groups.

4.3.2 Species-specific abundances

The abundance of gnathiid isopods (*Gnathia* spp.) infecting *S. doliatus* differed among habitats and was significantly higher on *S. doliatus* caged in rubble (adjusted mean = 0.3 ± 0.2 SE parasites per fish) than those in coral habitats (adjusted mean = 0.02 ± 0.02 SE parasites per fish; $P < 0.05$) with intermediate densities on *S. doliatus* caged over macroalgae (adjusted mean = 0.1 ± 0.1 SE parasites per fish; Figure 4-4a). The abundance of *Gnathia* spp. infecting caged *S.*

doliatus also differed between islands, with greater abundances of *Gnathia* spp. on *S. doliatus* caged at Pelorus relative to Fantome Island ($P < 0.01$; Figure 4-4b). The abundance of the ancyrocephalid, *Pseudohaliotrema* sp. 1, infecting caged *S. doliatus* did not differ among habitats (coral adjusted mean = 0.55 ± 0.14 SE parasites per fish; macroalgae = 0.79 ± 0.17 SE; rubble = 0.8 ± 0.18 SE; $P = 0.88$) or islands ($P = 0.18$; Figure 4-4c-d).

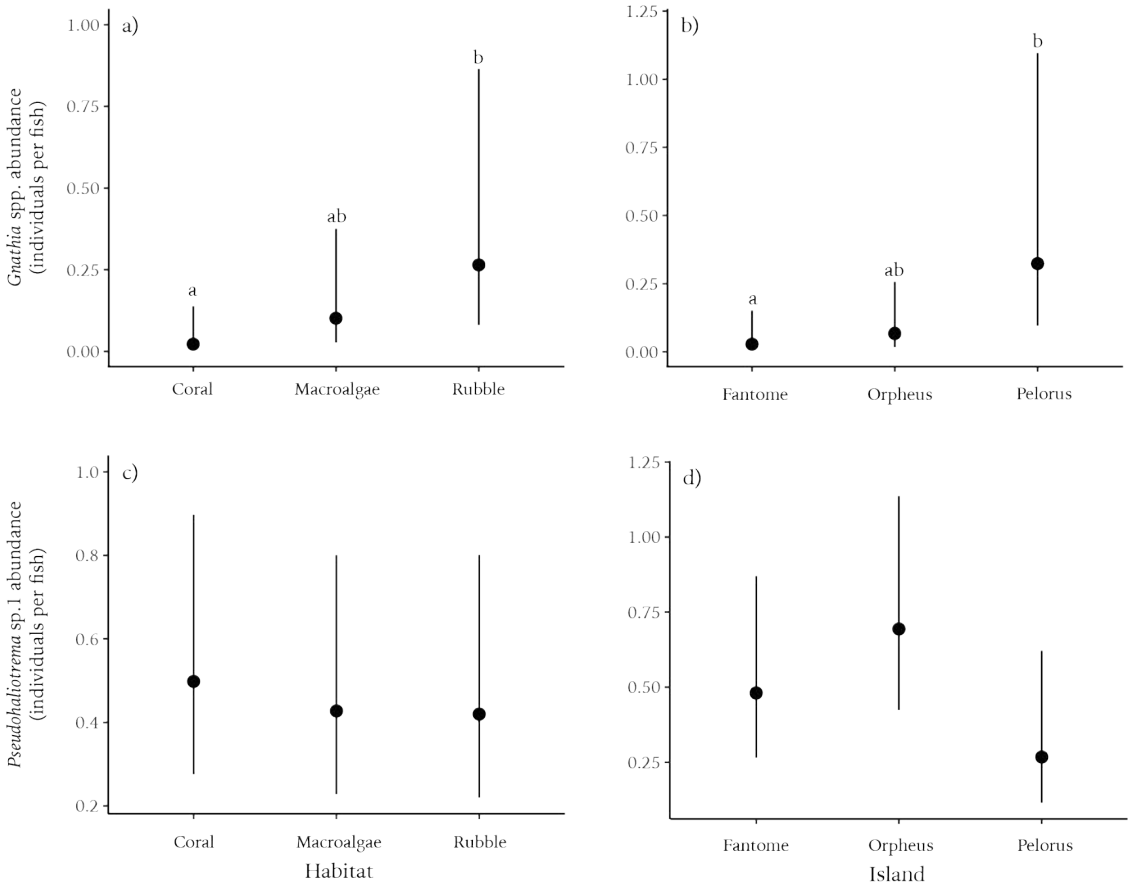

Figure 4-4: Differences in the abundance of a, b) *Gnathia* spp. and c, d) *Pseudohaliotrema* sp.1 infecting caged *Siganus doliatus* among coral, macroalgae and rubble habitats and Fantome, Orpheus and Pelorus island of the central Great Barrier Reef. Lines represent 95% confidence intervals; black circles represent adjusted means. Letters represent significant differences between groups.

4.3.3 Ectoparasite richness and community composition

The number of putative parasite species infecting individual *S. doliatus* after three days on the reef ranged from 0 to 2 putative species per fish and did not differ among habitats (coral adjusted mean = 0.5 ± 0.1 SE putative species per fish; macroalgae = 0.8 ± 0.2 SE; rubble = 0.8 ± 0.2 SE; P = 0.34; Figure 4-3c) or islands (Figure 4-3d; P = 0.38). The community composition of ectoparasites infecting *S. doliatus* after three days on the reef did not differ among coral, macroalgae or rubble habitats (PERMANOVA: P = 0.3), with broad overlap in the composition of ectoparasite communities infecting caged *S. doliatus* among the three habitats (Figure 4-5). However, ectoparasite communities did differ among islands (PERMANOVA: $P < 0.05$).

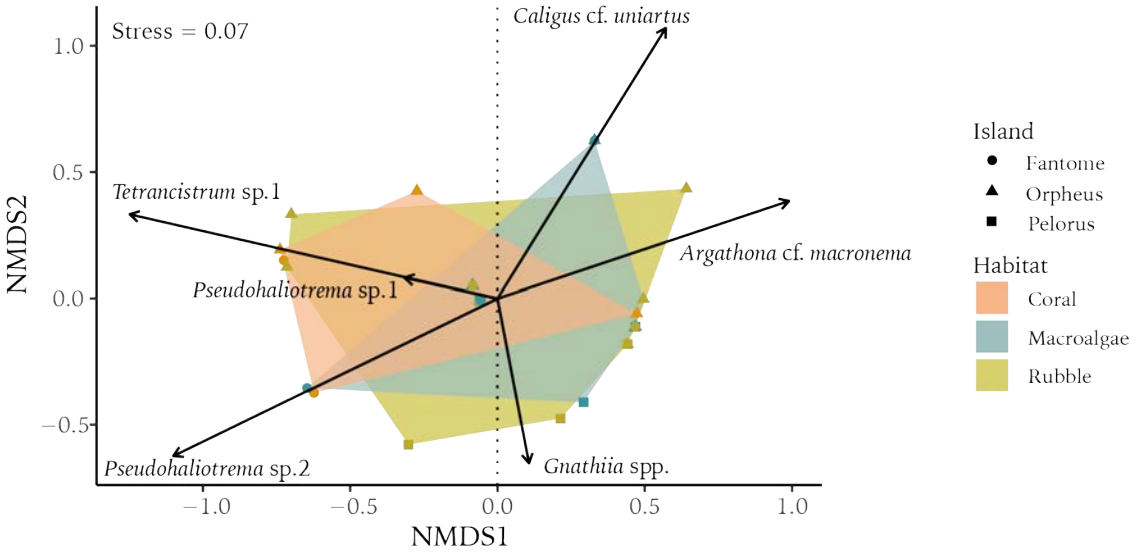

Figure 4-5: Two-dimensional solution from non-metric multidimensional scaling, showing the differences in the ectoparasite community composition of caged *Siganus doliatus* among coral, macroalgae or rubble habitats at Fantome, Orpheus and Pelorus island within the central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed species abundance data. Each point represents an individual infected *Siganus doliatus* (n = 42). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (putative ectoparasite species) with two dimensions. Vector length is proportional to the degree of correlation between the species and the ordination.

4.4 Discussion

Establishing the effect of coral reef benthic composition on the abundance and composition of ectoparasite communities infecting coral reef fish is important in understanding how habitat degradation may affect coral reef ecosystems into the future. Comparisons of ectoparasite communities infecting *S. doliatus* held in coral, macroalgae and rubble habitats for three days revealed a significantly higher abundance of ectoparasites, namely gnathiid isopods (*Gnathia* spp.), on those fish held over rubble habitat, compared to those in live coral habitat. Contrary to expectations and findings from several terrestrial ecosystems (e.g., forest: Kiene et al., 2020); lowland: Froeschke et al., 2013); urban: Ancillotto et al., 2018), among-habitat differences in the species richness and composition of ectoparasite communities infecting *S. doliatus* were not detected. As coral reefs are predicted to be exposed to an increasing frequency and intensity of disturbance, habitats dominated by live coral are likely to be increasingly replaced by habitats dominated by macroalgae and rubble (Cheal et al., 2010; Hughes, 1994; Mumby, 2009). These results indicate that the increased cover of rubble on coral reefs may result in increased abundances of generalist ectoparasite species, such as gnathiid isopods, on coral reef habitat generalists such as *S. doliatus*.

4.4.1 Ectoparasite abundance

The abundance of ectoparasites infecting *S. doliatus* held over rubble habitat was greater than those held in coral habitat and was driven by differences in infection by gnathiid isopods, *Gnathia* spp. Gnathiid isopods are host-generalists, infecting at least 39 coral reef fish species on the GBR (Grutter & Poulin, 1998). Gnathiid isopods can lower host blood volume (Grutter et al., 2008; C. M. Jones & Grutter, 2005), cause tissue damage (Heupel & Bennett, 1999) and even host mortality (Penfold et al., 2008). Therefore, the increased abundance of gnathiid isopods in rubble-dominated habitats may yield significant health repercussions for multiple fish species, in particular habitat generalists and species that utilise rubble habitats. Previous research in the Caribbean and in the Philippines has also shown that the abundance of gnathiid isopods emerging

from the benthos is greater in rubble than in coral habitats (Artim et al., 2020; Santos & Sikkel, 2019), and is likely related to the predation of gnathiid isopods by scleractinian corals (Artim & Sikkel, 2013; Paula et al., 2021). Indeed, several coral species (Caribbean: *Porites* sp. and *Montastraea* sp.; GBR: *Pocillopora damicornis* and *Goniopora lobata*) have been found to consume gnathiid isopods in aquaria, with gnathiid isopods also found to actively avoid live coral substrata (Artim & Sikkel, 2013; Paula et al., 2021). Additionally, the lower infections of gnathiid isopods on *S. doliatus* within coral habitats may also be related to the relative abundance of the cleaner wrasse *Labroides dimidiatus*, which is generally most abundant in coral-dominated habitats (Berkström et al., 2012). Removal of *L. dimidiatus* from coral-dominated patch reefs has been shown to increase gnathiid abundance, thus the lower abundance of gnathiid isopods on fish caged in coral habitats may be related to the direct effects of predation by corals and coral-associated cleaner organisms, and/or the avoidance of areas where these predators are most abundant (Grutter 1999; Grutter 2018). With a shift from live coral- to rubble-dominated habitats (e.g., following a severe storm or cyclone), we may find increased abundances of gnathiid isopods infecting *S. doliatus* and potentially other reef fishes, with potentially significant health repercussions.

Despite the moderate complexity and lack of live coral in macroalgal habitats, the abundance of *Gnathia* spp. infecting *S. doliatus* held in macroalgae habitats were intermediate to, but not significantly different from, those recorded in coral and rubble habitats. Positive relationships between species abundance and habitat complexity are widespread in ecology (Bracewell et al., 2018). Even though such relationships have been well documented for the physical complexity provided by live corals (Graham & Nash, 2013; Gratwicke & Speight, 2005), few studies have investigated the influence of the flexible structure provided by macroalgae on associated communities. Canopy-forming macroalgae such as *Sargassum* spp. can harbour high abundances of copepods, gnathiids and other, potentially parasitic epifauna (Tano et al., 2016), and the complexity of *Sargassum* beds has been shown to influence the behaviour and abundance of

associated fish assemblages (Tang et al., 2020). However, the effect of smaller and less structurally complex macroalgae, such as *Hypnea* spp. and *Laurencia* spp. that dominated macroalgal habitats across all islands in the current study, on fish and ectoparasite communities is largely unknown (Sambrook et al., 2020). Interestingly, gnathiid isopods have been found to preferentially select a macroalga (*Dictyota* sp.) as a substratum under controlled conditions (Artim & Sikkel, 2013), and macroalgal growth is encouraged in the maintenance of gnathiid cultures (Grutter et al., 2020). Under natural conditions however, macroalgal cover has been found to have a negative effect on gnathiid abundance (Artim et al., 2020). This is hypothesised to result from the unsuitability of macroalgae as an attachment surface, its potential toxicity, the risk of accidental consumption by herbivores in macroalgae habitats and risk of predation by macroalgae-associated cryptobenthic fauna (Artim et al., 2020).

Surprisingly, habitat type had no detectable effect on the abundance of the monogenean, *Pseudohaliotrema* sp.1. If corals are capable of consuming gnathiid isopods (<1 cm in body length; Tanaka, 2007), then it is reasonable to assume they also have the capacity to consume the pelagic eggs and larvae (oncomiracidia) of *Pseudohaliotrema* spp. (eggs approx. 42-90 μm in length; Yamaguti, 1953). The lack of detectable differences in the abundance of *Pseudohaliotrema* sp.1 among habitats may be due to the egg morphology and transmission of these parasites, allowing for their dispersal among multiple coral reef habitats and potential retention of eggs in all habitats. The eggs of *Pseudohaliotrema* spp., like many oviparous monogenean species, typically possess a long filament that often become entangled with one another to form egg masses and/or the substratum, enhancing retention within the hosts' habitat (Kearn, 1986; Lim, 2002). Oncomiracidia can swim (1-5 mm/sec), and although typically short-lived (<48 hr; Rohde, 2005), they have capacity for dispersal among habitats.

4.4.2 Ectoparasite community composition and species richness

Of the limited number of studies that have investigated the effect of habitat degradation (i.e., due to fishing and anthropogenic activity and landuse change) on the composition of parasite

communities in coastal and aquatic ecosystems, most have found habitat degradation to have an indirect, negative effect on parasite species richness (Dzikowski et al., 2003a; Huspeni & Lafferty, 2004; Keas & Blankespoor, 1997; Lafferty, Shaw, et al., 2008; Wood et al., 2014). Most studies have typically focused on endoparasite communities and have generally attributed changes in endoparasite communities to the negative effect of habitat degradation on host abundance (Huspeni & Lafferty, 2004; Lafferty, Shaw, et al., 2008; Wood et al., 2014). However, very few studies have investigated the effect of habitat on the species richness or composition of ectoparasite communities. While numerous studies have reported declines in the abundance and species richness of reef fish (i.e., potential hosts) as coral reefs transition from coral to macroalgae and rubble substrata (Graham et al., 2006; Wilson et al., 2006), no significant difference in ectoparasite species composition or richness among coral, macroalgae and rubble habitats were found in the present study.

The inability to detect among habitat differences in the richness and composition of ectoparasite communities in the present study may be due to the short (three day) duration of the study. While the short duration of the caging was necessary to prevent fish health from being compromised, it may have resulted in the low prevalence, abundance and richness of ectoparasites detected, and therefore the similarity of ectoparasite communities among habitats. Previous experiments to quantify infection on coral reef fish have caged fish from 3-12 hours to obtain gnathiid isopod infections (Santos & Sikkel, 2019); to up to 154 days testing host resistance (Benesh & Kalbe, 2016; Karvonen et al., 2004a). In this study, caging duration was limited, where possible, because of potential declining health and mortality of caged fish and the intent to return animals unharmed. To obtain a higher abundance, richness and prevalence of ectoparasitic infections, a longer caging duration may be valuable, however this will be governed by the intended endpoint of the experimental hosts.

4.4.3 Summary

With threats to and pressures on coral reef ecosystems projected to increase in the future from increased coastal populations, human activity and climate change impacts, the cover of macroalgae and rubble on formerly coral-dominated reefs is projected to increase (Osipova et al., 2020). While shifts to macroalgal-dominance appeared to have little effect on the ectoparasite communities of *S. doliatus*, shifts to rubble-dominated habitat are likely to result in increased ectoparasitic infections. Therefore, habitat generalists, such as *S. doliatus,* may suffer increased ectoparasitism because of shifts from coral to rubble substrata. For those species that do not interact as closely with the substratum, or that specialise in non-rubble habitats, we may see no shift in ectoparasite abundance as a result of habitat degradation. However, this assumes that coral reef substrata do not have a direct effect upon coral reef parasites, a relationship that has only been tested with *Gnathia* spp. (Artim & Sikkel, 2013; Paula et al., 2021).

This appears to be the first study to investigate the effect of different coral reef habitats on the transmission of coral reef ectoparasites and infection of a coral reef fish. In doing so, the likely effect of habitat degradation on the abundance and composition of ectoparasites infecting a common herbivorous reef fish can be determined. The findings of this study highlight the importance of including parasitism and infection parameters in understanding how habitat degradation may affect the biodiversity, structure and function of coral reef ecosystems into the future. Further research, featuring multiple coral reef fish species and capturing a broader diversity of ectoparasites, is required to understand the range of responses of coral reef ectoparasites to, and ultimately the ecological ramifications of, habitat degradation.

Chapter 5: Coral reef substrata impact hatching success of a common fish ectoparasite

5.1 Introduction

Climate change and anthropogenic activity are causing the degradation of habitats and ecosystems globally (IPCC, 2014), with shifts in the composition of foundation species becoming increasingly common (e.g., arctic permafrost: Swindles et al., 2015; equatorial savannahs: Burrell et al., 2020; shallow lakes: Scheffer et al., 1993; coral reefs: Hughes, Kerry, et al., 2018). These effects of climate change are perhaps most apparent on coral reefs, where increases in the severity and frequency of marine heatwaves have directly led to reductions in live coral cover and increases in other benthic substrata such as macroalgae and rubble (Adam et al., 2021; Contreras-Silva et al., 2020; Vieira, 2020). This has altered the physical structure (Pisapia et al., 2020) and chemical environment (McCormick et al., 2017) of reef habitats, the composition, richness and abundance of reef-associated species they support (Pratchett et al., 2018; Wilson et al., 2019), and interactions among coral reef species (Boström-Einarsson et al., 2018; Coker et al., 2009).

One of the most common biotic interactions on coral reefs are those between parasites and their hosts, with each coral reef fish species estimated to host approximately 20 parasite taxa (metazoan and protozoan; Justine, 2010; Rohde, 1976a). This high level of diversity has led to suggestions that parasites play a key role in ecosystem function through their influence on energy transfers (Dunne et al., 2013), predator-prey interactions (Allan et al., 2020), as well as the fitness (Binning et al., 2013), mortality (Grutter et al., 2008) and population dynamics of their hosts (Finley & Forrester, 2003; Sun et al., 2012). Despite their potential importance in ecological processes, few studies have investigated the relationship between coral reef parasites, host-parasite interactions and benthic composition (but see Chapters 3 and 4). Due to the ongoing degradation of coral reef habitats, understanding the effects of different coral reef substrata on host-parasite interactions is becoming increasingly important.

Among coral reef habitats, differences in parasite community composition, abundance (Chapter 3) and rates of infection (Chapter 4) have been observed. However, the mechanism of these changes (i.e., effects of habitat on development, life cycle and transmission of parasites) is uncertain. Previous experimental studies suggest that polar (i.e., water soluble) compounds from the alga *Ulva* spp. and *Asparagopsis taxiformis* may influence the embryonation and hatching success of *Neobenedenia girellae* (Hutson et al., 2012). However, whether this holds for other algal taxa, especially those that are often dominant on inshore and degraded coral reefs (e.g., *Sargassum*) is largely unknown. Corals, like macroalgae, are also known to produce chemicals to reduce biofouling and to prevent competition from other benthic organisms (Chadwick & Morrow, 2011; Koh & Sweatman, 2000), and these chemicals may similarly affect the embryonation and hatching success of parasites. Interestingly, chemical cues from dead coral have been suggested to influence the infection of juvenile coral reef fishes by gnathiid isopods, with infection rates being greater for fish held in water from dead coral than those in water from live coral (Narvaez et al., 2021). Therefore, through waterborne interactions, coral reef substrata may have the capacity to influence parasite life cycles and host-parasite interactions on coral reefs. Yet, I am not aware of any studies that have investigated the effect of coral reef substrata that proliferate in degraded habitats (e.g., *Sargassum* spp.; Fox & Bellwood, 2007; McCook, 1996) on the life cycles of marine ectoparasites.

The aim of this study was to determine how different coral reef substrata, representing a gradient of coral reef health (i.e., live branching coral, macroalgae and coral rubble substrata), influence the development, hatching, and infection success of a common coral reef ectoparasite, the monogenean flatworm, *Neobenedenia girellae.* I hypothesise that experimental hatching and infection success will be lowest in coral, intermediate in macroalgae and greatest in rubble treatments, in concordance with previously observed differences in ectoparasite abundance (Chapter 3) and transmission (Chapter 4) in the wild.

5.2 Methods

This research was conducted under JCU ethics approval A2449, A1989, GBRMPA permit G16/38425.1 and in accordance with section 20 of the Great Barrier Reef Marine Park Regulations 2019.

5.2.1 Host-parasite system

The blue-lined rabbitfish, *Siganus doliatus* (Guérin-Méneville 1829-1838), and monogenean flatworm, *Neobenedenia girellae* (Hargis 1955) Yamaguti, 1963, were selected as the host-parasite model. *Neobenedenia* spp. are common ectoparasites of coral reef fishes (Brazenor et al., 2018; Bullard et al., 2000; Jenkins et al., 2020), and are well-suited to laboratory-based experiments as they have a short generation time (Hirazawa et al., 2010), infect a wide range of host species (Brazenor et al., 2018), require only a single host to complete their life cycle, and are readily cultured under laboratory conditions (Hutson et al., 2018). *Neobenedenia girellae* eggs and oncomiracidia (larvae) were obtained from an established monoculture maintained at the Marine Parasitology Lab at James Cook University, Townsville (Hutson et al., 2018). *Siganus doliatus* was selected as the model host, as this species is susceptible to infection by capsalid monogeneans (Grutter, 1994) and is a common, herbivorous fish on inshore reefs of the Great Barrier Reef (GBR), where it is found associated with a range of habitats, including coral, macroalgae and rubble (Hoey et al., 2013). Forty *S. doliatus* (mean total length = 207.2 cm; range 14.7-24.3 cm) were sourced from an aquatic wildlife supplier (Cairns Marine).

5.2.2 Experimental setup

Three coral reef substrata live coral, macroalgae and rubble were used to test the effect of habitat on the life cycle of *N. girellae* and its maturation and infection success on the coral reef fish host, *S. doliatus*. Forty *S. doliatus* were randomly assigned to one of the four treatments (live coral, macroalgae, rubble and a no-substratum control; ten fish per treatment), and each placed in an individual 110 L aquarium. The ten aquaria for each treatment were supplied water from a 2,000

L sump, with each treatment maintained on a separate system to prevent mixing of bioactives among treatments (total water volume ~ 3,100 L per treatment). Each 110 L aquaria was provided with supplemental aeration and recirculating filtered seawater. Water from each aquarium was filtered through a 25 μm bag filter and a UV steriliser (> 490 J m^{-2}) before being recirculated. Water temperature was maintained at ambient (mean = 24.05 ± 0.02 °C SE), consistent with the collection location of the substrata (mean = 23.55 ± 0.02 °C SE). Each sump contained bioballs (K1 Kaldnes Type Media) for biological filtration and protein skimmers (Aqua One ProSkim G220) for removal of organic waste. Aquaria were subjected to a 12:12 hour light-dark cycle. LED lights were positioned above each aquarium to deliver 150 PAR (Photosynthetically Active Radiation), equivalent to the PAR recorded at 1.9 m depth from reefs in the central GBR. All *S. doliatus* were fed daily with a mixture of *Ulva turpida, Ulva ohnoi, Caulerpa* spp. (defrosted from frozen), Fish Fuel Co. Marine Green cubes and commercially supplied, dried *Pyropia* sp. (i.e., nori). A plastic hide (25 cm length of 26cm diameter pipe) was provided for shelter in each aquarium.

Sargassum spp. was selected as the macroalgae substratum, as it is the dominant alga on inshore reefs of the GBR (Fox & Bellwood, 2007; McCook, 1996), and often proliferates following coral loss (Done, 1992; Hughes, 1994; Rasher et al., 2013) *Porites cylindrica* was selected as the live coral substratum, as it is a common coral species in sheltered habitats on the GBR and co-occurs in habitats alongside *Sargassum* spp. on inshore reefs of the GBR (Veron et al., 2016). As such, these taxa are likely commonly encountered by *S. doliatus* on inshore reefs of the GBR. All substrata (*P. cylindrica*: fifteen fragments <30 cm length each; *Sargassum*: < 0.02 m^3 total; rubble: < 2 m^3) were collected from Pioneer Bay, on the leeward side of Orpheus Island in the central GBR.

To prevent epifaunal organisms associated with each substratum from being incidentally introduced to the system, *Sargassum* spp. and coral rubble were subjected to a series of washes with filtered seawater and subsequent short-term air-exposure (60 mins) before being introduced

to the system. For *P. cylindrica*, only fragments that were free of algae and had no obvious signs of biofouling were used in experiments. To standardise the size of each substratum across and within treatments, equal volumes (1.5 L) of each substratum were allocated to their respective treatment aquaria. Prior to being introduced to the system, *S. doliatus* were held in a weak formalin bath (0.01 L formalin: 50 L filtered seawater) with supplemental aeration for one hour to remove any existing ectoparasites. *Siganus doliatus* were allowed to acclimate within each treatment for approximately four weeks prior to commencing experiments.

5.2.3 Egg embryonation and hatching success

To investigate the effect of different coral reef substrata on the hatching success of *N. girellae,* twenty *N. girellae* eggs were placed in a cavity block filled with seawater sourced from one of the four treatment sumps (live coral, macroalgae, rubble or control), with ten replicate cavity blocks per treatment (n = 200 eggs per treatment, n = 800 eggs total). A glass cover was placed on top of the cavity block to reduce evaporation and to ensure that eggs and hatched oncomiracidia were not trapped in the surface water tension. Eggs were monitored every 24 h under a dissection microscope at 6.7 to 45x magnification for (i) development of eye spots and (ii) hatching (i.e., opened operculum; Figure 5-1). One third of the water within each cavity block was exchanged with seawater from the respective treatment sump every 24 h, whilst ensuring that eggs remained submerged. Eggs were monitored until no hatching was evident across all treatments for two consecutive days (i.e., after 10 days).

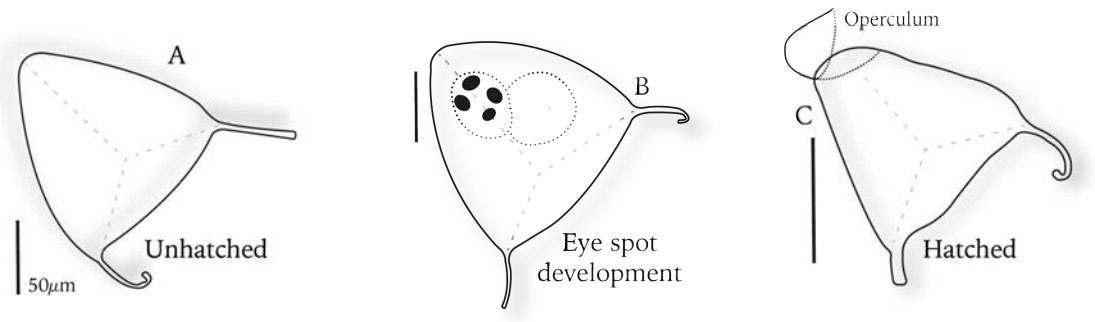

Figure 5-1: Developmental stages of *Neobenedenia girellae* embryos; A = unhatched, B = eye spot development, C = hatched (open operculum). Scale bars = 50 μm.

5.2.4 Infection success of Neobenedenia girellae

To determine the effect of different substrata on the infection success of *N. girellae*, 40 *S. doliatus* were randomly allocated to one of the four treatments, a control (no substratum) and three coral reef substrata: live coral (*P. cylindrica*), macroalgae (*Sargassum* spp.) and coral rubble, and each fish placed into an individual 110L aquarium. In the days prior to infection, newly laid *N. girellae* eggs were collected from the laboratory culture and acclimated in Petri dishes (one dish per day) to water from the control sump (2 x 50% water exchanges conducted within approx. 12 hours). Eggs were monitored at the same time daily (08:00) for signs of development and transferred into a new Petri dish so that recently hatched oncomiracidia (i.e., larvae) were selected for the infection experiment. On the morning of infection, oncomiracidia up to three hours old were counted and placed in 40 x 5 mL vials filled with seawater from the control treatment. Water flow and aeration to the aquaria was turned off prior to introduction of oncomiracidia to prevent them from being caught in the surface water tension and/or lost from the system. Water levels were also reduced by approximately 80% to increase the probability of the oncomiracidia encountering the fish. These conditions were maintained for approximately two hours, as *N. girellae* is capable of infecting fish within 15 minutes (Trujillo-González et al., 2015). Two hours after the oncomiracidia were introduced to the aquaria, water flow and aeration were resumed, and water levels returned to their initial levels. This was repeated every day for three days, with a total of 80 *N. girellae* oncomiracidia added to each aquarium (Day 1: n = 40 oncomiracidia per aquarium; Day 2: n = 20; Day 3: n = 20). The mean water temperature (24.05 ± 0.02 °C) and salinity (35 ‰) of our aquaria have been shown to result in *N. girellae* reaching sexual maturity and commence egg laying after approximately 11 days (Brazenor & Hutson, 2015). Egg collectors (squares of fine mesh ~ 6 cm x 6 cm) were placed in each aquaria on day eight of infection to capture any *N. girellae* eggs produced and were inspected every 24 hrs under the

microscope at 6.7 to 45x magnification for the presence of eggs. New egg collectors were replaced each day to detect consistent egg production within aquaria. Eggs were observed on mesh squares from day 11 (post day 1 of infection). On day 17 post-infection, fish were bathed in dechlorinated freshwater to kill and dislodge *N. girellae* that had infected the fish (Grutter, 1995; Harms, 1996; Kaneko II et al., 1988). Freshwater baths were filtered through a 63 μm mesh to capture adult *N. girellae*, which were counted and preserved in 70% ethanol.

5.2.5 Statistical analysis

All statistical analyses were performed using R software version 3.5.1 (R Core Team, 2018).

Eye spot development and hatching success of *N. girellae* eggs were compared among control, coral, macroalgae and rubble treatments using survival analysis within the '*survival'* package in R. This method allows data from eggs that had desiccated (n = 16 of 800; 2%) during the experiment, or that did not hatch, to be included within the analysis up until the day they became desiccated. Those eggs that failed to hatch were censored on day ten (i.e., 48 hours after hatching was last observed within any treatment) to show that hatching had not occurred. Kaplan–Meier survival curves were generated for each treatment as a function of time (days) using the '*survminer'* package in R, and the effect of treatment upon survival curves for eye spot development and hatching success was determined using a log rank test (Bewick et al., 2004). Post-hoc comparisons were made using the Benjamini-Hochberg procedure. A Kruskal-Wallis rank sum test was used to test the effect of treatment upon the proportion (%) of eggs that hatched (i.e., hatching success) within each treatment. Post-hoc comparisons were made between treatments using the Dunn test (Appendix D; Table S5.1).

Candidate models of the number of adult *N. girellae* recovered from *S. doliatus* were created using both Poisson and negative binomial distributions, as these are typically used for count data. To inform the appropriate error structure for each model, residual diagnostics, over-dispersion and zero-inflation were tested using the '*DHARMa*' package to determine model fit and assumptions of the error distributions were examined using residual plots, Chi square goodness-

of-fit tests and dispersion (the ratio of the variance to the mean). The error distribution with the best model fit and satisfaction of model assumptions was selected for the analysis. The number of adult *N. girellae* recovered from each *S. doliatus* was compared among treatments using a generalised linear model (GLM) following a negative binomial distribution (used for over-dispersed count data), and the presence of *N. girellae* eggs and adults within each aquarium were compared among treatments using a GLM following a binomial distribution.

5.3 Results

5.3.1 Eye spot development

There was no significant difference in the timing or frequency of eye spot development in *N. girellae* eggs among the four treatments, with 79% of *N. girellae* eggs developing eye spots on day five ($P = 0.57$; Figure 5-2).

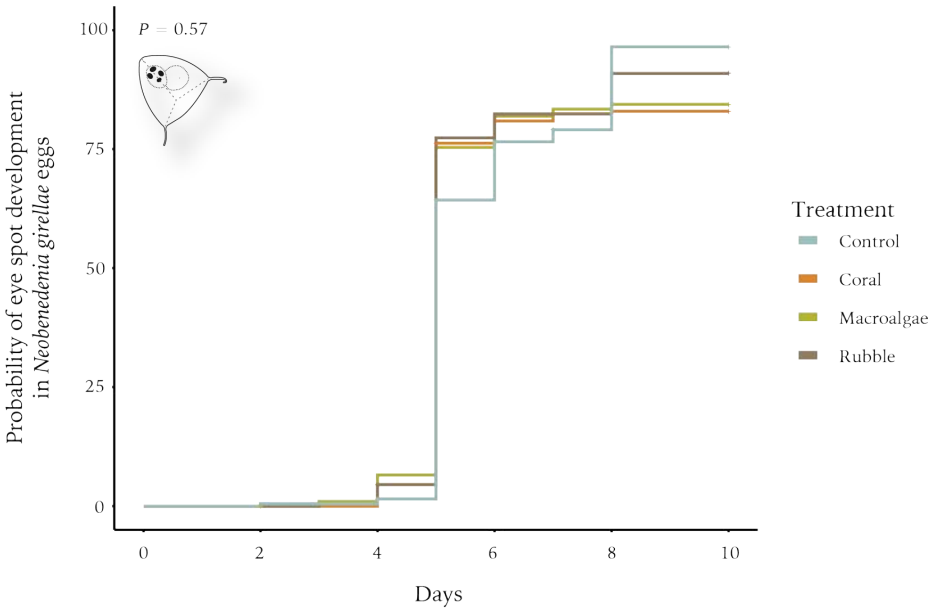

Figure 5-2: Kaplan–Meier survival curves showing the probability of eye spot development in *Neobenedenia girellae* eggs from each water treatment (n = 200 eggs per treatment; control, live coral, macroalgae and rubble) as a function of time (days). Vertical lines represent 'censored' data in which eye spot development has not yet occurred (i.e., eggs failed to develop) or is not known to have occurred (i.e., eggs were desiccated or unrecoverable). *P*-value is derived from a

log rank test, testing whether there is a difference among the survival curves of the different treatments.

5.3.2 Hatching success

There was a significant effect of treatment water upon the hatching success of *N. girellae* eggs (P = 0.01; Figure 5-3a). Specifically, a lower proportion of eggs hatched in the live coral (n = 168/200 hatched; P = 0.02) and macroalgae (n = 164/200 hatched; P = 0.03) treatments relative to those maintained within the control (n = 186/200 hatched). Due to high variability between replicates, hatching success within the rubble treatment (n = 180/200 hatched) was not significantly different to controls (P = 0.28) or the coral (P = 0.16) and macroalgae (P = 0.24) treatments. Survival (i.e., 'hatching') curves differed between the control and the three substrata treatments, with a greater proportion of eggs hatching a day earlier in the coral, macroalgae and rubble treatments (day seven) than the controls (day eight; P = 6e-09*;* Figure 5-3b).

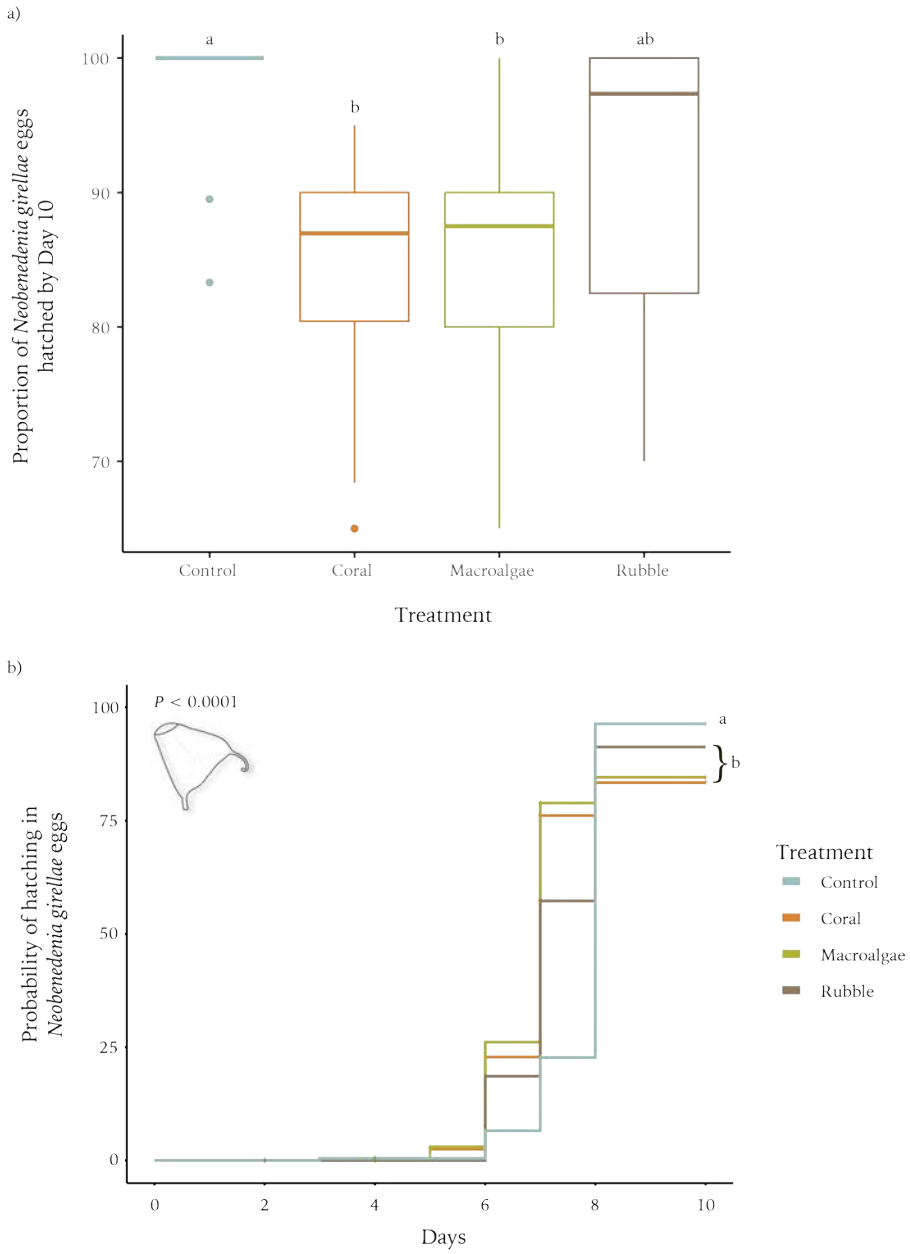

Figure 5-3: a) The proportion of *Neobenedenia girellae* eggs that hatched in the control (no substratum) and coral reef substrata treatments (water conditioned with live coral, macroalgae or rubble substrata; n = 200 eggs per treatment) at the close of the experiment (day 10); b) Kaplan–Meier survival curves showing the probability of hatching in *N. girellae* eggs from each water treatment (n = 200 eggs per treatment; control, live coral, macroalgae and rubble) as a function of time (days). Vertical lines represent 'censored' data in which hatching has not yet occurred (i.e., eggs failed to develop) or is not known to have occurred (i.e., eggs were desiccated or

unrecoverable). *P*-value is derived from a log rank test, testing whether there is a difference among the survival curves of the different treatments. Letters represent significant differences between treatments.

5.3.3 Abundance of Neobenedenia girellae adults

There was a slightly higher mean number of *N. girellae* adults infecting *S. doliatus* in the macroalgae treatment (adjusted mean = 0.67 ± 0.4 SE *N. girellae* adults per fish) than the three other treatments (control adjusted mean = 1.5e-9 ± 7.5e-6 SE *N. girellae* adults per fish; live coral = 0.1 ± 0.1 SE; rubble = 0.3 ± 0.2 SE), however these differences were not significant (*P* = 0.99; Figure 5-4). This lack of a significant difference was likely related to the high proportion of uninfected fish within each treatment. In line with the numbers of adult *N. girellae* infecting *S. doliatus* among treatments, there was no significant difference in the presence/absence of eggs or adults among treatments (*P* = 0.99).

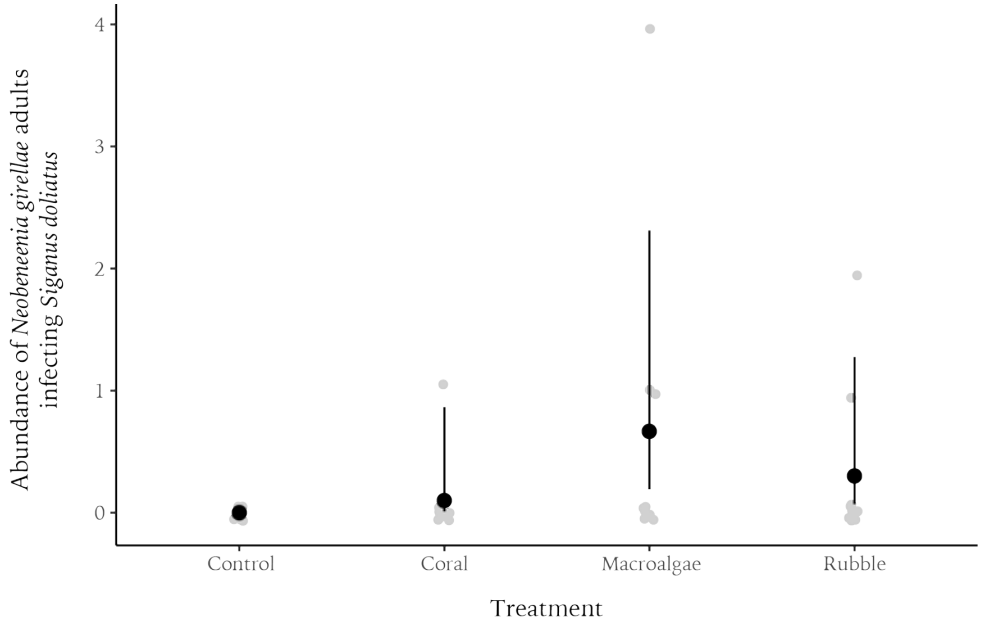

Figure 5-4: Differences in the abundance of *Neobenedenia girellae* adults infecting *S. doliatus* (n = 10 per treatment) from either control aquaria containing no substratum, or aquaria containing live coral, macroalgae or rubble substrata. Lines represent 95% confidence intervals; black circles represent adjusted means; grey circles represent raw data.

5.4 Discussion

Identifying the effects of coral reef substrata on parasite life histories and host-parasite interactions is key to understanding the basis for observed changes in parasite communities among habitats and predicting potential future changes under ongoing habitat degradation. In the present study, coral (*Porites cylindrica*), macroalga (*Sargassum* sp.) and rubble substrata significantly reduced the hatching success, but not eye spot development, of *N. girellae* relative to controls that lacked substrata. However, contrary to the initial hypothesis, there were no detectable differences in hatching success among the three coral reef substrata examined. Infection of *Siganus doliatus* by adult *N. girellae* was also comparable among the three coral reef substrata. This is concurrent with findings of Hutson et al. (2012), in which the embryonic development and hatching success of *N. girellae* larvae were significantly reduced when developed in polar extracts from two marine macroalgal species (*Ulva* spp. and *Asparagopsis taxiformis*), yet infection success of larvae and survival of *N. girellae* adults was unaffected. The reduced hatching success of *N. girellae* in water conditioned with the live coral, macroalga or rubble may be due to polar (i.e., water-soluble) chemicals released by corals, macroalgae, and biofouling taxa associated with rubble and/or their associated microbial communities. Several genera of algae (*Asparagopsis, Sargassum, Ulva*) have been found to kill or inhibit embryonic development in marine species through polar chemicals (i.e., water soluble surface extracts) and/or bacterial activity (Annelida, Echinodermata, Mollusca; Platyhelminthes; Hutson et al., 2012; Thabard et al., 2011). Therefore, in the present study, the direct negative influence of all three substrata treatments upon the life cycle of *N. girellae* is likely through chemical and/or microbial action.

5.4.1 Chemical and/or microbial reduction of egg-hatching success

In the present study, embryonic development and hatching success of the monogenean ectoparasite, *N. girellae,* were reduced by water conditioned with *Sargassum* sp., a canopy-forming brown alga abundant on inshore and degraded reefs (Bauman et al., 2017; Hughes et al.,

2007). Similar effects were observed when *N. girellae* embryos were reared in polar chemicals derived from the red alga *Asparagopsis taxiformis* and green alga *Ulva* sp. (Hutson et al., 2012). Surface extracts (algae dipped in hexane) of several sargassum species (*S. polyceratium, S. muticum*, and *S. horneri*) have also been found to negatively affect invertebrate embryos, larvae and larval settlement, causing 100% embryo mortality in the sea urchin, *Diadema antillarum,* bivalve, *Codakia orbicularis* (Thabard et al., 2011) and 80% mortality in larvae of the bryozoan, *Bugula neritina* (Schwartz et al., 2017). However, the effect of *Sargassum*'s chemical activity on embryos and larvae of coral reef species is variable, with surface extracts of *S. polyceratium* having no effect upon embryos of the annelid, *Pseudonereis* sp. (see Thabard et al., 2011) and *S. fusiforme* having no effect on the survival of *B. neritina* larvae (Schwartz et al., 2017).

Alternatively, the effects of *Sargassum* on embryos of *N. girellae* in the present study may be due to bacterial activity, with marine macroalgae typically hosting rich and abundant epiphytic bacterial communities (Barott et al., 2011; Egan et al., 2013). The bacterial communities of marine macroalgal species have been found repeatedly and extensively to affect marine invertebrates: reducing the survivorship and settlement of invertebrates such as coral (e.g., *Montipora capitata,* Vermeij et al., 2009) and bryozoan larvae (e.g., *B. neritina*; Rao et al., 2007). To the best of my knowledge however, the effects of macroalgal bacterial communities have not been tested upon the embryonic development of tropical marine species. However, in a similar study water conditioned with *Sargassum echinocarpum* and *S. polyphyllum*, from shallow, tropical reef systems, inhibited larval settlement of the bryozoan, *B. neritina*, and polychaete, *Hydroides elegans* (see Walters et al., 1996). In addition, the prevalence and abundance of the monogenean, *Lamellodiscus* sp., infecting white sea bream (*Diplodus sargus*) in an aquaculture setting has been shown to be negatively related to the density of the macroalga *Ulva* spp. (Cunha et al., 2019). In both studies, the precise mechanism (i.e., chemical or microbial activity) was not determined, therefore it is possible that the invertebrate and parasite species used may be responding to different chemical and or microbial activity of the algal species. Moreover, as

macroalgae forms part of a holobiont (Barott et al., 2011; Egan et al., 2013), other components of their holobiont / biofilm, such as diatoms, fungi, and protozoa may have also caused the results observed through the release of molecular cues or deterrents (Wahl et al., 2012).

Corals, like macroalgae, are known to use chemical and microbial activity to reduce biofouling and to prevent competition from other benthic organisms (Chadwick & Morrow, 2011; Koh & Sweatman, 2000). However, despite the capacity of the scleractinian coral holobiont to create a suite of antimicrobial, antibacterial, antiplasmodial and antiviral compounds (Sang et al., 2019), I am not aware of any studies that have directly tested the effect of these compounds on the embryonic development of marine species. This study found a significant reduction in the hatching success of the monogenean ectoparasite, *N. girellae*, when developed in water conditioned with the hard coral, *P. cylindrica*. Whilst the chemical inhibition of embryonation appears not to have been tested in hexacorals such as the Scleractinia, the chemical inhibition of embryonation in the tropical, freshwater zebrafish, *Danio rerio,* has been observed using solutions of marine natural compounds extracted from octocorals (i.e., soft corals and gorgonians; Bai et al., 2016). The tropical reef species of gorgonian, *Subergorgia mollis* and *Anthogorgia caerulea,* also inhibited larval settlement of the barnacle, *Balanus amphitrite*, through solutions of marine natural compounds (Bai et al., 2016). Moreover, bacteria from the biofilms of the soft coral *Dendronephthya* sp. have been shown to reduce the larval settlement success of the polychaete, *H. elegans,* and bryozoan, *B. neritina* (Dobretsov & Qian, 2004). However, the effects on hatching success may depend upon the hard coral species tested, as observed with species of macroalgae (Hutson et al., 2012), as well as other components of the holobiont, with solutions of marine natural compounds extracted from gorgonian and soft-coral derived fungi also found to inhibit embryonic development in zebrafish and larval settlement of the barnacle, *B. amphitrite* (Bai et al., 2016).

Contrary to hypotheses, *N. girellae* embryos developed in rubble-conditioned water hatched earlier and had a lower overall probability of hatching than those held in control water (i.e., water

without a substratum). The difference in the timing and hatching success of *N. girellae* from rubble conditioned water may be due to the presence of small quantities of multiple biofouling taxa that use coral rubble as a substratum, with solutions of marine natural compounds extracted from tunicates and sponges found to inhibit embryonic development of zebrafish (Bai et al., 2016). At the end of the experiment, the proportion of eggs that hatched was comparable between the rubble and control treatments and among rubble-, coral- and macroalgae-conditioned water. The similar effects of water conditioned with coral, macroalgae and rubble suggest there may be a common mechanism reducing the hatching success of *N. girellae* among these substrata.

5.4.2 Infection success of Neobenedenia girellae

The comparable infection success of *N. girellae* among control and substratum treatments suggests that once *N. girellae* larvae have contacted the host, the prevailing environmental conditions becomes less prohibitive. *Neobenedenia girellae* larvae may also initially bury under the epidermis to escape an undesirable environment, providing protection and increasing their likelihood of reaching sexual maturity (Buchmann & Lindenstrøm, 2002; Trujillo-González et al., 2015). Whilst attached, larvae obtain nutrition from the host which increases their resilience to environmental factors; without a host, larvae cannot persist for more than a few days in the environment (Brazenor & Hutson, 2015). In the present study, few adult *N. girellae* were recovered from all three substratum treatments while none were recovered from the control. In a previous study, the mean infection intensity of *N. girellae* infecting fish was found to decrease following 48 hours of infection (Trujillo-González et al., 2015). This was attributed to fish potentially developing an immune response to the parasites; to a natural attrition of parasites; and/or optimal experimental conditions for the fish, which potentially explains the low numbers of *N. girellae* recovered from *S. doliatus* in the present study. Moreover, *S. doliatus* used within the experiment were wild-captured and sourced from a commercial supplier and so may have had acquired immunity.

5.4.3 Future research

In the present study, all aquaria for a treatment were connected to a single sump due to the space and resource requirements of replicating each of the four treatments sumps. While the water from each aquaria was filtered and UV-sterilised before returning to the sump, some water-borne chemicals may have transferred among aquaria within each treatment. Future studies would benefit from increasing the replication of the treatment sumps. The results of the present study may have been influenced by prior exposure of the *S. doliatus* individuals to *Neobenedenia* spp., and hence acquired immunity to infection. An alternative fish model, such as captive-bred marine coral fishes (e.g., *Acanthochromis polyacanthus* or *Amphiprion percula*) would ensure naivety to infection and avoid potential effects of acquired immunity. Moreover, as model species were used to represent the interactions between coral reef parasites and substrata, and the action of benthic bacterial and chemical activity are species-specific, further research to see how different species of coral reef parasite and substrata may interact would be beneficial to up-scale these findings to a broader, ecosystem-wide scale. Lastly, efforts to determine the specific mechanisms within and components of the holobiont responsible for these effects (e.g., through the use of antibiotics) would greatly further our understanding of the mechanisms between host-parasite interactions among coral reef substrata.

5.4.4 Summary

This study was the first to find potential evidence of reduced egg-hatching success in a marine species due to chemical and/or microbial activity in hard corals. In doing so, I found that chemical/microbial activity of *P. cylindrica* (coral), *Sargassum* sp. (macroalga) and rubble substrata had comparably negative effects upon the hatching success of *N. girellae*. Therefore, whilst the proportion of *N. girellae* embryos surviving to adulthood may be reduced in these habitats, the similarity in development and survivorship among these three coral reef substrata suggest that the degradation of reef habitats from branching *P. cylindrica* to those dominated by rubble or *Sargassum* sp. may have little influence on the local population dynamics of capsalid

monogeneans, such as *N. girellae*. However, the effect of host abundance, also affected by shifts in coral reef benthic composition, on transmission; and the effect of environmental factors, such as water movement, on the residence time and local concentrations of bioactives must also be taken into consideration. Moreover, this study used only a single taxon to represent each coral reef substrata. Therefore, future research into the bioactivity of different coral reef benthic and habitat-forming taxa and their effect on the life cycles and infection success of marine ectoparasites should consider multiple taxa within each of the benthic categories. Lastly, understanding the specific compounds involved in these interactions is necessary to improve our understanding of the effects of habitat degradation upon parasite life cycles and infection success on coral reefs.

Chapter 6: General Discussion

Since the industrial revolution, the physical integrity of extensive areas of habitat have been either compromised or lost (Airoldi et al., 2008; Hansen et al., 2014). Understanding how infectious agents, such as parasites, are affected by habitat loss, degradation and loss of reef-building corals on coral reefs is of growing importance due to continued climatic change and anthropogenic stress. With the potential for habitat degradation to increase the prevalence of degraded macroalgae- and rubble-dominated habitats on coral reefs (Adam et al., 2021; Contreras-Silva et al., 2020; Vieira, 2020), previous studies have established a positive relationship between gnathiid isopod abundance and rubble habitats on coral reefs (Artim et al., 2020; Artim & Sikkel, 2013; Narvaez et al., 2021; Paula et al., 2021; Santos & Sikkel, 2019).

The objective of this thesis was to investigate the effect of coral reef substrata, representing a gradient of reef health (coral, macroalgae and rubble), on the parasite communities and host-parasite interactions of herbivorous coral reef fishes on inshore reefs of the Great Barrier Reef. Building on limited knowledge of the parasite communities of coral reef fishes, this thesis is the first to characterise the parasite communities of three common, co-occurring, herbivorous coral reef fishes on the GBR. Parasite communities and parasitism varied significantly among the three species. The larger-bodied *Siganus doliatus* was infected with the greatest abundance of parasites overall but was infected with similar taxon richness and ectoparasite abundances as *Pomacentrus wardi*. *Pomacentrus wardi* and *Pomacentrus adelus* had broadly overlapping parasite communities, sharing many of the same ecto- and endo-parasite families, yet they differed significantly in overall parasite abundance, richness and in levels of ectoparasitism. Whilst phylogeny plays a key role in determining a species' parasite community, differences in ectoparasitism may also be due to differences in body size, age, mobility and gregariousness of the three species. Differences in endoparasitism were likely related to differences in diet and feeding ecology among the three fish species (**Chapter 2**).

Diet and feeding ecology may also be important in determining endoparasitism of *P. wardi* in response to reef habitat degradation. *P. wardi* was infected with higher abundances of endoparasites in coral, relative to macroalgae and rubble habitats, potentially due to differences in the abundance of intermediate invertebrate hosts among coral, macroalgae and rubble habitats. Ectoparasite community composition and prevalence also varied among coral reef habitats, with ectoparasite prevalence greatest in rubble, intermediate in coral and lowest in macroalgae habitats. Predator abundance (i.e., live coral and cleaner wrasses) may have resulted in intermediate ectoparasite prevalence in coral habitats. Habitat degradation may have affected the ability of *P. wardi* to avoid or reduce ectoparasitic infection in rubble habitats, possibly through differences in the complexity and availability of shelter in rubble relative to coral and macroalgae habitats and in the chemical and olfactory landscape of degraded, rubble habitats (**Chapter 3)**. This is especially relevant as ectoparasites, particularly gnathiid isopods, were more abundant in rubble habitats, likely due to the reduced risk of predation by corals and cleaner wrasses (**Chapter 4**). Whilst coral reef substrata may indirectly influence coral reef parasite communities through host and habitat availability, live coral, macroalgae and coral rubble substrata might also influence ectoparasitism through chemical and microbial interactions, with water conditioned with live coral or macroalgae observed to reduce the hatching success of a common coral reef ectoparasite, *Neobenedenia girellae* (**Chapter 5**).

In **Chapters 3 and 4**, an apparent link between degraded coral reef habitats (i.e., macroalgae- or rubble-dominated habitats) and the increased abundance of crustacean parasites was observed. Specifically, ectoparasitic arthropods (Caligidae and Pennellidae) were in higher relative abundance in macroalgae habitats (**Chapter 3, 4**) and gnathiid isopods were most abundant in rubble and intermediate in macroalgae habitats (**Chapter 4**) relative to coral habitats. Previous research has shown rubble habitats support higher abundances of crustaceans (parasitic and non-parasitic) relative to sand, epilithic algal matrix and branching live coral habitats (Kramer et al., 2014). However, macroalgae habitats are also important habitats for epifauna such as gnathiid

isopods and copepods (mainly harpacticoid copepods) (Nakamura & Sano, 2005; Tano et al., 2016), compared to the Pennellidae and Caligidae (i.e., siphonostomatoid copepods) that characterised ectoparasite communities of *P. wardi* in macroalgal habitats (**Chapter 3**). Both rubble and macroalgal habitats support reduced abundances of live coral, known predators of gnathiid isopod larvae (Artim & Sikkel, 2013; Paula et al., 2021), and potentially other crustaceans such as Pennellidae and Caligidae. Further investigation is required to understand the potential link between degraded coral reef habitats and crustacean parasite abundance, diversity and community composition, utilising multiple fish species to capture the response of a range of crustacean parasites.

The avoidance of specific (micro)habitats to reduce exposure to parasites is a behaviour observed in freshwater (Karvonen et al., 2004b) and marine ecosystems (Poulin & FitzGerald, 1989a). Whilst several studies have suggested that coral reef fishes may avoid particular habitats, and/or favour other coral reef habitats, to reduce their exposure to ectoparasites (Artim & Sikkel, 2013; Paula et al., 2021), very few studies have tested the use of these behaviours in coral reef systems. However, there is some evidence to suggest that ectoparasitism of coral reef fishes may be affected by availability of shelter, allowing fish to potentially evade and reduce ectoparasitic infection (Sikkel et al., 2006). This is particularly important, as with continued declines in live coral cover, reduced structural complexity of coral reefs may reduce the capacity for such avoidance behaviours. Therefore, the use of habitat avoidance behaviour to reduce parasite exposure and infection in coral reef fishes is an area that requires further research.

6.1 Future research directions: habitat fragmentation and fragment connectivity

This study was the first to quantify and compare fish parasite communities and parasitic infection of coral reef fishes among coral reef habitats and substrata. These findings provide insight into the potential changes we may observe among coral reef habitats and the mechanisms that may be responsible. However, the conclusions of this study apply to a single study location and three species of coral reef fish. Further research into a broader diversity of fish families, functions,

ecologies and from different reef locations will allow us to understand the potential generality of these findings.

This thesis investigated the effects of habitat degradation, specifically shifts in benthic composition, on the parasite communities and parasitic infection of coral reef fishes. However, habitat fragmentation is also a common result of habitat loss and degradation (Fischer & Lindenmayer, 2007). Habitat fragmentation is the break-up of a habitat into smaller, more isolated habitat patches (Fahrig, 2003). These patches consequently have higher rates of species extinctions, lower immigration rates and result in a loss of biodiversity (Fischer & Lindenmayer, 2007; Haddad et al., 2015). Through reductions in biodiversity, habitat fragmentation may have the greatest impact on heteroxenous parasites (i.e., parasite taxa that require multiple hosts to complete their life cycle; Sala et al., 2000).

Habitat fragmentation can also affect parasitism within degraded systems through affecting the density and movement of hosts within and between habitats (McCallum, 2008; Thies et al., 2008). For example, when a habitat area is reduced through fragmentation and exhibits low connectivity between fragments, isolated 'island ecosystems' can form (Haila, 2002). This isolation can also result in a crowding effect in which a reduction in habitat area causes the density of the host population to increase within a patch. This can have knock-on effects to parasitism, as high host population densities can increase host-to-host transmission and local parasite densities, causing increased parasitism and vulnerability of host populations within fragmented landscapes (Deem et al., 2001; Holmes, 1996; McCallum, 2008). For example, for farmlands in northern Germany, the reduction of rape crops resulted in the crowding and increased density of pollen beetles. The increase in host density resulted in the increased transmission of pollen beetle parasites, increasing the infection intensity and mortality of pollen beetles (Thies et al., 2008). This crowding effect is a particular problem within aquaculture, where high densities of fish held in sea cages can result in high parasite transmission and outbreaks of parasites within commercial fish farms (Krkošek, 2010).

Connectivity is a key component in regulating parasitism within fragmented habitat patches, as it determines the level of host (and subsequently parasite) movement between patches, thereby regulating biodiversity and host and parasite densities within them (Hess, 1994, 1996). If habitat patches remain connected, mobile host species and the dispersal of parasite larvae may act as a source of parasites to nearby patches via host-host and trophic transmission, maintaining host biodiversity, replenishing parasite populations and sustaining infection within these patches. If habitat patches are poorly connected, the risk of extinction is likely to increase and the abundance of heteroxenous parasites may decrease due to reduced host diversity (Fischer & Lindenmayer, 2007; Haddad et al., 2015). In this way, high connectivity between fragments helps to alleviate extinction pressure from smaller patches, as well as maintaining host and parasite biodiversity alongside low, sustained levels of parasitism within patches (McCallum & Dobson, 2002).

To date, very few studies have investigated the effects of habitat fragmentation and fragment connectivity on parasitism and parasite communities in aquatic environments. It is possible that host-parasite interactions of larger, mobile species may not be affected by habitat fragmentation due to their capacity to move large distances between fragmented habitats. However, for small-bodied, site-attached species that occupy lower trophic levels, their home range may be too small to allow movement between patches. Species density, host-host transmission and consequently parasitism may therefore increase within small-bodied species due to habitat fragmentation. Habitat fragmentation and the crowding effect may therefore result in altered food web structure due to potentially elevated levels of parasitism within lower trophic levels. However, much of this research has been conducted within terrestrial systems.

To gain insight into the effects of habitat degradation on coral reef parasites and parasitism, it is important to also understand the role of habitat fragmentation and fragment connectivity. Whilst future research is required into the effect of fragmentation and connectivity among coral patches, coral reef habitats do not exist in isolation but are highly connected to other marine benthic systems such as seagrass meadows and mangroves. These systems provide habitat, nursery sites,

foraging opportunities and are widely used by multiple species of coral reef fishes (Sambrook et al., 2019; Sievers et al., 2020). Therefore, it is important for future studies to consider the spatial configuration of coral reef habitats and the broader effect of seascape and habitat connectivity on parasitism.

Habitat degradation and shifts in benthic composition on coral reefs are attributable to multiple anthropogenic and climatic stressors. Increases in sea surface temperature are one of the most immediate threats to coral reef organisms (Hughes et al., 2017), with global sea surface temperatures projected to increase by approximately 1.0 °C by 2050 under a 'worst case' emissions scenario (IPCC, 2019). The effects of increasing global temperatures on parasitism have been well-studied in many terrestrial (e.g., Wu et al., 2021), intertidal (e.g., Studer et al., 2010) and aquatic systems (Marcogliese, 2008). Specific coral reef organisms (Allan et al., 2017; Bernal et al., 2018; Scott et al., 2017) and parasite species (Brazenor et al., 2020; Brazenor & Hutson, 2015; Morales-Serna et al., 2021; Shodipo et al., 2020) have been the focus of considerable climate change research. However, few, if any, studies have investigated how the abundance and composition of parasites infecting coral reef fish vary with projected increases in sea surface temperature. Species-specific responses of both parasites and their hosts to increased sea surface temperatures highlight the need to understand the effects of increased sea surface temperatures on host-parasite interactions on coral reefs. Moreover, marine heatwaves such as those observed globally in 2016-2017 are projected to increase in frequency and severity, causing extensive coral mortality and reduced periods of recovery (Hughes et al., 2018). In response to the 2016-17 marine heatwave and subsequent global bleaching event, the abundance of gnathiid isopods in emergence traps was significantly reduced in warmer months relative to non-bleaching years (Sikkel et al., 2019). Therefore, the effects of such marine heatwaves upon parasite communities, infection and transmission (e.g., Sikkel et al., 2019; Claar & Wood 2020) would considerably improve our ability to predict the structure and composition of parasite communities in the future.

6.2 The future of host-parasite-environment interactions on coral reefs

Habitat degradation and coral loss can reduce the fitness (Thompson et al., 2019), growth (Feary et al., 2009), oxygen uptake (Downie et al., 2021), cautionary behaviour (McCormick et al., 2017), escape response (McCormick & Allan, 2017) and increase the metabolic rate (Norin et al., 2018) of coral reef fishes. The increased abundances of ectoparasites observed in rubble habitats in the present study are likely insufficient to cause mortality or significant decreases in host health and fitness. Yet, increased ectoparasitism in degraded habitats may contribute to the suite of sub-lethal effects that compromise the health and reduce the resilience of coral reef fishes. It is imperative that the effects of habitat degradation on parasitism and parasite communities on coral reefs and the health and fitness consequences of these changes are understood, particularly in herbivorous coral reef fishes that play a key role in the function and resilience of these systems. Finally, the complexity of these systems means that there is no one single cause nor one single effect. On the contrary, the multiplicity of host-parasite-environment interactions mean that the effect of habitat degradation on parasite communities and parasitism is multifaceted.

The effects of coral reef substrata and changes in the benthic composition of coral reefs may affect host-parasite interactions and parasite communities through effects to host diet and host behaviour, as well as parasite habitats, transmission and life history (**Chapter 2-5**). Shifts in the benthic composition of coral reefs potentially affected endoparasite communities and endoparasitism likely through shifts in intermediate host abundance (**Chapter 3**). Ectoparasite communities were affected by different coral reef habitats presumably through changes in habitat availability, suitability and predator abundance (**Chapter 3-5**). Coral reef parasites are affected by habitat degradation as a direct consequence of their habitat associations and the ways in which coral reef substrata can alter their reproductive, transmission and life history strategies (**Chapter 2-5**). This study provides supporting evidence that, due to reductions in intermediate host abundance, heteroxenous parasites may be more susceptible to habitat degradation, owing to their complex multi-host life cycle, relative to monoxenous parasites that use only a single host

(**Chapter 3;** Diamant et al., 1999; Dzikowski et al., 2003a, 2003b; Kiene et al., 2021; Pérez-del Olmo et al., 2007). Whilst reduced endoparasitism in degraded habitats may be perceived as a health benefit to the host, the reduced abundance of heteroxenous parasites with complex life cycles may represent the broader degradation of the habitat and trophic system overall (D'Amelio & Gerasi, 1997; Mackenzie, 1999; Marcogliese, 2005; Sures et al., 2017). Host specialists may be more dependent on, and therefore limited by, host availability than host generalists (**Chapter 3, 4**). Hence, in degraded habitats with reduced host abundance, the parasite community may become dominated by directly transmitted host- and habitat-generalist species, such as gnathiid isopods (Artim et al., 2020), with connections between degraded coral reef habitats and crustacean ectoparasite abundance in need of further research.

Due to the trait- and taxa-specific responses of parasites to habitat degradation, it is important to consider parasite communities to understand the full range of responses to habitat degradation, the mechanisms driving these responses and to reduce taxa bias towards those parasites that are the most abundant or easily sampled. At present, our understanding of parasite life cycles on coral reefs, their life histories, the effects of coral reef degradation on each life stage and on intermediate hosts are limited. Future studies may consider recording changes and differences in host and parasite populations and communities in response to habitat degradation, to unequivocally relate these changes to specific causal factors.

References

Adam, T. C., Burkepile, D. E., Holbrook, S. J., Carpenter, R. C., Claudet, J., Loiseau, C., Thiault, L., Brooks, A. J., Washburn, L., & Schmitt, R. J. (2021). Landscape-scale patterns of nutrient enrichment in a coral reef ecosystem: implications for coral to algae phase shifts. *Ecological Applications*, *31*(1), Article e2227.

Aeby, G. (2002). Trade-offs for the butterflyfish, *Chaetodon multicinctus,* when feeding on coral prey infected with trematode metacercariae. *Behavioural Ecology and Sociobiology, 52,* 158-165.

Afrane, Y. A., Zhou, G., Lawson, B. W., Githeko, A. K., & Yan, G. (2006). Effects of microclimatic changes caused by deforestation on the survivorship and reproductive fitness of *Anopheles gambiae* in western Kenya highlands. *The American Journal of Tropical Medicine and Hygiene*, *74*(5), 772–778.

Afrane, Y. A., Zhou, G., Lawson, B. W., Githeko, A. K., & Yan, G. (2007). Life-table analysis of *Anopheles arabiensis* in western Kenya highlands: effects of land covers on larval and adult survivorship. *The American Journal of Tropical Medicine and Hygiene*, *77*(4), 660–666. https://doi.org/10.4269/ajtmh.2007.77.660

Airoldi, L., Balata, D., & Beck, M. W. (2008). The gray zone: relationships between habitat loss and marine diversity and their applications in conservation. *Journal of Experimental Marine Biology and Ecology*, *366*(1–2), 8–15.

Al-Jahdali, M. O., & Hassanine, R. M. E.-S. (2012). The life cycle of *Gyliauchen volubilis* Nagaty, 1956 (Digenea: Gyliauchenidae) from the Red Sea. *Journal of Helminthology*, *86*(2), 165–172.

Allan, B. J. M., Domenici, P., Watson, S. A., Munday, P. L., & McCormick, M. I. (2017). Warming has a greater effect than elevated CO2 on predator–prey interactions in coral reef

fish. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1857). https://doi.org/https://doi.org/10.1098/rspb.2017.0784

Allan, B. J. M., Illing, B., Fakan, E. P., Narvaez, P., Grutter, A. S., Sikkel, P. C., McClure, E. C., Rummer, J. L., & McCormick, M. I. (2020). Parasite infection directly impacts escape response and stress levels in fish. *Journal of Experimental Biology*, *223*(16). https://doi.org/https://doi.org/10.1242/jeb.230904

Allen, G. R., Steene, R., Humann, P., & Deloach, N. (2015). *Reef fish identification: tropical Pacific* (Second). New World Publications Incorporated.

Alvarez-Filip, L., Dulvy, N. K., Gill, J. A., Côté, I. M., & Watkinson, A. R. (2009). Flattening of Caribbean coral reefs: region-wide declines in architectural complexity. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1669), 3019–3025.

Ancillotto, L., Studer, V., Howard, T., Smith, V. S., McAlister, E., Beccaloni, J., Manzia, F., Renzopaoli, F., Bosso, L., & Russo, D. (2018). Environmental drivers of parasite load and species richness in introduced parakeets in an urban landscape. *Parasitology Research*, *117*(11), 3591–3599.

Anderson, R. M., & May, R. M. (1978). Regulation and stability of host-parasite population interactions: I. Regulatory processes. *The Journal of Animal Ecology*, *47*(1), 219–247.

Aponte, V., Locke, S. A., Gentes, M.-L., Giroux, J.-F., Marcogliese, D. J., McLaughlin, D., & Verreault, J. (2014). Effect of habitat use and diet on the gastrointestinal parasite community of an avian omnivore from an urbanized environment. *Canadian Journal of Zoology*, *92*(7), 629–636.

Arneberg, P. (2002). Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. *Ecography*, *25*(1), 88–94.

Arneberg, P., Folstad, I., & Karter, A. J. (1996). Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology*, *112*(2), 213–219.

Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London B: Biological Sciences*, *265*(1403), 1283–1289.

Artim, J. M., Nicholson, M. D., Hendrick, G. C., Brandt, M., Smith, T. B., & Sikkel, P. C. (2020). Abundance of a cryptic generalist parasite reflects degradation of an ecosystem. *Ecosphere*, *11*(10), e03268.

Artim, J. M., & Sikkel, P. C. (2013). Live coral repels a common reef fish ectoparasite. *Coral Reefs*, *32*(2), 487–494.

Bai, H., Kong, W.-W., Shao, C.-L., Li, Y., Liu, Y.-Z., Liu, M., Guan, F.-F., & Wang, C.-Y. (2016). Zebrafish embryo toxicity microscale model for ichthyotoxicity evaluation of marine natural products. *Marine Biotechnology*, *18*(2), 264–270.

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, *10*(2), 131–165.

Barker, S. C., Cribb, T. H., Bray, R. A., & Adlard, R. D. (1994). Host-parasite associations on a coral reef: Pomacentrid fishes and digenean trematodes. *International Journal for Parasitology*, *24*(5), 643–647.

Barott, K. L., Rodriguez-Brito, B., Janouškovec, J., Marhaver, K. L., Smith, J. E., Keeling, P., & Rohwer, F. L. (2011). Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. *Environmental Microbiology*, *13*(5), 1192–1204.

Bauman, A. G., Hoey, A. S., Dunshea, G., Feary, D. A., Low, J., & Todd, P. A. (2017). Macroalgal browsing on a heavily degraded, urbanized equatorial reef system. *Scientific Reports*, *7*(1), 1–8.

Behie, A. M., Kutz, S., & Pavelka, M. S. (2014). Cascading effects of climate change: Do hurricane-damaged forests increase risk of exposure to parasites? *Biotropica*, *46*(1), 25–31.

Behringer, D. C., Karvonen, A., & Bojko, J. (2018). Parasite avoidance behaviours in aquatic environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1751). https://doi.org/http://dx.doi.org/10.1098/rstb.2017.0202

Bellwood, D. R., Hoey, A. S., Ackerman, J. L., & Depczynski, M. (2006). Coral bleaching, reef fish community phase shifts and the resilience of coral reefs. *Global Change Biology*, *12*(9), 1587–1594.

Benesh, D. P., & Kalbe, M. (2016). Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly. *Journal of Animal Ecology*, *85*(4), 1004–1013.

Berkström, C., Jones, G. P., McCormick, M. I., & Srinivasan, M. (2012). Ecological versatility and its importance for the distribution and abundance of coral reef wrasses. *Marine Ecology Progress Series*, *461*, 151–163. https://doi.org/https://doi.org/10.3354/meps09788

Bernal, M. A., Donelson, J. M., Veilleux, H. D., Ryu, T., Munday, P. L., & Ravasi, T. (2018). Phenotypic and molecular consequences of stepwise temperature increase across generations in a coral reef fish. *Molecular Ecology*, *27*(22), 4516–4528.

Beuret, J., Scott, D. A., & Pearson, J. C. (2000). The cercaria of *Galactosomum bearupi* Pearson, 1973 (Trematoda: Heterophyidae) at Heron and Masthead Islands, Great Barrier Reef, Australia. *Systematic Parasitology*, *46*(1), 69–77.

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 12: survival analysis. *Critical Care*, *8*(5), 389–394. https://doi.org/10.1186/cc2955

Binning, S. A., Roche, D. G., & Layton, C. (2013). Ectoparasites increase swimming costs in a coral reef fish. *Biology Letters*, *9*(1). https://doi.org/http://dx.doi.org/10.1098/rsbl.2012.0927

Boström-Einarsson, L., Bonin, M. C., Munday, P. L., & Jones, G. P. (2018). Loss of live coral compromises predator-avoidance behaviour in coral reef damselfish. *Scientific Reports*, *8*(1), 1–9.

Bracewell, S. A., Clark, G. F., & Johnston, E. L. (2018). Habitat complexity effects on diversity and abundance differ with latitude: an experimental study over 20 degrees. *Ecology*, *99*(9), 1964–1974.

Brandl, S. J., & Bellwood, D. R. (2013). Pair formation in the herbivorous rabbitfish *Siganus doliatus*. *Journal of Fish Biology*, *82*(6), 2031–2044.

Bray, R. A., & Cribb, T. H. (1998). Lepocreadiidae (Digenea) of Australian coastal fishes: new species of *Opechona* Looss, 1907, *Lepotrema* Ozaki, 1932 and *Bianium* Stunkard, 1930 and comments on other species reported for the first time or poorly known in Australian waters. *Systematic Parasitology*, *41*(2), 123–148.

Bray, R. A., & Cribb, T. H. (2000). The status of the genera *Hysterolecithoides* Yamaguti, 1934, *Neotheletrum* Gibson & Bray, 1979 and *Machidatrema* León-Règagnon, 1998 (Digenea: Hemiuroidea), including a description of *M. leonae* n. sp. from Australian waters. *Systematic Parasitology*, *46*(1), 1–22.

Bray, R. A., Cribb, T. H., & Barker, S. C. (1993). The Hemiuroidea (Digenea) of pomacentrid fishes (Perciformes) from Heron Island, Queensland, Australia. *Systematic Parasitology*, *24*(3), 159–184.

Brazenor, A. K., Bertozzi, T., Miller, T. L., Whittington, I. D., & Hutson, K. S. (2018). DNA profiling reveals *Neobenedenia girellae* as the primary parasitic monogenean in global fisheries and aquaculture. *Molecular Phylogenetics and Evolution*, *129*, 130–137.

Brazenor, A. K., Francis, D. S., Conlan, J. A., Carton, A. G., & Hutson, K. S. (2020). Temperature alters reproduction and maternal provisioning in a fish ectoparasite. *International Journal for Parasitology*, *50*(10–11), 839–849.

Brazenor, A. K., & Hutson, K. S. (2015). Effects of temperature and salinity on the life cycle of *Neobenedenia* sp. (Monogenea: Capsalidae) infecting farmed barramundi (*Lates calcarifer*). *Parasitology Research*, *114*(5), 1875–1886.

Breder, C. M., & Rosen, D. E. (1966). *Modes of reproduction in fishes*. T.F.H. Publications.

Brunner, F. S., & Eizaguirre, C. (2016). Can environmental change affect host/parasite-mediated speciation? *Zoology*, *119*(4), 384–394. https://doi.org/https://doi.org/10.1016/j.zool.2016.04.001

Buchmann, K., & Lindenstrøm, T. (2002). Interactions between monogenean parasites and their fish hosts. *International Journal for Parasitology*, *32*(3), 309–319.

Bullard, S. A., Benz, G. W., Overstreet, R. M., Williams Jr, E. H., & Hemdal, J. (2000). Six new host records and an updated list of wild hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). *Comparative Parasitology*, *67*(2), 190–196.

Burkepile, D. E., & Hay, M. E. (2008). Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. *Proceedings of the National Academy of Sciences*, *105*(42), 16201–16206.

Burkett-Cadena, N. D., & Vittor, A. Y. (2018). Deforestation and vector-borne disease: Forest conversion favors important mosquito vectors of human pathogens. *Basic and Applied Ecology*, *26*, 101–110.

Burrell, A. L., Evans, J. P., & de Kauwe, M. G. (2020). Anthropogenic climate change has driven over 5 million km^2 of drylands towards desertification. *Nature Communications*, *11*(1), 3853. https://doi.org/10.1038/s41467-020-17710-7

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of Parasitology*, *83*(4), 575–583.

Bush, S. E., & Clayton, D. H. (2018). Anti-parasite behaviour of birds. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1751). https://doi.org/https://doi.org/10.1098/rstb.2017.0196

Campbell, R. A., Haedrich, R. L., & Munroe, T. A. (1980). Parasitism and ecological relationships among deep-sea benthic fishes. *Marine Biology*, *57*(4), 301–313.

Caro, A., Combes, C., & Euzet, L. (1997). What makes a fish a suitable host for Monogenea in the Mediterranean? *Journal of Helminthology*, *71*(3), 203–210.

Casatti, L., Langeani, F., & Ferreira, C. P. (2006). Effects of physical habitat degradation on the stream fish assemblage structure in a pasture region. *Environmental Management*, *38*(6), 974–982.

Ceccarelli, D. M. (2007). Modification of benthic communities by territorial damselfish: a multi-species comparison. *Coral Reefs*, *26*(4), 853–866.

Ceccarelli, D. M., Hughes, T. P., & McCook, L. J. (2006). Impacts of simulated overfishing on the territoriality of coral reef damselfish. *Marine Ecology Progress Series*, *309*, 255–262.

Ceccarelli, D. M., Jones, G. P., & McCook, L. J. (2005). Effects of territorial damselfish on an algal-dominated coastal coral reef. *Coral Reefs*, *24*(4), 606–620.

Chadwick, N. E., & Morrow, K. M. (2011). Competition among sessile organisms on coral reefs. In Z. Dubinsky & N. Stambler (Eds.), *Coral Reefs: An Ecosystem in Transition* (pp. 347–371). Springer. https://doi.org/https://doi.org/10.1007/978-94-007-0114-4_20

Chambers, C. B., & Ernst, I. (2005). Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. *Aquaculture*, *250*(1–2), 60–69.

Chapman, J. M., Marcogliese, D. J., Suski, C. D., & Cooke, S. J. (2015). Variation in parasite communities and health indices of juvenile *Lepomis gibbosus* across a gradient of watershed land-use and habitat quality. *Ecological Indicators*, *57*, 564–572.

Cheal, A. J., Emslie, M., Miller, I., & Sweatman, H. (2012). The distribution of herbivorous fishes on the Great Barrier Reef. *Marine Biology*, *159*(5), 1143–1154.

Cheal, A. J., MacNeil, M. A., Cripps, E., Emslie, M. J., Jonker, M., Schaffelke, B., & Sweatman, H. (2010). Coral–macroalgal phase shifts or reef resilience: links with diversity and functional roles of herbivorous fishes on the Great Barrier Reef. *Coral Reefs*, *29*(4), 1005–1015.

Chivers, D. P., McCormick, M. I., Allan, B. J. M., & Ferrari, M. C. O. (2016). Risk assessment and predator learning in a changing world: understanding the impacts of coral reef degradation. *Scientific Reports*, *6*(1), 1–7.

Claar, D. C., & Wood, L. C. (2020). Pulse Heat Stress and Parasitism in a Warming World. *Trends in Ecology & Evolution, (35)*8, 704-715.

Clarke, K. R., Somerfield, P. J., & Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, *330*(1), 55–80.

Clements, K. D., & Choat, J. H. (1995). Fermentation in tropical marine herbivorous fishes. *Physiological Zoology*, *68*(3), 355–378.

Coker, D. J., Pratchett, M. S., & Munday, P. L. (2009). Coral bleaching and habitat degradation increase susceptibility to predation for coral-dwelling fishes. *Behavioral Ecology*, *20*(6), 1204–1210. https://doi.org/10.1093/beheco/arp113

Contreras-Silva, A. I., Tilstra, A., Migani, V., Thiel, A., Pérez-Cervantes, E., Estrada-Saldívar, N., Elias-Ilosvay, X., Mott, C., Alvarez-Filip, L., & Wild, C. (2020). A meta-analysis to

assess long-term spatiotemporal changes of benthic coral and macroalgae cover in the Mexican Caribbean. *Scientific Reports*, *10*(1), 1–12.

Côté, I. M., & Molloy, P. P. (2003). Temporal variation in cleanerfish and client behaviour: does it reflect ectoparasite availability? *Ethology*, *109*(6), 487–499.

Cribb, T. H., Adlard, R. D., & Bray, R. A. (1998). A DNA-based demonstration of a three-host life-cycle for the Bivesiculidae (Platyhelminthes: Digenea). *International Journal for Parasitology*, *28*(11), 1791–1795.

Cribb, T. H., Bott, N. J., Bray, R. A., McNamara, M. K. A., Miller, T. L., Nolan, M. J., & Cutmore, S. C. (2014). Trematodes of the Great Barrier Reef, Australia: Emerging patterns of diversity and richness in coral reef fishes. *International Journal for Parasitology*, *44*(12), 929–939. https://doi.org/10.1016/j.ijpara.2014.08.002

Cribb, T. H., & Bray, R. A. (2010). Gut wash, body soak, blender and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. *Systematic Parasitology*, *76*(1), 1–7.

Cribb, T. H., Bray, R. A., & Barker, S. C. (1994). Bivesiculidae and Haplosplanchnidae (Digenea) from fishes of the southern Great Barrier Reef, Australia. *Systematic Parasitology*, *28*(2), 81–97.

Cribb, T. H., Bray, R. A., Barker, S. C., & Adlard, R. D. (1996). Taxonomy and biology of *Mitotrema anthostomatum* Manter, 1963 (Digenea: Cryptogonimidae) from fishes of the southern Great Barrier Reef, Australia. *Journal of the Helminthological Society of Washington*, *63*(1), 110–115.

Cribb, T. H., Bray, R. A., Barker, S. C., Adlard, R. D., & Anderson, G. R. (1994). Ecology and diversity of digenean trematodes of reef and inshore fishes of Queensland. *International Journal for Parasitology*, *24*(6), 851–860. https://doi.org/https://doi.org/10.1016/0020-7519(94)90011-6

Cunha, M. E., Quental-Ferreira, H., Parejo, A., Gamito, S., Ribeiro, L., Moreira, M., Monteiro, I., Soares, F., & Pousão-Ferreira, P. (2019). Understanding the individual role of fish, oyster, phytoplankton and macroalgae in the ecology of integrated production in earthen ponds. *Aquaculture*, *512*. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.734297

Curtis, P. G., Slay, C. M., Harris, N. L., Tyukavina, A., & Hansen, M. C. (2018). Classifying drivers of global forest loss. *Science*, *361*(6407), 1108–1111.

Cvitanovic, C., & Hoey, A. S. (2010). Benthic community composition influences within-habitat variation in macroalgal browsing on the Great Barrier Reef. *Marine and Freshwater Research*, *61*(9), 999–1005.

Dairain, A., Legeay, A., & de Montaudouin, X. (2019). Influence of parasitism on bioturbation: from host to ecosystem functioning. *Marine Ecology Progress Series*, *619*, 201–214. https://doi.org/10.3354/meps12967

D'Amelio, S., & Gerasi, L. (1997). Evaluation of environmental deterioration by analysing fish parasite biodiversity and community structure. *Parassitologia*, *39*(3), 237–241.

Dáttilo, W., Barrozo-Chávez, N., Lira-Noriega, A., Guevara, R., Villalobos, F., Santiago-Alarcon, D., Neves, F. S., Izzo, T., & Ribeiro, S. P. (2020). Species-level drivers of mammalian ectoparasite faunas. *Journal of Animal Ecology*, *89*(8), 1754–1765.

Deem, S. L., Karesh, W. B., & Weisman, W. (2001). Putting theory into practice: wildlife health in conservation. *Conservation Biology*, *15*(5), 1224–1233.

Derby, C. D., & Sorensen, P. W. (2008). Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans. *Journal of Chemical Ecology*, *34*(7), 898–914.

di Santo, V., O'Boyle, L. A., Saylor, R. K., Dabruzzi, T. F., Covell, M. A., Kaack, K., Scharer, R., Seger, K., Favazza, N., & Pomory, C. M. (2020). Coral loss alters guarding and farming behavior of a Caribbean damselfish. *Marine Biology*, *167*(8), 1–9.

Diamant, A., Banet, A., Paperna, I., Westernhagen, H. v, Broeg, K., Krüner, G., Koerting, W., & Zander, S. (1999). The use of fish metabolic, pathological and parasitological indices in pollution monitoring. *Helgoland Marine Research*, *53*(3–4), 195–208.

Diamant, A., & Paperna, I. (1986). The parasites of wild Red-Sea rabbitfish (Siganus spp.)(Teleostei: Perciformes) as potential pathogens in mariculture. *Pathology in Marine Aquaculture. European Aquaculture Society, Special Publication*, *9*, 71–83.

Dobretsov, S., & Qian, P.-Y. (2004). The role of epibotic bacteria from the surface of the soft coral *Dendronephthya* sp. in the inhibition of larval settlement. *Journal of Experimental Marine Biology and Ecology*, *299*(1), 35–50.

Dobson, A. P., & Hudson, P. J. (1986). Parasites, disease and the structure of ecological communities. *Trends in Ecology & Evolution*, *1*(1), 11–15.

Dobson, A. P., Lafferty, K. D., Kuris, A. M., Hechinger, R. F., & Jetz, W. (2008). Homage to Linnaeus: How many parasites? How many hosts? *Proceedings of the National Academy of Sciences*, *105*, 11482–11489. http://www.pnas.org/content/early/2008/08/08/0803232105.abstract

Done, T. J. (1992). Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia*, *247*(1–3), 121–132.

Downie, A. T., Phelps, C. M., Jones, R., Rummer, J. L., Chivers, D. P., Ferrari, M. C. O., & McCormick, M. I. (2021). Exposure to degraded coral habitat depresses oxygen uptake rate during exercise of a juvenile reef fish. *Coral Reefs*, *40*, 1361–1367.

Dunne, J. A., Lafferty, K. D., Dobson, A. P., Hechinger, R. F., Kuris, A. M., Martinez, N. D., McLaughlin, J. P., Mouritsen, K. N., Poulin, R., & Reise, K. (2013). Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology*, *11*(6), Article e1001579.

Duong, B., Blomberg, S. P., Cribb, T. H., Cowman, P. F., Kuris, A. M., McCormick, M. I., Warner, R. R., Sun, D., & Grutter, A. S. (2019). Parasites of coral reef fish larvae: its role in the pelagic larval stage. *Coral Reefs*, *38*(2), 199–214.

Dzikowski, R., Paperna, I., & Diamant, A. (2003a). Multi-annual changes in the parasite communities of rabbitfish Siganus rivulatus (Siganidae) in the Gulf of Aqaba, Red Sea. *Helgoland Marine Research*, *57*(3), 228–235.

Dzikowski, R., Paperna, I., & Diamant, A. (2003b). Use of fish parasite species richness indices in analyzing anthropogenically impacted coastal marine ecosystems. *Helgoland Marine Research*, *57*(3–4), 220–227.

Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T. (2013). The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiology Reviews*, *37*(3), 462–476.

Emslie, M. J., Logan, M., Ceccarelli, D. M., Cheal, A. J., Hoey, A. S., Miller, I., & Sweatman, H. P. A. (2012). Regional-scale variation in the distribution and abundance of farming damselfishes on Australia's Great Barrier Reef. *Marine Biology*, *159*(6), 1293–1304. https://doi.org/10.1007/s00227-012-1910-0

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 487–515.

Feary, D. A., McCormick, M. I., & Jones, G. P. (2009). Growth of reef fishes in response to live coral cover. *Journal of Experimental Marine Biology and Ecology*, *373*(1), 45–49.

Finley, R. J., & Forrester, G. E. (2003). Impact of ectoparasites on the demography of a small reef fish. *Marine Ecology Progress Series*, *248*, 305–309.

Fischer, J., & Lindenmayer, D. B. (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography*, *16*(3), 265–280.

Fox, R. J., & Bellwood, D. R. (2007). Quantifying herbivory across a coral reef depth gradient. *Marine Ecology Progress Series*, *339*, 49–59.

Fox, R. J., Sunderland, T. L., Hoey, A. S., & Bellwood, D. R. (2009). Estimating ecosystem function: contrasting roles of closely related herbivorous rabbitfishes (Siganidae) on coral reefs. *Marine Ecology Progress Series*, *385*, 261–269.

Froeschke, G., van der Mescht, L., McGeoch, M., & Matthee, S. (2013). Life history strategy influences parasite responses to habitat fragmentation. *International Journal for Parasitology*, *43*(14), 1109–1118.

Gillespie, T. R., & Chapman, C. A. (2008). Forest fragmentation, the decline of an endangered primate, and changes in host–parasite interactions relative to an unfragmented forest. *American Journal of Primatology*, *70*(3), 222–230.

Gosnell, J. S., DiPrima, J. B., & Gaines, S. D. (2012). Habitat complexity impacts persistence and species interactions in an intertidal whelk. *Marine Biology*, *159*(12), 2867–2874.

Graham, N. A. J., & Nash, K. L. (2013). The importance of structural complexity in coral reef ecosystems. *Coral Reefs*, *32*(2), 315–326.

Graham, N. A. J., Wilson, S. K., Jennings, S., Polunin, N. V. C., Bijoux, J. P., & Robinson, J. (2006). Dynamic fragility of oceanic coral reef ecosystems. *Proceedings of the National Academy of Sciences*, *103*(22), 8425–8429.

Granath Jr, W. O. (2015). Habitat alteration and parasite transmission: is it a one-way street? *Comparative Parasitology*, *82*(2), 161–174.

Gratwicke, B., & Speight, M. R. (2005). The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology*, *66*(3), 650–667.

Grutter, A. S. (1994). Spatial and temporal variations of the ectoparasites of seven reef fish species from Lizard Island and Heron Island, Australia. *Marine Ecology Progress Series*, *115*, 21–30.

Grutter, A. S. (1995). Comparison of methods for sampling ectoparasites from coral reef fishes. *Marine and Freshwater Research*, *46*(6), 897–903.

Grutter, A. S., Blomberg, S. P., Box, S., Bshary, R., Ho, O., Madin, E. M. P., McClure, E. C., Meekan, M. G., Murphy, J. M., & Richardson, M. A. (2019). Changes in local free-living parasite populations in response to cleaner manipulation over 12 years. *Oecologia*, *190*(4), 783–797.

Grutter, A. S., Feeney, W. E., Hutson, K. S., McClure, E. C., Narvaez, P., Smit, N. J., Sun, D., & Sikkel, P. C. (2020). Practical methods for culturing parasitic gnathiid isopods. *International Journal for Parasitology*, *50*(10–11), 825–837.

Grutter, A. S., & Hendrikz, J. (1999). Diurnal variation in the abundance of juvenile parasitic gnathiid isopods on coral reef fish: implications for parasite-cleaner fish interactions. *Coral Reefs*, *18*(2), 187–191.

Grutter, A. S., Pickering, J. L., McCallum, H., & McCormick, M. I. (2008). Impact of micropredatory gnathiid isopods on young coral reef fishes. *Coral Reefs*, *27*(3), 655–661.

Grutter, A. S., & Poulin, R. (1998). Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. *Marine Ecology Progress Series*, *164*, 263–271.

Gunter, N. L., & Adlard, R. D. (2008). Bivalvulidan (Myxozoa: Myxosporea) parasites of damselfishes with description of twelve novel species from Australia's Great Barrier Reef. *Parasitology*, *135*(10), 1165–1178.

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., Lovejoy, T. E., Sexton, J. O., Austin, M. P., & Collins, C. D. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, *1*(2), Article e1500052.

Hagen, N. T. (1992). Macroparasitic epizootic disease: a potential mechanism for the termination of sea urchin outbreaks in Northern Norway? *Marine Biology*, *114*(3), 469–478.

Haila, Y. (2002). A conceptual genealogy of fragmentation research: from island biogeography to landscape ecology. *Ecological Applications*, *12*(2), 321–334.

Hall, K. A. (2004). *The taxonomy, systematics and evolutionary biology of the Gyliauchenidae Fukui, 1929 (1918) (Platyhelminthes: Digenea)*. [Doctoral dissertation, The University of Queensland]. UQ espace.

Hall, K. A., & Cribb, T. H. (2004). *Ptychogyliauchen*, a new genus of Gyliauchenidae (Platyhelminthes: Digenea) from siganid fishes of the Indo-West Pacific. *Invertebrate Systematics*, *18*(5), 607–625.

Hall, K. A., & Cribb, T. H. (2005). Family Gyliauchenidae Fukui, 1929. In A. Jones, R. Bray, & D. Gibson (Eds.), *Keys to the Trematoda* (vol. 2, pp. 665–678). CABI Publishing and the Natural History Museum.

Hall, K. A., & Cribb, T. H. (2008). Revision of *Flagellotrema* Ozaki, 1936 (Digenea, Gyliauchenidae Fukui, 1929), including the description of two species from acanthuroid fishes from the Great Barrier Reef, Queensland, Australia. *Zootaxa*, *1718*(1), 1–35.

Hansen, A. J., Piekielek, N., Davis, C., Haas, J., Theobald, D. M., Gross, J. E., Monahan, W. B., Olliff, T., & Running, S. W. (2014). Exposure of US National Parks to land use and climate change 1900–2100. *Ecological Applications*, *24*(3), 484–502.

Harms, C. A. (1996). Treatments for parasitic diseases of aquarium and ornamental fish. *Seminars in Avian and Exotic Pet Medicine*, *5*(2), 54–63.

Harris, T., Yang, Z., & Hardin, J. W. (2012). Modeling underdispersed count data with generalized Poisson regression. *The Stata Journal*, *12*(4), 736–747.

Harrison, X. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, *2*, Article e616. https://doi.org/10.7717/peerj.616

Hassanine, R. M. E.-S., & Al-Jahdali, M. O. (2007). Ecological comments on the intestinal helminths of the rabbitfish Siganus rivulatus (Teleostei, Siganidae) from the northern Red Sea. *Acta Parasitologica*, *52*(3), 278–285.

Hassanine, R. M. E.-S., Al-Zahrani, D. A., Touliabah, H. E.-S., & Youssef, E. M. (2016). The life cycle of *Hexangium sigani* Goto & Ozaki, 1929 (Digenea: Microscaphidiidae) from the Red Sea. *Journal of Helminthology*, *90*(5), 539–546.

Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2006). How parasites affect interactions between competitors and predators. *Ecology Letters*, *9*(11), 1253–1271.

Hervé, M. (2020). *RVAideMemoire: Testing and plotting procedures for biostatistics* (R package version 0.9-75) [Computer software]. The Comprehensive R Archive Network. https://cran.r-project.org/package=RVAideMemoire

Hess, G. R. (1994). Conservation corridors and contagious disease: a cautionary note. *Conservation Biology*, *8*(1), 256–262.

Hess, G. R. (1996). Linking extinction to connectivity and habitat destruction in metapopulation models. *The American Naturalist*, *148*(1), 226–236.

Heupel, M. R., & Bennett, M. B. (1999). The occurrence, distribution and pathology associated with gnathiid isopod larvae infecting the epaulette shark, Hemiscyllium ocellatum. *International Journal for Parasitology*, *29*(2), 321–330.

Hirazawa, N., Takano, R., Hagiwara, H., Noguchi, M., & Narita, M. (2010). The influence of different water temperatures on *Neobenedenia girellae* (Monogenea) infection, parasite

growth, egg production and emerging second generation on amberjack *Seriola dumerili* (Carangidae) and the histopathological effect of this parasite on fish skin. *Aquaculture*, *299*(1–4), 2–7.

Hoey, A. S., Brandl, S. J., & Bellwood, D. R. (2013). Diet and cross-shelf distribution of rabbitfishes (f. Siganidae) on the northern Great Barrier Reef: implications for ecosystem function. *Coral Reefs*, *32*(4), 973–984. https://doi.org/10.1007/s00338-013-1043-z

Holling, C. S. (1992). Cross-scale morphology, geometry, and dynamics of ecosystems. *Ecological Monographs*, *62*(4), 447–502.

Holmes, J. C. (1996). Parasites as threats to biodiversity in shrinking ecosystems. *Biodiversity and Conservation*, *5*(8), 975–983.

Hudson, P. J., Dobson, A. P., & Lafferty, K. D. (2006). Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, *21*(7), 381–385.

Hudson, P. J., Dobson, A. P., & Newborn, D. (1992). Do parasites make prey vulnerable to predation? Red grouse and parasites. *Journal of Animal Ecology*, *61*(3), 681–692.

Hudson, P. J., Gould, E., Laurenson, K., Gaunt, M., Reid, H., Jones, L., Norman, R., MacGuire, K., & Newborn, D. (1997). The epidemiology of louping-ill, a tick-borne infection of red grouse (*Lagopus lagopus scoticus*). *Parassitologia*, *39*(4), 319–323. http://europepmc.org/abstract/MED/9802086

Hughes, T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science*, *265*(5178), 1547–1551.

Hughes, T. P., Anderson, K. D., Connolly, S. R., Heron, S. F., Kerry, J. T., Lough, J. M., Baird, A. H., Baum, J. K., Berumen, M. L., & Bridge, T. C. (2018). Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science*, *359*(6371), 80–83.

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B. C., Kleypas, J., van de Leemput, I. A., Lough, J. M., & Morrison, T. H. (2017). Coral reefs in the Anthropocene. *Nature*, *546*(7656), 82.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., & Berkelmans, R. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, *543*(7645), 373.

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., Heron, S. F., Hoey, A. S., Hoogenboom, M. O., Liu, G., McWilliam, M. J., Pears, R. J., Pratchett, M. S., Skirving, W. J., Stella, J. S., & Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492–496. https://doi.org/10.1038/s41586-018-0041-2

Hughes, T. P., Rodrigues, M. J., Bellwood, D. R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., Moltschaniwskyj, N., Pratchett, M. S., Steneck, R. S., & Willis, B. (2007). Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology*, *17*(4), 360–365.

Hughes-Stamm, S. R., Cribb, T. H., & Jones, M. K. (1999). Structure of the tegument and ectocommensal microorganisms of *Gyliauchen nahaensis* (Digenea: Gyliauchenidae), an inhabitant of herbivorous fish of the Great Barrier Reef, Australia. *The Journal of Parasitology*, *85*(6), 1047–1052.

Huspeni, T. C., & Lafferty, K. D. (2004). Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. *Ecological Applications*, *14*(3), 795–804.

Huston, D. C., Cutmore, S. C., & Cribb, T. H. (2018). *Isorchis cannoni* n. sp. (Digenea: Atractotrematidae) from Great Barrier Reef rabbitfishes and the molecular elucidation of its life cycle. *Journal of Helminthology*, *92*(5), 604–611.

Hutson, K. S., Brazenor, A. K., Vaughan, D. B., & Trujillo-González, A. (2018). Monogenean parasite cultures: current techniques and recent advances. *Advances in Parasitology*, *99*, 61–91.

Hutson, K. S., Ernst, I., Mooney, A. J., & Whittington, I. D. (2007). Metazoan parasite assemblages of wild *Seriola lalandi* (Carangidae) from eastern and southern Australia. *Parasitology International*, *56*(2), 95–105.

Hutson, K. S., Mata, L., Paul, N. A., & de Nys, R. (2012). Seaweed extracts as a natural control against the monogenean ectoparasite, *Neobenedenia* sp., infecting farmed barramundi (*Lates calcarifer*). *International Journal for Parasitology*, *42*(13–14), 1135–1141.

IPCC. (2014). *Climate Change 2014: Synthesis Report Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Core Writing Team, R.K. Pachauri, & L.A. Meyer, Eds.). IPCC, Geneva, Switzerland.

IPCC. (2018). *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change* (V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P. R. Shukla, Pirani. A., W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, Maycock, M. Tignor, & T. Waterfield, Eds.). https://doi.org/10.1017/CBO9781107415324

IPCC. (2019). Summary for Policymakers. In H.-O. Pörtner, D. C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold, B. Rama, & N. M. Weyer (Eds.), *IPCC Special Report on the Ocean and Cryosphere in a Changing Climate*. In Press.

Jenkins, W. G., Demopoulos, A. W. J., Nicholson, M. D., & Sikkel, P. C. (2020). Stable isotope dynamics of herbivorous reef fishes and their ectoparasites. *Diversity*, *12*(11).

Jones, C. M., & Grutter, A. S. (2005). Parasitic isopods (*Gnathia* sp.) reduce haematocrit in captive blackeye thicklip (Labridae) on the Great Barrier Reef. *Journal of Fish Biology*, *66*(3), 860–864.

Jones, C. M., Nagel, L., Hughes, G. L., Cribb, T. H., & Grutter, A. S. (2007). Host specificity of two species of *Gnathia* (Isopoda) determined by DNA sequencing blood meals. *International Journal for Parasitology*, *37*(8–9), 927–935.

Jones, M. K., Hughes-Stamm, S. R., East, R. M., & Cribb, T. H. (2000). Ultrastructure of the digestive tract of Gyliauchen nahaensis (Platyhelminthes, Digenea), an inhabitant of the hindgut of herbivorous fishes. *Journal of Morphology*, *246*(3), 198–211.

Justine, J.-L. (2010). Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia. *Belgian Journal of Zoology*, *140*(Suppl.), 155–190.

Kaneko II, J. J., Yamada, R., Brock, J. A., & Nakamura, R. M. (1988). Infection of tilapia, *Oreochromis mossambicus* (Trewavas), by a marine monogenean, *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its treatment. *Journal of Fish Diseases*, *11*(4), 295–300.

Karvonen, A., Seppälä, O., & Valtonen, E. T. (2004a). Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology*, *129*(4), 473–478.

Karvonen, A., Seppälä, O., & Valtonen, E. T. (2004b). Parasite resistance and avoidance behaviour in preventing eye fluke infections in fish. *Parasitology*, *129*(2), 159–164.

Kearn, G. C. (1986). The Eggs of Monogeneans. In J. R. Baker & R. Muller (Eds.), *Advances in Parasitology* (25th ed., pp. 175–262). Academic Press Inc.

Keas, B. E., & Blankespoor, H. D. (1997). The prevalence of cercariae from *Stagnicola emarginata* (Lymnaeidae) over 50 years in northern Michigan. *The Journal of Parasitology*, *83*(3), 536–540.

Kiene, F., Andriatsitohaina, B., Ramsay, M. S., Rakotondramanana, H., Rakotondravony, R., Radespiel, U., & Strube, C. (2020). Forest edges affect ectoparasite infestation patterns of small mammalian hosts in fragmented forests in Madagascar. *International Journal for Parasitology*, *50*(4), 299–313.

Kiene, F., Andriatsitohaina, B., Ramsay, M. S., Rakotondravony, R., Strube, C., & Radespiel, U. (2021). Habitat fragmentation and vegetation structure impact gastrointestinal parasites of small mammalian hosts in Madagascar. *Ecology and Evolution*, *11*(11), 6766–6788.

King, K. C., Gendron, A. D., McLaughlin, J. D., Giroux, I., Brousseau, P., Cyr, D., Ruby, S. M., Fournier, M., & Marcogliese, D. J. (2008). Short-term seasonal changes in parasite community structure in northern leopard froglets (*Rana pipiens*) inhabiting agricultural wetlands. *Journal of Parasitology*, *94*(1), 13–22.

Klemme, I., & Karvonen, A. (2016). Learned parasite avoidance is driven by host personality and resistance to infection in a fish–trematode interaction. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1838). https://doi.org/https://doi.org/10.1098/rspb.2016.1148

Koh, E. G. L., & Sweatman, H. (2000). Chemical warfare among scleractinians: bioactive natural products from *Tubastraea faulkneri* Wells kill larvae of potential competitors. *Journal of Experimental Marine Biology and Ecology*, *251*(2), 141–160.

Køie, M., & Gibson, D. I. (1991). Aspects of the life-cycle of *Magnibursatus caudofilamentosa* (Reimer, 1971) Gibson & KØie, 1991 (digenea, hemiuroidea, derogenidae). *Ophelia*, *34*(2), 117–127.

Kramer, M. J., Bellwood, D. R., & Bellwood, O. (2014). Benthic Crustacea on coral reefs: a quantitative survey. *Marine Ecology Progress Series*, *511*, 105–116.

Kramer, M. J., Bellwood, O., & Bellwood, D. R. (2013). The trophic importance of algal turfs for coral reef fishes: the crustacean link. *Coral Reefs*, *32*(2), 575–583.

Kritsky, D. C., Galli, P., & Yang, T. (2007). Dactylogyrids (Monogenoidea) parasitising the gills of spinefoots (Teleostei: Siganidae): proposal of *Glyphidohaptor* n. gen., with two new species from the Great Barrier Reef, Australia, and *G. plectocirra* n. comb. from Ras Mohammed National Park. *Journal of Parasitology*, *93*(1), 39–46. https://doi.org/10.1645/GE-3580.1

Krkošek, M. (2010). Host density thresholds and disease control for fisheries and aquaculture. *Aquaculture Environment Interactions*, *1*(1), 21–32.

Kuiter, R. H., & Tonozuka, T. (2001). *Pictorial guide to Indonesian reef fishes. Part 3. Jawfishes–sunfishes, Opistognathidae–Molidae*. Zoonetics, Melbourne, Vic, Australia.

Kuris, A. M., Hechinger, R. F., Shaw, J. C., Whitney, K. L., Aguirre-Macedo, L., Boch, C. A., Dobson, A. P., Dunham, E. J., Fredensborg, B. L., & Huspeni, T. C. (2008). Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature*, *454*(7203), 515–518.

Lafferty, K. D. (1997). Environmental parasitology: what can parasites tell us about human impacts on the environment? *Parasitology Today*, *13*(7), 251–255.

Lafferty, K. D., Allesina, S., Arim, M., Briggs, C. J., de Leo, G., Dobson, A. P., Dunne, J. A., Johnson, P. T. J., Kuris, A. M., Marcogliese, D. J., Martinez, N. D., Memmott, J., Marquet, P. A., McLaughlin, J. P., Mordecai, E. A., Pascual, M., Poulin, R., & Thieltges, D. W. (2008). Parasites in food webs: the ultimate missing links. *Ecology Letters*, *11*(6), 533–546. https://doi.org/10.1111/j.1461-0248.2008.01174.x

Lafferty, K. D., Dobson, A. P., & Kuris, A. M. (2006). Parasites dominate food web links. *Proceedings of the National Academy of Sciences*, *103*(30), 11211–11216. https://doi.org/10.1073/pnas.0604755103

Lafferty, K. D., & Kuris, A. M. (2005). Parasitism and environmental disturbances. In F. Thomas, F. Renaud, & J.-F. Guégan (Eds.), *Parasitism and Ecosystems* (pp. 113–123). Oxford University Press Inc.

Lafferty, K. D., & Kuris, A. M. (2009). Parasitic castration: the evolution and ecology of body snatchers. *Trends in Parasitology*, *25*(12), 564–572.

Lafferty, K. D., Shaw, J. C., & Kuris, A. M. (2008). Reef fishes have higher parasite richness at unfished Palmyra Atoll compared to fished Kiritimati Island. *EcoHealth*, *5*(3), 338–345.

Larsen, B. B., Miller, E. C., Rhodes, M. K., & Wiens, J. J. (2017). Inordinate fondness multiplied and redistributed: the number of species on earth and the new pie of life. *The Quarterly Review of Biology*, *92*(3), 229–265.

Lester, R. J. G., & Sewell, K. B. (1989). Checklist of Parasites From Heron Island, Great Barrier Reef. *Australian Journal of Zoology*, *37*(1), 101–128.

Lim, L. H. S. (2002). Three new species of *Pseudohaliotrema* Yamaguti, 1953 (Monogenea: Ancyrocephalidae) from *Siganus* species (Siganidae) and the description of a mechanism for cross-insemination. *Journal of Natural History*, *36*(14), 1639–1660.

Lo, C. M., Morand, S., & Galzin, R. (1998). Parasite diversity\host age and size relationship in three coral-reef fishes from French Polynesia. *International Journal for Parasitology*, *28*(11), 1695–1708.

Locke, S. A., McLaughlin, J. D., Lapierre, A. R., Johnson, P. T. J., & Marcogliese, D. J. (2011). Linking larvae and adults of *Apharyngostrigea cornu*, *Hysteromorpha triloba*, and *Alaria mustelae* (Diplostomoidea: Digenea) using molecular data. *Journal of Parasitology*, *97*(5), 846–851. https://doi.org/10.1645/GE-2775.1

Lockyer, A. E., Olson, P. D., & Littlewood, D. T. J. (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes):

implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, *78*(2), 155–171.

Lõhmus, M., & Björklund, M. (2015). Climate change: what will it do to fish–parasite interactions? *Biological Journal of the Linnean Society*, *116*(2), 397–411.

Mackenzie, K. (1999). Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin*, *38*(11), 955–959.

Marcogliese, D. J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, *79*(8), 1331–1352.

Marcogliese, D. J. (2004). Parasites: small players with crucial roles in the ecological theatre. *EcoHealth*, *1*(2), 151–164.

Marcogliese, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology*, *35*(7), 705–716.

Marcogliese, D. J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Scientific and Technical Review, 27*(2), 467-484.

Martens, E., & Moens, J. (1995). The metazoan ecto-and endoparasites of the rabbitfish, Siganus sutor (Cuvier & Valenciennes, 1835) of the Kenyan coast. I. *African Journal of Ecology*, *33*(4), 405–416.

McCallum, H. (2008). Landscape Structure, Disturbance, and Disease Dynamics. In R. S. Ostfeld, V. T. Eviner, F. Keesing, R. S. S. Ostfeld, & V. T. T. Eviner (Eds.), *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems*. Princeton University Press.

McCallum, H., & Dobson, A. P. (2002). Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*(1504), 2041–2049.

McCook, L. J. (1996). Effects of herbivores and water quality on *Sargassum* distribution on the central Great Barrier Reef: cross-shelf transplants. *Marine Ecology Progress Series*, *139*, 179–192.

McCormick, M. I., & Allan, B. J. M. (2017). Interspecific differences in how habitat degradation affects escape response. *Scientific Reports*, *7*(1), 1–8.

McCormick, M. I., Chivers, D. P., Allan, B. J. M., & Ferrari, M. C. O. (2017). Habitat degradation disrupts neophobia in juvenile coral reef fish. *Global Change Biology*, *23*(2), 719–727.

McPherson, M. L., Finger, D. J. I., Houskeeper, H. F., Bell, T. W., Carr, M. H., Rogers-Bennett, L., & Kudela, R. M. (2021). Large-scale shift in the structure of a kelp forest ecosystem co-occurs with an epizootic and marine heatwave. *Communications Biology*, *4*(1), 1–9.

Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well-being: Synthesis.* Island Press, Washington DC.

Monod, T. (1926). Les Gnathiidae. Essai monographique (morphologie, biologie, systématique). *Mémoires de La Société Des Sciences Naturelles Du Maroc*, *13*, 1–668.

Morales-Serna, F. N., Olivas-Padilla, L., Marín-Enriquez, E., Osuna-Cabanillas, J. M., Aguirre-Villaseñor, H., & Hernández-Covarrubias, V. (2021). Copepod and monogenean infection of fish under sea surface temperature anomalies. *Journal of Sea Research*, *175*, Article 102098.

Moravec, F. (2001). *Trichinelloid nematodes parasitic in cold-blooded vertebrates.* Academia, Praha, Czech Republic.

Mordue, A. J., & Birkett, M. A. (2009). A review of host finding behaviour in the parasitic sea louse, Lepeophtheirus salmonis (Caligidae: Copepoda). *Journal of Fish Diseases*, *32*(1), 3–13.

Mouritsen, K. N. (2002). The parasite-induced surfacing behaviour in the cockle *Austrovenus stutchburyi*: a test of an alternative hypothesis and identification of potential mechanisms. *Parasitology*, *124*(5), 521–528.

Mouritsen, K. N., & Haun, S. C. B. (2008). Community regulation by herbivore parasitism and density: trait-mediated indirect interactions in the intertidal. *Journal of Experimental Marine Biology and Ecology*, *367*(2), 236–246.

Mouritsen, K. N., & Poulin, R. (2005). Parasites boosts biodiversity and changes animal community structure by trait-mediated indirect effects. *Oikos*, *108*(2), 344–350.

Mumby, P. J. (2009). Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. *Coral Reefs*, *28*(3), 761–773.

Muñoz, G., Grutter, A. S., & Cribb, T. H. (2007). Structure of the parasite communities of a coral reef fish assemblage (Labridae): testing ecological and phylogenetic host factors. *Journal of Parasitology*, *93*(1), 17–30. https://doi.org/10.1645/GE-969R.1

Mwita, C., & Nkwengulila, G. (2008). Determinants of the parasite community of clariid fishes from Lake Victoria, Tanzania. *Journal of Helminthology*, *82*(1), 7–16.

Nagelkerken, I., & Munday, P. L. (2016). Animal behaviour shapes the ecological effects of ocean acidification and warming: moving from individual to community-level responses. *Global Change Biology*, *22*(3), 974–989.

Nakamura, Y., & Sano, M. (2005). Comparison of invertebrate abundance in a seagrass bed and adjacent coral and sand areas at Amitori Bay, Iriomote Island, Japan. *Fisheries Science*, *71*(3), 543–550.

Narvaez, P., Morais, R. A., Hutson, K. S., McCormick, M. I., & Grutter, A. S. (2021). Habitat degradation drives increased gnathiid isopod ectoparasite infection rate on juvenile but not adult fish. *Coral Reefs*, *40*, 1867–1877. https://doi.org/10.1007/s00338-021-02166-y

Nolan, M. J., & Cribb, T. H. (2006). An exceptionally rich complex of Sanguinicolidae von Graff, 1907 (Platyhelminthes: Trematoda) from Siganidae, Labridae and Mullidae (Teleostei: Perciformes) from the Indo-west Pacific Region. *Zootaxa*, *1218*(1), 1–80.

Norin, T., Mills, S. C., Crespel, A., Cortese, D., Killen, S. S., & Beldade, R. (2018). Anemone bleaching increases the metabolic demands of symbiont anemonefish. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1876).

Norström, A. V, Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs: beyond coral–macroalgal phase shifts. *Marine Ecology Progress Series*, *376*, 295–306.

Oksanen, J., Blanchett, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *vegan: Community Ecology Package* (R package version 2.5-7) [Computer software]. The Comprehensive R Archive Network. https://CRAN.R-project.org/package=vegan

Olson, P. D., & Littlewood, D. T. J. (2002). Phylogenetics of the Monogenea – evidence from a medley of molecules. *International Journal for Parasitology*, *32*(3), 233–244.

Olson, S. H., Gangnon, R., Silveira, G. A., & Patz, J. A. (2010). Deforestation and malaria in Mancio Lima county, Brazil. *Emerging Infectious Diseases*, *16*(7), 1108–1115.

Osipova, E., Emslie-Smith, M., Osti, M., Murai, M., Åberg, U., & Shadie, P. (2020). *IUCN World Heritage Outlook 3: A conservation assessment of all natural World Heritage sites, November 2020*. https://doi.org/https://doi.org/10.2305/IUCN.CH.2020.16.en.

Overstreet, R. M. (2005). Family Haploporidae Nicoll, 1914. In A. Jones, R. A. Bray, & D. I. Gibson (Eds.), *Keys to the Trematoda* (pp. 129–165). The Natural History Museum, Wallingford.

Overstreet, R. M. (2007). Effects of a hurricane on fish parasites. *Parassitologia* , *49*(3), 161–168.

Patz, J. A., Graczyk, T. K., Geller, N., & Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology*, *30*(12–13), 1395–1405. https://doi.org/10.1016/s0020-7519(00)00141-7

Paula, J. R., Sun, D., Pissarra, V., Narvaez, P., Rosa, R., Grutter, A. S., & Sikkel, P. C. (2021). The role of corals on the abundance of a fish ectoparasite in the Great Barrier Reef. *Coral Reefs*, *40*, 535–542.

Penfold, R., Grutter, A. S., Kuris, A. M., McCormick, M. I., & Jones, C. M. (2008). Interactions between juvenile marine fish and gnathiid isopods: predation versus micropredation. *Marine Ecology Progress Series*, *357*, 111–119.

Pérez-del Olmo, A., Raga, J. A., Kostadinova, A., & Fernández, M. (2007). Parasite communities in *Boops boops* (L.) (Sparidae) after the *Prestige* oil-spill: Detectable alterations. *Marine Pollution Bulletin*, *54*(3), 266–276.

Phoenix, G. K., & Press, M. C. (2005). Linking physiological traits to impacts on community structure and function: the role of root hemiparasitic Orobanchaceae (ex-Scrophulariaceae). *Journal of Ecology*, *93*(1), 67–78.

Pisapia, C., Edmunds, P. J., Moeller, H., Riegl, B. M., McWilliam, M., Wells, C. D., & Pratchett, M. S. (2020). Projected shifts in coral size structure in the Anthropocene. In B. M. Riegl (Ed.), *Population Dynamics of the Reef Crisis* (Vol. 87, pp. 31–61). Academic Press. https://doi.org/https://doi.org/10.1016/bs.amb.2020.07.003

Post, R. J., & Millest, A. L. (1991). Sample size in parasitological and vector surveys. *Parasitology Today*, *7*(6), 141–141.

Poulin, R. (1995). Phylogeny, Ecology, and the Richness of Parasite Communities in Vertebrates: Ecological Archives M065-001. *Ecological Monographs*, *65*(3), 283–302.

Poulin, R. (1999). The functional importance of parasites in animal communities: many roles at many levels? *International Journal for Parasitology*, *29*(6), 903–914.

Poulin, R., & FitzGerald, G. J. (1989a). Risk of parasitism and microhabitat selection in juvenile sticklebacks. *Canadian Journal of Zoology*, *67*(1), 14–18.

Poulin, R., & FitzGerald, G. J. (1989b). Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). *Behavioral Ecology and Sociobiology*, *24*(4), 251–255.

Poulin, R., & Leung, T. L. F. (2010). Taxonomic resolution in parasite community studies: are things getting worse? *Parasitology*, *137*(13), 1967–1973. https://doi.org/DOI: 10.1017/S0031182010000910

Poulin, R., Marcogliese, D. J., & McLaughlin, J. D. (1999). Skin-penetrating parasites and the release of alarm substances in juvenile rainbow trout. *Journal of Fish Biology*, *55*(1), 47–53.

Pratchett, M. S., Hoey, A. S., Wilson, S. K., Messmer, V., & Graham, N. A. J. (2011). Changes in biodiversity and functioning of reef fish assemblages following coral bleaching and coral loss. *Diversity*, *3*(3), 424–452.

Pratchett, M. S., Thompson, C. A., Hoey, A. S., Cowman, P. F., & Wilson, S. K. (2018). Effects of coral bleaching and coral loss on the structure and function of reef fish assemblages. In M. van Oppen & J. Lough (Eds.), *Coral Bleaching. Ecological Studies (Analysis and Synthesis)* (Vol. 233, pp. 265–293). Springer.

Preston, D. L., Mischler, J. A., Townsend, A. R., & Johnson, P. T. J. (2016). Disease ecology meets ecosystem science. *Ecosystems*, *19*(4), 737–748.

Price, P. W. (1977). General Concepts on the Evolutionary Biology of Parasites. *Evolution*, *31*(2), 405–420. https://doi.org/10.1111/j.1558-5646.1977.tb01021.x

Pywell, R. F., Bullock, J. M., Walker, K. J., Coulson, S. J., Gregory, S. J., & Stevenson, M. J. (2004). Facilitating grassland diversification using the hemiparasitic plant *Rhinanthus minor*. *Journal of Applied Ecology*, *41*(5), 880–887.

R Core Team. (2018). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. https://www.r-project.org/

Ramírez-Hernández, G., Suazo-Ortuño, I., Alvarado-Díaz, J., Escalera-Vázquez, L. H., Maldonado-López, Y., & Tafolla-Venegas, D. (2019). Effects of habitat disturbance on parasite infection and stress of the endangered Mexican stream salamander *Ambystoma ordinarium*. *SALAMANDRA*, *55*(3), 160–172.

Rao, D., Webb, J. S., Holmström, C., Case, R., Low, A., Steinberg, P., & Kjelleberg, S. (2007). Low densities of epiphytic bacteria from the marine alga *Ulva australis* inhibit settlement of fouling organisms. *Applied and Environmental Microbiology*, *73*(24), 7844–7852.

Rasher, D. B., Hoey, A. S., & Hay, M. E. (2013). Consumer diversity interacts with prey defenses to drive ecosystem function. *Ecology*, *94*(6), 1347–1358.

Reynolds, P. L., Stachowicz, J. J., Hovel, K., Boström, C., Boyer, K., Cusson, M., Eklöf, J. S., Engel, F. G., Engelen, A. H., & Eriksson, B. K. (2018). Latitude, temperature, and habitat complexity predict predation pressure in eelgrass beds across the Northern Hemisphere. *Ecology*, *99*(1), 29–35.

Richardson, L. E., Graham, N. A. J., Pratchett, M. S., Eurich, J. G., & Hoey, A. S. (2018). Mass coral bleaching causes biotic homogenization of reef fish assemblages. *Global Change Biology*, *24*(7), 3117–3129.

Rohde, K. (1976a). *Marine parasitology in Australia.* (Vol. 7). Search, Sydney.

Rohde, K. (1976b). Species diversity of parasites on the Great Barrier Reef. *Parasitology Research*, *50*(1), 93–94.

Rohde, K. (1984). Ecology of marine parasites. *Helgoländer Meeresuntersuchungen*, *37*(1), 5–33.

Rohde, K. (2005). *Marine parasitology*. CSIRO Publishing, Victoria, Australia.

Rohde, K., & Hobbs, R. P. (1988). Rarity in marine Monogenea: does an Allee-effect or parasite-induced mortality explain truncated frequency distributions? *Biologisches Zentralblatt*, *107*(3), 327–338.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., & Kinzig, A. (2000). Global biodiversity scenarios for the year 2100. *Science*, *287*(5459), 1770–1774.

Sambrook, K., Bonin, M. C., Bradley, M., Cumming, G. S., Duce, S., Andréfouët, S., & Hoey, A. S. (2020). Broadening our horizons: seascape use by coral reef-associated fishes in Kavieng, Papua New Guinea, is common and diverse. *Coral Reefs*, *39*, 1187–1197.

Sambrook, K., Hoey, A. S., Andréfouët, S., Cumming, G. S., Duce, S., & Bonin, M. C. (2019). Beyond the reef: The widespread use of non-reef habitats by coral reef fishes. *Fish and Fisheries*, *20*(5), 903–920.

Sang, V. T., Dat, T. T., Vinh, L. B., Cuong, L. C., Oanh, P. T., Ha, H., Kim, Y. H., Anh, H. L., & Yang, S. Y. (2019). Coral and Coral-Associated Microorganisms: A Prolific Source of Potential Bioactive Natural Products. *Marine Drugs*, *17*(8). https://doi.org/https://doi.org/10.3390/md17080468

Santos, T. R. N., & Sikkel, P. C. (2019). Habitat associations of fish-parasitic gnathiid isopods in a shallow reef system in the central Philippines. *Marine Biodiversity*, *49*(1), 83–96.

Scheffer, M., Hosper, S. H., Meijer, M. L., Moss, B., & Jeppesen, E. (1993). Alternative equilibria in shallow lakes. *Trends in Ecology & Evolution*, *8*(8), 275–279.

Schwartz, N., Rohde, S., Dobretsov, S., Hiromori, S., & Schupp, P. J. (2017). The role of chemical antifouling defence in the invasion success of *Sargassum muticum*: a comparison of native and invasive brown algae. *PLoS ONE*, *12*(12), Article e0189761.

Scott, M., Heupel, M., Tobin, A., & Pratchett, M. (2017). A large predatory reef fish species moderates feeding and activity patterns in response to seasonal and latitudinal temperature variation. *Scientific Reports*, *7*(1), 1–9.

Shirakashi, S., Hirano, C., binti Asmara, A., Ishimaru, K., & Miyashita, S. (2013). Shading reduces *Neobenedenia girellae* infection on cultured greater amberjack *Seriola dumerili*. *Fish Pathology*, *48*(1), 25–28.

Shodipo, M. O., Duong, B., Graba-Landry, A., Grutter, A. S., & Sikkel, P. C. (2020). Effect of acute seawater temperature increase on the survival of a fish ectoparasite. *Oceans*, *1*(4), 215–236.

Sievers, K. T., McClure, E. C., Abesamis, R. A., & Russ, G. R. (2020). Non-reef habitats in a tropical seascape affect density and biomass of fishes on coral reefs. *Ecology and Evolution*, *10*(24), 13673–13686.

Sikkel, P. C., Cheney, K. L., & Côté, I. M. (2004). In situ evidence for ectoparasites as a proximate cause of cleaning interactions in reef fish. *Animal Behaviour*, *68*(2), 241–247.

Sikkel, P. C., Fuller, C. A., & Hunte, W. (2000). Habitat/sex differences in time at cleaning stations and ectoparasite loads in a Caribbean reef fish. *Marine Ecology Progress Series*, *193*, 191–199.

Sikkel, P. C., Nemeth, D., McCammon, A., & Williams Ernest H, J. (2009). Habitat and species differences in prevalence and intensity of *Neobenedenia melleni* (Monogenea: Capsalidae) on sympatric Caribbean surgeonfishes (Acanthuridae). *Journal of Parasitology*, *95*(1), 63–68.

Sikkel, P. C., Richardson, M. A., Sun, D., Narvaez, P., Feeney, W. E., & Grutter, A. S. (2019). Changes in abundance of fish-parasitic gnathiid isopods associated with warm-water bleaching events on the northern Great Barrier Reef. *Coral Reefs*, *38*(4), 721–730.

Sikkel, P. C., Schaumburg, C. S., & Mathenia, J. K. (2006). Diel infestation dynamics of gnathiid isopod larvae parasitic on Caribbean reef fish. *Coral Reefs*, *25*(4), 683–689.

Sikkel, P. C., Sears, W. T., Weldon, B., & Tuttle, B. C. (2011). An experimental field test of host-finding mechanisms in a Caribbean gnathiid isopod. *Marine Biology*, *158*(5), 1075–1083.

Sol, D., Jovani, R., & Torres, J. (2003). Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. *Oecologia*, *135*(4), 542–547.

Staver, A. C., Archibald, S., & Levin, S. A. (2011). The global extent and determinants of savanna and forest as alternative biome states. *Science*, *334*(6053), 230–232.

Stella, J. S., Pratchett, M. S., Hutchings, P. A., & Jones, G. P. (2011). Coral-associated invertebrates: Diversity, ecological importance and vulnerability to disturbance. *Oceanography and Marine Biology: An Annual Review*, *49*, 43–116.

Steneck, R. S. (1988). Herbivory on coral reefs: a synthesis. In J. H. Choat, D. Barnes, MA. Borowitzka, J. C. Coll, P. Davies, P. Flood, B. G. Hatcher, D. Hopley, P. Hutchings, D. Kinsey, G. R. Orme, M. Pichon, P. F. Sale, P. Sammarco, C. C. Wallace, C. Wilkinson, E. Wolanski, & O. Bellwood (Eds.), *6th International Coral Reef Symposium* (pp. 37–49).

Strohm, E., Laurien-Kehnen, C., & Bordon, S. (2001). Escape from parasitism: spatial and temporal strategies of a sphecid wasp against a specialised cuckoo wasp. *Oecologia*, *129*(1), 50–57.

Studer, A., Thieltges, D. W., & Poulin, R. (2010). Parasites and global warming: net effects of temperature on an intertidal host-parasite system. *Marine Ecology Progress Series, 415*, 11-22.

Sun, D., Blomberg, S. P., Cribb, T. H., McCormick, M. I., & Grutter, A. S. (2012). The effects of parasites on the early life stages of a damselfish. *Coral Reefs*, *31*(4), 1065–1075.

Sures, B., Nachev, M., Selbach, C., & Marcogliese, D. J. (2017). Parasite responses to pollution: what we know and where we go in 'Environmental Parasitology.' *Parasites & Vectors*, *10*(1), 1–19.

Swindles, G. T., Morris, P. J., Mullan, D., Watson, E. J., Turner, T. E., Roland, T. P., Amesbury, M. J., Kokfelt, U., Schoning, K., & Pratte, S. (2015). The long-term fate of permafrost peatlands under rapid climate warming. *Scientific Reports*, *5*(1), 1–6.

Tanaka, K. (2007). Life history of gnathiid isopods-current knowledge and future directions. *Plankton and Benthos Research*, *2*(1), 1–11.

Tang, S., Graba-Landry, A., & Hoey, A. S. (2020). Density and height of *Sargassum* influence rabbitfish (f. Siganidae) settlement on inshore reef flats of the Great Barrier Reef. *Coral Reefs*, *39*(2), 467–473.

Tano, S., Eggertsen, M., Wikström, S. A., Berkström, C., Buriyo, A. S., & Halling, C. (2016). Tropical seaweed beds are important habitats for mobile invertebrate epifauna. *Estuarine, Coastal and Shelf Science*, *183*, 1–12. https://doi.org/https://doi.org/10.1016/j.ecss.2016.10.010

Tchoumbou, M. A., Mayi, M. P. A., Malange, E. N. F., Foncha, F. D., Kowo, C., Fru-cho, J., Tchuinkam, T., Awah-Ndukum, J., Dorazio, R., Nota Anong, D., Cornel, A. J., & Sehgal, R. N. M. (2020). Effect of deforestation on prevalence of avian haemosporidian parasites and mosquito abundance in a tropical rainforest of Cameroon. *International Journal for Parasitology*, *50*(1), 63–73. https://doi.org/https://doi.org/10.1016/j.ijpara.2019.10.006

Thabard, M., Gros, O., Hellio, C., & Maréchal, J.-P. (2011). *Sargassum polyceratium* (Phaeophyceae, Fucaceae) surface molecule activity towards fouling organisms and embryonic development of benthic species. *Botanica Marina*, *54*(2), 147–157.

Thies, C., Steffan-Dewenter, I., & Tscharntke, T. (2008). Interannual landscape changes influence plant–herbivore–parasitoid interactions. *Agriculture, Ecosystems & Environment*, *125*(1), 266–268.

Thomas, F., Guegan, J.-F., Renaud, F., & Guegan, J.-F. (2005). *Parasitism and ecosystems*. Oxford University Press Inc., New York.

Thomas, F., Poulin, R., de Meeüs, T., Guégan, J.-F., & Renaud, F. (1999). Parasites and ecosystem engineering: what roles could they play? *Oikos*, *84*(1), 167–171.

Thompson, C. A., Matthews, S., Hoey, A. S., & Pratchett, M. S. (2019). Changes in sociality of butterflyfishes linked to population declines and coral loss. *Coral Reefs*, *38*(3), 527–537.

Tompkins, D. M., Dobson, A. P., Arneberg, P., Begon, M. E., Cattadori, I. M., Greenman, J. v, Heesterbeek, J. A. P., Hudson, P. J., Newborn, D., & Pugliese, A. (2002). Parasites and host population dynamics. *The Ecology of Wildlife Diseases*, 45–62.

Tompkins, D. M., Draycott, R. A. H., & Hudson, P. J. (2000). Field evidence for apparent competition mediated via the shared parasites of two gamebird species. *Ecology Letters*, *3*(1), 10–14. https://doi.org/10.1046/j.1461-0248.2000.00117.x

Trieu, N., Cutmore, S. C., Miller, T. L., & Cribb, T. H. (2015). A species pair of *Bivesicula* Yamaguti, 1934 (Trematoda: Bivesiculidae) in unrelated Great Barrier Reef fishes: implications for the basis of speciation in coral reef fish trematodes. *Systematic Parasitology*, *91*(3), 231–239.

Trujillo-González, A., Constantinoiu, C. C., Rowe, R., & Hutson, K. S. (2015). Tracking transparent monogenean parasites on fish from infection to maturity. *International Journal for Parasitology: Parasites and Wildlife*, *4*(3), 316–322.

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García, D., Gómez, J. M., & Jordano, P. (2015). Beyond species loss: the extinction of ecological interactions in a changing world. *Functional Ecology*, *29*(3), 299–307.

Vercelloni, J., Liquet, B., Kennedy, E. V, González-Rivero, M., Caley, M. J., Peterson, E. E., Puotinen, M., Hoegh-Guldberg, O., & Mengersen, K. (2020). Forecasting intensifying disturbance effects on coral reefs. *Global Change Biology*, *26*(5), 2785–2797.

Vermeij, M. J. A., Smith, J. E., Smith, C. M., Thurber, R. V., & Sandin, S. A. (2009). Survival and settlement success of coral planulae: independent and synergistic effects of macroalgae and microbes. *Oecologia*, *159*(2), 325–336.

Veron, J. E. N., Stafford-Smith, M. G., Turak, E., & DeVantier, L. M. (2016). *Corals of the World*. http://www.coralsoftheworld.org/page/home/

Vieira, C. (2020). *Lobophora*–coral interactions and phase shifts: summary of current knowledge and future directions. *Aquatic Ecology*, *54*(1), 1–20.

Vignon, M., & Sasal, P. (2010). Multiscale determinants of parasite abundance: a quantitative hierarchical approach for coral reef fishes. *International Journal for Parasitology*, *40*(4), 443–451.

Vitone, N. D., Altizer, S., & Nunn, C. L. (2004). Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates. *Evolutionary Ecology Research*, *6*(2), 183–199.

Wahl, M., Goecke, F., Labes, A., Dobretsov, S., & Weinberger, F. (2012). The second skin: ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology*, *3*, 292.

Walters, L. J., Hadfield, M. G., & Smith, C. M. (1996). Waterborne chemical compounds in tropical macroalgae: positive and negative cues for larval settlement. *Marine Biology*, *126*(3), 383–393.

Wang, W., Ren, G., He, Y., & Zhu, J. (2008). Habitat Degradation and Conservation Status Assessment of Gallinaceous Birds in the Trans-Himalayas, China. *The Journal of Wildlife Management*, *72*(6), 1335–1341.

Wilson, S. K., Graham, N. A. J., Pratchett, M. S., Jones, G. P., & Polunin, N. V. C. (2006). Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? *Global Change Biology*, *12*(11), 2220–2234.

Wilson, S. K., Robinson, J. P. W., Chong-Seng, K., Robinson, J., & Graham, N. A. J. (2019). Boom and bust of keystone structure on coral reefs. *Coral Reefs*, *38*(4), 625–635.

Wood, C. L., Baum, J. K., Reddy, S. M. W., Trebilco, R., Sandin, S. A., Zgliczynski, B. J., Briggs, A. A., & Micheli, F. (2015). Productivity and fishing pressure drive variability in fish parasite assemblages of the Line Islands, equatorial Pacific. *Ecology*, *96*(5), 1383–1398.

Wood, C. L., & Lafferty K. D. 2015. How have fisheries affected parasite communities? *Parasitology*, *142*(1), 134-144.

Wood, C. L., Sandin, S. A., Zgliczynski, B., Guerra, A. S., & Micheli, F. (2014). Fishing drives declines in fish parasite diversity and has variable effects on parasite abundance. *Ecology*, *95*(7), 1929–1946.

Wu, Q., Miles, D. B., Richard, M., Rutschmann, A., & Clobert, J. (2021). Intraspecific diversity alters the relationship between climate change and parasitism in a polymorphic ectotherm. *Global Change Biology, 28*(4), 1301-1314.

Yamaguti, S. (1953). Parasitic worms mainly from Celebes. Part 2. Monogenetic trematodes of fishes. *Acta Medicinae Okayama*, *8*(3), 203–265.

Yasuoka, J., & Levins, R. (2007). Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *The American Journal of Tropical Medicine and Hygiene*, *76*(3), 450–460.

Appendix A. Supplementary Material for Chapter 2:

Variation in the parasite communities of three co-occurring herbivorous coral reef fishes

Methods

Fish necropsies

Gills were removed and placed in seawater for inspection. Meanwhile, fish were placed individually in freshwater for a minimum of 5 mins to dislodge any ectoparasites. Parasitological analyses of internal organs (i.e., heart, liver, spleen, gall bladder, white muscle, brain, stomach and intestines) were conducted following Hutson et al. (2007) and Cribb & Bray (2010). For both Pomacentrid species, a sample of white muscle tissue (from the ribs) and the entire heart, liver, gall, spleen and brain were squashed onto slides, forming a tissue layer approximately one cell thick. Slides were examined for infection under a compound microscope at 200x magnification (400x magnification for the gall bladder). For *S. doliatus*, due to its larger organs, a sample of the white muscle tissue (from the ribs), heart, liver and spleen was consistently removed from the same area, squashed onto a slide and examined as above. The remainder of the organs were dissected and inspected for parasites under a dissecting microscope at 6.7 to 45x magnification. The brain and gall bladder of *S. doliatus* were squashed as whole organs. Due to the size of *S. doliatus'* digestive tract, the stomach and intestines were each cut open, sectioned and shaken vigorously in physiological saline and the settled contents and tissues examined under dissection microscope (Cribb & Bray, 2010). For the two pomacentrid species, the stomach and intestines were dissected and inspected under a dissecting microscope at 6.7 to 45x magnification. All trematodes and cestodes found were killed in near-boiling saline. Organ and parasite samples were preserved in 70% ethanol.

Parasite identification

Caligid copepods were cleared in lactophenol for visualization of diagnostic morphological features. Gnathiid isopods were grouped as a single species: '*Gnathia* sp.*',* because identification

of gnathiid isopods to species-level is achieved with molecular analysis; or through rearing the parasitic larvae to adulthood and using morphological characteristics of the males. These methods were beyond the scope of this study.

Ancyrocephalid species were digested using a 1:90 µl solution of Proteinase-K and ATL buffer, to liberate sclerotised structures from the specimens. Images of the sclerotised parts of the parasite haptor (anchors, connective bars, marginal hooks) and reproductive organs (male copulatory organs) were used for identification.

Trematodes were stained and mounted on glass slides and morphological features were used for species identification. Specimens were stained in Mayer's Haematoxylin, dehydrated in a series of increasing concentrations of ethanol, cleared in Methyl Salicylate and mounted onto slides using Canada balsam.

Two novel species were discovered including Capsalidae sp. nov. and Faustulidae sp. nov. Only two specimens of Capsalidae sp. nov. were found, and suboptimal quality of the specimens precluded a description of the species. A formal description of Faustulidae sp. nov., alongside molecular and morphological analysis is proposed by Berilin Duong, the University of Queensland.

Table S2.1: Summary of model design used in univariate statistical analyses. OLRE = Observation-level random effects.

Response	Predictor	Distribution	Analysis
Total parasite abundance	Host sp. + (1| OLRE)	Poisson	GLMM
Ectoparasite abundance	Host sp.	Negative Binomial	GLM
Endoparasite abundance	Host sp. + (1| OLRE)	Poisson	GLMM
Pennellidae abundance	Host sp. + offset (log (Total length))	Negative Binomial	GLM
Graffillidae abundance	Host sp.	Negative Binomial	GLM
Total parasite prevalence	Host sp.	Binomial	GLM
Ectoparasite prevalence	Host sp.	Binomial	GLM
Endoparasite prevalence	Host sp.	Binomial	GLM
Taxonomic richness	Host sp.	Poisson	GLM
Total parasite community composition	Species	N/A	PERMANOVA

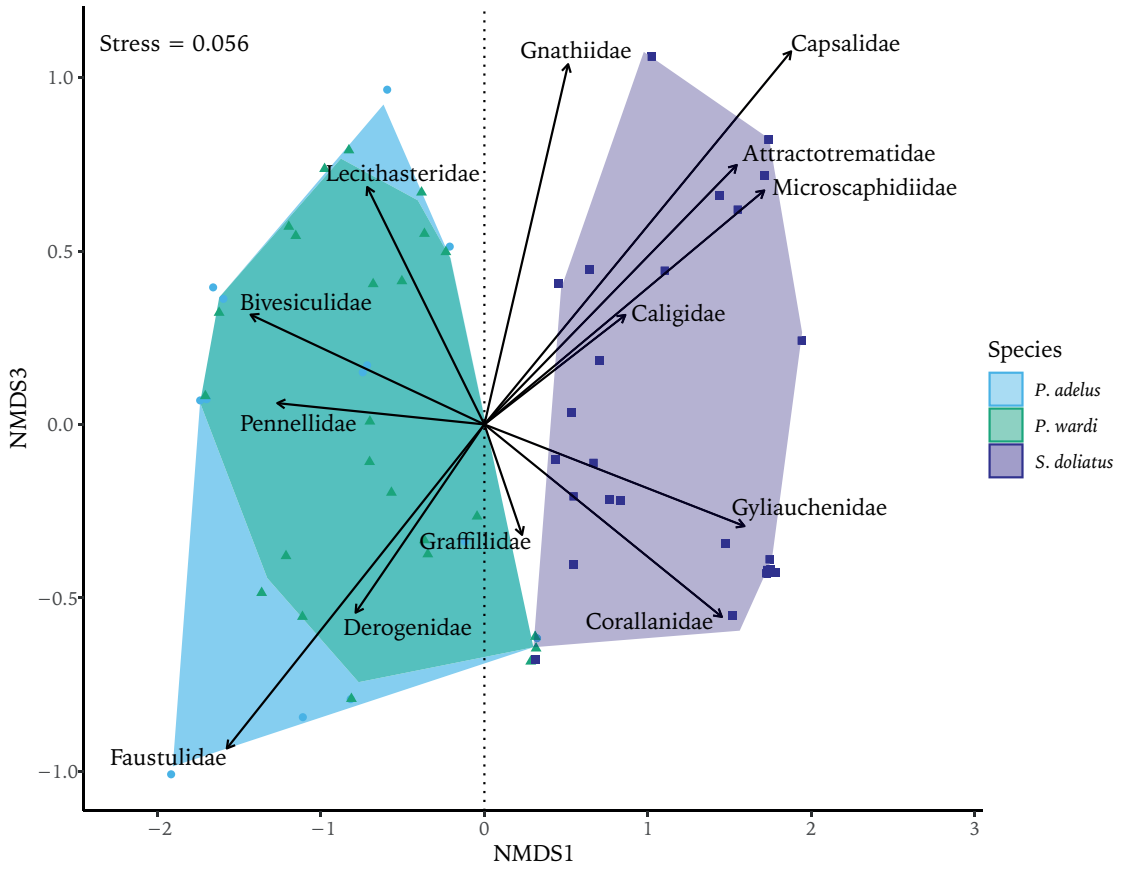

Figure S2.1: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the overall parasite communities of *Siganus doliatus* (n = 25), *Pomacentrus wardi* (n = 24) and *Pomacentrus adelus* (n = 13) from Pioneer Bay, Orpheus Island, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed infection intensity data (i.e., only infected hosts). Each point represents individual fish and polygons represent each fish species. Vectors represent the partial regression coefficients of the original variables (parasite species) with the two dimensions. Vector length is proportional to the degree of correlation between the parasite family and the ordination.

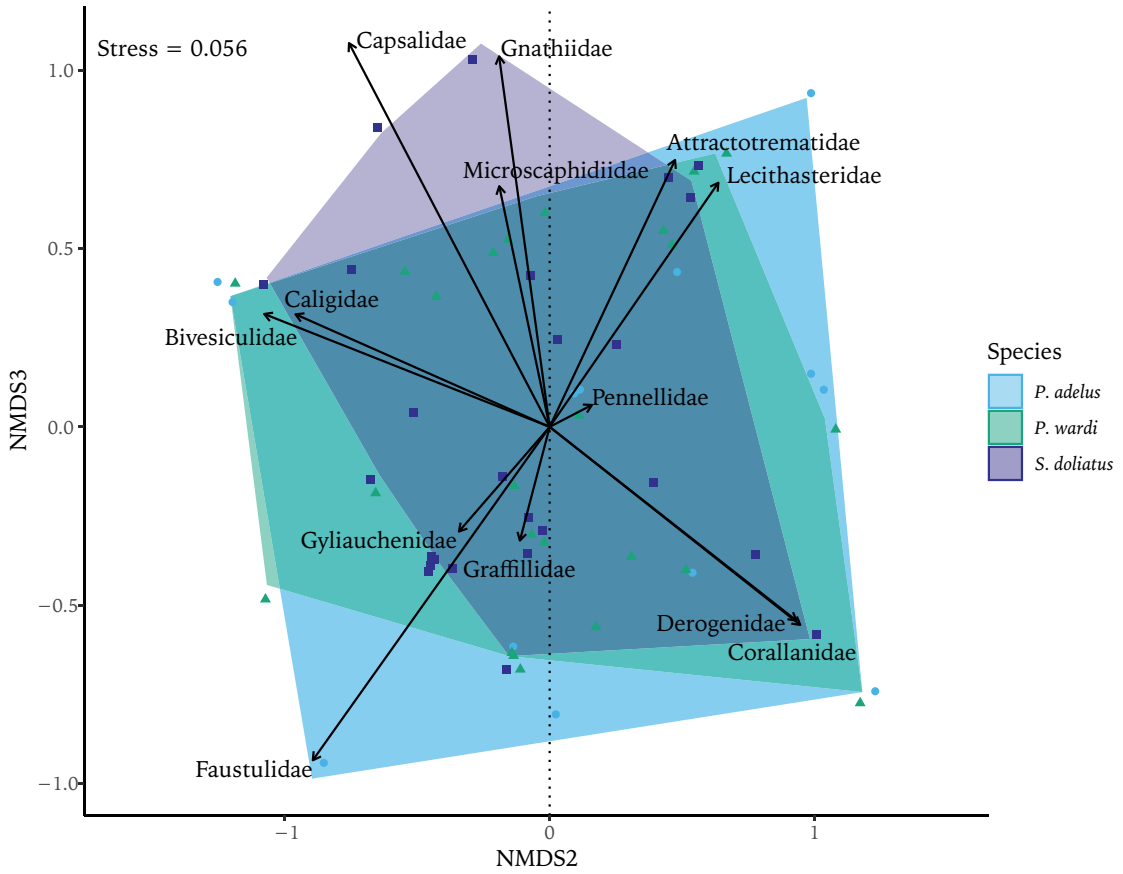

Figure S2.2: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the overall parasite communities of *Siganus doliatus* (n = 25), *Pomacentrus wardi* (n = 24) and *Pomacentrus adelus* (n = 13) from Pioneer Bay, Orpheus Island, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed infection intensity data (i.e., only infected hosts). Each point represents individual fish and polygons represent each fish species. Vectors represent the partial regression coefficients of the original variables (parasite species) with the two dimensions. Vector length is proportional to the degree of correlation between the parasite family and the ordination.

Table S2.2: Tukey's pairwise comparisons of overall parasite prevalence between *Siganus doliatus*, *Pomacentrus wardi* and *Pomacentrus adelus*.

Contrast	Odds ratio	Std. Error	z ratio	Pr(>|z|)
P. adelus / P. wardi	1.72 e-01	1.95 e-01	-1.56	0.27
P. adelus / S. doliatus	2.00 e-08	3.12 e-05	-0.01	1.00
P. wardi / S. doliatus	9.00 e-08	1.81 e-04	-0.01	1.00

Table S2.3: Tukey's pairwise comparison of endoparasite prevalence between *Siganus doliatus*, *Pomacentrus wardi* and *Pomacentrus adelus*.

Contrast	Odds ratio	Std. Error	z ratio	Pr(>|z|)
P. adelus / P. wardi	0.55	3.52 e-01	-0.99	0.62
P. adelus / S. doliatus	0.00	1.72 e-05	-0.01	1.00
P. wardi / S. doliatus	0.00	3.12 e-04	-0.01	1.00

Appendix B. Supplementary Material for Chapter 3:

Benthic habitat composition affects parasite communities of a common coral reef fish, Pomacentrus wardi

Methods

Site surveys

At each of the nine sites, the benthic community was quantified using four replicate 10 m point-intercept transects, with the substratum immediately beneath the transect line identified and recorded every 20 cm (i.e., 50 points per transect). The substratum was categorised as hard coral (identified to genus); soft coral; rubble; macroalgae (i.e., complex algal forms > 10 mm in height; Steneck, 1988); turf algae (i.e., filamentous algae < 10 m in height; Steneck, 1988); sand; other abiotic (i.e., bare rock) and other biotic (e.g., sponges, and clams).

Benthic community composition analysis

The composition of the benthic community was compared among islands and habitats using PERMANOVA, with post-hoc comparisons conducted using the package '*RVAideMemoire*' (Hervé 2020). Non-metric multidimensional scaling (nMDS) was used to ordinate dissimilarities in benthic composition among islands and habitats. Raw count data was standardised within the model using Wisconsin double standardisation, and dissimilarities calculated using the Bray-Curtis dissimilarity matrix and '*vegan*' package in R.

Results

Benthic community composition results

The benthic community of each habitat varied significantly among sites (PERMANOVA: $F_{4,35} = 4.67$; $P < 0.01$). However, a clear separation of habitat types is observable, with coral sites characterised by hard and soft coral, turf algae and other biotic substrata; macroalgae sites

typified by macroalgae and other abiotic substrata; and rubble sites characterised by rubble and sand (Fig. S3.1).

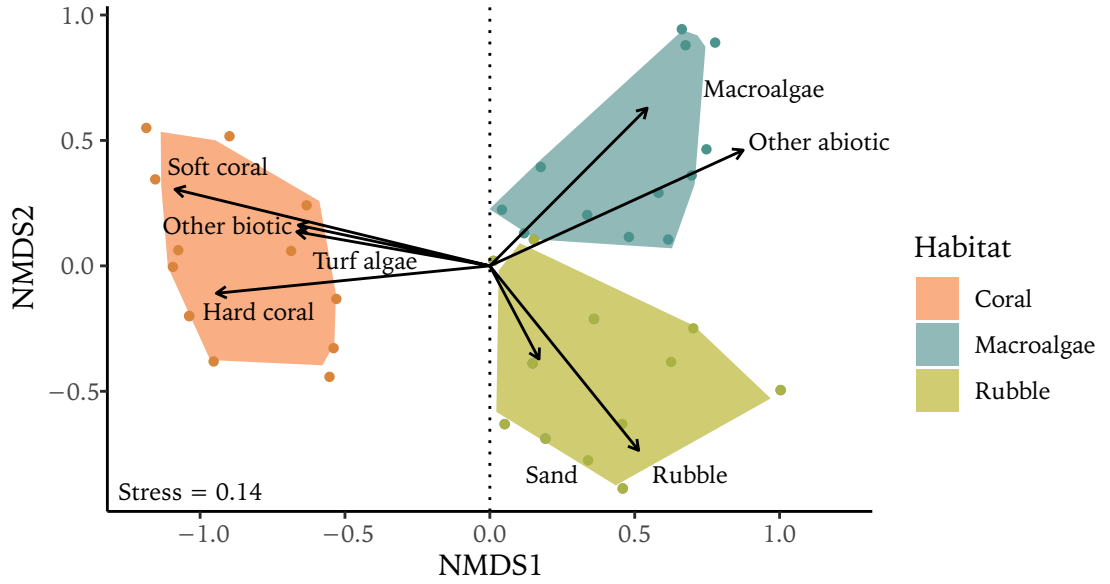

Figure S3.1: Two-dimensional solution from non-metric multidimensional scaling showing the differences in the benthic composition of coral, macroalgae and rubble habitats, at Fantome, Orpheus and Pelorus Islands within the Palm Island Group, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed benthic community data. Each point represents a 10 m point intercept transect (n = 36). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (substratum type) with two dimensions. Vector length is proportional to the degree of correlation between the substratum and the ordination.

Fish abundance, taxonomic richness and community composition

Fish abundance was significantly greater in coral habitats (adjusted mean = 259.4 ± 31.8 SE fish 20 m^{-2}; Fig. S3.2a), relative to macroalgae (adjusted mean = 74.9 ± 9.4 SE fish 20 m^{-2}; $P < 0.01$) and rubble habitats (adjusted mean = 53.2 ± 6.8 SE fish 20 m^{-2}; $P < 0.01$), but comparable between macroalgae and rubble habitats ($P = 0.14$). Fish abundances at Fantome Island (adjusted mean = 69.6 ± 8.8 SE; Fig. S3.2b) were significantly lower than those observed at Orpheus

(adjusted mean = 137.2 ± 17.0 SE; $P < 0.01$) and Pelorus Island (adjusted mean = 108.4 ± 13.6 SE; $P < 0.01$), but comparable between Pelorus and Orpheus Island ($P = 0.37$).

The richness of fish families was significantly higher in coral habitats (adjusted mean = 6.0 ± 0.7 SE fish families) relative to rubble (adjusted mean = 3.4 ± 0.5 SE fish families; $P < 0.01$) and macroalgal habitats (adjusted mean = 4.0 ± 0.6 SE fish; $P < 0.05$; Fig. S3.2c). No significant difference in the richness of fish families was observed between rubble and macroalgal habitats ($P = 0.74$).

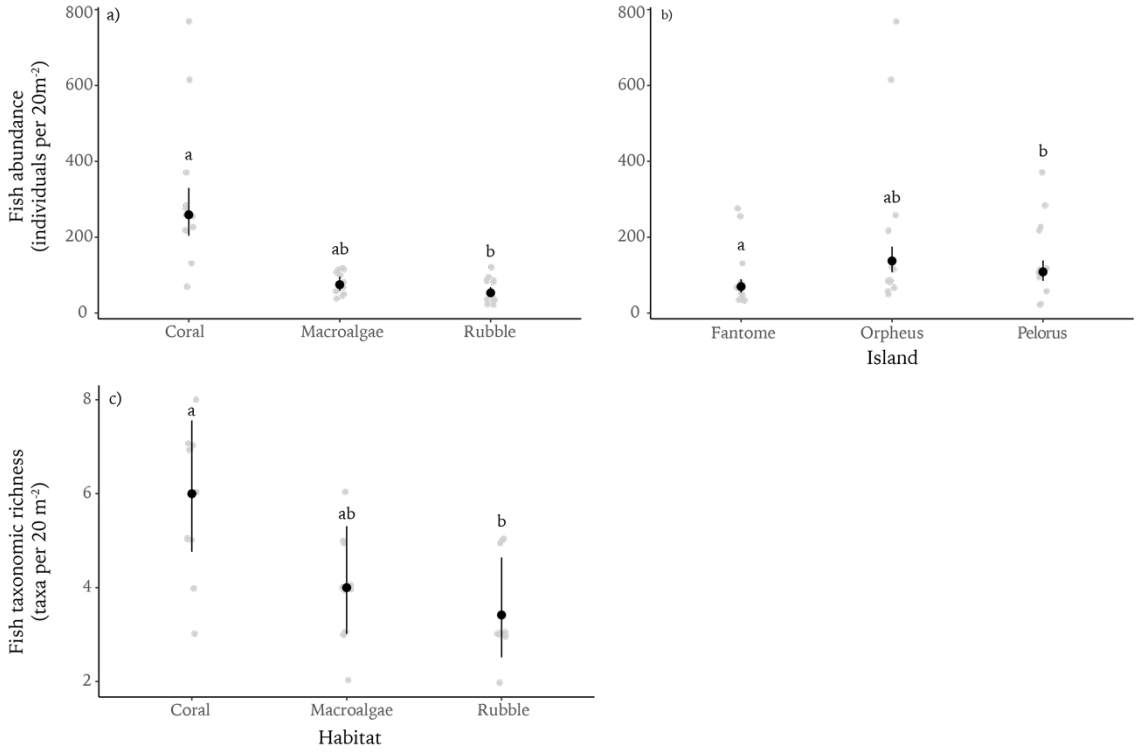

Figure S3.2: Differences in the a, b) abundance and c) taxonomic richness of diurnally active, non-cryptic fish per 10 x 2 m belt transect among coral, macroalgae and rubble habitats of Fantome, Orpheus and Pelorus island of the central Great Barrier Reef. Lines represent 95% confidence intervals; black circles represent adjusted means and; grey points represent raw data. Letters represent significant differences between groups.

For fish community composition, a significant interaction between habitat and island was found (PERMANOVA: $F_{4,35} = 1.78$; $P < 0.01$; Fig. S3.3). With the effect of habitat upon fish

community composition depending upon the island sampled. Coral sites at Fantome, Orpheus and Pelorus were typified by families such as Caesionidae, Pomacanthidae, Pomacentridae and Acanthuridae. Macroalgae sites were characterised mainly by Blennidae and Belonidae, with Orpheus Macro typified by Apogonidae and Siganidae. Rubble sites were characterised by Gobiidae, Pinguipedidae and Balistidae.

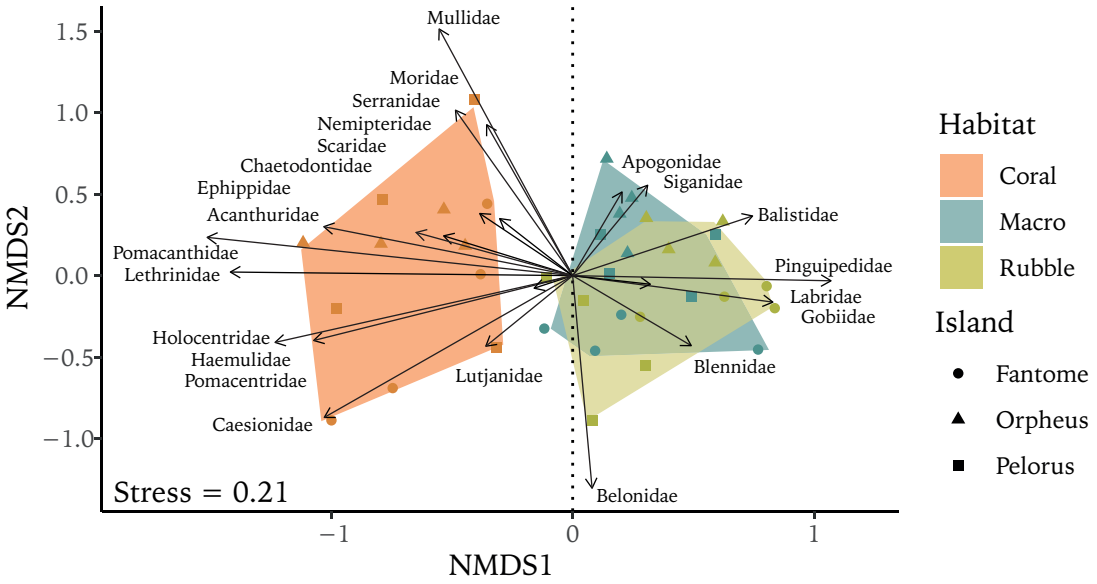

Figure S3.3: Two-dimensional solution from non-metric multidimensional scaling showing the differences in fish community composition among coral, macroalgae and rubble habitats, at Fantome, Orpheus and Pelorus Islands within the Palm Island Group, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed fish community data. Each point represents a 2 x 10 m transect (n = 36). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (fish families) with two dimensions. Vector length is proportional to the degree of correlation between the substratum and the ordination.

Table S3.1: Summary of model design used in univariate statistical analyses. 'Fresh' refers to whether the individual fish was dissected as fresh or preserved. PA - Pomacentrid abundance; OLRE - Observation level random effect.

Response	Predictor	Distribution	Analysis
Ectoparasites:			
Abundance	Habitat * Island + offset (log (TL))	Neg. Binomial	GLM
Prevalence	Habitat + offset (log (TL))	Binomial	GLM
Taxon richness	Habitat + offset (log (TL))	Poisson	GLM
Community composition	Habitat + Island	N/A	PERMANOVA
Endoparasites:			
Abundance	Habitat + Island + offset (log (TL)) + PA+ Method	Neg. Binomial	GLM
Prevalence	Habitat + Island + offset (log (TL)) + Method	Binomial	GLM
Taxon richness	offset (log (TL))	Poisson	GLM
Community composition	Habitat * Island	N/A	PERMANOVA
Site surveys:			
Fish abundance	Habitat + Island + (1|OLRE)	Poisson	GLMM
Fish richness	Habitat	Poisson	GLM
Fish community composition	Habitat * Island	N/A	PERMANOVA
Benthic community	Habitat * Island	N/A	PERMANOVA

Table S3.2: Candidate models of ecto- and endo-parasite abundance, prevalence and richness. Values highlighted in bold were the best candidate models used within the analysis. 'AICc' refers to Akaike's Information Criterion adjusted for small sample sizes. 'H' refers to Habitat; 'I' - Island; 'PA' - Pomacentrid abundance; 'HA' - Host abundance; 'TL' - Total length; 'M' - Dissection method. *The additive model was simplest.

Model variables	Ectoparasite						Endoparasite					
	Abundance		Prevalence		Richness		Abundance		Prevalence		Richness	
	AICc	df	AICc	df	AICc	df	AICc	df	AICc	df	AICc	df
H + I + H:I + TL + HA + PA+ M	511.88	12	331.84	11	388.82	11	1638.47	12	216.89	11	820.69	11
H + I + H:I + TL + HA + PA	509.72	11	330.28	10	386.92	10	1655.70	11	222.47	10	819.56	10
H + I + H:I + TL + HA + M	511.88	12	331.84	11	388.82	11	1638.47	12	216.89	11	820.69	11
H + I + H:I + TL + HA	509.72	11	330.28	10	386.92	10	1655.70	11	222.47	10	819.56	10
H + I + H:I + TL + PA + M	510.08	11	331.59	10	390.03	10	**1636.34***	**8**	214.76	10	818.55	10
H + I + H:I + TL + PA	507.95	10	330.28	10	388.42	9	1653.88	10	220.40	9	817.45	9
H + I + H:I + TL + M	510.08	11	331.59	10	390.03	10	1636.39	11	**210.58***	**6**	818.55	10
H + I + H:I + TL	**507.95**	**10**	330.23	9	388.42	9	1653.88	10	220.40	9	817.45	9
H + TL + M	532.74	5	325.60	4	384.50	4	1683.14	5	212.64	4	814.68	4
H + TL	530.69	4	**324.19**	**3**	**382.87**	**3**	1701.51	4	219.11	3	813.85	3

I + TL + M	530.6	5	331.19	4	388.00	4	1653.14	5	212.37	4	813.17	4
I + TL	528.55	4	330.21	3	386.47	3	1676.53	4	217.95	3	812.88	3
TL + M	544.59	3	329.27	2	385.73	2	1691.44	3	214.21	2	812.31	2
TL	542.85	2	328.05	1	384.14	1	1713.22	2	221.27	1	**812.20**	**1**

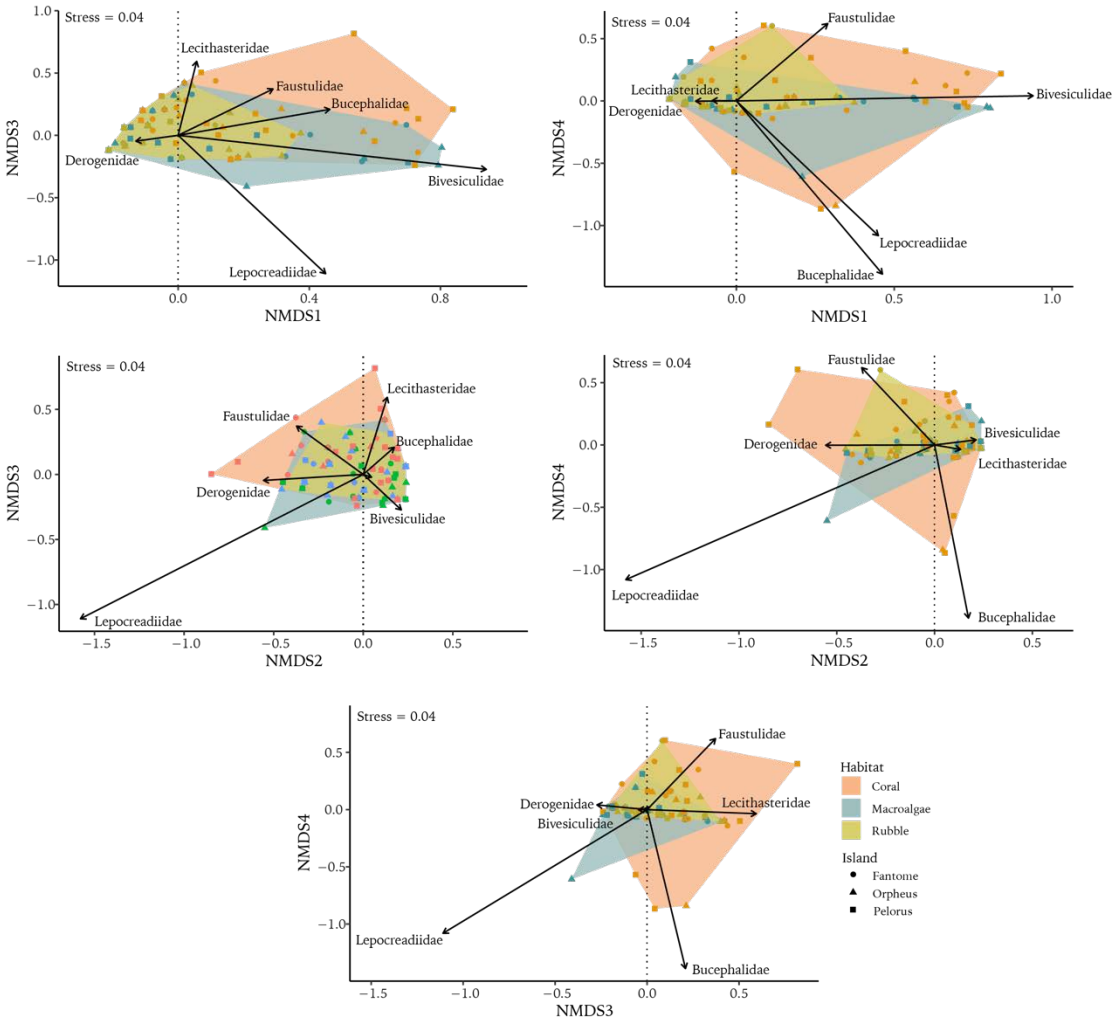

Figure S3.4: Axis combinations from a two-dimensional solution using non-metric multidimensional scaling. Axis combinations show the difference in endoparasite community composition of nine sites categorised as coral, rubble or macroalgae habitats, at Fantome, Orpheus and Pelorus Islands within the Palm Island Group, central Great Barrier Reef. The solution is based on Bray-Curtis dissimilarities of square root transformed species abundance data. Each point represents an individual infected *P. wardi* (n = 198). Polygons represent each habitat. Vectors represent the partial regression coefficients of the original variables (parasite taxa) with two dimensions. Vector length is proportional to the degree of correlation between the species and the ordination.

Appendix C. Supplementary Material for Chapter 4:

Reef habitat affects ectoparasite colonisation of a coral reef fish

Table S4.1: Summary of all known ectoparasite records for *Siganus doliatus* from the Great Barrier Reef. Taxa identified in the present study are in bold. Records from the present study that may be the same species as those identified in previous studies are indicated by (*). Locations are abbreviated as follows: 'HI' = Heron Island, 'LI' = Lizard Island, 'PI' = Palm Island Group, 'GI' = Green Island.

Class	Family	Taxon	Record	Location
Neoophora	Piscinquilinidae	*Ichthyophaga* sp.	Lockyer et al., 2003	GI
	Piscinquilinidae / Graffillidae	*Ichthyophaga* sp. / *Paravortex* sp.	Grutter, 1994	LI; HI
	Graffillidae	*Paravortex* sp.	Chapter 2	PI
Monogenea	**Ancyrocephalidae**	*Glyphidohaptor sigani*	Kritsky et al., 2007	HI
		*Pseudohaliotrema sphincteroporus**	P. D. Olson & Littlewood, 2002	GI
		Pseudohaliotrema* sp. 1	**Chapter 2 & 4**	**PI**
		***Pseudohaliotrema* sp. 2**	**Chapter 2 & 4**	**PI**
		***Tetrancistrum* sp.**	**Chapter 2 & 4**	**PI**
	Capsalidae	Capsalidae n. sp.	Chapter 2	PI
		'Benedeninae'	Grutter, 1994	HI
Trematoda	Transversotrematidae	*Transversotrema licinum*	Grutter, 1994	LI
Hexanauplia	**Caligidae**	*Lepeophtheirus* sp.	Grutter, 1994	LI; HI
		Caligus sp.*	Grutter, 1994	LI; HI
		Caligus* cf. *uniartus*	**Chapter 2 & 4**	**PI**
	Bomolochidae	*Acanthocolax / Orbitacolax* sp. nov.	Grutter, 1994	LI; HI
	Corallanidae	***Argathona* cf. *macronema***	**Chapter 2 & 4**	**PI**
Malacostraca	Cymothoidae	*Anilocra* sp.	Grutter, 1994	GBR

Gnathiidae	***Gnathia* spp.***	Grutter, 1994	LI; HI
		Chapter 2 & 4	**PI**
	*Gnathia falcipines**	C. M. Jones et al., 2007	LI

Table S4.2: Summary of model design used in univariate statistical analyses

Response	Predictor	Distribution	Analysis
Benthic Cover	Habitat * Island	N/A	PERMANOVA
Ectoparasite community composition	Habitat * Island	N/A	PERMANOVA
Total ectoparasite abundance	Habitat + Island + offset (log (Total length)) + (1|Observation level Random Effect)	Poisson	GLMM
Ectoparasite species richness	Habitat + Island + offset (log (Total length))	Neg. Binomial	GLM
Gnathia spp. abundance	Habitat + Island + offset (log (Total length)) + (1|Observation level Random Effect)	Poisson	GLMM
Pseudohaliotrema sp.1 abundance	Habitat + Island + offset (log (Total length))	Neg. Binomial	GLM

Table S4.3: The percentage of *Siganus doliatus* individuals infected with ectoparasites at each habitat and island after caging.

	Coral	Rubble	Macroalgae	**# Caged per Island**
Fantome	44.4	44.4	44.4	**27**
Orpheus	60.0	77.8	70.0	**29**
Pelorus	0.0	66.7	75.0	**22**
# Caged per habitat	**27**	**24**	**27**	**Total = 78**

Appendix D. Supplementary Material for Chapter 5:

Coral reef substrata impact hatching success of a common fish ectoparasite

Table S5.1: Summary of model design used in statistical analyses.

Response	Predictor	Distribution	Analysis
Hatching curve	Treatment	N/A	Log rank test
Hatching success	Treatment	N/A	Kruskal-Wallis rank sum
Eyespot development curve	Treatment	N/A	Log rank test
Number of adults	Treatment	Negative binomial	Linear regression
Adult presence/absence	Treatment	Binomial	Generalised linear regression
Egg presence/absence	Treatment	Binomial	Generalised linear regression