THE TROPHIC ECOLOGY OF REEF FISHES:

THE CNIDARIAN CHALLENGE

Thesis submitted by

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Statement of the Contribution of Others

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Chapter 2: "Mucus-secreting lips offer protection to suction-feeding corallivorous fishes"

Victor Huertas: conception of study, processing of samples, data collection, data analysis, figure preparation, writing of manuscript

David Bellwood: conception of the study, writing of manuscript, supervision

Chapter 3: "Feeding innovations and the first coral-feeding fishes"

Victor Huertas: conception of study, processing of samples, data collection, data analysis, figure preparation, writing of manuscript

David Bellwood: conception of study, writing of manuscript, supervision

Chapter 4: "Trophic separation in planktivorous reef fishes: A new role for mucus?"

Victor Huertas: conception of study, processing of samples, data collection, data analysis, figure preparation, writing of manuscript

David Bellwood: conception of study, writing of manuscript, supervision

Chapter 5: "Food partitioning in planktivorous reef fishes"

Victor Huertas: conception of study, sample processing, data analysis, figure preparation, writing of manuscript

Renato Morais: data analysis, writing of manuscript

Veronica Radice: conception of study, data analysis, writing of manuscript

David Bellwood: conception of study, writing of manuscript, supervision

Chapter 6: "Parrotfish corallivory on stress-tolerant corals in the Anthropocene"

Victor Huertas: conception of study, data collection, data analysis, figure preparation, writing of manuscript

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Roberta Bonaldo: data collection, writing of manuscript

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Abstract

The feeding anatomy of animals is often associated with their diet. Through gradual variation and natural selection, the feeding apparatus is moulded by performance needs, sometimes resulting in striking modifications from its ancestral state. In fishes, one of the most diverse group of vertebrates, the predator-prey arms race has underpinned numerous extreme feeding mechanisms; from the reef-crushing beaks of parrotfishes to the alien-like raptorial jaws in the throat of moray eels. Many examples highlight how highly modified feeding mechanisms can facilitate feeding and contribute to the evolutionary success of reef fishes. However, the vast majority of studies have concentrated on fish musculature and hard structures (i.e., bones and teeth). Indeed, the role of the soft anatomy of the mouths of fishes remains poorly understood. Yet, extraordinary examples of modified features in the soft anatomy of reef fishes provide an excellent opportunity to investigate the role of soft tissues in fish feeding, the most striking of which include the lips of coral-feeding tubelip wrasses. To date, however, the functions of these structures remain unclear.

On tropical reefs, corals provide a ubiquitous supply of food for reef fishes. It is therefore surprising that only a small fraction of fishes regularly feed on them, and an even smaller fraction specialize on this diet. One key explanation may be the presence of defensive nematocysts; these are also present in other cnidarians, including anemones, hydroids, and a broad range of organisms that contribute to the gelatinous zooplankton, such as jellyfish and siphonophores. How do fishes overcome these cnidarian defences?

In this thesis, I investigated the trophic ecology of wrasses (family Labridae) that feed on corals and gelatinous zooplankton to understand how these fishes overcome the difficulties associated with feeding on cnidarian prey. Specifically, the two main aims were:

1) to investigate the mechanisms enabling tubelip and fairy wrasses to feed on cnidarian prey (corals and gelatinous zooplankton) (Chapters 2 to 5), and

2) to assess the trophic dynamics between coral-grazing parrotfishes and corals on rapidly changing Anthropocene reefs (Chapter 6).

Firstly, I evaluated the anatomy and functional role of the lips of the coral-feeding tubelip wrasse *Labropsis australis* (**chapter 2**). Tubelip wrasses have some of the most remarkable lips in the animal kingdom. However, the potential functional role of these oral structures was unknown. Using histological sections, scanning electron microscopy, and high-speed video imaging, I showed that the lips of *L. australis* contain a large amount of densely-packed goblet cells in the skin of an unusual, highly-folded lip. My findings suggest that abundant mucous secretions on the lips of *L. australis* may protect their skin from the corals' nematocysts during feeding strikes, providing a feeding mechanism that enables this fish to suck coral material unharmed.

In **chapter 3**, I incorporated a broader range of labrids from the Great Barrier Reef, including two additional genera of tubelip wrasses – *Labrichthys* and *Diproctacanthus*. The objectives of this chapter were: a) to assess the taxonomic extent of the unusual lip innovation unveiled in chapter 2 and, b) to determine the evolutionary origin of mucus-secreting lips and its potential evolutionary significance. Using a phylogenetic principal components analysis of 27 morphological traits, I quantified the morphological disparity of lips across 15 genera of wrasses. My results revealed a clear separation between the three corallivorous genera (i.e., *Labropsis, Labrichthys,* and *Diproctacanthus*) and the non-corallivorous genera. This separation was primarily driven by the presence of numerous folds containing mucus-secreting cells (as described in chapter 2) in tubelip wrasse genera, suggesting that this lip innovation may underpin corallivory within this clade. More importantly, the presence of mucus-secreting lips in *Labrichthys* places the origin of this functional innovation approximately 20 million years ago, indicating that mucus-secreting lips constitute a significant breakthrough that likely enabled reef fishes to feed on corals for the first time.

Other cnidarians found on coral reefs such as jellyfish also possess nematocysts. This raises the question: Is feeding on nematocyst-bearing zooplankton also enabled by enhanced mucus secretion? To answer this question, in **chapter 4** I shifted my focus to planktivorous labrids to assess the mucus secretion ability in the buccal cavity of fishes feeding on gelatinous zooplankton. I measured four key anatomical traits of the mucosa of the buccal cavity of 19 species of labrids, including seven planktivores. Then, I compared the morphological results with gut content data to assess whether the ability to secrete mucus in the buccal cavity is correlated with the proportion of amorphous organic matter (AOM) in their gut (AOM likely comprises gelatinous planktonic material). My results showed that labrids with a greater ability to secrete mucus in their buccal cavity had the largest proportion of AOM in their guts. Notably, the plankton-feeding fairy wrasses (genus *Cirrhilabrus*) had larger and more numerous goblet cells throughout their buccal cavity than any other labrid species evaluated. Interestingly, in contrast to *Cirrhilabrus*, planktivores with a lower ability to secrete mucus primarily fed on micro-crustaceans and had very little AOM in the guts. This suggests a mucus-based food partitioning between labrids feeding in the water column, and that mucus secretion may be a common mechanism used by cnidarian-feeding labrids to reduce nematocyst damage.

In **chapter 5**, I incorporated stable isotope data to study whether the trophic separation between fairy wrasses and other planktivorous labrids suggested in chapter 4 reflects distinct trophic niches. In this chapter, I evaluated 13 labrid species that included planktivores, mobile invertebrate feeders, a corallivore, and a cleaner. Based on bulk stable isotope data ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) analysed in a Bayesian framework, I calculated a 62% probability that the isotopic niches of *Cirrhilabrus* (AOM feeders) and other planktivores that primarily feed on crustaceans had very limited (less than 8%) overlap. The congruence between morphology, gut contents, and stable isotope signals is indicative of a robust and sustained food partitioning between coral reef planktivores, and reveals a degree of trophic structuring among planktivorous reef fishes that had previously not been recognized.

My findings from chapters 2 through to 5 highlight the difficulties labrids face when feeding on cnidarians. In addition to the natural defences of cnidarian prey, coral predators may now face a new challenge as reefs rapidly shift to new configurations in the Anthropocene. In **chapter 6** I set out to investigate how parrotfishes cope with the changing availability of corals. This study was conducted on a coral reef at Lizard Island that has experienced a dramatic decline in coral cover in the last decade. The specific goal of this study was to examine whether the intensity of parrotfish corallivory on massive *Porites* has shifted in response to severe reef degradation. To do this, I quantified the composition of the benthos, conducted parrotfish surveys, and counted parrotfish scars on massive *Porites* colonies across four reef habitats (slope, crest, flat, and back reef). Then, I compared my results to a previous dataset collected in 2008, before two cyclones and two back-to-back mass coral bleaching events severely impacted Lizard Island reefs. I found that coral predation rates appeared to have diminished, despite small changes in parrotfish densities. However, when comparing bite scar densities, I detected higher values on small *Porites* colonies, which may reflect colony size-specific scarring rates. The overall reduced parrotfish corallivory observed in this study may be due to fewer small *Porites* colonies or changing foraging opportunities for parrotfishes. This chapter underscores the need to consider the ecological context when evaluating trophic interactions.

In this thesis, I show that labrids have developed two highly modified oral structures to feed on cnidarians: fleshy, highly-folded, tube-shaped lips in tubelip wrasses; and a specialized buccal mucosa in fairy wrasses. Both structures represent striking modifications of the soft anatomy and confer fishes an extraordinary ability to secrete copious amounts of oral mucus. Indeed, mucus appears to be the key for preying on cnidarians for fishes that lack hard structures, such as fused beaks or long bristle-like teeth, to access cnidarian tissues. These findings underscore the need to consider the soft anatomy of the feeding apparatus in fish feeding studies. I also show that in addition to coping with their prey's defences, coral predators now face significant changes in the availability of corals due to reef degradation. Overall, cnidarians present

reef fishes with a widespread but challenging dietary resource, one that labrids have managed to access thanks to elaborate modifications of the soft anatomy in their mouths.

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Resumen (Abstract in Spanish)

La anatomía oral de los animales está a menudo asociada con su dieta. Mediante variaciones graduales y selección natural, el aparato digestivo es moldeado por la necesidad de mejorar el rendimiento, en ocasiones dando lugar a llamativas modificaciones del estado ancestral. En peces, uno de los grupos de vertebrados más diversos, la carrera de armamento ha originado numerosos mecanismos de alimentación excepcionales; desde los picos de los peces loro, capaces de pulverizar porciones de arrecife, a las mandíbulas internas en la garganta de las morenas, similares a las de una criatura alienígena. Multitud de ejemplos destacan cómo los mecanismos de alimentación altamente modificados pueden facilitar la alimentación y contribuir al éxito evolutivo de los peces de arrecife. Sin embargo, la vasta mayoría de estudios se han concentrado en la musculatura y estructuras rígidas (i.e., huesos y dientes). En efecto, el papel de las partes anatómicas blandas (por ejemplo, labios y mucosas) de las bocas de los peces permanece en gran parte desconocido. Aun así, ejemplos extraordinarios de rasgos modificados en la anatomía blanda de los peces de arrecife, como los casos de los labios de los lábridos de labio de tubo y la mucosa oral de los lábridos hada, proporcionan una excelente oportunidad para investigar el papel de los tejidos blandos en la alimentación. Hasta la fecha, no obstante, las funciones de estas estructuras permanecen sin investigar.

En arrecifes tropicales, los corales proporcionan un suministro ubicuo de alimento a los peces de arrecife. Por lo tanto, es sorprendente que tan sólo una pequeña fracción de peces se alimenta de ellos de forma regular, y que una fracción incluso más pequeña se especializa en esta dieta. Una explicación clave puede ser la presencia de nematocistos defensivos; éstos están también presentes en otros cnidarios que incluyen anémonas, hidrozoos hidroides y un amplio grupo de organismos que forman parte del plancton gelatinoso, como las medusas y los sifonóforos. ¿Cómo superan los peces estas defensas de los cnidarios?

En esta tesis, investigué la ecología trófica de los lábridos (familia Labridae) que se alimentan de corales y zooplancton gelatinoso para entender cómo estos peces superan las dificultades asociadas con comer cnidarios. Específicamente, los dos objetivos principales fueron:

3) investigar los mecanismos que permiten a los lábridos de labio de tubo y los lábridos hada alimentarse de cnidarios (corales y zooplancton gelatinoso) (Capítulos 2 al 5), y

4) evaluar las dinámicas tróficas entre peces loro y corales en los cambiantes arrecifes del Antropoceno (Capítulo 6).

En primer lugar, evalué la anatomía y el rol funcional de los labios del lábrido de labio de tubo *Labropsis australis* (**capítulo 2**). Los lábridos de labio de tubo (especie coralívora) poseen unos de los labios más singulares del reino animal. Sin embargo, el potencial rol funcional de estas estructuras orales era desconocido. Usando secciones histológicas, microscopía de escaneo de electrones, y videos de alta velocidad, demostré que los labios de *L. australis* contienen una gran cantidad de células caliciformes comprimidas en un labio inusual con numerosos pliegues. Mis hallazgos sugieren que las abundantes secreciones mucosas en los labios de *L. australis* posiblemente protegen su piel de los nematocistos de los corales mientras se alimenta, proporcionando un mecanismo de alimentación que permite a este pez succionar material producido por los corales ileso.

En el **capítulo 3**, incorporé un mayor abanico de lábridos de la Gran Barrera de Coral, incluidos dos géneros adicionales de lábridos de labio de tubo – *Labrichthys* y *Diproctacanthus*. Los objetivos de este capítulo fueron: a) evaluar el alcance taxonómico de la inusual innovación en los labios desvelada en el capítulo 2 y, b) determinar el origen evolutivo de los labios secretores de mucosidad y su potencial importancia evolutiva. Usando un análisis de componentes principales filogenético de 27 rasgos morfológicos, cuantifiqué la disparidad morfológica de los labios de 15 géneros de lábridos. Mis resultados revelaron una clara separación entre los tres géneros coralívoros (i.e., *Labropsis, Labrichthys,* and *Diproctacanthus*) y los géneros no coralívoros. Esta

separación fue debida principalmente a la presencia de numerosos pliegues con células secretoras de mucosidad (descritas en el capítulo 2) en los géneros de lábridos de labio de tubo, sugiriendo que esta innovación en estos labios podría facilitar coralivoría en este clado. Lo que es más importante, la presencia de labios secretores de mucosidad en *Labrichthys* ubica el origen de esta innovación funcional en aproximadamente 20 millones de años atrás, lo cual sugiere que los labios secretores de mucosidad constituyen un avance significativo que permitió a los peces de arrecifes alimentarse de corales por primera vez.

Otros cnidarios en arrecifes de coral como las medusas también poseen nematocistos. Esto plantea la pregunta: ¿Está la dieta basada en zooplancton que posee nematocistos también facilitada por elevadas secreciones de moco? Para resolver esta pregunta, en el **capítulo 4** dirigí mi atención a lábridos planctívoros para evaluar la capacidad de secreción de mucosidad en la cavidad bucal de peces que se alimentan de zooplancton gelatinoso. Medí cuatro rasgos anatómicos claves de la mucosa de la cavidad bucal de 19 especies de lábridos, con siete planctívoros incluidos. A continuación, comparé los resultados morfológicos con los datos de los contenidos intestinales para evaluar si la capacidad de secretar mucosidad en la cavidad bucal está correlacionada con la proporción de materia orgánica amorfa (AOM por sus siglas en inglés, "amorphous organic matter") en su tubo digestivo. (la AOM probablemente contiene material del zooplancton gelatinoso). Mis resultados mostraron que los lábridos capaces de secretar una mayor cantidad de mucosidad en la cavidad bucal contenían la mayor proporción de AOM en su tubo digestivo. De forma destacada, los lábridos hada (género *Cirrhilabrus*), planctívoros, presentaron un mayor número de células caliciformes, y de mayor tamaño, a lo largo de su cavidad bucal que cualquier otra especie evaluada. Interesantemente, contrario a *Cirrhilabrus*, los planctívoros con una menor capacidad para secretar mucosidad se alimentaron principalmente de micro-crustáceos y presentaron muy poca AOM en su tubo digestivo. Esto sugiere una partición alimenticia basada en mucosidad entre lábridos que se alimentan en la columna de agua, y que la

secreción de mucosidad podría ser un mecanismo común usado por lábridos que se alimentan de cnidarios para reducir el daño provocado por los nematocistos.

En el **capítulo 5**, incorporé datos de isótopos estables para estudiar si la separación trófica entre lábridos hada y los otros planctívoros sugerida en el capítulo 4 está reflejada en distintos nichos tróficos. En este capítulo, evalué 13 especies de lábridos que incluyeron planctívoros, peces que se alimentan de invertebrados móviles, un coralívoro, y un pez limpiador. Basado en datos de isótopos estables ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) analizados en un marco Bayesiano, calculé una probabilidad del 62% de que la superposición de los nichos isotópicos de *Cirrhilabrus* (género que se alimentan de AOM) y otros planctívoros que se alimentan principalmente de crustáceos sea muy limitada (menos del 8%). La congruencia entre la morfología, los contenidos intestinales, y las señales de los isótopos estables indica una partición alimenticia robusta y sostenida entre planctívoros en arrecifes de coral, y revela un grado de estructuración trófica entre peces de arrecife planctívoros que no había sido previamente reconocido como tal.

Mis hallazgos en los capítulos 2 al 5 destacan las dificultades que los lábridos enfrentan cuando se alimentan de cnidarios. Además de las defensas naturales propias de los cnidarios, puede que los depredadores de coral enfrenten ahora un nuevo desafío a medida que los arrecifes cambian rápidamente hacia nuevas configuraciones en el Antropoceno. En el **capítulo 6** investigué cómo los peces loro hacen frente a la cambiante disponibilidad de corales. Este estudio fue llevado a cabo en un arrecife de coral en Lizard Island que ha experimentado un declive dramático en la cobertura de coral en la última década. El objetivo específico de este estudio fue examinar si la intensidad de la coralivoría producida por peces loro en *Porites* masivos ha cambiado en respuesta a la degradación de arrecife severa. Para ello, cuantifiqué la composición del bentos, realicé censos de peces loro, y conté las cicatrices producidas por peces loro en colonias de *Porites* masivas en cuatro hábitats arrecifales (pendiente, cresta, planície, y zona arrecifal posterior).

A continuación, comparé mis resultados con los de una colección de datos previa recopilada en 2008, antes de que dos ciclones y dos eventos de blanqueo de coral consecutivos impactaran severamente los arrecifes de Lizard Island. Encontré que las tasas de depredación de coral parecen haber disminuido, a pesar de pequeños cambios en densidades de peces loro. Sin embargo, al comparar densidades de cicatrices, detecté valores más elevados en colonias de *Porites* pequeñas, lo cual puede reflejar tasas de cicatrices específicas del tamaño de las colonias. La reducida coralivoría por parte de peces loro en términos globales observada en este estudio puede ser debida a una menor presencia de colonias pequeñas de *Porites* o a cambios en las oportunidades de alimentación para peces loro. Este capítulo resalta la necesidad de considerar el contexto ecológico al evaluar interacciones tróficas.

En esta tesis muestro que los lábridos han desarrollado dos estructuras orales altamente modificadas para consumir cnidarios: unos labios gruesos, con numerosos pliegues, en lábridos de labio de tubo; y una mucosa bucal especial en lábridos hada. Ambas estructuras representan llamativas modificaciones de la anatomía blanda y otorgan a estos peces una capacidad extraordinaria para secretar abundantes cantidades de mucosidad oral. En efecto, esta mucosidad parece ser la clave para consumir los tejidos de los cnidarios. Estos hallazgos destacan la necesidad de tomar en cuenta la anatomía blanda del aparato alimenticio en estudios de los hábitos alimenticios de los peces. También muestro que además de hacer frente a las defensas de sus presas, los depredadores de coral afrontan ahora cambios significativos en la disponibilidad de los corales debido al deterioro de los arrecifes. En general, los cnidarios suponen un amplio pero difícil recurso alimenticio para los peces de arrecife, uno al cual los lábridos han logrado acceder gracias a elaboradas modificaciones de la anatomía blanda de sus bocas.

Table of Contents

List of Tables

List of Figures

Chapter 1

GENERAL INTRODUCTION

The variation in the form and function of the feeding structures amongst teleost fishes, and the diversity of their feeding mechanisms, suggests that functional innovations of the feeding apparatus were key to the success of this group (Siqueira, Morais, Bellwood, & Cowman, 2020; Wainwright & Longo, 2017). This morphological diversity primarily originated in the late Cretaceous (Bellwood, Goatley, Cowman, & Bellwood, 2015; M. Friedman, 2010) when a series of morphological breakthroughs paved the way for one of the most important vertebrate radiations on record (Near et al., 2013). Today, the elevated number of species and the diversity of skull morphologies and feeding modes make fishes an excellent group for studying feeding systems.

There is broad recognition that the shape and mechanics of the elements comprising the feeding apparatus are closely intertwined with trophic functions (Wainwright & Bellwood, 2002). Understanding how the structure of the feeding apparatus enables feeding on specific items can help to elucidate how reef fishes are able to perform key tasks. Ecomorphological studies address these questions by examining the links between morphological attributes and how organisms use available resources (Wainwright & Bellwood, 2002; Wainwright & Richard, 1995). To date, however, the majority of studies have focused on the osteology and myology of the skull, while the soft tissues remain poorly understood. For example, of all the sophisticated feeding modes used by reef fishes, we still do not know how fishes that lack a hardened oral structure (e.g., a beak or long, protruding teeth) are capable of feeding on cnidarians. Is it possible that soft tissues are involved? This thesis explores this hypothesis to identify potential mechanisms that may help explain how reef fishes feed on nematocyst-laden prey using the soft anatomy of their mouths.

1.1. Why is feeding on cnidarians so challenging?

Cnidarians comprise a diverse group of organisms that include corals, hydroids, anemones, jellyfish, and siphonophores (Santhanam, 2020). A defining feature of all cnidarians is the possession of nematocysts: capsules that have a thread with a barb at the end that delivers a paralyzing sting (Tardent, 1995). Nematocysts are embedded in the tissues but they can also be present in the surface mucous layer that coats cnidarians, and they are used to immobilize prey and as a defense mechanism to deter would-be predators (Bullard & Hay, 2002; Gochfeld, 2004; Shanks & Graham, 1988). Predators, however, can have modified anatomical structures that facilitate the consumption of cnidarians. The oral cavity and esophagus of the leatherback turtle (*Dermochelys coriacea*), for example, are lined with numerous conical, heavily keratinized papillae oriented posteriorly (Dunlap, 1955; Wyneken, 2015). These cornified papillae may not only assist with the transport of the prey down the esophagus but also help discharge the nematocysts of their noxious prey prior to digestion (Howe, 1993).

On tropical coral reefs, cnidarians are an abundant prey for reef fishes. Corals, for example, are a common component of coral reefs and occupy a significant portion of the reef substratum (Bellwood, Hughes, Folke, & Nyström, 2004). However, only 128 of the approximately 6,000 species of reef fishes feed on corals (Cole, Pratchett, & Jones, 2008; Rotjan & Lewis, 2008). The small proportion of corallivorous fishes, considering prey availability, suggests that feeding on corals is particularly difficult. In addition to having stinging nematocysts, corals (and other cnidarians) provide limited nutrients (Arai, 1988; Elliott & Bellwood, 2003; Gregson, Pratchett, Berumen, & Goodman, 2008; Tricas, 1989) and their tissues and mucous coat are spread on razor-sharp skeletons of calcium carbonate. This, combined with nematocysts, which reduce their palatability, makes corals a particularly challenging prey.

1.2. Thesis aims and outline

The goal of this thesis is to explore the trophic ecology of labrids that feed on corals and other cnidarians. Specifically, I aim to determine how labrids cope with the challenges imposed by a diet comprised of prey laden with nematocysts and whether changes in the availability of corals due to reef degradation pose a growing challenge for these fishes.

Firstly (in **chapter 2**), I conduct an in-depth morphological analysis of the lips of the coral-feeding tubelip wrasse *Labropsis australis*. Many organisms use their lips to procure food, from grazing terrestrial mammals (Venter, Vermeulen, & Brooke, 2019) to freshwater fishes (Baumgarten, Machado-Schiaffino, Henning, & Meyer, 2015; Miller & Evans, 1965), but very little is known about how reef fishes use their lips during feeding. In this chapter, I use high-speed video footage to describe the mechanism underpinning its unusual feeding mode. Since this fish has protruding lips and feeds on live coral, the purpose of this chapter is to investigate if its distinctive lips play a role in its feeding biology. In this chapter, therefore, I explore the morphological differences between the lips of *L. australis* and those of a typical labrid (represented by the yellowtail wrasse *Coris gaimard*). The outcome of this analysis will lay the ground for the phylogenetic comparative analysis conducted in **chapter 3**, where I evaluate the taxonomic extent of their specialized oral anatomy to determine the evolutionary origins of this functional innovation and the prevalence of this trait.

The careful evaluation of the morphology, functional use, and evolutionary origin of soft tissue specialization in the mouths of coral-feeding tubelip wrasses revealed other changes in the buccal anatomy of wrasses. I therefore shifted my focus to labrids that feed on gelatinous zooplankton to study whether similar morphological diversification also occurs among plankton-feeding fishes. In **chapter 4**, I investigate the relationship between the anatomy of the buccal cavity of fairy wrasses (genus *Cirrhilabrus*) and their diet. To do this, I use data collected on 19 species of labrids from the Great Barrier Reef and the Coral Sea, including three genera of planktivores (*Cirrhilabrus*, *Pseudocoris*, and *Thalassoma* [*amblycephalum*]). Along the mucosa of the

buccal cavity, I measure four key traits that, combined, are indicative of mucus secreting ability, and using a principal components analysis and a phylogenetic generalised least squares regression, I evaluate the correlation between the buccal morphology and the proportion of amorphous organic matter in the gut (from gut content analyses). The goal of this chapter is to explore potential patterns of association between the ability to secrete mucus in the buccal cavity and the diet of planktivorous labrids that target planktonic cnidarians. I further explore the disparity in the diet of planktivorous wrasses (in **chapter 5**) by coupling gut contents analyses with stable isotope analyses. The goal is to delineate resource use amongst labrids feeding in the water column and, specifically, to explore both short term (gut contents) and long term (stable isotope) approaches, to critically evaluate the potential for resource partitioning among planktivorous fishes on coral reefs.

Finally, in **chapter 6**, I aimed to explore how changes in benthic communities at Lizard Island (Great Barrier Reef) following two cyclones and two back-to-back mass coral bleaching events in the last decade may have affected parrotfish predation on massive *Porites* corals. The focus in this chapter is to assess whether rapid disruptions in coral communities impact parrotfish corallivory. Overall, the goal of this thesis is to examine how intrinsic (e.g., morphology, histology) and extrinsic (e.g., habitat degradation) factors may shape the trophic ecology of cnidarian-feeding labrids.

Chapter 2

MUCUS-SECRETING LIPS OFFER PROTECTION TO SUCTION-FEEDING CORALLIVOROUS FISHES

Of the 6,000 reef fish species, only 128 feed on corals (Cole et al., 2008; Rotjan & Lewis, 2008). Despite being widely available on tropical reefs, corals appear to represent a particularly challenging trophic resource, with mucus and nematocyst-laden tissues spread over a sharp coral skeleton. Coral-feeding tubelip wrasses use highly modified lips to suck material from the coral surface. These lips have a specialized mushroom-like lamellar epithelium that secretes mucus. This mucus may facilitate suction and reduce damage by nematocysts in a manner akin to anemonefishes. The remarkable lip specializations observed in tubelip wrasses highlight the potential role of soft tissues in shaping the trophic ability of fishes.

Fishes exploit almost every available ecological niche, with coral-feeding being one of the most specialized diets. Of all coral-feeders, tubelip wrasses appear to have modified their mouth the most to meet this dietary challenge. Their teeth and jaw bones resemble those of other wrasses (Wainwright, Bellwood, Westneat, Grubich, & Hoey, 2004), but their ability to feed on corals appears to derive primarily from the structure of their lips. Our understanding of the functional role of fish lips is in its infancy, although the diversity of lips in reef fishes offers an exciting opportunity to explore the potential roles of lips in feeding. On coral reefs, wrasses (family Labridae) are one of the most conspicuous and diverse groups of fishes (Wainwright et al., 2004), and of all wrasses, the lips of tubelip wrasses appear to be the most distinctive (Figure 2.1.A-B). This raises the question: how do tubelip wrasses feed?
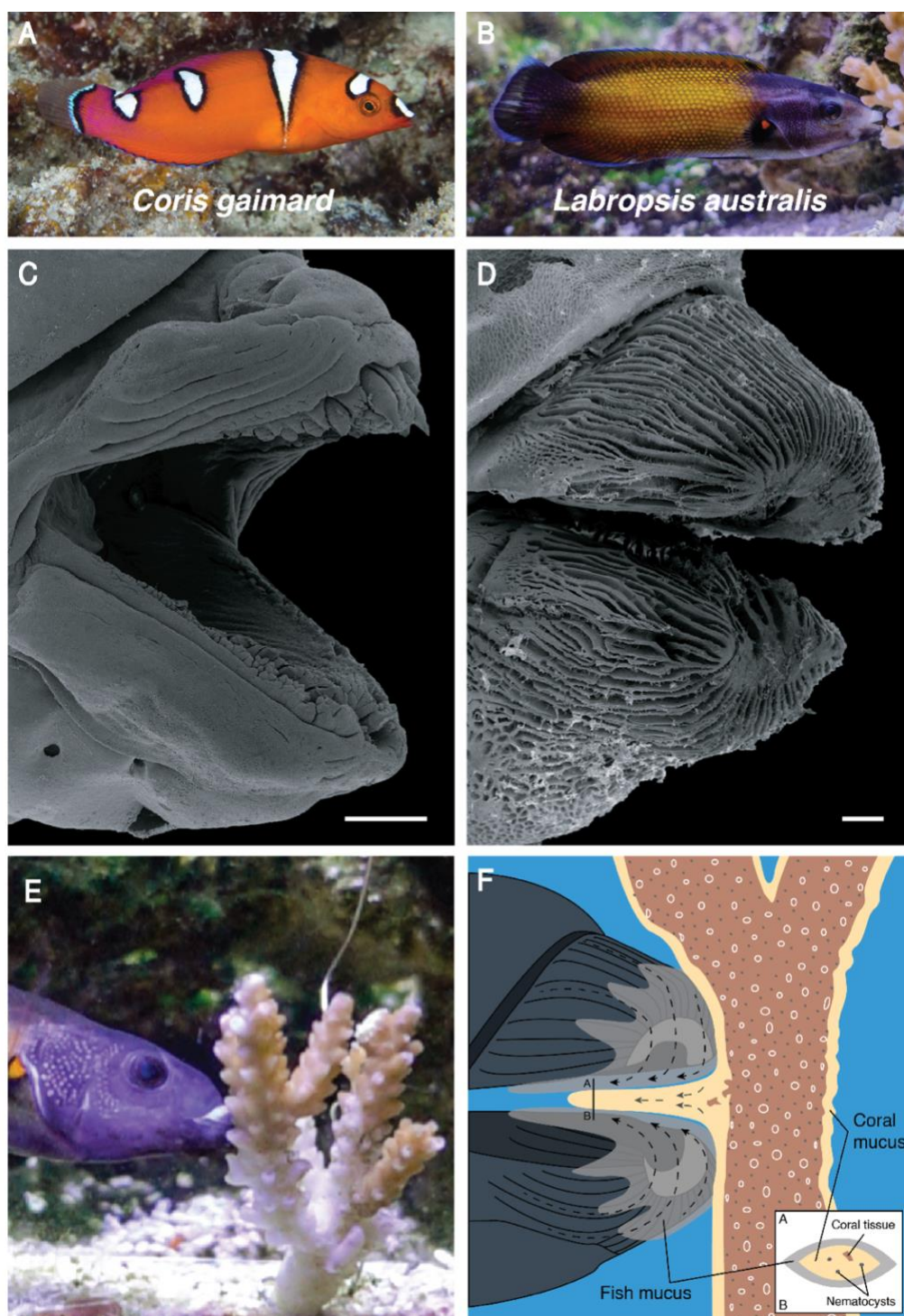
Figure 2.1. The unusual lips of tubelip wrasses. (A) A typical wrasse *Coris gaimard*; **(B)** a tubelip wrasse *Labropsis australis*; **(C)** scanning electron micrograph images of the lateral view of the lips of *C. gaimard*, and **(D)** *L. australis*; **(E)** still image of *L. australis* feeding on coral (*Acropora* sp.); **(F)** a schematic diagram of the potential feeding mechanism of tubelip wrasses to feed on coral mucus and tissues. The mucus on the lips may offer protection from nematocysts, help the lips seal with the uneven surface during suction, and help enclose dislodged mucus and tissues. Dashed arrows indicate the flow of the mucus during the suction. Scale bars = 500 µm. See also figure 2.2. Photo 2.1.A: João Paulo Krajewski.

Scanning electron micrographs reveal the remarkable differences between the external morphology of the lips of the tubelip wrasse *Labropsis australis*, and those of *Coris gaimard*, a typical non-corallivorous wrasse (Figure 2.1.C-D). The lips of *C. gaimard* are thin and smooth, with the teeth protruding slightly (Figure 2.1.C). By contrast, the lips of *L. australis* are fleshy, protruding, and form a tube when the mouth is closed, covering all the teeth (Figure 2.1.D). The most prominent characteristic of tubelip wrasse lips, however, is the presence of numerous thin lamellae (40-50 μm thick) arranged radially. The lamellae originate on the internal side of the lip and typically bifurcate near the external margins.

Histological sections reveal that the lips of *C. gaimard* are characterized by a thin, non-keratinized, stratified epithelium composed of multiple layers of cuboidal cells (Figure 2.2.A). Mucus-secreting cells (goblet cells), if present, occur in low numbers and very rarely aggregate into multicellular glandular structures. By contrast, tubelip wrasses have a highly convoluted oral epithelium (Figure 2.2.B), with numerous grooves that appear to function as high-productivity mucous glands. The lining of the lip epithelia contains numerous goblet cells that stain positive with Alcian blue-PAS, i.e., containing secretory vesicles filled with acid and/or neutral mucopolysaccharides (Figure 2.2.C). Goblet cells typically occupy from one quarter to half the thickness of the epithelium.

Figure 2.2. (see pages 8-9) Histological sections of the lips of tropical wrasses. (A) Masson's trichrome-stained cross section of the lips of *Coris gaimard*, a non-corallivorous wrasse. **(B)** Masson's trichrome-stained cross-section of the highly modified lips of the corallivorous tubelip wrasse *Labropsis australis*. **(C)** Alcian blue and PAS-stained cross-section of the lips of *L. australis*. Note the goblet cells filled with reactive mucopolysaccharides (dark purple) along the lip epithelium (light purple).
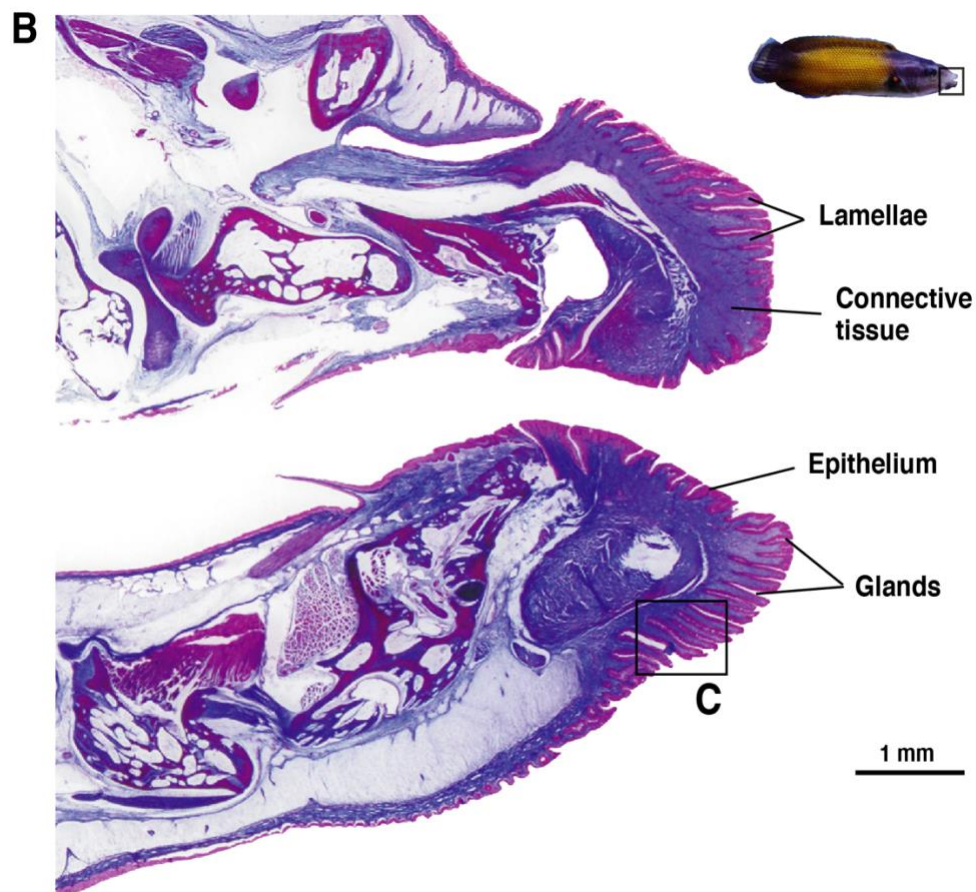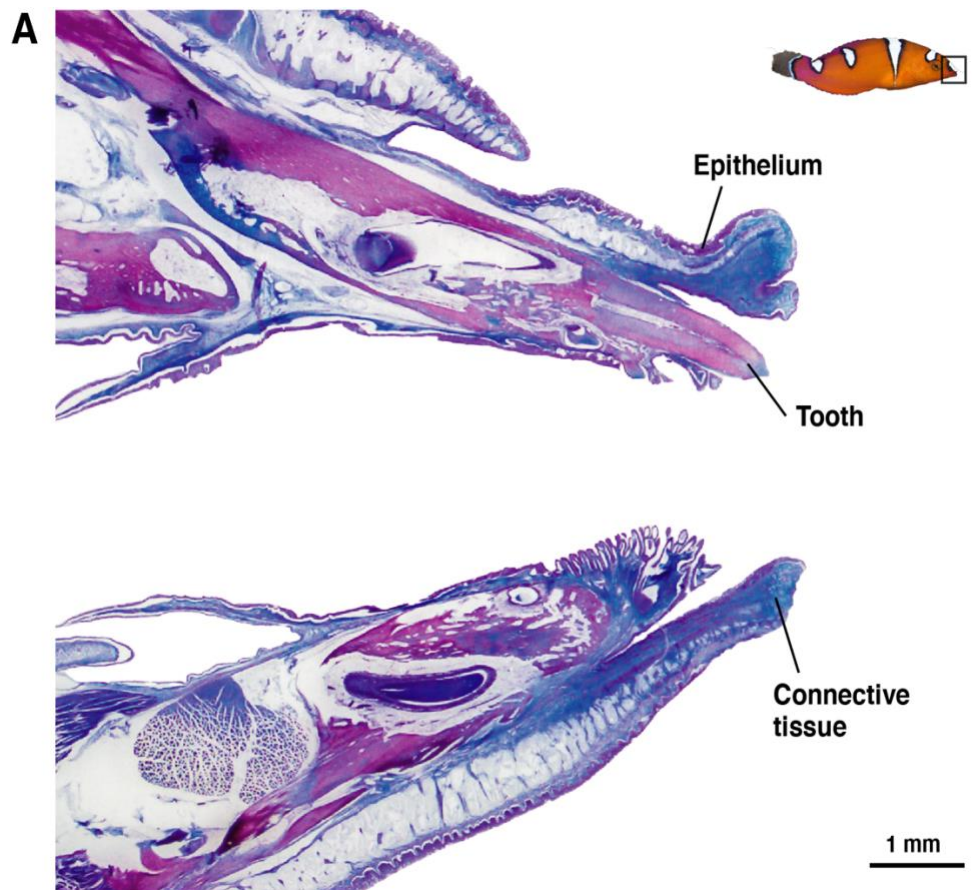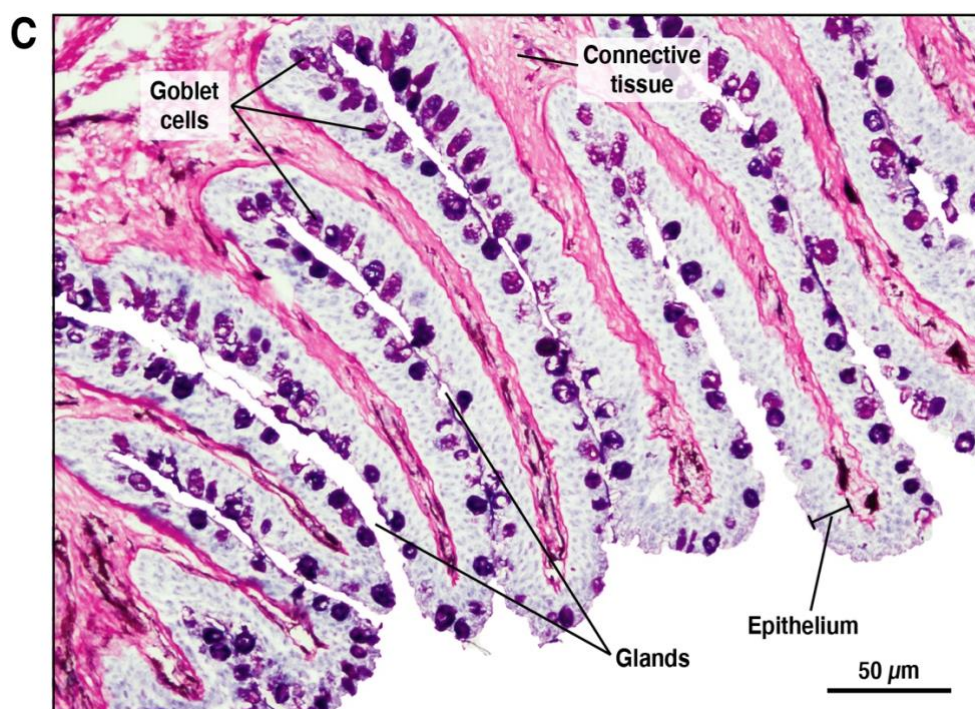
Fish-based corallivory is an important feeding mode that has the potential to play a significant role in coral reef dynamics (Cole, Pratchett, & Jones, 2010; Rotjan & Lewis, 2008). The effect of corallivory has been widely investigated, especially in butterflyfishes (Cole et al., 2008; Rotjan & Lewis, 2008). Unlike butterflyfishes, however, tubelip wrasses appear to routinely use their lips when removing material from coral. High-speed video image analyses indicated that *L. australis* briefly placed their lips in contact with the coral prior to a powerful suck. The lips did not grab or hold coral material, rather they appeared to be used for sealing the mouth over a small localized area, presumably to increase suction-feeding efficiency (Supplementary Movies 1.1 and 1.2). The relatively small proportion of sucks with visual evidence of coral tissue removal suggests that *Labropsis* might predominantly feed on coral mucus. Tubelip wrasses have been reported to prefer feeding on damaged areas of the coral (Cole, Pratchett, & Jones, 2009; McIlwain & Jones, 1997), where abundant mucus is produced. This suggests that these fishes may target coral mucus. It appears that the fleshy lips of tubelip wrasses play a critical role in feeding, and that both fish and coral mucus are likely to be involved.

The highly modified lips of coral-feeding tubelip wrasses are fundamentally different from those of non-corallivorous wrasses and, to our knowledge, have not been reported previously. The structure of these lips strongly suggests that mucus secretion is the key factor that enables these fishes to feed on corals by providing protection, a seal for suction (the thick mucus and soft lips providing a seal on the uneven coral surface during suction) and, potentially, a means of mucus ingestion (Figure 2.1.F) (details in Appendix A). Indeed, video observations reveal that tubelip wrasses feed using short sharp 'kisses' to suck mucus and occasionally tissue off the coral surface (Figures 2.1.E-F; Supplementary Movies 1.1 and 1.2). Feeding strikes were often associated with an audible 'tuk'.

The production of mucus in fishes is widespread and serves different purposes. Most fishes secrete small amounts of epidermal mucus that aids locomotion (Bernadsky, Sar, & Rosenberg, 1993). Epidermal mucus also contains UV-absorbing compounds that provide protection from ultraviolet radiation (Eckes, Siebeck, Dove, & Grutter, 2008). It may also, as in the production of a mucous cocoon by parrotfishes, reduce nocturnal predation or parasitism (Grutter, Rumney, Sinclair-Taylor, Waldie, & Franklin, 2011). However, the most widely documented use of mucous protection is in anemonefishes (Amphiprioninae), where skin secretions enable them to shelter amongst the tentacles of sea anemones. It has been suggested that anemonefishes are protected by a thick layer of epidermal mucus that acts as a physical barrier, protecting the fish from nematocysts. It appears that the mucus does not impair the anemone's ability to sting; it merely prevents the fish from being stung due to the protective mucous coating (Mebs, 1994).

In tubelip wrasses, the histological analyses suggest that their lips may provide a similar protective mucous coat. Although the surface of the lips of tubelip wrasses appears smooth to the naked eye, our examinations revealed a system of lamellae that has not been previously described in fish lips. This convoluted epithelium, lined with goblet cells that secrete mucus onto

the external surface of the lip, bears more resemblance to the epithelium of a fish gut than to the lips of other reef-dwelling fishes. These grooves increase the lip surface area in a manner comparable to the gills of mushrooms and toadstools. The resultant mucus probably both protects the lip tissues from coral nematocysts when the lips are in close contact with the coral surface and serves as a sealant to enhance in-contact suction feeding. Tubelip wrasses appear to exploit an abundant but challenging food resource, corals, by using mucus-secreting lips.

Chapter 3

FEEDING INNOVATIONS AND THE FIRST CORAL-FEEDING FISHES

3.1. Abstract

Tubelip wrasses were probably the first modern fish group to feed on corals, an ability that has been linked to their unusual lips. However, the only detailed account of these lips is based on a qualitative description of one tubelip wrasse species. Here, we provide the first quantitative evaluation of the lips of a broad range of wrasses and offer insights into the functional role of lips in coral-feeding fishes. A phylogenetic principal components analysis of 27 morphological traits revealed a clear differentiation between the lips of three coral-feeding tubelip wrasse genera (*Labrichthys*, *Labropsis*, and *Diproctacanthus*) and 12 non-corallivorous genera. This separation among taxa was based primarily on the presence of a glandular lip epithelium in tubelip wrasses. Our findings support the hypothesis that mucus secretion in the lips of tubelip wrasses plays a key role in their coral-feeding ecology and highlight the value of soft anatomy in enabling fishes to exploit novel trophic resources.

3.2. Introduction

Modern fishes, and reef fishes in particular, display remarkable morphological diversity (M. Friedman, 2010; Wainwright & Bellwood, 2002). Over the last 60 million years, extreme modifications of their feeding apparatus have resulted in multiple functional innovations that have revolutionized their ecological roles (Bellwood, Hoey, Bellwood, & Goatley, 2014; Wainwright & Longo, 2017). There have been two major phases of expansion in reef fish ecology, with associated changes in feeding guilds (Bellwood, Goatley, & Bellwood, 2017). In the first phase (Eocene), many of the basic feeding modes had already arisen, including herbivory and durophagy. The rise of corallivory, however, occurred during the second wave of trophic innovation during the Oligocene-Miocene (Cowman, Bellwood, & van Herwerden, 2009). At this time, corallivorous fishes emerged as a prominent functional group capable of influencing coral reef dynamics (Cole et al., 2008; Rotjan & Lewis, 2008).

Corallivory, i.e. predation on live corals, is potentially one of the most significant reef-shaping processes due to its impact on reef-building corals (Bellwood, Hoey, & Choat, 2003; Bonaldo & Bellwood, 2011; Cole et al., 2008; Rotjan & Lewis, 2008). Corallivorous reef fishes impact the growth and survival of individual coral colonies, potentially altering the structure of coral communities (Bonaldo & Bellwood, 2011; Rotjan & Lewis, 2008). However, corals are a particularly difficult resource to feed on. Coral tissues are protected by stinging nematocysts. They are also spread thinly over a sharp, calcified skeleton. These features are likely to limit the fishes' access to this otherwise widely available food resource. Nevertheless, at least 128 reef fish species are known to feed on live corals (Cole et al., 2008).

The most important coral-feeding fishes in terms of their abundance, geographical range, and impact on coral populations are all restricted to three major lineages: the butterflyfishes (family Chaetodontidae), the giant bumphead parrotfish (*Bolbometopon muricatum*), and the tubelip wrasses (tribe Labrichthyinii). Butterflyfishes have a highly modified feeding apparatus.

However, the modified oral structures that are typically associated with butterflyfishes (i.e., bristle-toothed) are not specifically associated with feeding on live coral. Indeed, this trait probably arose to feed on a broad range of benthic invertebrates (Bellwood et al., 2010; Konow & Ferry-Graham, 2013). The key trait that appears to enable butterflyfishes to exploit a coral-based diet is a long gut (Berumen, Pratchett, & Goodman, 2011; Elliott & Bellwood, 2003; Konow & Ferry-Graham, 2013; Konow, Price, Abom, Bellwood, & Wainwright, 2017). This trait may be indicative of an evolutionary response to the challenges associated with assimilating coral material (Elliott & Bellwood, 2003; Konow et al., 2017).

Numerous parrotfishes occasionally bite live corals (Bonaldo & Bellwood, 2011; Bruckner & Bruckner, 1998; Bruggemann, van Oppen, & Breeman, 1994; Francini-Filho, Moura, Ferreira, & Coni, 2008; Rotjan & Lewis, 2006), but while they may impact corals significantly, corals generally represent a minimal part of their diet. Only the giant bumphead parrotfish (*Bolbometopon muricatum*), the world's largest excavating reef fish, bites extensively on living corals (Bellwood et al., 2003). Although *Bolbometopon* may get most of the nutrients from the microbial community that grows on or within the reef matrix (Clements, German, Piché, Tribollet, & Choat, 2017), it is nevertheless one of the most important corallivores, taking approximately half of its bites from corals, and removing an estimated 13.5 kg m^{-2} of live coral and 5.7 tonnes of carbonate per individual each year (Bellwood et al., 2003).

All coral-feeders have highly specialized jaws. However, while their highly derived feeding structures enable the fishes to feed on corals, in most cases these features did not emerge in response to corallivory (i.e., they are exaptations). So far, the tubelip wrasse *Labropsis australis*, with its tube-shaped mucus-secreting lips, appears to be the only fish with a modified oral structure specifically associated with the consumption of coral material (Huertas & Bellwood, 2017 [chapter 2 in this thesis]).

The highly specialized lips reported in *Labropsis* may thus have represented a major anatomical breakthrough that provided access to a novel resource. Yet, we have only just begun to investigate the relationships between lip morphology and food preferences (Huertas & Bellwood, 2017 [chapter 2 in this thesis]). The taxonomic extent of this unusual lip structure among tubelip wrasses, for example, is currently unknown. Furthermore, the published phylogenetic evidence (Bellwood et al., 2010; Cowman et al., 2009) strongly suggests that tubelip wrasses may have been the first fishes to feed on corals.

Here, we provide a detailed, quantitative evaluation of the lips of tubelip wrasses, and compare them to those of a broad spectrum of other wrasse genera. Our analysis aims to answer two key questions: 1) Are the mucus-secreting folds described in *Labropsis australis* a shared trait across tubelip wrasses? And 2) Is this structure present in *Labrichthys*, and therefore, in the earliest lineage of tubelip wrasses that diverged approximately 20 million years ago?

3.2. Materials and Methods

3.2.1. *Study species*

We examined the lips of species from three of the four tubelip wrasse genera (*Labrichthys unilineatus*, *Labropsis australis,* and *Diproctacanthus xanthurus*). These three species are all obligate corallivores as adults (Cole et al., 2010), belong to the Labrichthyinii (Figure 3.1) (Cowman et al., 2009) and possess distinctive, fleshy, tube-shaped lips. We compared the lips of adult individuals of these tubelip wrasses with those of species from 12 additional genera that encompass the broad phylogenetic diversity of the family (Cowman et al., 2009). In total, we included 30 fishes from 15 species of wrasses in this study (Supplementary Table 3.1).
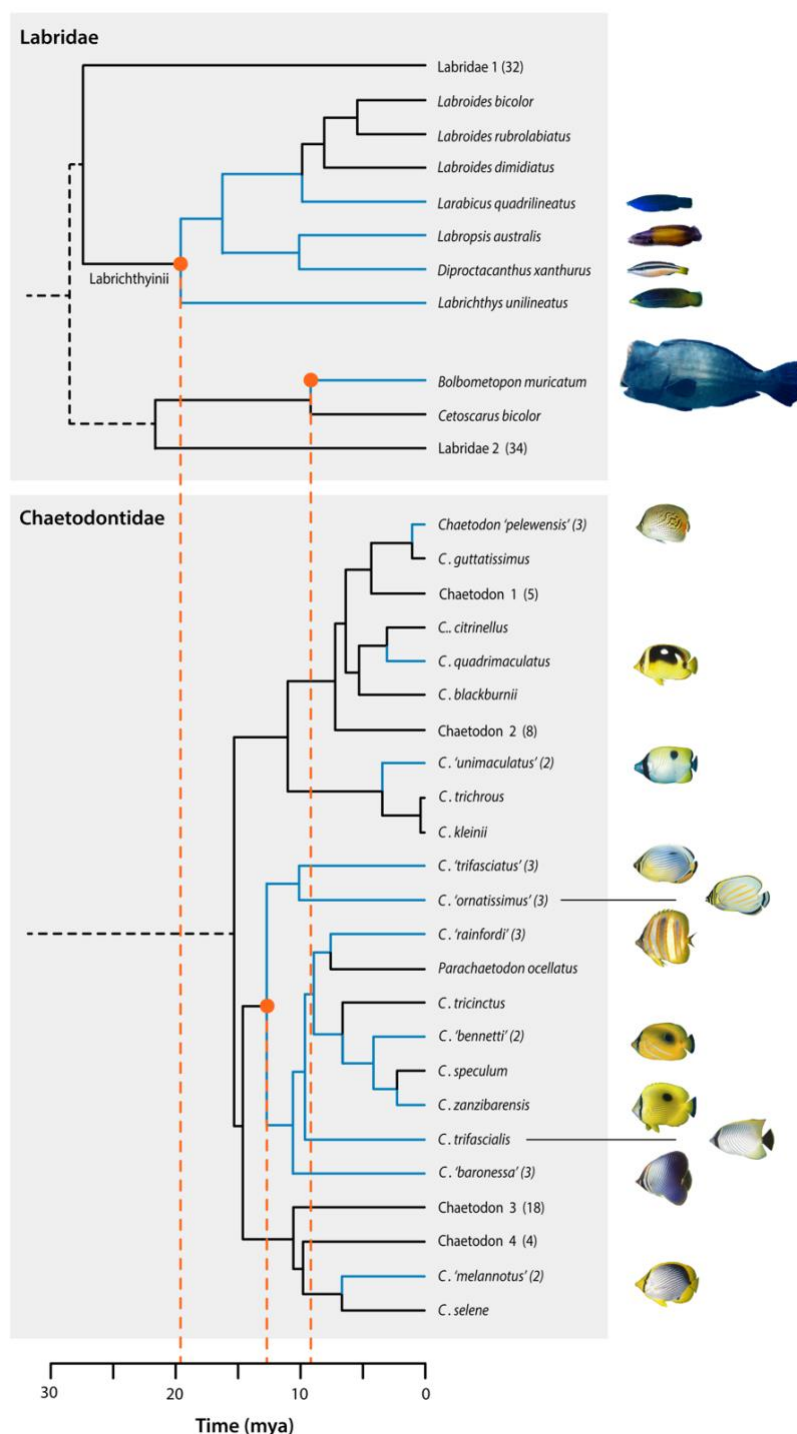
Figure 3.1. Chronograms of the three main lineages of coral-feeding fishes: tubelip wrasses (Labrichthyinii), the giant bumphead parrotfish (*Bolbometopon muricatum*), and coral-feeding butterflyfishes (Chaetodontidae). All chaetodontids shown belong to the genus *Chaetodon* with the exception of *Parachaetodon ocellatus*. Branches in blue indicate lineages of coral-feeding species with ancestral state reconstruction following Floeter et al. (2018). Branches in black indicate non-corallivorous lineages. Parentheses indicate number of species grouped in each clade. Photo credits: Victor Huertas, Scott Johnson, Ronald Kuiter, François Libert, and Robert Linsdell.

3.2.2. Sample preparation

Fishes were anaesthetized using clove oil and euthanized in a clove-oiled ice-water slurry. Next, the standard length (SL) of each individual was measured. Lip sample preparation for both light and scanning electron microscopy (SEM) followed standard protocols (Huertas & Bellwood, 2017 [chapter 2 in this thesis]). Thirty sagittal sections (one from each of the 30 fishes used in this study) were used for morphological analyses. Sections were close to the midline but slightly offset to avoid the symphyseal region as it is invested in collagenous tissue and, therefore, it may not be morphologically representative of the entire lip structure. The stain of choice for morphological analysis was Masson's trichrome stain because this technique provides a clear contrast between the epithelium and the underlying connective tissue. The potential presence of mucus-secreting goblet cells in the outer margin of the lips was evaluated using the Alcian Blue (pH 2.5) - Periodic Acid Schiff (PAS) staining technique (Yamabayashi, 1987). Photomicrographs were analysed using Macnification v1.8 (Orbicule, Heverlee, Belgium).

3.2.3. Morphological data

A comprehensive set of 27 measurements was developed to quantify the main morphological features of the lips (Figure 3.2; Table 3.1). All measurements were straight-line distances except those that measured perimeters and length of glands. The lip maximum length (UL-L_{max} and LL-L_{max}) was defined as the straight-line distance from the perpendicular projection of the skin fold (SF) to each lip's anteriormost point. The lip maximum thickness (UL-T_{max} and LL-T_{max}) was defined as the maximum straight-line distance between the inner and outer surface of the lip. The protrusion of the lips (UL-PM_{dist} and LL-DT_{dist}) was determined by measuring the minimum distance from the anteriormost point of the UL/LL to the premaxilla/dentary, respectively. If this straight-line measurement from the lip's anteriormost point did not intersect with the oral jaw bones but a tooth, the distance to the tooth was recorded. The epithelium

thickness (ULET and LLET) was estimated by measuring the distance from the outermost layer of the epithelium, perpendicular to the surface of the lip, down to the basal membrane. The epithelium thickness along the lip can be highly variable. Hence, a value for each lip was averaged from five measurements taken at haphazard points around the lip. When glands were present, their number was recorded. The length of the longest gland (ULG_{max} and LLG_{max}) was estimated with a curved measurement following the glandular grooves. Additionally, up to five glands were selected haphazardly and the mean value for gland thickness was estimated for each lip (ULGT and LLGT). For each gland, the thickness was calculated by measuring the distance between the base of the epithelial layer at opposite sides of the gland, at half the length of the gland. Three perimeters were measured: the total perimeter of the epithelium (ULE and LLE), the perimeter of the glandular epithelium (ULGE-I and LLGE-I), and the macroscopic (outer) perimeter of the glandular epithelium (ULGE-II and LLGE-II). To ensure that the ULE and LLE measurements remained consistent across species regardless of lip morphology, the skin fold (SF) between the RC and the UL, and the tip of the velum were set as reference points that defined the boundaries of the lip perimeter. Thus, the ULE was measured from the SF to the tip of the velum, and the LLE was measured from the tip of the velum to the intersection between the perpendicular projection of the SF and the lower lip's ventral surface (SF_V). The glandular epithelium was defined as the secretory portion of the epithelium that included all the glands from the oral jaws onwards and around the lip. The ULGE-I and LLGE-I runs along the entire surface of the glandular epithelium. The ULGE-II and LLGE-II, however, provide an estimation of the perimeter along the same section if no glands were present (i.e., without including the folds). Finally, two ratios were calculated to provide a measure of the mucus production capability. The ULE/ULGE-I and LLE/LLGE-I ratios were variables that indicate the extent of the perimeter where mucus was produced. The ULGE-I/ULGE-II and LLGE-I/LLGE-II ratios indicated the rugosity of the glandular epithelium and thus, could be interpreted as a proxy for mucus secretion capacity.

Table 3.1. List of measurements taken to quantify the lip morphology of wrasses. Letters to the left of the measurement labels refer to the measurements illustrated in Figure 3.2. RC = Rostral cap; SF = Skin fold between RC and upper lip; SF_V = intersection of the vertical projection of SF with ventral surface of lower lip; UL = Upper lip; LL = Lower lip. *Measurements not shown in Figure 3.2.

Trait		Description
A	Depth RC	Depth of rostral cap (RC) measured from tip of RC to the base of the skin fold (SF) between the RC and the upper lip (UL).
Upper lip		
B	ULE	Perimeter of upper lip's total epithelium
C	UL-SF$_{dist}$	Minimum distance from SF to intersection with UL-L$_{max}$
D	UL-L$_{max}$	Upper lip's maximum length
E	UL-T$_{max}$	Upper lip's maximum thickness
F	UL-PM$_{dist}$	Min. distance from premaxilla to anteriormost point of UL
G	ULG$_{max}$	Length of upper lip's longest gland
H	ULGE -I	Perimeter of upper lip's glandular epithelium
I	ULGE-II	Macroscopic (outer) perimeter of ULGE-I
-	ULE/ULGE-I*	Ratio of total epithelium vs glandular epithelium in UL
-	ULGE-I/ULGE-II*	Ratio of glandular epithelium vs macroscopic perimeter of glandular epithelium in UL
-	#ULG*	Number of upper lip's glands
-	ULET*	Upper lip's epithelium thickness
-	ULGT*	Upper lip's gland thickness
Lower lip		
J	LLE	Perimeter of lower lip's total epithelium (LLE)
K	LL-SF$_{Vdist}$	Minimum distance from SFV to intersection with LL-L$_{max}$
L	LL-T$_{max}$	Lower lip's maximum thickness
M	LL-DT$_{dist}$	Minimum distance from dentary to anteriormost point of LL
N	LL-L$_{max}$	Lower lip's maximum length
O	LLGE-I	Perimeter of lower lip's glandular epithelium
P	LLGE-II	Macroscopic (outer) perimeter of LLGE-I
Q	LLG$_{max}$	Length of lower lip's longest gland
-	LLE/LLGE-I*	Ratio of total epithelium vs glandular epithelium in LL
-	LLGE-I/LLGE-II*	Ratio of glandular epithelium vs macroscopic perimeter of glandular epithelium in LL
-	#LLG*	Number of lower lip's glands
-	LLET*	Lower lip's epithelium thickness
-	LLGT*	Lower lip's gland thickness
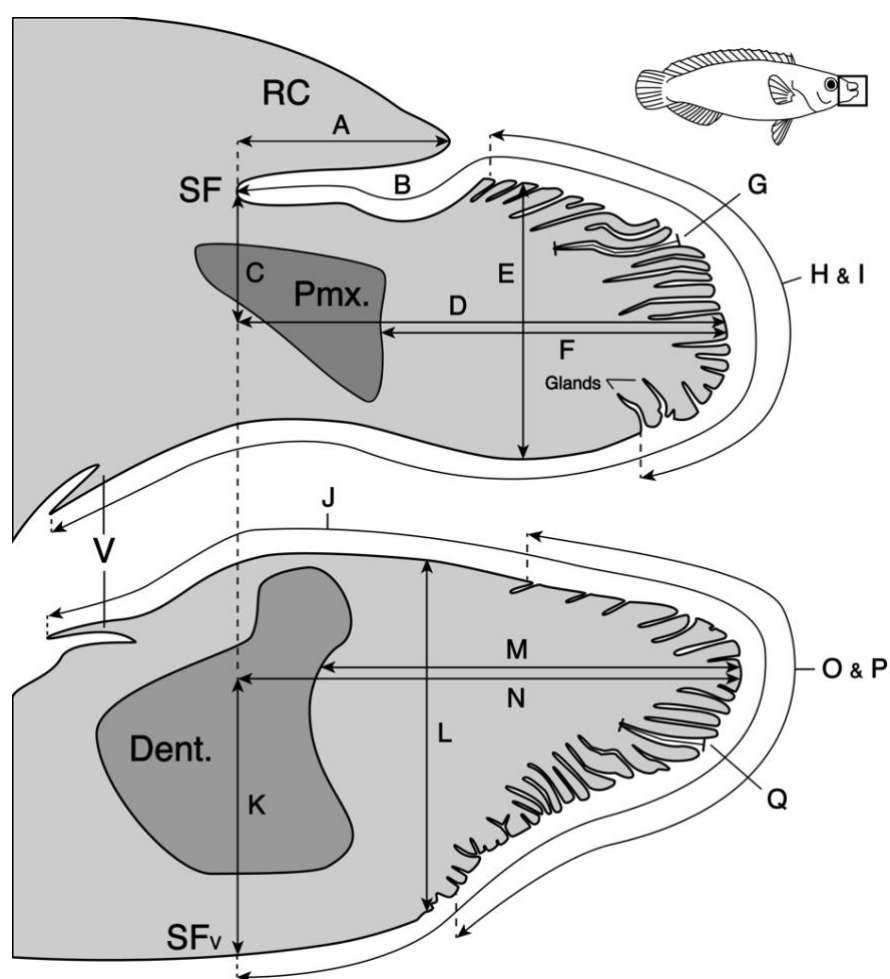
Figure 3.2. Vertical section of the lips of the tubelip wrasse *Labropsis australis* showing the measurements taken to quantify the lip morphology. RC = Rostral cap; SF = Skin fold between RC and upper lip; SFV = intersection of the vertical projection of SF with ventral surface of lower lip; Pmx. = Premaxilla; Dent. = Dentary; V = Velum. Solid lines indicate measurements and dashed lines indicate reference lines. Additional variables not represented in this diagram include the number of glands, mean epithelium thickness, mean gland thickness, proportion of glandular epithelium vs total epithelium, and proportion of glandular epithelium vs macroscopic portion lip perimeter between first and last gland. See Supplementary Table 3.1 in Appendix B for description of measurements.

3.2.4. Data analysis

We conducted a phylogenetic Principal Components Analysis (pPCA) on a correlation

matrix to investigate the morphological diversity of wrasse lips while accounting for phylogenetic

relationships among species. This analysis was conducted using the function 'phyl.pca' in the 'phytools' package in R (Revell, 2012) and a phylogenetic tree that we pruned from (Cowman et al., 2009). To account for minor differences in size among individuals (residual analyses were not required (Wainwright et al., 2004)); we standardized linear values by dividing them by the standard length of the fish. We used mean values for each species where possible. The analysis was conducted using the 'ape' (Paradis, Claude, & Strimmer, 2004) and 'phytools' (Revell, 2012) packages in R (R Development Core Team 2017).

3.3. Results

The anatomy of the lips of typical wrasses (e.g., *Halichoeres*, *Coris*, *Thalassoma*) resembled that of the skin around the body. The epidermis was not vascularized, and it was composed of multiple layers of epithelial cells that were sustained by the basal membrane. Mucous cells were sometimes present in the epidermis, although they were typically rare. Beneath the basal membrane, the dermis was largely made up of connective tissue rich in collagen. Their lips were small, thin, and in most cases, did not fully cover the protruding canine teeth (Figure 3.3.A). The lips of *Hemigymnus melapterus* followed the same structure (Figure 3.3.B) but they were larger. In addition, the lower lip of *H. melapterus* was characterized by a lobe that extended backward.

The most remarkable structural changes were observed in the lips of tubelip wrasses (*Labrichthys* (Figure 3.3.C), *Labropsis*, and *Diproctacanthus*). These lips were characterized by a tube-like morphology and a densely packed series of radial lamellae, oriented parallel to the antero-posterior axis of the fish. Notably, the epidermis along the outer margin of the thick, tube-shaped lips of *Labrichthys unilineatus*, *Diproctacanthus xanthurus*, and *Labropsis australis*, all contained numerous mucus-secreting cells.
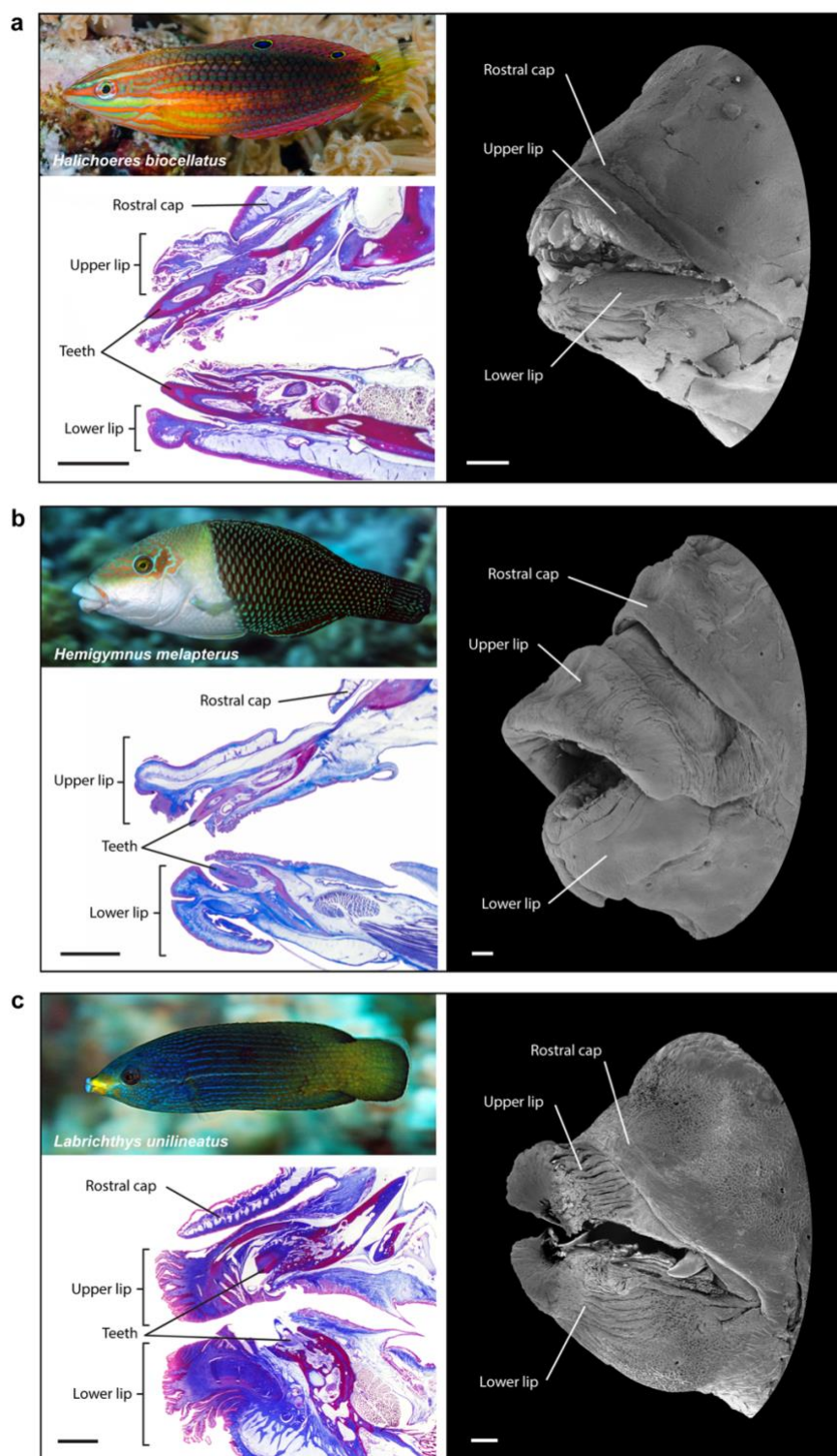
Figure 3.3. Cross sections and scanning electron microscopy (SEM) images of the lips of (A) the red-lined wrasse *Halichoeres biocellatus*, (B) the thicklip wrasse *Hemigymnus melapterus*, and (C) the tubelip wrasse *Labrichthys unilineatus*. Histological sections are stained using Masson's trichrome. The lip epithelium lining the outer surface of the lip is stained in magenta while the underlying connective tissue is stained in blue. All scale bars 1 mm. Photo credits: François Libert (A), Jeanette Johnson (B), and Scott Johnson (C).

22

These observations were clearly reflected in a pPCA of the 27 traits included in the analysis. The first two principal components accounted for 79.6% of the total variance and revealed a clear separation between the lips of coral-feeding tubelip wrasses and those of typical, non-corallivorous wrasses (Figure 3.4). This separation was explained mainly by PC1. Specifically, the position of tubelip wrasses was driven primarily by a greater perimeter of the lips (ULE and LLE) and traits associated with the presence of numerous glandular folds in the outer margin of their lips (ULG$_{max}$, ULGE-I, ULGT, LLGE-I, LLGE-II, LLG$_{max}$, LLGE-I/LLGE-II, and LLGT) (Table 3.2; Supplementary Figure 3.1, Supplementary Table 3.2). *Hemigymnus melapterus* had large lips but smooth surface features characteristic of non-corallivorous wrasses. Its position in the phylomorphospace was therefore largely driven by the length (UL-L$_{max}$ and LL-L$_{max}$), projection (UL-PM$_{dist}$ and LL-DT$_{dist}$), and large perimeter of the lips (high values of ULE/ULGE-I and LLE/LLGE-I) (Table 3.2; Supplementary Figure 3.1, Supplementary Table 3.2).

Overall, the histological and morphometric analyses showed three main types of lips: the thin, typical lips of most wrasses; the enlarged (and smooth) lips of *H. melapterus*; and the fleshy, mucus-secreting lips of tubelip wrasses. Of these, the lips of coral-feeding tubelip wrasses were the only ones that exhibited a folded outer surface lined with numerous mucus-secreting goblet cells.

Table 3.2. Principal components loadings for 27 traits and variance explained for the first two axes of a phylogenetic principal components analysis based on standard length-standardized measurements. Boldfaced values have a principal component (PC) loading over the cut-off of ± 0.9 to highlight the variables with the greatest influence.

Trait	PC1	PC2
Depth RC	-0.693	0.369
ULE	**-0.938**	0.022
UL-SF$_{dist}$	-0.672	-0.430
UL-L$_{max}$	-0.693	-0.633
UL-T$_{max}$	-0.729	-0.345
UL-PM$_{dist}$	-0.439	-0.574
ULG$_{max}$	**-0.931**	-0.046
ULGE -I	**-0.906**	0.406
ULGE-II	-0.870	0.327
ULE/ULGE-I	-0.533	-0.521
ULGE-I/ULGE-II	-0.898	0.128
#ULG	-0.877	0.465
ULET	-0.255	-0.742
ULGT	**-0.942**	-0.126
LLE	**-0.937**	-0.007
LL-SF$_{Vdist}$	-0.619	0.372
LL-T$_{max}$	-0.778	-0.293
LL-DT$_{dist}$	-0.712	-0.410
LL-L$_{max}$	-0.797	-0.498
LLGE-I	**-0.903**	0.409
LLGE-II	**-0.934**	0.301
LLG$_{max}$	**-0.958**	0.236
LLE/LLGE-I	-0.617	-0.658
LLGE-I/LLGE-II	**-0.903**	0.380
#LLG	-0.884	0.451
LLET	-0.609	-0.221
LLGT	**-0.983**	0.021
% Variance explained	63.716	15.927
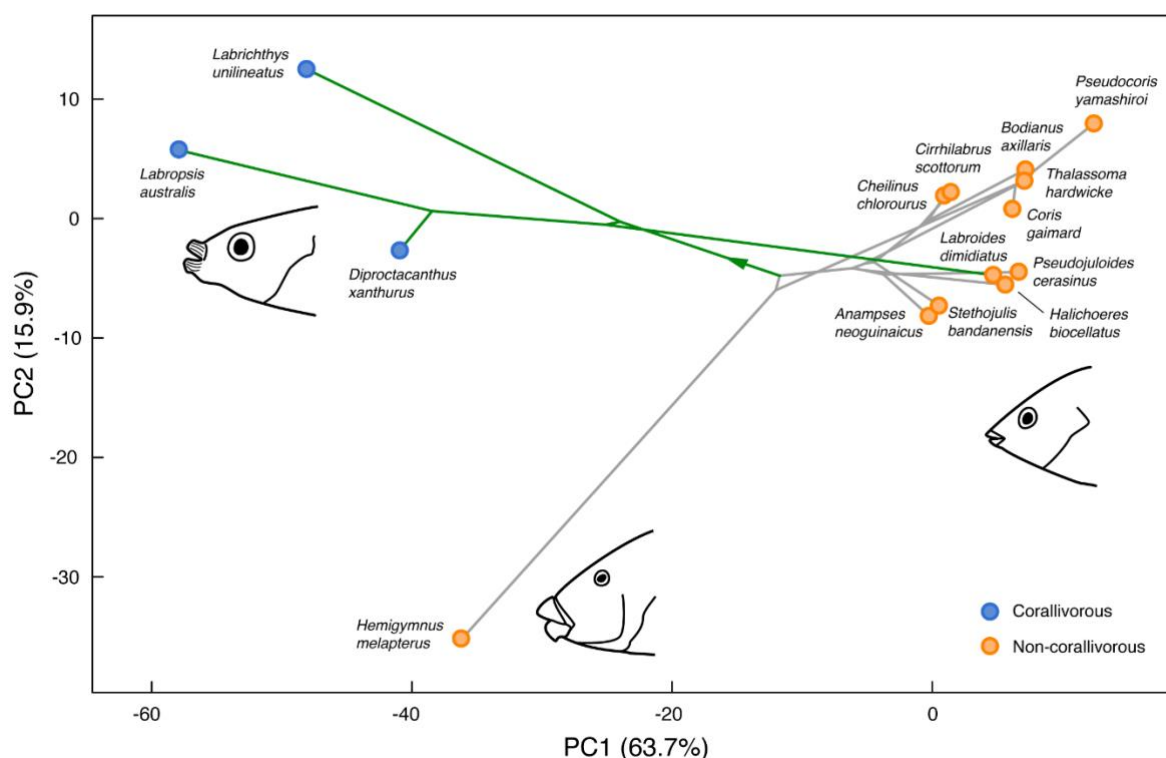
Figure 3.4. Phylomorphospace for 15 labrid species based on 27 lip morphological traits with a pruned labrid tree superimposed. Blue circles represent corallivorous tubelip wrasses with a highly folded secretory epithelium; orange circles represent non-corallivorous wrasses with a non-secretory lip. Green lines in the tree connect species within the tribe Labrichtyinii. Arrow indicates the diversion of the most recent common ancestor of the labrichthyines. Note the position of the cleaner wrasse *Labroides dimidiatus* clustered together with the wrasses with a typical lip configuration despite its close phylogenetic association with tubelip wrasses. In the phylogenetic reconstruction for this pPCA we used *Bodianus mesothorax* as a replacement for *B. axillaris* following Floeter et al. (2018).

3.4. Discussion

Our analysis showed that the unusual lip structure previously documented in *Labropsis australis* is also present in both *Labrichthys unilineatus* and *Diproctacanthus xanthurus*. Thus, this functional innovation appears to be a shared (and exclusive) trait of all corallivorous tubelip wrasses rather than a species-specific attribute. The presence of this lamellate structure in *L. unilineatus* is particularly important because, aside from being the largest of these obligate corallivores (up to 17.5 cm) (Randall, Allen, & Steene, 1997), it is also the earliest diverging

tubelip wrasse lineage (Figure 3.1). Mucus-secreting lips, therefore, are the ancestral condition within the labrichthyines, indicating that this may have been the first group of fishes to specialize on coral feeding around 20 million years ago (Figure 3.1). It is also important to note that since this type of lip is present in the tubelip wrasses' most recent common ancestor, it is probably present in all four genera (i.e., *Labrichthys*, *Labropsis*, *Diproctacanthus*, and *Larabicus*). Thus, using this unusual lip morphology, tubelip wrasses are able to feed on at least 17 different species of corals (Cole et al., 2010; McIlwain & Jones, 1997) across the Indo-Pacific region, from the Red Sea to the Central Pacific islands.

Interestingly, although the cleaner wrasse *Labroides dimidiatus* is phylogenetically nested within the labrichthyines (Figure 3.1), its lips lacked the numerous lamellae and glandular epithelium observed in tubelip wrasses. Indeed, they were morphologically more similar to those of typical wrasses (Figure 3.4, Supplementary Figure 3.2). This phenotypic divergence represents a secondary return to the 'typical' labrid morphology, and it may be the result of a shift in their diet. *Labroides dimidiatus* evolved from coral mucus feeders (Cowman et al., 2009) that rely upon lip mucus secretion to remove and consume coral mucus (Huertas & Bellwood, 2017 [chapter 2 in this thesis]). In *L. dimidiatus*, an obligate cleaner, the loss of the ability to secrete mucus likely occurred in response to a shift away from corallivory. This raises the question of whether the lips of tubelip wrasses that exhibit cleaning behaviour as juveniles experience a reconfiguration of the lips when they undergo an ontogenetic shift in diet to corallivory.

Coral feeding is a relatively recent breakthrough in the evolution of fishes (Cowman et al., 2009; Floeter et al., 2018), and the timing appears to reflect the Miocene expansion of reef habitat (Bellwood et al., 2017) that preceded the more recent expansion of *Acropora* corals (Renema et al., 2016). Although corallivory has emerged multiple times, of the three main groups of corallivorous fishes, the butterflyfishes, tubelip wrasses, and excavating parrotfishes (i.e., *Bolbometopon*), all had to circumvent the corals' nematocysts and overcome the challenge of

processing and assimilating coral material. Neither butterflyfishes with their long, bristle-like teeth (Motta, 1989), nor the giant bumphead parrotfish (*Bolbometopon muricatum*) with its powerful jaws (Bellwood, 1994) modified the morphology of their oral structure to feed specifically on corals. Both retain the 'typical' morphology of their respective clades. The only modification in butterflyfishes is in the gut (Berumen et al., 2011; Konow et al., 2017), which like tubelip wrasses, are long and exceptionally narrow (Elliott & Bellwood, 2003; Konow & Ferry-Graham, 2013). Remarkably, it was only the tubelip wrasses, the earliest coral feeders, that modified their oral structure to feed on corals.

Other reef fishes have thick, fleshy lips (e.g., *Hemigymnus*, *Coradion*, *Plectorhinchus*), but tubelip wrasses are the only fishes known to use tube-shaped lips to feed on corals (although the big-lipped damselfish *Cheiloprion labiatus* needs further investigation). The ability of tubelip wrasses to feed on coral mucus and tissues, however, is not simply a product of the shape of their lips. Rather, it appears to be a combination of the tubed lips, a highly folded epithelium, and the ability to secrete copious amounts of mucus, that enables them to exploit coral mucus (Huertas & Bellwood, 2017 [chapter 2 in this thesis]). Together, these traits appear to represent a functional innovation that underpinned a new feeding guild: the coral mucus feeders.

We have shown that the mucus-secreting lips first described in *Labropsis* are a common trait among tubelip wrasses. Most importantly, our findings suggest that this functional innovation originated approximately 20 Mya, enabling tubelip wrasses to diversify their diet by feeding on coral mucus. Thus, despite all the evolutionary innovations in the oral jaws and the pharyngeal apparatus of wrasses (Ferry-Graham, Wainwright, Westneat, & Bellwood, 2002; Wainwright et al., 2004), the first clade to successfully feed on corals did so by what appears to be the first major trophic innovation based on lips (Huertas & Bellwood, 2017 [chapter 2 in this thesis]), highlighting the importance of soft anatomy in the evolution of fish feeding.

Chapter 4

**TROPHIC SEPARATION IN PLANKTIVOROUS REEF FISHES: A NEW ROLE
FOR MUCUS?**

4.1. Abstract

The feeding apparatus directly influences a species' trophic ecology. In fishes, our
understanding of feeding modes is largely derived from studies of rigid structures (i.e., bones,
teeth, gill rakers). The lip innovation described in chapter 2, however, highlighted the role of soft
anatomy in enabling specialized feeding modes. In this study, we explore whether similar
diversification may also occur in the soft anatomy of the buccal cavity. Using four key
anatomical traits to classify 19 species (14 genera) of wrasses, we evaluated the relationship
between anatomical specialization of the buccal cavity and diet. Our data revealed a previously
undocumented anatomical adaptation in the mouths of fairy wrasses (*Cirrhilabrus*): the mucosa
throughout the buccal cavity (i.e., anterior to the pharynx) is packed with goblet cells, enabling it
to secrete large quantities of mucus in this region; a new trait that, until now, it had not been
documented in wrasses. This disparity reflects diet differences, with mucus secretion found only
in planktivorous *Cirrhilabrus* that feed predominantly on amorphous organic material (potentially
gelatinous organisms). This suggests a cryptic mucus-based resource partitioning in
planktivorous wrasses.

4.2. Introduction

Understanding the link between the functional morphology of the feeding apparatus and diet is a critical first step in assessing a species' potential ecological role. The feeding apparatus of fishes is a remarkable example of a complex structure that has diversified greatly over time (Baliga & Mehta, 2015; Burress, 2014; Hulsey, Fraser, & Streelman, 2005). With few exceptions (Bellwood, Wainwright, Fulton, & Hoey, 2006), trophic morphology is strongly correlated with fish feeding mode (Hulsey & Wainwright, 2002; Wainwright & Bellwood, 2002). Among reef fishes, wrasses (family Labridae) are exceptionally diverse (Burress & Wainwright, 2018; Wainwright et al., 2004), and an excellent model for comparative studies. Coral reef wrasses feed on a remarkable diversity of prey, ranging from fishes and molluscs to crustaceans and corals (Bellwood, Wainwright, et al., 2006; Cole et al., 2010; Wainwright et al., 2004). This ability to feed on such diverse food resources is related to complex modifications of the skull (Hulsey & Wainwright, 2002; Wainwright & Bellwood, 2002; Wainwright et al., 2004; Westneat, 2003, 2004). The fused teeth and powerful pharyngeal mill of parrotfishes, which can grind chunks of reef into fine sand (Bellwood & Choat, 1990; Clements et al., 2017), and the exceptional feeding mechanism of the slingjaw wrasse *Epibulus insidiator*, with the longest jaw protrusion recorded in fishes (Westneat, 1991; Westneat & Wainwright, 1989), are just two of the notable examples of extreme modifications of the feeding apparatus of wrasses. The bulk of studies evaluating the shape and mechanics of the skull, however, focused on the musculoskeletal system. Only recently has the importance of soft tissues become apparent (Clements et al., 2017), with some emphasizing the importance of mucus in coral-feeding wrasses (Huertas & Bellwood, 2017 [chapter 2 in this thesis]).

The physical and chemical properties of mucus make it a highly versatile resource that fishes secrete for a broad range of functions (Shephard, 1994). In skin epidermis, mucus plays a major role in ion regulation (Shephard, 1994), and protection from ultraviolet radiation (Eckes et al., 2008), microbial pathogens (Salinas, 2015), would-be predators (Böni, Fischer, Böcker,

Kuster, & Rühs, 2016; Gratzer, Millesi, Walzl, & Herler, 2015), and parasites (Grutter et al.,

2011; Munday et al., 2003). Epidermal mucus also lubricates the skin, reducing drag (Daniel,

1981). Importantly, fish mucus plays a central role in the feeding ecology of some fishes (Huertas

& Bellwood, 2018 [chapter 3 in this thesis]; Sanderson et al., 1996). Parrotfishes and some

suspension-feeders, for example, trap food particles using a mucous layer secreted by goblet cells

in the pharyngeal region (Board, 1956; Clements et al., 2017; Gohar & Latif, 1961; Sanderson et

al., 1996). The folded epidermis of the lips of coral-feeding tubelip wrasses is also densely

populated with goblet cells, presumably to protect the lips from nematocysts (Huertas &

Bellwood, 2017 [chapter 2 on this thesis]). This suggests that reef fish groups with broad dietary

habits exhibit variation in their ability to secrete mucus. To test this hypothesis, we evaluated the

mucosa of the buccal cavity of a broad range of wrasses, a fish group known for exhibiting a

broad diversity of feeding modes (Cowman et al., 2009; Wainwright et al., 2004). Specifically, we

set out to answer two key questions: Does mucus secretion in the buccal cavity vary among

wrasses? And, if so, is this variation correlated with their diet?

4.3. Materials and Methods

We examined the buccal cavity and gut contents of 19 species representing 14 of the

most abundant genera of wrasses (family Labridae) on Indo-Pacific reefs, including members of

the genera *Cirrhilabrus, Thalassoma, Pseudocoris, Coris, and Halichoeres*. Species were sampled to

encompass a trophically diverse wrasse assemblage that includes planktivores, mobile

invertebrate feeders, corallivores, and one cleaner (Figure 4.1.A, Table 4.1). Specimens were

predominantly collected using fine barrier nets on the Great Barrier Reef (Australia). Only adult

fishes were used to avoid the influence of ontogenetic shift in diet in some species.

We examined the buccal cavity of 32 fishes. Specifically, the region evaluated comprised

the midsection of the oral cavity (delineated by the front end of the neurocranium dorsally and

the tip of the urohyal ventrally, back to the pharynx, which was excluded from the study). In this study, we refer to this region as the "buccal cavity". The head was removed, fixed in Bouin's fixative for 24 h, rinsed thoroughly, stored in 70% ethanol, then decalcified in Gooding and Stewart's fluid for 48 h. Samples were then divided along the midline and embedded in paraffin wax. Sagittal sections (5 μm thick) close to the midline were used for anatomical analysis. We used Alcian Blue (pH 2.5)-periodic acid Schiff (PAS) stain to detect mucus in the buccal cavity, focusing on the epidermal mucosa. Photomicrographs from one histological section from each fish were taken with an Olympus DP21 digital camera on an Olympus BX40 light microscope were combined using the photomerge tool in Adobe Photoshop CS6 (Adobe Systems, San Jose, USA), to provide a high-resolution view of the buccal cavity.

The methodology used in this chapter differed from that of Chapter 3 due to fundamental differences between the structure of fish lips and the buccal cavity. Here, to quantify the fishes' ability to secrete mucus we measured the thickness of the mucosa, mean goblet cell width, mean goblet cell length, and the number of goblet cells per 100 μm section of mucosa. Measurements were taken at 10 equidistant sampling locations placed along the upper and lower margins of the oral mucosa to give a total of 20 sampling locations within the buccal cavity (Supplementary Figure 4.1). The thickness of the mucosa represents the amount of space available for mucus-filled vacuoles. We used the mean width and mean length of goblet cells (GCs) as proxies of mucus secretion capability at the cellular level (i.e., we assumed that larger cells are capable of secreting more mucus). Lastly, the GC density was calculated by averaging the number of GCs within the 100 μm-long section. Where possible, measurements were averaged for each trait.
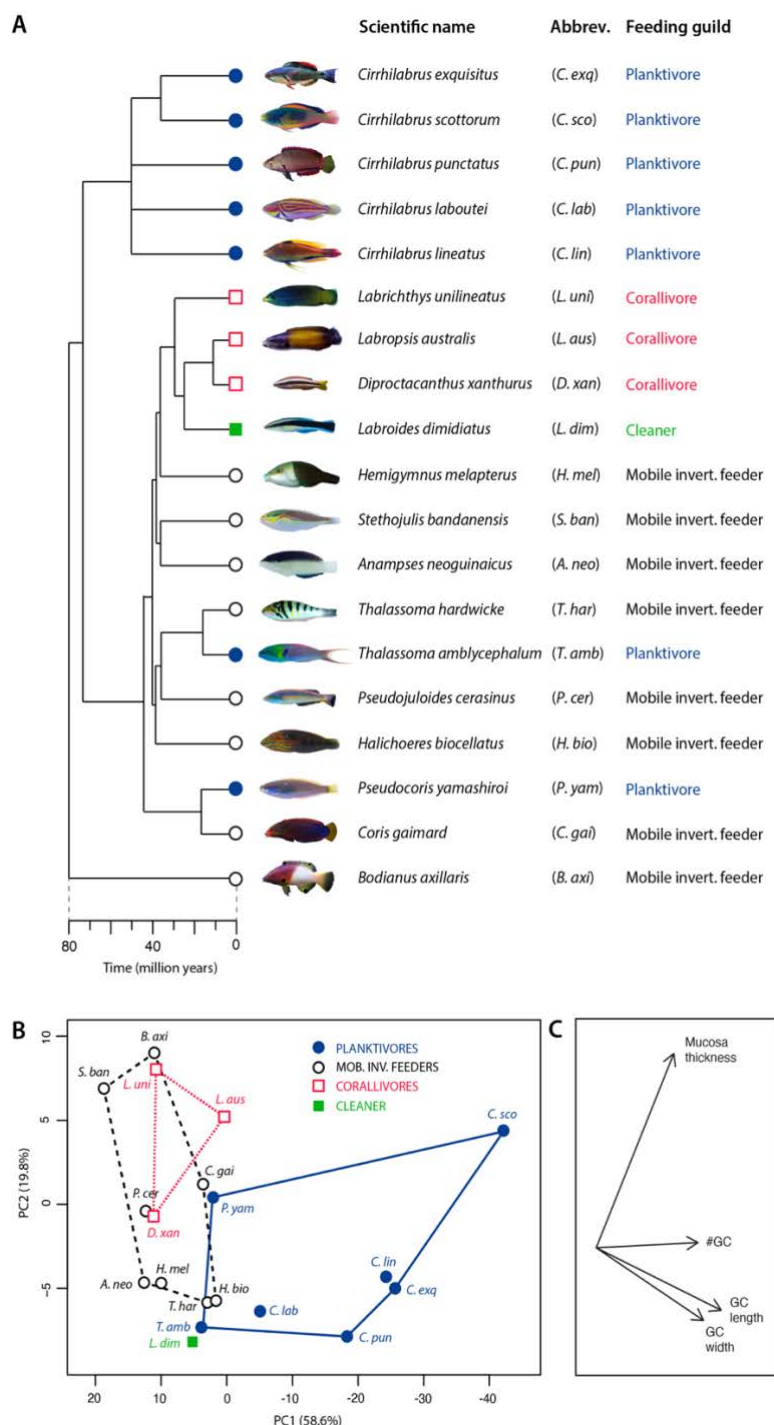
Figure 4.1. Reef-associated wrasses evaluated for morphological variation associated with mucus secreting ability in the buccal cavity. (A) Phylogenetic relationships of the 19 species of wrasses included in this study. Pruned tree from Rabosky et al. (2018). **(B)** Morphospace of mean values of goblet cell density, goblet cell length and width, and mucosa thickness. **(C)** Vectors representing the degree of correlation of each morphological trait with the first two main axes of variation. Filled blue circles: planktivores; Empty magenta squares: corallivores; Filled green square: cleaner; Empty black circles: mobile invertebrate feeders. GC: goblet cell. Photo credits: Christopher Hemingson, Jeanette Johnson, Scott Michael, Yi-Kai Tea.

Table 4.1. Mean values of morphological and gut content data used in this study. Goblet cell density is the number of goblet cells contained in a stretch of 100 μm along the mucosa. Gut content data was collected using the point intersect method [see Bellwood et al. (2006) for details on how this data was collected]. All specimens evaluated were adults (trophic guild classification was based on the diet of adults) and were predominantly collected on coral reefs from the Great Barrier Reef. Sample sizes and references to support trophic guild classification are in Supplementary Table 4.1 in Appendix C. Mob. invert. feeder = mobile invertebrate feeder; GC = Goblet cell; AOM = Amorphous organic matter

| Species | Trophic guild | Morphological data | | | | Gut content data (%) | | | | | | | |
		Mucosa thickness (μm)	GC width (μm)	GC length (μm)	GC density	Micro-crustacea (<3 mm)	Macro-crustacea (>3 mm)	AOM	Polychaeta	Sediment	Fish material	Coral organic matter	Mollusca
Cirrhilabrus exquisitus	Planktivore	33.13	8.88	23.44	14.10	60.09	0.00	38.39	0.27	0.63	0.09	0.00	0.18
Cirrhilabrus laboutei	Planktivore	21.30	7.78	13.42	6.38	17.50	0.00	70.17	0.00	9.83	0.00	0.00	0.83
Cirrhilabrus lineatus	Planktivore	33.11	9.98	19.57	14.80	27.30	0.00	71.70	0.20	0.60	0.00	0.00	0.00
Cirrhilabrus punctatus	Planktivore	26.14	9.12	19.40	11.40	53.02	0.15	43.90	0.15	1.54	0.00	0.00	0.29
Cirrhilabrus scottorum	Planktivore	50.09	10.05	24.64	27.88	20.27	0.00	70.14	0.74	5.74	0.00	0.00	0.34
Pseudocoris yamashiroi	Planktivore	20.44	4.58	7.68	16.20	83.33	1.91	14.29	0.00	0.12	0.00	0.00	0.36
Thalassoma amblycephalum	Planktivore	14.44	6.95	8.73	5.98	75.83	0.00	12.00	0.25	4.67	3.33	0.00	0.33
Thalassoma hardwicke	Mob. invert. feeder	16.36	6.93	8.45	7.28	15.75	29.25	16.33	0.75	3.58	9.50	0.00	15.00
Anampses neoguinaicus	Mob. invert. feeder	12.73	5.06	5.82	3.55	44.52	13.93	11.91	11.67	14.88	0.00	0.00	1.43
Bodianus axillaris	Mob. invert. feeder	30.11	3.74	4.70	4.70	7.80	11.90	6.60	0.10	1.40	4.00	0.00	58.50
Coris gaimard	Mob. invert. feeder	25.42	6.42	7.40	5.78	13.45	12.38	4.41	0.83	5.00	0.00	0.00	61.43
Halichoeres biocellatus	Mob. invert. feeder	18.56	7.79	8.73	5.20	16.98	4.69	26.15	0.31	7.60	0.00	0.00	39.48
Hemigymnus melapterus	Mob. invert. feeder	14.19	6.12	5.26	4.90	56.37	13.79	10.73	2.42	8.23	0.73	0.00	6.86
Pseudojuloides cerasinus	Mob. invert. feeder	19.01	4.97	5.69	1.68	38.18	0.00	32.96	5.46	13.18	0.00	0.00	9.55
Stethojulis bandanensis	Mob. invert. feeder	23.39	2.55	2.99	1.55	77.50	0.19	3.27	3.17	12.69	0.00	0.00	3.08
Labroides dimidiatus	Cleaner	12.33	6.95	7.72	6.15	42.10	0.00	45.00	0.00	0.00	12.18	0.00	0.00
Labropsis australis	Corallivore	33.48	7.56	7.01	4.18	0.74	0.00	1.32	0.00	1.62	0.00	99.83	0.00
Labrichthys unilineatus	Corallivore	30.27	4.69	4.39	2.45	0.00	0.00	4.90	0.00	1.77	0.00	96.32	0.00
Diproctacanthus xanthurus	Corallivore	18.58	5.07	5.60	3.45	0.00	0.00	0.00	0.00	0.00	0.00	93.33	0.00

To evaluate the relationship of the buccal anatomy among wrasses, we conducted a phylogenetic principal components analysis (pPCA) (Revell, 2009) on a multivariate dataset of four key traits associated with mucus secretion ability (i.e., GC density, GC length, GC width, and mucosa thickness). This pPCA was based on a correlation matrix assuming a Brownian motion model of evolution. A pruned tree from the literature (Rabosky et al., 2018) (Figure 4.1.A) was incorporated in the analysis to account for autocorrelation due to shared ancestry (Revell, 2009).

We used a subset of the gut content data previously published by Bellwood et al. (2006). Specifically, we compiled data from 480 fishes (Supplementary Table 4.1). The gut contents were examined using the 'point intercept' method. The main item category was recorded at 40 randomly allocated quadrats spread on a Petri dish containing the gut contents. These values ranging from zero to 40 were then transformed into percentages. Only food items comprising at least 10% of the gut content in at least one species were included in the analysis. Food items were classified into eight broad trophic categories (Table 4.1). Gut content composition was visualized with a non-metric multidimensional scaling (nMDS) ordination using Bray-Curtis distances based on a square-root-transformed matrix of gut content data. Next, we used phylogenetic generalized least squares models (PGLS) (Grafen, 1989) to determine if the anatomy of the buccal cavity was correlated with the proportion of the main dietary items found in the gut of wrasses. In this case, the buccal anatomy was represented as the PC1 scores from a non-phylogenetic PCA based on the anatomical dataset. The phylogeny was incorporated in the calculation of the correlation matrix for the PGLS using the pruned tree from Rabosky et al. (2018) and assuming a Brownian motion model of evolution. To test each model's strength, we used adjusted R^2 values. However, because R^2 values in generalized least squares techniques cannot be computed as in ordinary least squares regression (Symonds & Blomberg, 2014), we calculated an R^2 by fitting a linear regression of our raw data with the values predicted by each PGLS (Morais & Bellwood, 2018) and then used the adjusted R^2 values from these regressions to

34

determine the best fit. All statistical analyses were conducted using the software R (R Core Team, 2019) with the packages *ape* v.5.2 (Paradis et al., 2004), *nlme* v.3.1-137 (Pinheiro, Bates, DebRoy, & Sarkar, 2018), *phytools* v.0.6-60 (Revell, 2012), and *vegan* v.2.5-4 (Oksanen et al., 2013).

4.4. Results

In all species, the mucosa of the buccal cavity was characterized by a stratified squamous epithelium interspersed with mucus-secreting goblet cells. However, sections revealed varying degrees of mucus secretion ability reflected by the thickness of the mucosa, the maximum width and length of goblet cells, and goblet cell density. Collectively, *Cirrhilabrus* consistently had the highest values (Table 4.1). When mucus was present, the Alcian Blue-PAS stains revealed the presence of both neutral and acidic mucus.

The phylogenetic principal components analysis (pPCA) of 19 wrasse species (Figure 4.1.A) separated the five *Cirrhilabrus* species from the remaining 14 wrasses along PC1 (Figure 4.1.B). PC1 was positively correlated with goblet cell length and density, whereas PC2 was mainly correlated with the thickness of the mucosa and goblet cell width (Figure 4.1.C, Table 4.2). Combined, PC1 and PC2 accounted for 78.4% of the variance. The pPCA clearly distinguished the morphospaces of mobile invertebrate feeders, coral-mucus feeders, and the cleaner wrasse. However, planktivores, which occupied a much larger morphospace, overlapped with the other groups. This was due to the buccal anatomy of *Pseudocoris yamashiroi* and *Thalassoma amblycephalum*, which contained smaller GC, and in the case of *T. amblycephalum*, a much lower density of GC than the average *Cirrhilabrus* (Table 4.1). Consequently, these wrasses appear near typical wrasses such as *Coris* and *Halichoeres* along PC1, an axis of variation that is primarily driven by the density and size of goblet cells (Table 4.2).

Table 4.2. Loadings for the four morphological traits used in a phylogenetic principal components analysis based on measurements. Values in bold indicate moderate correlations over ± 0.50 between traits and principal components.

Measurement	PC1	PC2	PC3	PC4
Mucosa thickness	0.44	**0.78**	0.44	0.00
Goblet cell width	0.49	**-0.58**	0.43	0.49
Goblet cell length	**0.55**	-0.22	0.00	**-0.81**
Goblet cell density	**0.52**	0.12	**-0.79**	0.31
% Variance explained	*58.6*	*19.8*	*15.3*	*5.4*

Overall, the most common item found in the guts of the wrasses we evaluated were micro-crustaceans (< 3 mm) (mean 34.2 ± 6.3 % [±SE]). However, the proportion of micro-crustaceans varied considerably across species (Table 4.1). For example, micro-crustaceans were absent in the guts of tubelip wrasses, which were almost entirely filled with coral organic matter (average 93.3%, 96.3%, and 99.8% of the gut content in *Diproctacanthus xanthurus*, *Labrichthys unilineatus*, and *Labropsis australis* respectively). Although the nMDS ordination separated the four feeding guilds (Figure 4.2), there was considerable variation among planktivores. The diet of *Cirrhilabrus* had the highest proportion of amorphous organic matter (AOM) (58.9 ± 7.3 %, Figure 4.2) while micro-crustaceans accounted for 35.6 ± 8.8 %. Conversely, the guts of *P. yamashiroi* and *T. amblycephalum* primarily contained micro-crustaceans (83.3% and 75.8%, respectively) with a much lower AOM content (14.3% and 12.0%, respectively).
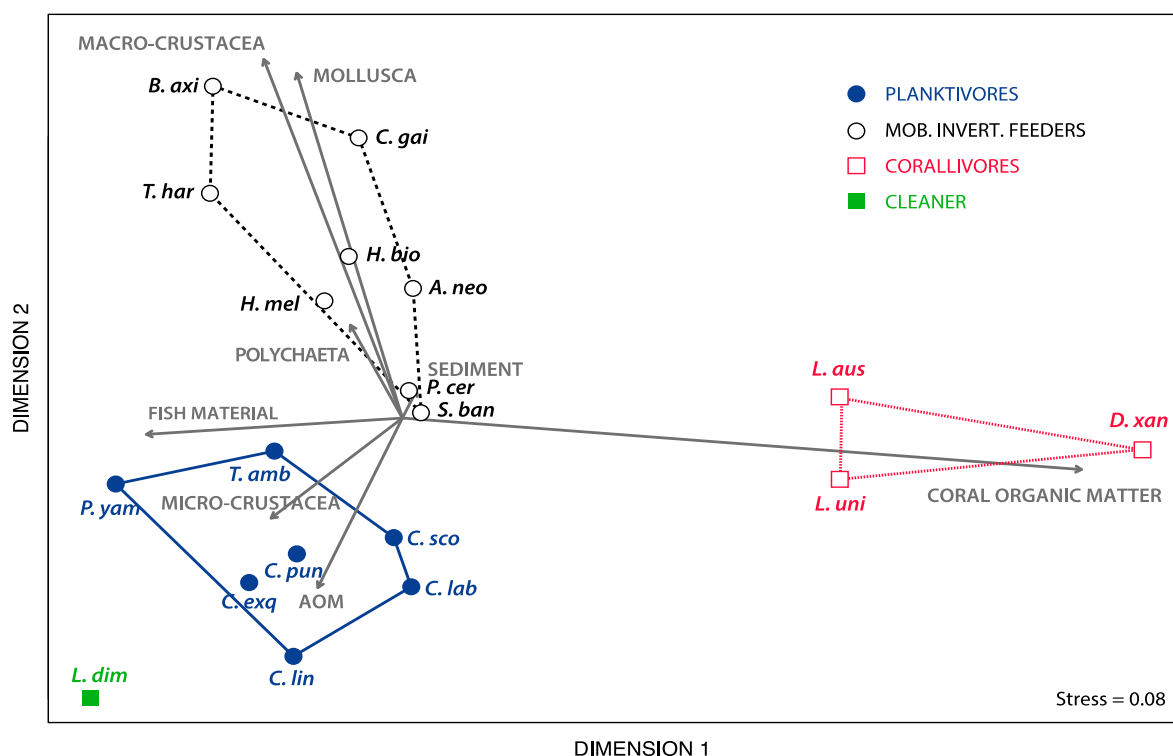
Figure 4.2. Non-metric multidimensional scaling ordination of the gut contents of 19 coral reef wrasses. Polygons encompass distinct feeding guilds. Arrows (grey) represent gradients in the proportion of the eight primary components recorded in gut content samples. Filled blue circles: planktivores; Empty magenta squares: corallivores; Filled green square: cleaner; Empty black circles: mobile invertebrate feeders. Mob. Invert. Feeders: mobile invertebrate feeders. Species abbreviations are listed in Figure 4.1.A.

Of the eight predominant items in the gut in planktivorous fishes, AOM and micro-crustaceans accounted for the largest proportions (Table 4.1, Figure 4.2). Due to their importance to planktivores, we evaluated the relationship between buccal mucus secretion ability and the proportion of micro-crustaceans and AOM, respectively. Phylogenetic generalized least squares (PGLS) regression failed to show a clear statistical relationship between the proportion of micro-crustaceans in the gut and the anatomy of the buccal cavity (adjusted $R^2 = 0.04$, P = 0.196). However, we found the proportion of AOM to be positively correlated with the presence of numerous and larger goblet cells throughout the buccal cavity (adjusted $R^2 = 0.55$, P < 0.0002, Figure 4.3).
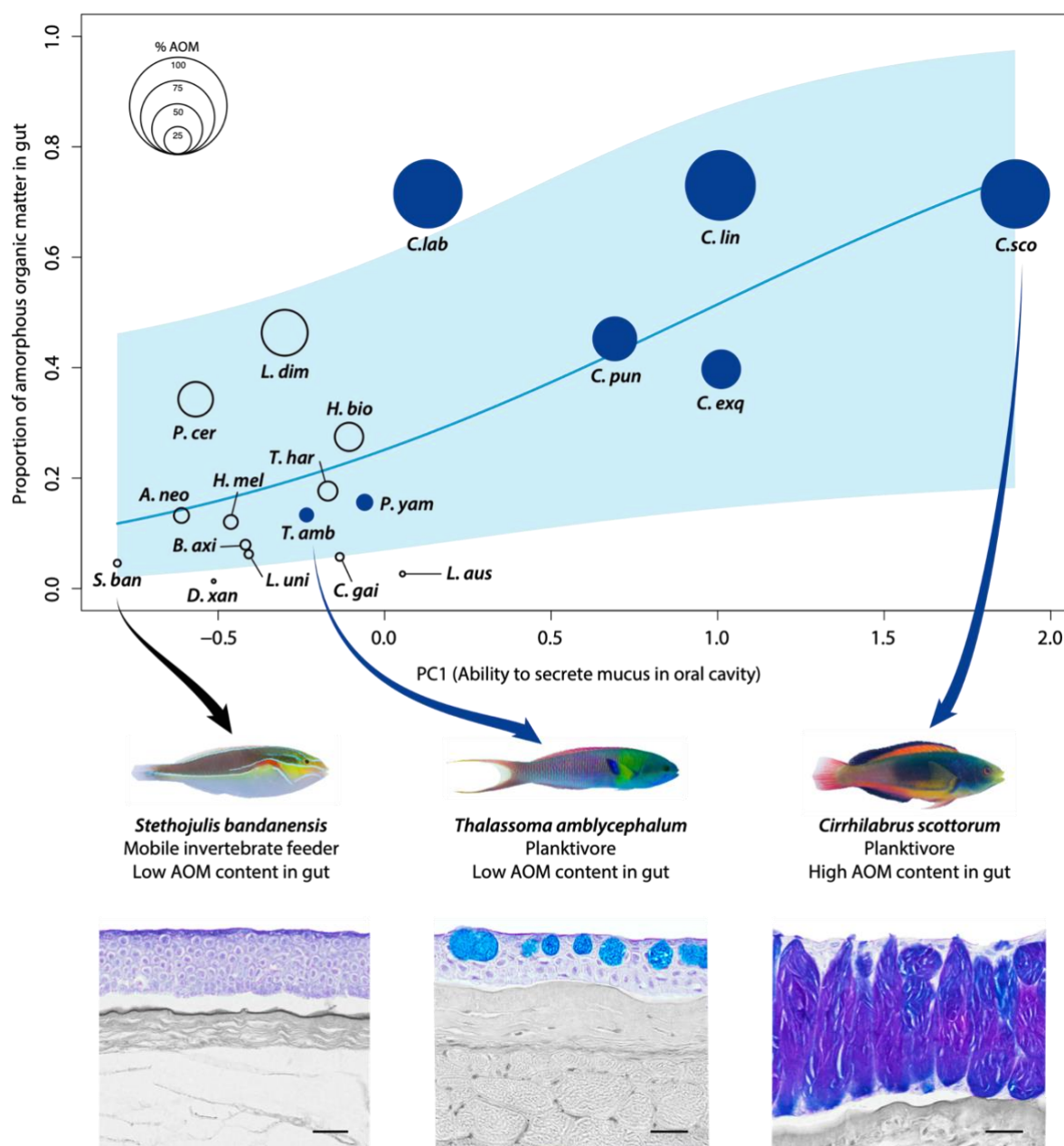
Figure 4.3. Relationship between the morphology of the buccal cavity and the diet of coral reef wrasses. Scatterplot showing the relationship between the first principal component from the anatomical dataset and the proportion of amorphous organic matter (AOM) in the gut. Planktivores are shown in blue filled bubbles. Bubble size represents the percentage of gut content corresponding to amorphous organic matter (AOM). Blue line represents the PGLS regression model. Shaded area represents 95% confidence interval. Species abbreviations are listed in Figure 4.1.A. The connective tissue in the histological sections is desaturated to highlight the buccal mucosa (left coloured from the original Alcian Blue-PAS stain). See Supplementary Figure 4.2 in Appendix C for full sections of the buccal cavity of these three species. Scale bars = 20 μm. Photos of fishes: Christopher Hemingson.

4.5. Discussion

We documented remarkable variation in buccal mucus secretion ability prior to the pharyngeal region within a community of wrasses. Of 19 species of wrasses encompassing 14 different genera, fairy wrasses (genus *Cirrhilabrus*) consistently scored the highest values in goblet cell density, goblet cell size, and mucosa thickness. Combined, these traits provide a measure of the quantity of mucus produced in the buccal cavity. The larger quantity of mucus observed in fairy wrasses indicates that these fishes are capable of secreting large amounts of mucus throughout their buccal cavity. To the best of our knowledge, this ability has not been previously documented in reef fishes.

Remarkably, this trait was not shared among all planktivores. Although *Pseudocoris yamashiroi* and *Thalassoma amblycephalum* also feed on mid-water zooplankton (Randall et al., 1997), we found that they lack the ability to secrete large quantities of mucus in their mouths. Indeed, the buccal cavities of these species resemble those of typical wrasses (e.g., *Halichoeres, Coris,* and *Stethojulis*). This suggests that mucus secretion in the buccal cavity of *Cirrhilabrus* may be associated with feeding on a specific component of the plankton. We investigated this hypothesis and found that the variation in the ability to secrete mucus in the buccal cavity among planktivorous wrasses is consistent with differences in gut content composition. Specifically, our gut content analysis showed that all the planktivorous wrasses evaluated targeted AOM and/or micro-crustaceans (< 3 mm). However, while *Pseudocoris yamashiroi* and *Thalassoma amblycephalum* mainly fed on micro-crustaceans, the diet of *Cirrhilabrus* was dominated by amorphous organic matter (particularly in *C. laboutei*, *C. lineatus*, and *C. scottorum*).

The potential for trophic niche partitioning becomes more evident if we consider the probable origin of AOM ingested by *Labroides*. Since the gut contents were examined visually, we were unable to distinguish the amorphous material found in the guts of *Labroides* from the amorphous material found in the guts of *Cirrhilabrus*. Thus, it was classified as 'AOM' in both

cases. On coral reefs, AOM typically refers to clumps of sinking particles that are composed of decayed plankton, faecal pellets, coral mucus, bacteria, and other reef-derived material (Huettel, Wild, & Gonelli, 2006; Johannes, 1967; Marshall, 1968). We know, however, that *Labroides* targets the mucus secreted in the skin of their clients (Grutter & Bshary, 2003, 2004), which also appears as amorphous material in the gut. Therefore, we presume that most of the amorphous matter found in *Labroides* is, in fact, fish mucus. Among wrasses, therefore, *Cirrhilabrus* appears to represent a major consumer of non-fish AOM.

Importantly, we found that having a mucosa with a greater density of larger goblet cells in the buccal cavity was positively correlated with high loads of AOM in the gut, suggesting that: (1) the feeding ecology of *Cirrhilabrus* is influenced by their ability to secrete mucus in the buccal cavity, and (2) that mucus may be involved in the ingestion of AOM. It is important to note, however, that although we found buccal mucus secretion to be positively correlated with the proportion of AOM ingested, this does not provide evidence of a causal relationship (although ingested mucus will contribute to the AOM). However, the literature provides clues that suggest that this relationship is not a coincidence. So why would a diet of AOM require buccal mucus?

Planktivorous fishes feed in the water column, either by filtering the water to retain planktonic particles or, like *Cirrhilabrus*, by plucking small suspended prey/material with precise suction strikes (Lazzaro, 1987; Wainwright & Bellwood, 2002). Traits that have been associated with picking plankton from the water column include: small terminal mouths with protrusible jaws (Aguilar-Medrano, Frédérich, De Luna, & Balart, 2011; Cooper, Carter, Conith, Rice, & Westneat, 2017), small teeth (S. T. Friedman, Price, Hoey, & Wainwright, 2016), high visual acuity (Schmitz & Wainwright, 2011), a reduction of the adductor mandibulae musculature (Wainwright & Bellwood, 2002; Wainwright & Richard, 1995), and streamlined bodies that facilitate swimming in current-swept water (S. T. Friedman et al., 2016; Wainwright, Bellwood, & Westneat, 2002). Some planktivorous fishes may have also evolved longer gill rakers that

enhance plankton retention (Schmitz & Wainwright, 2011). Nevertheless, gill rakers alone are probably unable to retain small particles such as bacteria (Beveridge, Sikdar, Frerichs, & Millar, 1991). Likewise, the loose consistency of AOM may make it difficult to retain this material with a filtering mechanism consisting of a series of thin appendages such as gill rakers. Suspension-feeders such as tilapia (family Cichlidae) and the common carp (family Cyprinidae) may retain particulate material using a substantial amount of mucus in the oropharyngeal region (Beveridge et al., 1991; Sanderson et al., 1996). Indeed, experimental research has shown that the addition of a film of mucus to the gill arches enhances particle retention (J. C. Smith & Sanderson, 2007). The pharyngeal valve of parrotfishes (f. Labridae) has also been hypothesized to function in this manner (Clements et al., 2017). If having long gill rakers coated in mucus enhances particle retention, the characteristic short gill rakers of *Cirrhilabrus* (Randall & Kuiter, 1989; Randall & Lubbock, 1982; Randall & Pyle, 1988; J. L. B. Smith, 1957) suggest that the mucus in *Cirrhilabrus* is not primarily related to particle retention. We posit that another plausible role for this film of mucus may be to aid in the ingestion of gelatinous zooplankton.

The use of nematocysts as a chemical defence against predators is common among gelatinous organisms (Bullard & Hay, 2002; Shanks & Graham, 1988). The mucus secreted by *Cirrhilabrus* in their mouths may block nematocyst discharge facilitating access to this widespread resource, functioning in a similar way as the thick coat of mucus on the skin of anemonefishes (Lubbock, 1980) and the mucus produced on the lips of coral-feeding tubelip wrasses (Huertas & Bellwood, 2017 [chapter 2 in this thesis]). While this interpretation must be speculated given the data presented, we hope our findings will stimulate new research that use novel techniques, such as molecular markers, to elucidate the diet of fairy wrasses in greater detail and to test the hypothesis of gelatinous zooplankton-feeding in *Cirrhilabrus*.

By feeding primarily on amorphous organic matter, *Cirrhilabrus* may be targeting a specific portion of suspended material in the water column. If so, this dietary specialization sets

Cirrhilabrus apart from other planktivorous wrasses and the typical reef-associated wrasses, which feed predominantly on small mobile crustaceans (Kramer, Bellwood, Fulton, & Bellwood, 2015). The material targeted by *Cirrhilabrus* may also include a diversity of gelatinous organisms, many of which bear stinging nematocysts. However, this type of material is digested quickly (Arai, Welch, Dunsmuir, Jacobs, & Ladouceur, 2003) and is most likely under-represented in gut content analyses (Hays, Doyle, & Houghton, 2018). We posit that the enhanced mucus secretion ability in the mouths of *Cirrhilabrus* is likely involved in the retention of loose particulate and gelatinous material. It is worth noting that given the nature of AOM, *Cirrhilabrus* can simultaneously be considered planktivores (because they feed on material suspended in the water column) and detritivores (because a significant portion of their diet is composed of AOM, which is often made up largely of decaying organic matter or detritus).

One of the main properties of mucus is its stickiness. Although this study focused on characterising the presence and distribution of mucus secreting cells in the buccal cavity of wrasses, we detected the presence of both neutral and acidic mucus in our sections. This opens up a promising avenue for future research that may reveal functional differences between sheets of largely neutral (low viscosity) mucus or acidic (high viscosity) mucus.

Our study showed that buccal mucus secretion is highly variable among wrasses and that it appears to be strongly linked with AOM-feeding in fairy wrasses. The specialized mucosa in the buccal cavity of *Cirrhilabrus* offers a new example of the role of soft tissues; a specialization that may have a central role in driving resource partitioning in coral reef planktivores and in the evolutionary success of *Cirrhilabrus* (with 61 spp. and high local abundances). Our findings reveal discrete food preferences in a feeding guild that has traditionally been thought to feed indiscriminately on plankton, highlighting the need to better assess trophic interactions to improve our understanding of food pathways on both coral reefs and in the water around them.

Chapter 5

FOOD PARTITIONING IN PLANKTIVOROUS REEF FISHES

This chapter is currently under review as:

Huertas, V., Radice, V. Z., Morais, R. A., Bellwood, D. R. Food partitioning in planktivorous reef fishes.

5.1. Abstract

The intricate network of trophic interactions in hyperdiverse systems has been the subject of intense research for over half a century. On coral reefs, however, much of the focus has concentrated on the trophic links between reef fishes and organisms growing on the reef substratum (particularly algae and corals). This has resulted in a thorough, but incomplete, picture of coral reef food webs as the pelagic component remains largely unexplored. Using a widespread and diverse group of reef fishes (wrasses) as a model, we investigated the trophic niches occupied by planktivores to assess potential differences in resource use within this trophic guild. We used two complementary methodological approaches: gut content analysis and stable isotope analysis. We examined 13 wrasse species including planktivores, mobile invertebrate feeders, a corallivore, and a cleaner. Overall, the two main items found in the guts were amorphous organic matter (AOM) and micro-crustaceans (< 3 mm). Interestingly, two groups of planktivores were detected based on gut contents: fairy wrasses (*Cirrhilabrus*) mainly contained AOM (~70 % of material ingested) whereas the other planktivores primarily ingested micro-crustaceans (~80 %). This dichotomy was also reflected in the stable isotope analysis. The isotopic niches of *Cirrhilabrus* and the other planktivorous wrasses had a 62% probability of limited (less than 8%) overlap. Integrating multiple lines of evidence, our findings strongly suggest that there is a robust and persistent food partitioning between coral reef planktivores, a group that has traditionally been presumed to feed non-selectively on material in the water column. Trophic interactions by planktivorous fishes may be more complex than previously thought.

5.2. Introduction

Coral reefs have one of the most complex networks of biotic interactions on our planet. Trophic interactions, in particular, are of great interest to reef ecologists because they represent key drivers of ecosystem functioning (Brandl, Rasher, et al., 2019). In this exceptionally diverse ecosystem, fishes constitute one of the most conspicuous and well-studied groups. Through feeding, reef fishes can induce changes in the reef substratum (Bonaldo, Hoey, & Bellwood, 2014; Hughes et al., 2007; Perry, Kench, O'Leary, Morgan, & Januchowski-Hartley, 2015); but they also represent key links in multiple trophic pathways (Brandl, Tornabene, et al., 2019; Morais & Bellwood, 2019). Although this topic has garnered a lot of interest among reef ecologists, a disproportionately large volume of literature concentrates on the feeding ecology of fishes that feed on the benthos when compared to other trophic pathways. This may result in a skewed perception of the diversity and importance of the roles that reef fishes play on coral reefs (Bellwood, Streit, Brandl, & Tebbett, 2019).

Multiple studies have found evidence of limited trophic overlap within species of herbivorous (Allgeier, Adam, & Burkepile, 2017; Brandl & Bellwood, 2014; Dromard, Bouchon-Navaro, Harmelin-Vivien, & Bouchon, 2015; Eurich, Matley, Baker, McCormick, & Jones, 2019) and corallivorous fishes (Bouchon-Navaro, 1986; Nagelkerken, Van Der Velde, Wartenbergh, Nugues, & Pratchett, 2009; Pratchett, 2005; Zekeria, Dawit, Ghebremedhin, Naser, & Videler, 2002). This indicates strong structuring of resource use among benthic-feeding reef fishes and suggests that there is limited dietary competition within this community (Burkepile & Hay, 2008; Hoey & Bellwood, 2009; Nicholson & Clements, 2021). This trophic segregation is widely recognized and is reflected in the division of 'benthic-feeding' fishes into trophic or functional groups based on their diet (e.g., herbivores, corallivores, detritivores) and/or feeding mode (e.g., browsers, scrapers, excavators, croppers, etc.) (Bellwood, Streit, et al., 2019).

In the water column, however, the term 'planktivore' rarely has any subdivision, with all planktivorous fishes typically placed in a single broad category which delineates both their trophic status (plankton-feeding) and feeding behaviour (in the water column). Evidence of a non-selective diet, however, is scant. Although planktivorous fishes are one of the most abundant and diverse feeding guilds on coral reefs (Siqueira, Morais, Bellwood, & Cowman, 2021), our understanding of the feeding ecology of this group continues to lag behind that of fishes feeding on the reef substratum (Bellwood, Streit, et al., 2019). Indeed, planktivores have been traditionally assumed to play a minor role in coral reef ecosystem dynamics. However, several studies have indicated that not only do planktivorous organisms fuel coral reef ecosystem functioning through the assimilation of energy and nutrient subsidies generated off the reef (Hamner, Jones, Carleton, Hauri, & Williams, 1988; Morais & Bellwood, 2019; Skinner et al., 2021; Wyatt, Waite, & Humphries, 2012); they may also be a major contributor of prey biomass (Brandl, Tornabene, et al., 2019; Skinner, Newman, Mill, Newton, & Polunin, 2019). In this study, we set out to investigate whether planktivorous fishes on coral reefs exploit the same pool of dietary resources or not.

Evidence from gut content analyses suggests that resource use by planktivorous reef fishes may be more complicated than we think. For example, some planktivores appear to target specific planktonic items such as oceanic copepods (Hanson, Schnarr, & Leichter, 2016) and gelatinous zooplankton (Hamner et al., 1988; Huertas & Bellwood, 2020 [chapter 4 in this thesis]). However, while gut content analyses can provide a detailed description of the diet of organisms, they are subject to a series of limitations. First and foremost, they can only provide a snapshot of the diet of an organism. This can have a significant influence on how we perceive the diet of organisms that feed opportunistically because only their most recent meal is reflected in the data. In addition, our ability to accurately detect the full range of items ingested is affected by the structural characteristics of the prey. For example, shelled organisms are likely to be over-represented in gut contents (Michener & Kaufman, 2008) whereas soft-bodied prey such as

gelatinous zooplankton tend to be underestimated because their tissues are digested quickly (Hays et al., 2018) and typically appear as an amorphous substance when examined visually under a microscope. To elucidate the trophic niches of planktivorous fishes while accounting for these limitations, we coupled gut content analyses with stable isotope analyses.

Stable isotope analyses (SIA) are common research tools that have been applied to diverse fields such as forensics, archaeology, and ecology. In ecological studies, SIA are useful for examining trophic niche breadth (Newsome, Martinez del Rio, Bearhop, & Phillips, 2007; Peterson & Fry, 1987). By evaluating the multivariate space composed of multiple stable isotope ratios (typically C and N), the isotopic niche space of species are used to represent trophic niches (Layman et al., 2012). Using this approach, the range of $\delta^{13}C$ values indicates the range of resource use whereas the range of $\delta^{15}N$ values provides an estimate of the trophic height of the food web. Further, sulphur stable isotopes ($\delta^{34}S$) can help to distinguish between fishes among different coral reef environments (Gajdzik, Parmentier, Sturaro, & Frédérich, 2016).

The purpose of this study was to disentangle food resource use among planktivorous fishes on a coral reef. To investigate the dietary preference, breadth of resource use, and extent of trophic niche overlap of sympatric planktivorous wrasses, we integrated gut content data (high resolution but short-term) with stable isotope data (integrates the food assimilated over weeks but lacks the fine resolution of gut content data). We then evaluated whether our data supports the placement of planktivorous wrasses in a single trophic group or, alternatively, if it supports the hypothesis of distinct plankton-feeding trophic groups on coral reefs as suggested by the buccal morphology previously noted in fairy wrasses by Huertas & Bellwood (2020) [chapter 4 in this thesis]. Specifically, we addressed two questions:

1) Do the trophic niches of planktivorous wrasses overlap?

2) Is the anatomical specialization documented in the mouths of fairy wrasses reflected in a distinct dietary or isotopic signature within this group of fishes?

5.3. Materials and Methods

We examined the diet of planktivorous wrasses (family Labridae) on a coral reef. To provide a broader context, we incorporated additional labrids from other trophic groups. In total, we evaluated 13 broadly distributed species representing nine common labrid genera from the Great Barrier Reef (GBR) and the Coral Sea. This assemblage includes seven species from three of the four labrid genera that forage in the water column on the GBR and Coral Sea region (*Cirrhilabrus exquisitus, C. laboutei, C. lineatus, C. scottorum, Pseudocoris heteroptera, P. yamashiroi*, and *Thalassoma amblycephalum*) and six labrid species of three additional trophic groups for comparison: *Stethojulis bandanensis, Anampses neoguinaicus, Halichoeres biocellatus*, and *Coris gaimard* (mobile invertebrate feeders); *Labroides dimidiatus* (a cleaner); and *Labropsis australis* (a corallivore).

We focused on *Cirrhilabrus* because although this genus represents one of the most diverse genera of labrids with 61 species described to date (Tea et al., 2021), little is known about their feeding ecology. The main purpose of this study was to evaluate whether dietary data provided by gut content analysis and stable isotope analysis are congruent and support the existence of trophic partitioning within planktivorous wrasses as suggested by anatomical adaptations (Huertas & Bellwood, 2020 [chapter 4 in this thesis]). Therefore, from here onwards we consider planktivores as belonging to one of two putative groups (identified based on morphology by Huertas & Bellwood (2020) [chapter 4 in this thesis]): AOM feeders and micro-crustacean feeders.

5.3.1. Gut content data

To characterize the diet of each species, we sourced gut content data from Bellwood, Wainwright, Fulton, & Hoey (2006). In total, we included data from 296 fishes, with an average of 24.7 fishes per species (range = 15-37) except for *Pseudocoris heteroptera*, where data were not available (Table 5.1). To avoid the effect of variable gut volume across fishes, data is presented in proportions based on 40 point intercepts of items distributed on a planar, hashed area, following Bellwood et al. (2006). These data were visualized with a network diagram using the function 'sankeyNetwork' in the package 'networkD3' (Allaire, Gandrud, Russell, & Yetman, 2017) in the software R.

5.3.2. Stable isotope sample collection and preparation

Samples from each species were collected for SIA (Table 5.1, Supplementary Table 5.1), with an aim to minimize differences in body size within a species as body size can affect isotopic values (Michener & Kaufman, 2008). All fishes for SIA were hand caught at Holmes Reef (Coral Sea) in September 2019 using barrier nets, between eight and 20 m of depth (except *Cirrhilabrus lineatus* fishes which were collected at ~35 m). Within four days (a period in which fishes were not fed), all fishes were anesthetized in clove oil, euthanized in an ice-water slurry, and tissue samples removed for SIA. Stable isotope ratios can vary among fish tissues. Thus, we chose to use approximately 4 cm^3 of dorsal muscle tissue from each fish, as muscle tissue better reflects long-term diet assimilation compared to red blood cells and plasma (Matley, Tobin, Simpfendorfer, Fisk, & Heupel, 2017; Pinnegar & Polunin, 1999). All muscle samples were stored at -80°C overnight and then dried in a freeze drier (Alpha 1-2 LDplus, Martin Christ, Germany) at -55°C in a vacuum for 48 h. Each dried sample was then homogenized for 1 minute using a ring mill; the ring mill container was thoroughly rinsed with Milli-Q water between each sample.

5.3.3. Stable isotope analysis

Bulk SIA of δ^{13}C, δ^{15}N, and δ^{34}S was conducted at the Stable Isotope Geochemistry Laboratory at The University of Queensland, Australia. Stable isotope ratios (i.e., ^{13}C/^{12}C, ^{15}N/^{14}N, and ^{34}S/^{33}S) are expressed in δ notation and calculated as:

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] * 1000$$

where X is the stable isotope evaluated and R represents the ratio of heavy to light isotope.

We used a vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to a PrecisION (Isoprime-Elementar, Manchester, UK) isotope ratio mass spectrometer to determine δ^{13}C and δ^{15}N simultaneously. Samples and standards were combusted at 950°C. Calibration was achieved via a two-point normalisation using USGS standards USGS40 and USGS41 with USGS43 analysed as an unknown. δ^{13}C$_{VPDB}$ are -26.39‰ and +36.55‰, and δ^{15}N$_{air}$ values are -4.52‰ and +47.55‰ for USGS40 and USGS41, respectively. Precision for δ^{13}C$_{VPDB}$ was ± 0.1‰ and for δ^{15}N$_{air}$ it was ± 0.2‰ at 1σ.

We took into consideration the potential effect of high lipid concentrations on δ^{13}C values. A high proportion of lipid content can introduce a substantial bias in δ^{13}C values because lipids are depleted in ^{13}C compared to proteins and carbohydrates (Post et al., 2007). Although white muscle tissue in fishes typically contains low amounts of lipids (Pinnegar & Polunin, 1999), we calculated the C:N ratio and determined that normalisation of δ^{13}C values was not necessary since ratios did not exceed 3.5 on any of our fish samples (Post et al., 2007).

To determine δ^{34}S we used an updated method following Baublys et al. (2004). The same vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) coupled to a PrecisION (Isoprime-Elementar, Manchester, UK) isotope ratio mass spectrometer

was used, but both instruments (EA-IRMS) were set to sulphur-only mode. Samples and standards were combusted at 1,150°C. Calibration was performed via 3-point normalization using international silver sulphide standards, S-2 and S-3 with USGS-43 hair. Laboratory pyrite standard WD-11 was analysed as an unknown and used for drift correction. $\delta^{34}S_{VCDT}$ values for USGS-43, S-2, and S-3 were 10.46‰, 22.67‰, and -32.55‰, respectively. Precision based on USGS-43 for this analytical run was ± 0.2‰ at 1σ.

5.3.4. Statistical data analyses

We visualized the composition of gut contents constructing a network diagram where nodes on the left represent the labrid taxa, nodes on the right represent the most prevalent items recorded in their gut contents, and the links connecting the nodes indicate the diversity of items ingested (number of links) and the relative proportion of each item (link thickness). To increase clarity, we grouped the gut contents into nine dietary categories which collectively accounted for a mean 98.1% (range 96% - 100%) of the contents recorded for each species (Table 5.1). The nine categories were: amorphous organic matter (AOM; marine snow or clumps of organic material that may include coral mucus, microbial and detrital aggregates, faecal pellets, and dead planktonic organisms), planktonic micro-crustaceans (< 3 mm), macro-crustaceans (> 3 mm), polychaetes, molluscs, fish scales, gnathiid isopods, coral organic matter, and sediment.

We plotted the mean stable isotope values ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) of reef-associated labrids to evaluate the regions occupied by each trophic group in δ-space, a measure of isotopic niche width (Newsome et al., 2007). We then focused on the fishes feeding in the water column to calculate the probability that the isotopic niches of the two putative groups of planktivorous labrids (AOM feeders and micro-crustacean feeders) overlap. Here, we interpret the isotopic niche as a proxy of trophic niche.

We did not consider estimates of total isotopic niche area based on convex hulls as these can be influenced by extreme values and tend to increase with sample size (Syväranta, Lensu, Marjomäki, Oksanen, & Jones, 2013). Instead, we used the package Stable Isotope Bayesian Ellipses in R (*SIBER* v.2.1.5) because this package uses a Bayesian framework to generate standard ellipses that reduces the influence of extreme values and small sample sizes (Jackson, Inger, Parnell, & Bearhop, 2011). The Bayesian estimates of Standard Ellipse Area (SEA_B) were calculated from $\delta^{13}C$ and $\delta^{15}N$ values and are expressed in $‰^2$. These estimates provide a bivariate measure that is equivalent to the standard deviation in univariate analyses.

Prior to analysis, we assessed the convergence of iterative Markov chain Monte Carlo (MCMC) simulations with the Gelman and Rubin's convergence diagnostic using the function 'gelman.diag' in the package *coda* v.0.19-4 (Plummer, Best, Cowles, & Vines, 2006). These tests indicated that our models converged (scale reduction factor values were between 1 and 1.1). Generating standard ellipse areas to represent isotopic niche width assumes that organisms feed randomly and therefore stable isotopic data should follow a normal distribution (Jackson et al., 2011). Therefore, we conducted a multivariate Shapiro-Wilk normality test using the function 'mshapiro.test' from the package *RVAideMemoire* v.0.9-78 (Herve, 2020) to assess normality of our stable isotope dataset. After verifying that the assumption of normality was not violated (p = 0.116) we characterized the isotopic niche width of the two groups of planktivorous labrids and calculated the overlap of the isospace occupied by each group. We fitted ellipses with a 95% prediction interval using a vaguely informative prior on the means and an Inverse-Wishart prior on the covariance matrix ($2x10^4$ posterior draws with 10^3 burn-in, thin=10, chains=3) (Plummer, 2019). We then used the posterior estimates of the ellipses to calculate the SEA_B for 'AOM feeders' and 'micro-crustacean feeders' and the probability of overlap. All data analyses and visualizations were conducted in R (R Core Team, 2019).

5.4. Results

We observed marked differences in gut content composition across the different trophic guilds (Figure 5.1, Table 5.1). The gut contents of planktivorous wrasses were almost entirely comprised of amorphous organic matter (AOM) and micro-crustaceans. Remarkably, species primarily targeted one dietary item or the other. The guts of fairy wrasses (genus *Cirrhilabrus*) mainly contained AOM (70% - 72% of the total gut content) whereas other planktivores such as *Pseudocoris yamashiroi* and *Thalassoma amblycephalum* mostly contained micro-crustaceans (83.3% and 75.8%, respectively). Fairy wrasses also contained micro-crustaceans in their gut, although they accounted for a much lower proportion of the material ingested (mean 17.5% - 27.3%). The exquisite fairy wrasse (*Cirrhilabrus exquisitus)* was the only species in the genus that contained more micro-crustaceans than AOM, though their guts still contained, on average, 2.6 times more AOM than those of *P. yamashiroi* and *T. amblycephalum*.

The main components in the gut contents of the invertebrate feeders *Stethojulis bandanensis* and *Anampses neoguinaicus* were micro-crustaceans (77.5% and 44.5% of the gut contents, respectively); while molluscs were the dominant item in the guts of *Coris gaimard* and *Halichoeres biocellatus* (61.4% and 39.5%, respectively). Additionally, all mobile invertebrate feeders included varying amounts of macro-crustaceans (> 3 mm), polychaetes, AOM, and sediment (Figure 5.1). The diet of the cleaner wrasse *Labroides dimidiatus* was comprised mainly of AOM (45.0%), gnathiid isopods (27.2%), with smaller amounts of micro-crustaceans (12.7%), fish scales (12.2%), and other material (3.0%). The tubelip wrasse *Labropsis australis* fed almost exclusively on coral organic matter (96.3%).
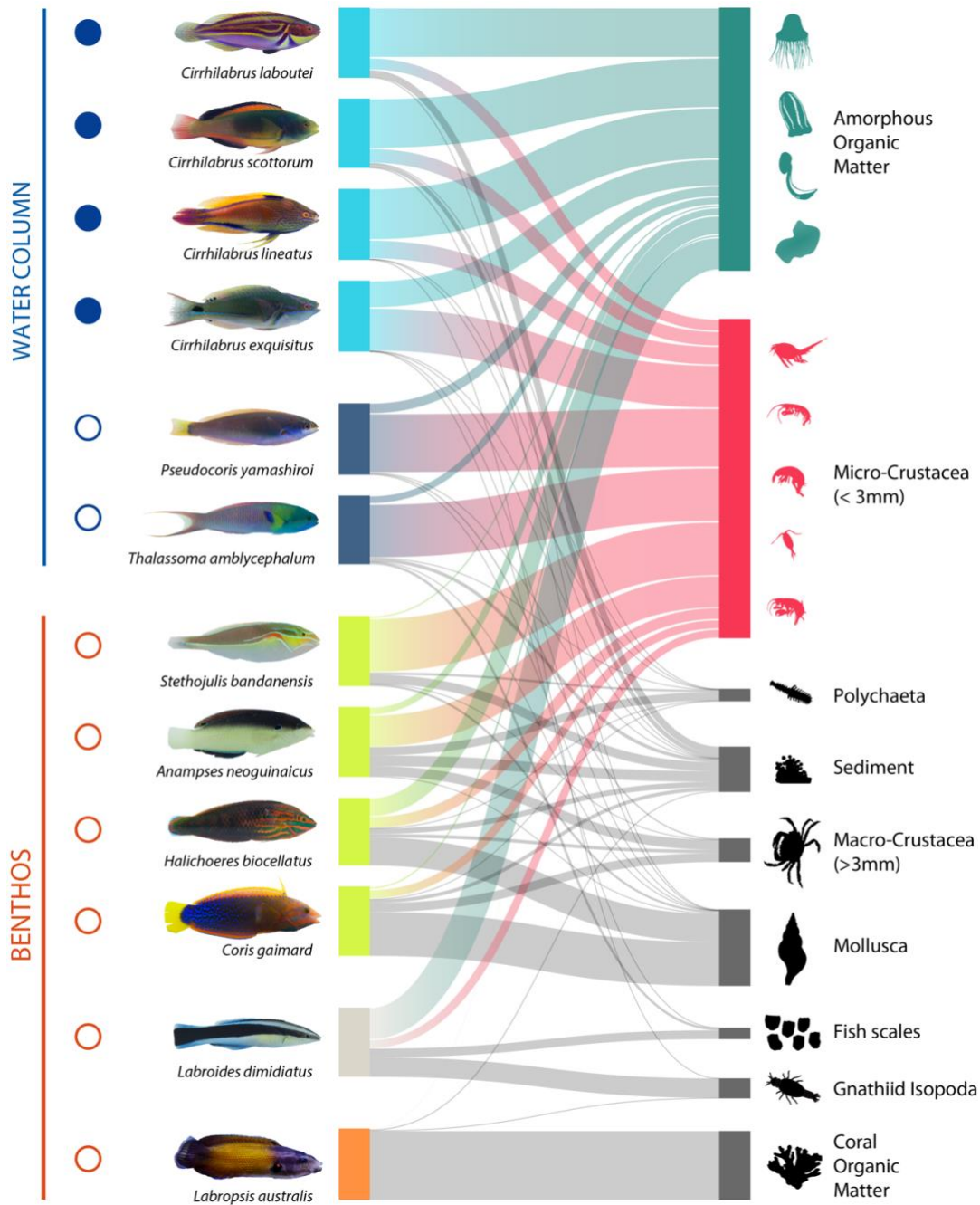
Figure 5.1. Diets of reef-associated wrasses on the Great Barrier Reef. Network diagram showing the average proportion of the nine primary items found in the guts of 12 species of wrasses. This assemblage includes wrasses that rely on the benthic and pelagic trophic pathways and encompasses four trophic guilds: planktivores (blue and cyan), mobile invertebrate feeders (yellow), a cleaner (grey), and a corallivore (orange). The two main prey items are coloured in green (amorphous organic matter or AOM) and red (micro-crustaceans). The thickness of the connecting paths is proportional to the mean contribution of each item to the species' gut contents. Circles indicate whether the species produces abundant mucus in the buccal cavity (solid circles) or not (empty circles). Credits: Chris Hemingson for fish photographs, and Pauline Narvaez, Collin Gross, Hans Hillewaert, and Qiang Ou for the gnathiid isopoda, micro-crustacean, and jellyfish silhouettes.

Table 5.1. Mean values of gut content composition (%) and stable isotopes (δ^{13}C, δ^{15}N, and δ^{34}S). Gut content data were collected using the point intercept method [see Bellwood et al. (2006) for additional information on this dataset]. Fishes were predominantly collected on coral reefs from the Great Barrier Reef. Sample sizes and references to support trophic guild classification are in Supplementary Table 5.1 in Appendix D. Mob. invert. feeder = mobile invertebrate feeder; AOM = Amorphous organic matter.

Species	Trophic guild	Gut contents (%)										Stable isotopes (‰)		
		AOM	Micro-crustacea (<3 mm)	Polychaeta	Sediment	Macro-crustacea (>3 mm)	Mollusca	Fish scales	Gnathid isopoda	Coral organic matter	Other	δ^{13}C	δ^{15}N	δ^{34}S
Cirrhilabrus laboutei	Planktivore (AOM feeder)	70.17	17.50	0.00	9.83	0.00	0.83	0.00	0.00	0.00	1.67	-18.4	8.0	21.0
Cirrhilabrus scottorum	Planktivore (AOM feeder)	70.14	20.27	0.74	5.74	0.00	0.34	0.00	0.00	0.00	2.84	-18.4	8.0	21.3
Cirrhilabrus lineatus	Planktivore (AOM feeder)	71.70	27.30	0.20	0.60	0.00	0.00	0.00	0.20	0.00	0.20	-18.5	7.8	21.1
Cirrhilabrus exquisitus	Planktivore (AOM feeder)	38.39	60.09	0.27	0.63	0.00	0.18	0.09	0.00	0.00	0.54	-18.5	8.2	21.8
Pseudocoris yamashiroi	Planktivore (micro-crustacean feeder)	14.29	83.33	0.00	0.12	1.91	0.36	0.00	0.00	0.00	0.00	-18.2	8.4	20.6
Pseudocoris heteroptera	Planktivore (micro-crustacean feeder)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	-18.2	8.8	20.2
Thalassoma amblycephalum	Planktivore (micro-crustacean feeder)	12.00	75.83	0.25	4.67	0.00	0.33	3.33	0.00	0.00	3.67	-18.1	8.4	21.2
Stethojulis bandanensis	Mob. invert. feeder	3.27	77.50	3.17	12.69	0.19	3.08	0.00	0.00	0.00	1.63	-12.9	7.3	20.9
Anampses neoguinaicus	Mob. invert. feeder	11.91	44.52	11.67	14.88	13.93	1.43	0.00	0.00	0.00	1.79	-12.9	8.3	19.3
Halichoeres biocellatus	Mob. invert. feeder	26.15	16.98	0.31	7.60	4.69	39.48	0.00	0.00	0.00	5.10	-12.6	7.3	17.8
Coris gaimard	Mob. invert. feeder	4.41	13.45	0.83	5.00	12.38	61.43	0.00	0.00	0.00	2.50	-12.7	7.5	18.5
Labroides dimidiatus	Cleaner	45.00	12.70	0.00	0.00	0.00	0.00	12.18	27.18	0.00	2.98	-13.0	10.5	20.7
Labropsis australis	Corallivore	1.32	0.74	0.00	1.62	0.00	0.00	0.00	0.74	96.32	0.00	-14.6	7.8	20.6

The δ^{13}C and δ^{15}N mean isotopic values indicated that the planktivorous wrasses occupied a distinct isotopic space in relation to other reef-associated wrasses (Figure 5.2.A). This separation was driven primarily along the δ^{13}C axis with planktivorous wrasses having the lowest mean δ^{13}C values (-18.5‰ to -18.1‰) relative to other trophic groups that feed on benthic organisms (-14.6‰ to -12.6 ‰). Mean δ^{15}N values ranged between 7.3‰ and 8.8‰ with the exception of *Labroides dimidiatus* which had the highest δ^{15}N values (10.5‰). The small range of δ^{15}N observed was expected since all of our study species are secondary consumers of similar body size, captured in the same area on a single trip.

Although all planktivores clustered in a relatively small isotopic space, AOM feeders (i.e., fairy wrasses) and micro-crustacean feeders appeared to occupy distinct isotopic niches (Figure 5.2.B). Based on the 95% prediction Bayesian estimates of standard ellipse areas (SEA$_B$) for AOM feeders and micro-crustacean feeders, we calculated that there was a 62.4% probability that the isotopic niches of these groups did not overlap or, if they did, the overlap was very limited (< 8% of the combined SEA$_B$ of both groups) (Figure 5.2.C).

Figure 5.2. (see next page) Isotopic niches of the two groups of planktivorous wrasses identified based on differences in gut content. (A) Biplot of mean δ^{13}C and δ^{15}N of dorsal muscle tissue of reef-associated wrasses. Error bars represent the standard error of the mean. **(B)** Isotopic space occupied by planktivorous wrasses. Points represent raw values for each planktivorous wrasse. Polygons delineate AOM feeders (cyan) and micro-crustacean feeders (blue). Values in Figures 5.2.A and 5.2.B are summarized in Table 5.1. **(C)** Density plot showing the probability of overlap between the 95% prediction Bayesian estimates of standard ellipse areas (SEA$_B$) of fairy wrasses (AOM feeders) and other planktivorous wrasses (micro-crustacean feeders). The probability is indicated by the proportion of 1,000 draws from the posterior distribution.
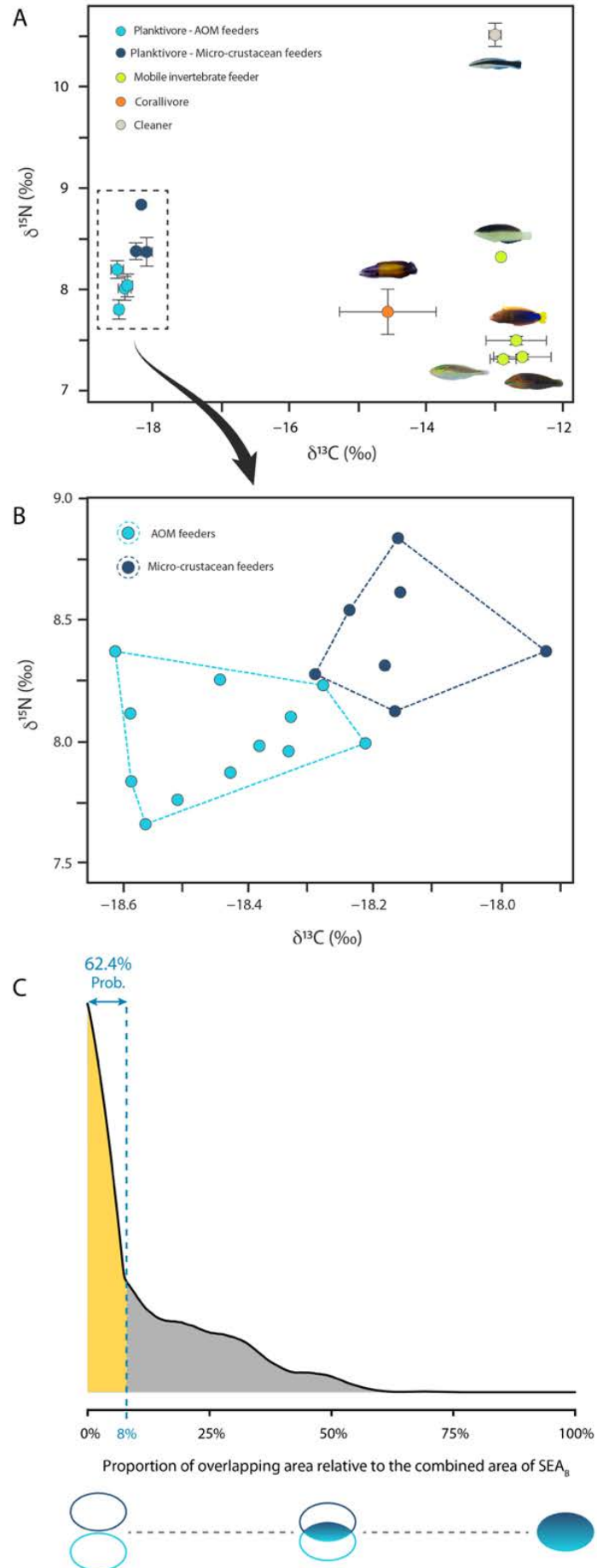
In addition to the most commonly used $\delta^{13}C$ and $\delta^{15}N$ bivariate isotopic space, we also analysed $\delta^{34}S$ and plotted these values together with $\delta^{13}C$ (Supplementary Figure 5.1). Interestingly, $\delta^{34}S$ values showed the most differentiation among the species within the benthic trophic pathway, especially within the mobile invertebrate feeder trophic guild (range from 17.8‰ to 20.9‰) (Supplementary Figure 5.1.A). The most depleted mean $\delta^{34}S$ values recorded were those of mobile invertebrate feeders *Coris gaimard* and *Halichoeres biocellatus* (18.5‰ and 17.8‰, respectively) whereas a diverse trophic assemblage encompassing all planktivores, *Labropsis australis* (corallivore), *Labroides dimidiatus* (cleaner), and *Stethojulis bandanensis* (mobile invertebrate feeder) had enriched $\delta^{34}S$ values ranging from 20.2‰ to 21.8‰ (Table 5.1). The isotopic niches of AOM feeders and micro-crustacean feeders also showed little overlap based on their $\delta^{34}S$ and $\delta^{13}C$ values (Supplementary Figure 5.1.B). However, in this case there was a lower probability of limited overlap of the SEA_B of each group (Supplementary Figure 5.1.C). For example, we calculated a 46.8% probability that the overlap of the isotopic niches of these groups equal or lower than 8% of the combined SEA_B of both groups (compared to 62.4% in the $\delta^{13}C$ and $\delta^{15}N$ analysis).

5.4. Discussion

Analyses of the gut contents and isotopic niches of seven species of sympatric planktivorous wrasses supported their subdivision in two distinct trophic groups: 'AOM feeders' and 'micro-crustacean feeders'. Contrary to the traditional generic label 'planktivore', our study showed that plankton-feeding fishes that pluck food items from the water column can be highly selective. Indeed, our data suggests that planktivorous wrasses have partitioned trophic niches, with *Cirrhilabrus* spp. exhibiting no (or very limited) dietary overlap with other planktivores.

The observed division agrees strongly with previous observations on the morphology of the buccal cavities of labrids that showed a correlation of buccal mucus secretion ability with elevated amounts of AOM in the gut (Huertas & Bellwood, 2020 [chapter 4 in this thesis]). The data herein shows this division is robust due to evidence from gut contents as well as a sustained trophic separation demonstrated by $\delta^{13}C$ and $\delta^{15}N$ isotope values. The $\delta^{34}S$ values across planktivores did not contribute to segregate species and suggested that, regardless of their specific prey items, planktivores had $\delta^{34}S$ values that generally correspond with sulphates produced in the water column (Peterson & Fry, 1987). In effect, there is now a clear consensus: the buccal morphology, dietary composition, and stable isotope values are all indicative of a sustained and presumably adaptive separation of planktivorous wrasses into two groups: AOM feeders and micro-crustacean feeders.

The separation between the isotopic niches occupied by these two groups suggests that there may be a trade-off that is reflected in a preference for different food sources. The gut contents showed that the main alternate items that were targeted were either AOM or micro-crustaceans. This raises the question: why would planktivores feed selectively on different food items that occur as mixed particles in the water column? Although the drivers of this resource partitioning are unclear, we posit two non-mutually exclusive hypotheses that could explain this separation: anatomical constraints or modifications involved in ingestion and digestion (e.g., mouth secretions, gut length), and habitat partitioning.

The amorphous material found in the guts of *Cirrhilabrus* likely contains elevated amounts of gelatinous zooplankton in addition to marine snow (Huertas & Bellwood, 2020). Gelatinous organisms can be an abundant source of food for coral reef fishes, although aggregations of gelatinous zooplankton are linked to episodic pulses influenced by environmental factors (W. M. Graham, Pagès, & Hamner, 2001; Hamner et al., 1988; Purcell, 2005). Despite their availability,

many of these organisms are armed with stinging nematocysts that may be more easily ingested and digested by predators with a defensive mechanism.

Reef fishes for which corals or pelagic cnidarians (and other gelatinous organisms) comprise a substantial portion of their diet appear to have modified their mouths and digestive tract. The secretion of a film of mucus appears to be an effective way of neutralizing nematocyst discharge (Greenwood, Garry, Hunter, & Jennings, 2004; Lubbock, 1980). Indeed, several reef fishes that routinely get in close contact with cnidarians, either because they seek shelter among them (e.g., anemonefishes) or because they prey on them (e.g., tubelip wrasses), are capable of coating the skin exposed to the nematocysts with large amounts of mucus (Huertas & Bellwood, 2017, 2018 [chapters 2 and 3 in this thesis]; Lubbock, 1980). Planktivores that likely ingest pelagic cnidarians appear to be no exception. *Cirrhilabrus*, for example, have been shown to contain a disproportionate number of mucous cells in the oral mucosa, a trait that may have enabled a specialized feeding mode in this genus, facilitating the retention of loose suspended particles and/or offering protection to the buccal cavity from nematocyst-bearing prey (Huertas & Bellwood, 2020 [chapter 4 in this thesis]).

The differences in diet between AOM feeders and crustacean feeders could also be related to habitat partitioning. Although all planktivorous fishes feed on suspended particles, their position in the water column and the underlying habitat differs (Figure 5.3). *Cirrhilabrus* tend to hover over rubble zones at the base of the reef slope (Kuiter, 2002; Randall et al., 1997). By contrast, schools of *Pseudocoris* are typically found high in the water column in areas exposed to moderate currents (Kuiter, 1996; Myers, 1999) while *Thalassoma amblycephalum* are primarily found on the reef crest (Fulton, Wainwright, Hoey, & Bellwood, 2017; Myers, 1999) sometimes mixed with the initial phase of *Pseudocoris heteroptera* (Randall & McCosker, 1993).
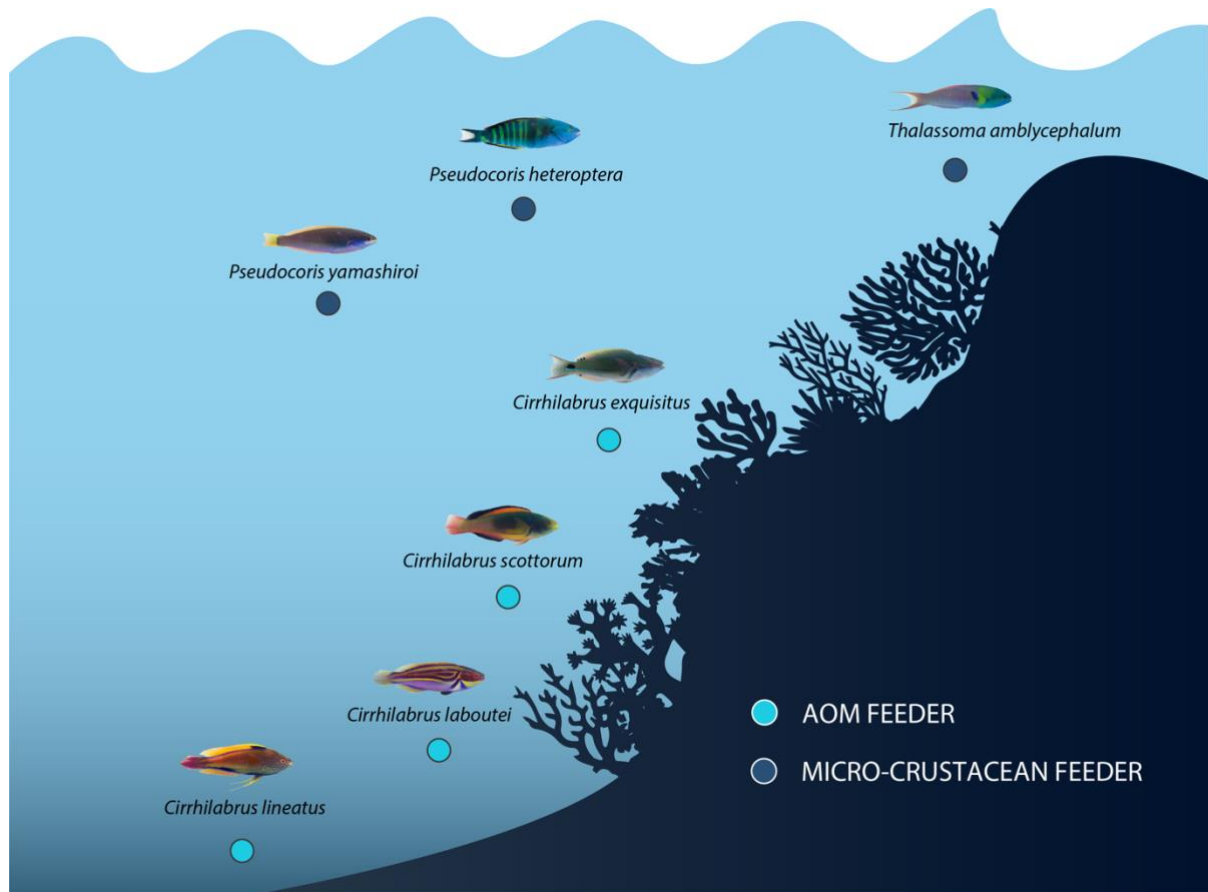
Figure 5.3. Cross-reef habitat distribution of planktivorous wrasses on the Great Barrier Reef. Schematic representation of the typical habitat distribution occupied by planktivorous wrasses based on the mode of the abundance of each species and accounts of their respective habitat in the literature (see discussion for details).

While planktivores do not participate in ecological processes that directly alter the structure of the reef substratum such as herbivory, corallivory, and bioerosion, they play a key role in pelagic-benthic trophic pathways on coral reefs (Morais & Bellwood, 2019). Traditionally, plankton-feeding has been thought to be a single pathway. The labrid assemblage evaluated herein, however, provides a clear indication of a fundamental division among reef-associated planktivores into AOM-feeding and crustacean-feeding trophic pathways. Food partitioning may alleviate competition for food, with planktivores being no exception (Leray et al., 2019). It is possible that resource partitioning is one of the contributing factors explaining the coexistence of

the exceptional diversity of planktivores found in the marine biodiversity hotspot in the Indo-Australian archipelago (Siqueira et al., 2021).

Our results expand the growing literature on the ecology of planktivorous fishes on coral reefs (Emslie, Logan, & Cheal, 2019; Huertas & Bellwood, 2020 [chapter 4 in this thesis]; Morais & Bellwood, 2019; Siqueira et al., 2021; Skinner et al., 2021) and highlight that plankton-feeding reef fish communities appear to be more complex and structured than previously thought. Promising avenues for future research include the incorporation of tools capable of providing a finer diet resolution such as DNA metabarcoding (Casey et al., 2019; Kartzinel et al., 2015; Leray et al., 2019) and the evaluation of a broader taxonomic diversity of planktivorous reef fishes to investigate the nature and extent of trophic specialization among planktivores. Future work monitoring and characterizing the different planktivore food sources and their relative proportions would help to better understand how food source availability affects planktivore trophic niches.

There may be no such thing as a typical planktivore; their trophic niches may be as diverse as those of their benthos-feeding counterparts. While our study reveals the existence of two trophic pathways among the planktivorous species evaluated, it is likely that additional groups will emerge as we learn more about the feeding behaviour of coral reef planktivores. There are already strong suggestions of a fundamental division within planktivores with off-reef AOM-feeding species including caesionids (Hamner et al., 1988), *Naso* (*annulatus/brevirostris/hexacanthus/lopezi/vlamingii*) (Choat, Clements, & Robbins, 2002), and possibly the labrid *Clepticus parrae* (Randall, 1967) in comparison to a much more crustacean-oriented group of reef-associated planktivores, including pomacentrids (Hobson, 1991) and the apogonid genus *Rhabdamia* (Hobson & Chess, 1978).

5.5. Conclusion

Despite significant advances in our understanding of the feeding ecology of reef fishes feeding on the benthos, our understanding of resource partitioning among those fishes that feed in the water column is in its infancy. Plankton-feeding fishes have been traditionally grouped into a single feeding guild. Here we show a significant and sustained partitioning of dietary resources among planktivorous wrasses, suggesting the existence of a major division between AOM and crustacean-based pathways. To our knowledge, this is the first study to identify two fundamentally different trophic pathways in the water column for coral reef fishes using multiple lines of evidence. It is likely that this division also applies to other taxa and represents access to alternate trophic food webs; a division that may require strong specialization or constraints, possibly due to distinct oral or intestinal morphology.

Chapter 6

PARROTFISH CORALLIVORY ON STRESS-TOLERANT CORALS IN THE ANTHROPOCENE

6.1. Abstract

Cumulative anthropogenic stressors on tropical reefs are modifying the physical and community structure of coral assemblages, altering the rich biological communities that depend on this critical habitat. As a consequence, new reef configurations are often characterized by low coral cover and a shift in coral species towards massive and encrusting corals. Given that coral numbers are dwindling in these new reef systems, it is important to evaluate the potential influence of coral predation on these remaining corals. We examined the effect of a key group of coral predators (parrotfishes) on one of the emerging dominant coral taxa on Anthropocene reefs: massive *Porites*. Specifically, we evaluate whether the intensity of parrotfish predation on this key reef-building coral has changed in response to severe coral reef degradation. We found evidence that coral predation rates may have decreased, despite only minor changes in parrotfish abundance. However, higher scar densities on small *Porites* colonies, compared to large colonies, suggests that the observed decrease in scarring rates may be a reflection of colony-size specific rates of feeding scars. Reduced parrotfish corallivory may reflect the loss of small *Porites* colonies, or changing foraging opportunities for parrotfishes. The reduction in scar density on massive *Porites* suggests that the remaining stress-tolerant corals may have passed the vulnerable small colony stage. These results highlight the potential for shifts in ecological functions on ecosystems facing high levels of environmental stress.

6.2. Introduction

The scale and severity of disturbances that coral reefs have endured in the last decade have altered their coral assemblages and caused profound changes to their composition and physical appearance (Hughes, Barnes, et al., 2017; Hughes, Kerry, et al., 2018). By 2020, the footprint of severe tropical storms and coral bleaching —two of the primary manifestations of climate change on coral reefs— have left an indelible mark on reefs throughout the tropics (Hughes, Anderson, et al., 2018; Skirving et al., 2019). Temperature-induced coral bleaching, the leading cause of coral mortality, has been associated with recent episodes of widespread loss of coral cover on the Great Barrier Reef (Hughes, Kerry, et al., 2017), and around the globe (Harrison et al., 2019; Monroe et al., 2018; Pisapia, Burn, & Pratchett, 2019; Sully, Burkepile, Donovan, Hodgson, & van Woesik, 2019). Furthermore, the uneven susceptibility of different coral taxa to stressors has driven extensive coral community changes. On the Great Barrier Reef, for example, an increase in the proportion of massive *Porites* has been reported relative to the remaining coral cover (Hughes, Kerry, et al., 2018; Zawada, Madin, Baird, Bridge, & Dornelas, 2019).

Changes in coral cover following acute disturbances are quickly apparent (Hughes, Anderson, et al., 2018), but reef degradation also has knock-on effects across the entire ecosystem (Pratchett, Hoey, Wilson, Messmer, & Graham, 2011; Przeslawski, Ahyong, Byrne, Wörheide, & Hutchings, 2008). Reef fish communities, for example, respond in complex ways to shifts in coral communities (Bellwood, Hoey, Ackerman, & Depczynski, 2006; Berumen & Pratchett, 2006; Ceccarelli, Emslie, & Richards, 2016; Cheal, Wilson, Emslie, Dolman, & Sweatman, 2008; Lowe, Evans, Williamson, Ceccarelli, & Russ, 2019; Morais et al., 2020; Pratchett et al., 2011; Robinson, Wilson, Jennings, & Graham, 2019; Triki & Bshary, 2019). Typically, these responses have been investigated from the perspective of fish abundance and community structure while the impact on the capacity of fishes to deliver key ecological functions has received less attention (Bellwood, Streit, et al., 2019). This is important because the impact of

climate change on coral reefs is expected to escalate (Heron, Maynard, Van Hooidonk, & Eakin, 2016; Hughes, Barnes, et al., 2017; Knutson et al., 2010). Thus, as coral reefs cope with a warming ocean, a critical question emerges: will the delivery of ecological functions by fishes change on Anthropocene reefs? In this study, we investigate this question by focusing on parrotfish predation on corals.

Although parrotfishes generally feed on algal turf-covered substrata, they also occasionally scrape the surface of live corals (Alwany, Thaler, & Stachowitsch, 2009; Bonaldo & Bellwood, 2011; Bruckner & Bruckner, 2015; Rotjan & Lewis, 2005). On the Great Barrier Reef, multiple parrotfish species have been reported to bite on massive *Porites*, including *Scarus flavipectoralis*, *S. niger*, *S. frenatus*, *S. rivulatus*, *Chlorurus microrhinos*, *C. spilurus*, *Cetoscarus ocellatus*, and *Bolbometopon muricatum* (Bellwood, 1985; Bellwood & Choat, 1990; Bonaldo & Bellwood, 2011). Indeed, parrotfish corallivory may, under specific circumstances, compromise the survival of corals, regulating their distribution and abundances (Bonaldo & Bellwood, 2011; Littler, Taylor, & Littler, 1989; Mumby, 2009; Rotjan et al., 2006; Rotjan & Lewis, 2005). Bonaldo and Bellwood (2011) provided the first quantitative assessment of parrotfish predation on massive *Porites* on the Great Barrier Reef (GBR). This study highlighted that parrotfish corallivory on the GBR primarily affects massive *Porites* colonies (Bonaldo & Bellwood, 2011). Welsh et al. (2015) provided further evidence for this negative impact showing that clustered bites can trigger partial mortality in *Porites* corals. Since these studies were published, however, coral cover at Lizard Island has decreased and strong compositional changes have been observed (Hughes, Kerry, et al., 2018; Madin et al., 2018). These changes may have impacted the extent of parrotfish predation on corals, potentially endangering the remaining massive *Porites* colonies.

The purpose of this study, therefore, was to investigate the footprint of parrotfish predation on massive *Porites* on Anthropocene reefs, where the proportion of substratum occupied by turf algae (their primary feeding microhabitat) is expected to have increased while

most corals, except *Porites*, have become less abundant (especially acroporid corals) (Madin et al., 2018). Specifically, we addressed two questions:

1) What is the distribution of parrotfish predation on massive *Porites* following reef degradation?

2) Has reef degradation affected the intensity of parrotfish predation?

6.3. Materials and methods

We conducted this study on the coral reef between Palfrey and South islands (S 14° 41' 57", E 145° 26' 55"), just south of Lizard Island (Figure 6.1.A), on the northern Great Barrier Reef (GBR). In the last decade, this reef has been affected by two cyclones (Ita in 2014 and particularly Nathan in 2015) and two consecutive mass bleaching episodes (2016 and 2017) (Madin et al., 2018), making it an appropriate location for investigating the effect of reef degradation on fish-coral interactions. Importantly, fishing in the area is prohibited (GBRMPA, 2016) and because it is located in the middle of the continental shelf, its exposure to land-based sediment inputs is limited (Tebbett, Goatley, & Bellwood, 2018).
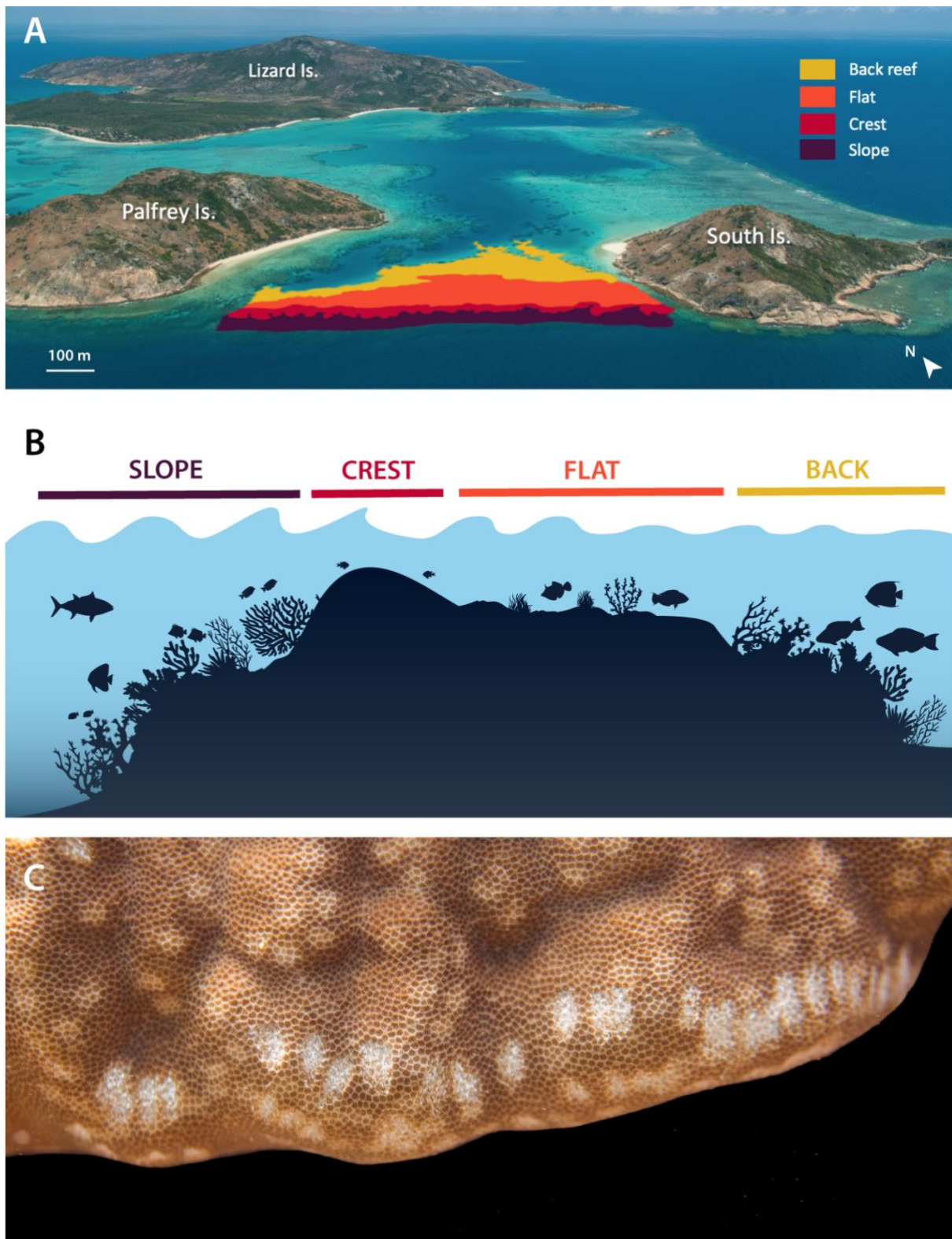
Figure 6.1. Study location between Palfrey and South islands, in the Lizard Island group, Great Barrier Reef (A), illustration representing the reef habitats across the reef profile at the study location **(B)**, and a photograph showing a series of *Scarus* parrotfish scars along the edge of a massive *Porites* coral **(C).** Photographs taken by Victor Huertas.

We selected four reef zones that differ in depth and wave exposure following Bonaldo and Bellwood (2011): slope (7-10 m deep), crest (0.7-2 m), flat (0.5-1 m), and back reef (5-8 m) (Figure 6.1.A-B). To quantify parrotfish predation on massive *Porites*, we counted the number of parrotfish scars on massive *Porites* (Figure 6.1.C). Parrotfish scars were distinguished by a pair of opposing oval-shaped marks on the coral surface with shape and depth varying depending on the size of the fish. The scars left by different parrotfish species cannot be separated except in separating whether they were inflicted by a scraper or an excavator, with excavators producing wider and deeper scars than scrapers (Bonaldo & Bellwood, 2011). In each reef zone we collected three sets of data to evaluate: a) the composition of the benthic substratum, b) parrotfish abundances, and c) the number of parrotfish scars on massive *Porites*. This study was conducted under approval from James Cook University's Animal Ethics Committee (Ethics permit A2627) and a Great Barrier Reef Marine Park Authority research permit (Permit G17/38142.1).

6.3.1. Benthic composition

We quantified the benthic composition using a photoquadrat method following the methodology used by Bonaldo and Bellwood (2011). A minimum of seven 20 m transects were laid in each reef zone (slope, crest, flat, and back reef; 41 transects). Images were taken from a distance of approximately 1.5 m that subsequently permitted a 1 m² quadrat to be overlaid on the image prior to analysis. In each transect, the substratum was photographed at every other meter (n = 410 frames). Next, we estimated the percent of live coral and the percent of massive *Porites* from 10 points laid on each photoquadrat (n = 4,100 points) via a stratified randomization process using the software photoQuad (Trygonis & Sini, 2012).

6.3.2. Parrotfish abundance

We conducted underwater visual surveys to determine parrotfish abundances. A team of two divers conducted a series of twelve 50 m x 2 m tape transects on each of the reef zones in 2018. To avoid underestimating parrotfish abundance by scaring the fish away (Emslie, Cheal, MacNeil, Miller, & Sweatman, 2018; Welsh & Bellwood, 2011), the 50 m tape was laid by the same diver simultaneously to the counts. Only those parrotfishes larger than 10 cm in length observed within 1 m on either side of the tape were recorded. The 2018 parrotfish counts were then compared with surveys using the same methods conducted on the same reef in 2008 (Bonaldo, Krajewski, & Bellwood, 2011), i.e., before the series of severe disturbances that affected this reef.

6.3.3. Parrotfish predation on massive Porites

We photographed massive *Porites* colonies from above with a Nikon Coolpix W300 digital camera with a scale ruler to calibrate the measurement of their horizontal planar surface area (subsequently termed "surface area"). All images were taken from the same position (i.e., directly above the colony) to ensure consistency across all colonies sampled. Dead areas of the colony were not included in the total surface area. We then recorded all clearly visible parrotfish scars [see examples of parrotfish scars in Bonaldo et al. (2011)]. During the image analysis, each scar was marked to avoid double-counting scars.

6.3.4. Statistical data analysis

Parrotfish abundance across the reef profile was examined using a generalised linear model (GLM). To account for the overdispersion and non-normality of our count data, we fitted our data with a negative binomial regression with year (2008 vs 2018) and reef zone (slope, crest,

flat, and back) as predictors. We assessed the fit and relevant assumptions of the model with residual plots, which were all satisfactory after we removed a single outlier that we considered to be an error. We also used the Akaike Information Criterion (AIC) in a model comparison framework to determine whether any subset of the predictors generated a more parsimonious model (Supplementary Table 6.1). Finally, we assessed differences in parrotfish abundance pre- and post-disturbances at each zone with pairwise comparisons using Tukey-adjusted p-values (function 'emmeans' in the R package 'emmeans').

We also investigated if parrotfish predation on massive *Porites* colonies was influenced by colony size. To determine (a) if the likelihood of being bitten or not (binary) changed depending on the colony's size, and whether it varied across zones, we fitted a GLM with a binomial error distribution. Next, (b) we focused on colonies that have been bitten (i.e., with at least one scar) to investigate the influence of colony size on the magnitude of parrotfish predation. In this case, we fitted a GLM using a negative binomial distribution to determine if there was a relationship between the number of scars on individual colonies, and colony surface area, as well as the reef zone in which they were located. Again, we performed model selection on subset of predictors based on AIC scores (Supplementary Table 6.2).

Finally, (c) we also modelled the relationship between the density of scars on individual colonies and their surface area and reef zone. Here, we fitted the data using a GLM with a gamma distribution. Although density can be decomposed in its two original components (number of scars and area) we did not include surface area as an offset in the model. Offsets allow for the removal of variability that arises from confounding dimensional factors (e.g., colony size) from the response variable. They do this by dividing each observation of the response variable by the corresponding observation of the offset variable. However, in this case, colony size was our predictor of interest, rather than a scaling variable. As colony size varies both within

and across the reef zones (Supplementary Figure 6.1), including it as an offset would have kept it at a constant value, thus constraining our ability to detect patterns that may emerge with it.

In both cases (b and c), colony surface area was log transformed prior to analysis. A pairwise post-hoc comparison was conducted to examine variation in the number of scars among reef zones. All statistical analyses were conducted in the software R v.3.6.1 (R Core Team, 2019) using the packages *ggplot2* (Wickham, 2016), *glmmTMB* (Brooks et al., 2017), *MASS* (Venables & Ripley, 2002), *MuMIn* (Barton, 2019), and *emmeans* (Lenth, 2019).

6.4. Results

6.4.1. Benthic composition

Between 2008 and 2018 the study site lost two thirds of its live coral cover, which fell from 22% to ~7% (Table 6.1). Live coral cover declined the least on the back reef (-25.0%) vs. -72.2% on the slope, -77.4% on the flat, and -86.2% on the crest (Figure 6.2, Table 6.1). Although the area occupied by live corals declined in all four reef zones, coral loss varied by taxa and resulted in a marked increase in the proportion of massive *Porites* among hard corals on the slope (from 30.6% to 58.9% of the total coral cover), the crest (4.9% to 20.0%), and the reef flat (9.4% to 70.6%) (Figure 6.2). In relative terms, the proportion of all corals represented by massive *Porites* tripled. Thus, *Porites* shifted from being a minor component of the hard coral assemblage (with ~30% of all corals on the slope, <5% on the crest, and <10% on the flat) to the dominant coral in two of these three habitats (Figure 6.2).

Table 6.1. Percentage of live hard coral cover in 2008 and 2018 at the study site, Lizard Island, on the Great Barrier Reef. Data for 2008 sourced from Bonaldo and Bellwood (2011).

	Massive *Porites*			Total live coral		
	2008	**2018**	**Change**	**2008**	**2018**	**Change**
Slope	7.31	3.91	-46.54	23.89	6.64	-72.22
Crest	1.77	1.00	-43.64	36.14	5.00	-86.16
Flat	0.65	1.09	69.09	6.83	1.55	-77.38
Back	15.59	6.75	-56.71	21.12	15.83	-25.02
Average	*6.33*	*3.19*	*-49.65*	*21.99*	*7.25*	*-67.02*
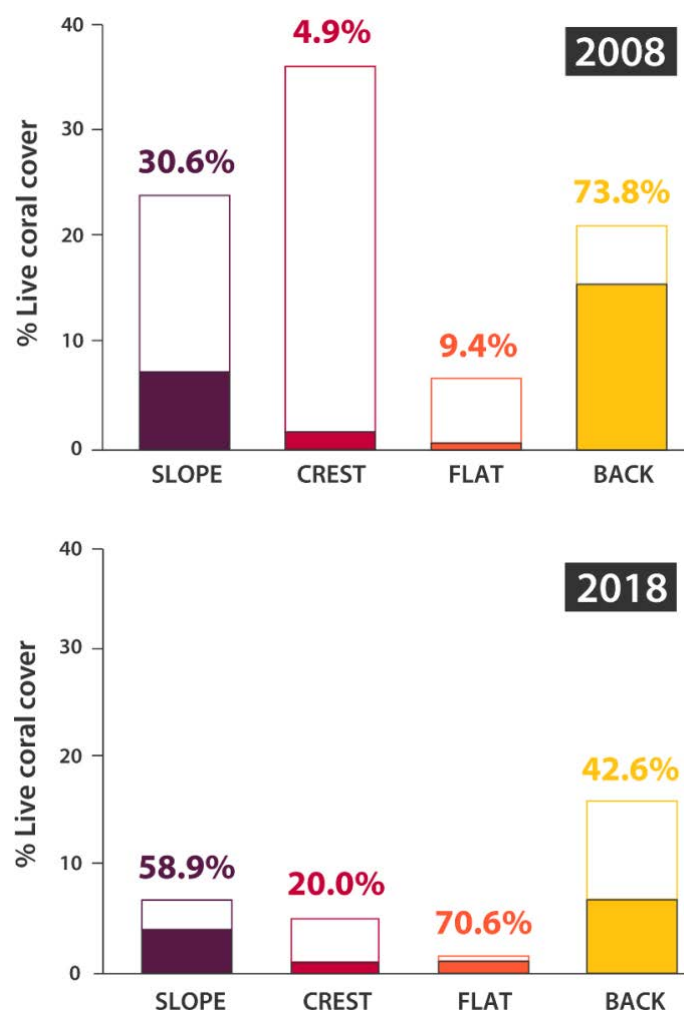
Figure 6.2. Changes in the benthic cover at Lizard Island, on the Great Barrier Reef. Percentage of reef substratum occupied by massive *Porites* (filled) and other hard corals (empty) in 2008 and 2018. Purple, red, orange, and yellow indicate the slope, crest, flat, and back reef habitats, respectively. Values above the bars indicate the percentage of massive *Porites* cover relative to total coral cover for the respective habitat.

6.4.2. Parrotfish abundance

The number of parrotfishes counted in 2018 relative to 2008 remained relatively stable with minor changes depending on the reef zone (Figure 6.3.A). We did not detect statistically significant differences in parrotfish abundance between the two time periods (pre and post-disturbances) on the slope or crest (GLM; slope effect size$_{[2008 \text{ vs } 2018]}$ = 1.63, CI$_{95}$ = 0.78-2.48, p = 0.063; crest effect size$_{[2008 \text{ vs } 2018]}$ = 1.47, CI$_{95}$ = 0.73-2.20, p = 0.130) (Supplementary Table 6.2), however, the number of parrotfish was lower on the reef flat in 2018 compared to 2008 (GLM; effect size$_{[2008 \text{ vs } 2018]}$ = 3.50, CI$_{95}$ = 1.75-5.25, p < 0.001, Supplementary Table 6.2) but was higher on the back reef in 2018 compared to 2008 (GLM; effect size$_{[2008 \text{ vs } 2018]}$ = 0.50, CI$_{95}$ = 0.25-0.75, p = 0.007, Supplementary Table 6.2).
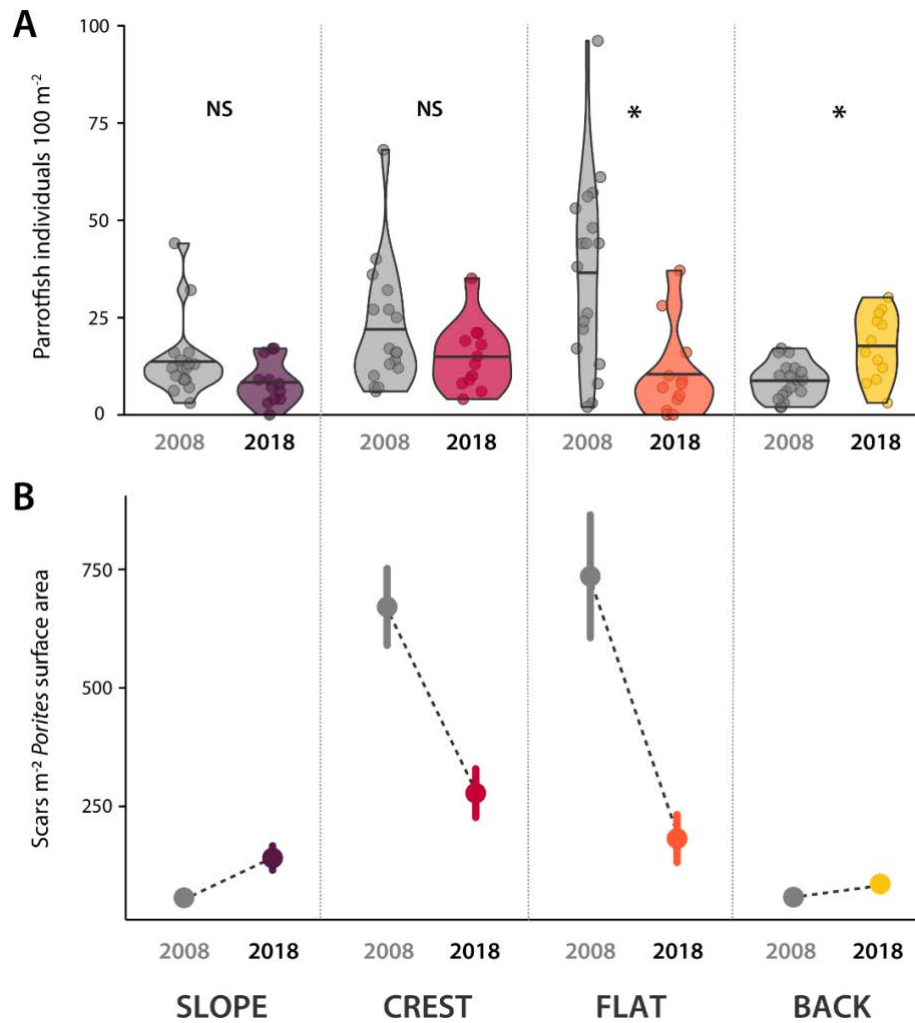
Figure 6.3. Parrotfish abundance and predation pressure on corals across the study location at Lizard Island, on the Great Barrier Reef. (A) Violin plots representing parrotfish abundance in 2008 (grey) and 2018 (coloured). Horizontal lines indicate the mean. Circles are individual samples. NS: statistically non-significant; *: statistically significant. **(B)** Predation pressure on massive *Porites* on the slope, crest, flat and back reef zones at the study site in 2008 (grey) and 2018 (coloured). Circles represent the mean number of scars m⁻² and lines indicate the standard error of the mean. Data from 2008 were sourced from Bonaldo & Bellwood (2011). Purple, red, orange, and yellow indicate the slope, crest, flat, and back reef habitats, respectively.

6.4.3. Parrotfish predation on massive Porites

Following the impact of cyclones Ita and Nathan, and the 2016 and 2017 mass coral bleaching events, we observed a marked reduction in the density of parrotfish scars on massive *Porites* at the study site in 2018 compared to 2008. This was mainly driven by a precipitous decline

in coral scar densities on the crest and the flat in 2018, the habitats that exhibited the vast majority of parrotfish scars in 2008 (Bonaldo & Bellwood, 2011). While these shallow habitats continued to support the largest densities of scars in 2018, parrotfish coral scars were only 40% and 25% of the 2008 values on the crest and the flat, respectively (Figure 6.3.B, Supplementary Table 6.3). The number of scars on the slope and the back reef in 2018 was slightly higher than in 2008, but these zones only accounted for 20.5% and 12.6% of the total number of scars present on the reef in 2018, respectively. To investigate the reason for this apparent reduction in parrotfish predation on massive *Porites* in 2018 (with limited overall change in parrotfish density), we looked at the potential effect of colony size on the pattern observed.

Across the four reef zones, the probability of a *Porites* colony being bitten by a parrotfish in 2018 did not vary depending on colony size (i.e., individual coral surface area available for predation) (Supplementary Table 6.4). Parrotfishes, therefore, did not choose whether to bite corals or not based on coral colony size. Among colonies that had scars, however, there was a clear colony size effect (Figure 6.4).

Figure 6.4. (see next page) Parrotfish predation pressure on massive *Porites* at Lizard Island, Great Barrier Reef. (A) The relationship between the number of scars and the size of massive *Porites* colonies. Fitted lines and bands are, respectively, generalised linear model fits and their 95% confidence interval. **(B)** The relationship between the density of parrotfish scars and the colony surface area. Note that axes are on a log scale and reveal the exceptionally high scar density on small colonies. **(C)** Examples of small (left) and large (right) massive *Porites* colonies at the study site. The same ruler is featured as a scale in both images. Scars are outlined in green and planar colony surface area in white. Scale bars = 5 cm. Purple, red, orange, and yellow indicate the slope, crest, flat, and back reef habitats, respectively.
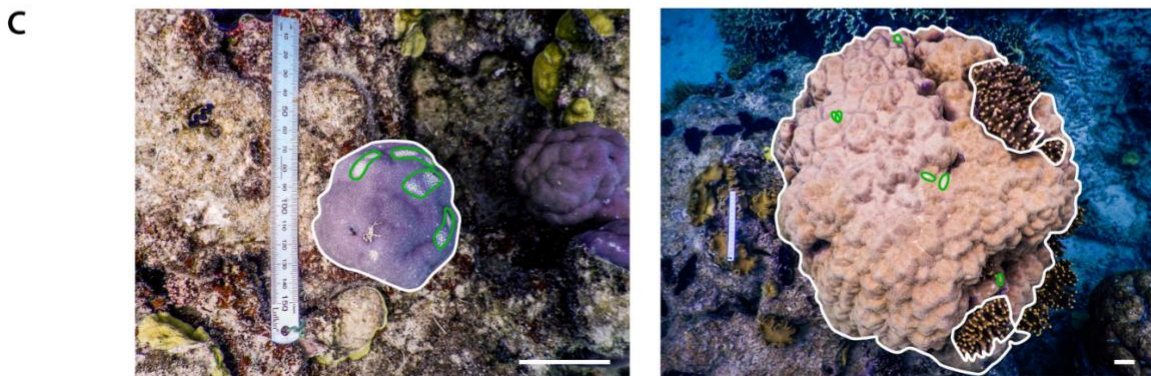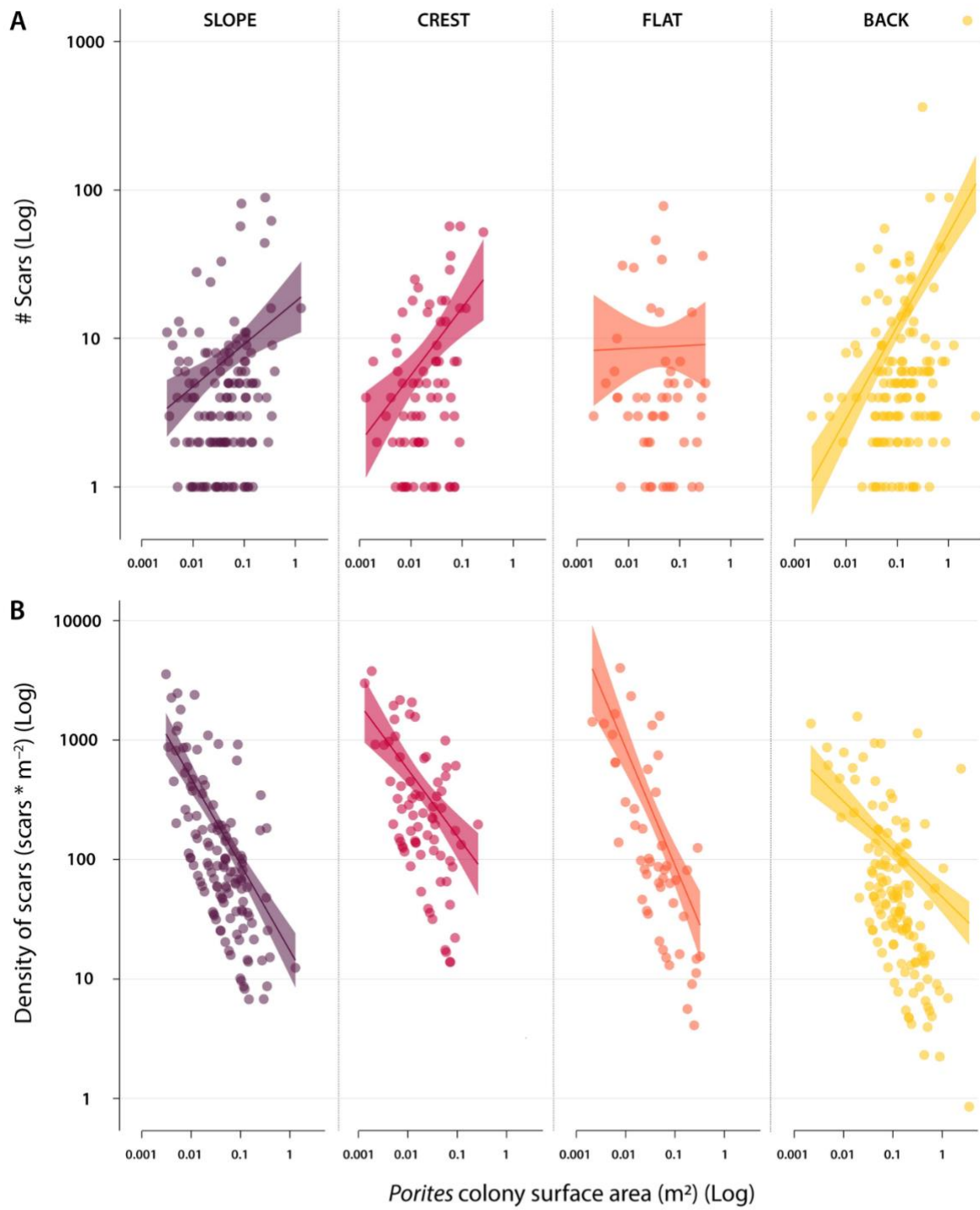
We found a strong positive relationship between the number of scars and the colony surface area on the slope (GLM Estimated coefficient = 0.65 ± 0.17), crest (GLM Estimated coefficient = 1.04 ± 0.31), and back reef (GLM Estimated coefficient = 1.44 ± 0.22) (Figure 6.4.A). We did not detect a relationship on the reef flat (GLM Estimated coefficient = 0.04 ± 0.37). Overall, of those colonies that had scars the number of scars was generally higher on larger colonies. However, the most revealing values were scar densities. We found a strong negative relationship between the density of scars and the *Porites* colony surface area for all four reef zones (Figure 6.4.B, Supplementary Table 6.5). Thus, the density of scars was far higher on small colonies and diminished rapidly as the size of the colony increased. This negative relationship was consistent across all four reef habitats, although it varied slightly in magnitude.

6.5. Discussion

Between 2008 and 2018, our Lizard Island study site lost two thirds of its coral cover, with live corals covering only ~7% of the substratum in 2018. During this period, the forereef habitat (i.e., slope and crest) was affected the most, with a coral cover decline of up to 86%. These results are congruent with other studies conducted at the same (exact) location (Baird et al., 2018; Madin et al., 2018; Zawada et al., 2019) and reflect the broader declines reported from the Great Barrier Reef (Hughes, Kerry, et al., 2017). Most of the coral mortality in the forereef was caused by damage from cyclone-generated waves, especially from cyclone Nathan in 2015 (Baird et al., 2018), but also by the severe bleaching events that followed in 2016 and 2017. The corals most heavily affected were habitat-forming *Acropora*, while keystone reef-builders like massive *Porites* endured the severe weather well (Madin et al., 2018). This uneven sensitivity of coral taxa to stressors resulted in an increase in the proportion of massive *Porites* in 2018, an effect that has been previously reported in other locations (Green, Edmunds, & Carpenter, 2008; van Woesik, Sakai, Ganase, & Loya, 2011) and this stems from the relative high resilience of

massive *Porites* to hydrodynamic forcing (Madin, Baird, Dornelas, & Connolly, 2014; Zawada et al., 2019) and heat stress (DeCarlo et al., 2019; Loya, Sakai, Nakano, & van Woesik, 2001; McClanahan et al., 2007; Putnam, Barott, Ainsworth, & Gates, 2017).

Despite the loss of most other corals, a decrease in absolute massive *Porites* cover, previous evidence that parrotfishes selectively target massive *Porites* (Bonaldo & Bellwood, 2011; Rotjan & Lewis, 2005), and stable parrotfish abundances, we did not detect an increase in parrotfish predation on massive *Porites* in 2018. Indeed, we found that the number of bites appears to have declined relative to 2008. The apparent reduction in predation pressure is best illustrated by the difference in the overall density of scars. Considering, for example, a typical section of reef from the study site (composed of 35.2% reef flat, 27.3% of back reef, 24.2% of reef slope, and 13.3% of crest; as measured from satellite images, Supplementary Methods in Appendix E), using the *Porites* cover (Table 6.1 and Figure 6.1, respectively) and assuming a uniform distribution of *Porites* colonies yields a ~40% reduction in the mean density of scars at the study location from 2008 to 2018 (from 676 to 400 scars per 100 m^2 of massive *Porites* planar surface area). We offer two hypotheses that may explain this phenomenon.

6.5.1. Changes in parrotfish abundance or behaviour in response to reef degradation

A decrease in parrotfish abundance after the disturbances could have contributed to a decline in parrotfish scars (Littler et al., 1989). However, the major disturbances had little effect on overall parrotfish abundances. One possible explanation for this lack of a response is that foraging parrotfishes predominantly feed on non-coral surfaces covered in algal turfs (Bellwood & Choat, 1990); coral-feeding is relatively infrequent (Bonaldo et al., 2014; Bruggemann et al., 1994; Rotjan & Lewis, 2005). Indeed, parrotfishes and other herbivorous fishes usually respond positively to extensive coral mortality, typically with a rapid growth in biomass (Morais et al., 2020; Perry, Morgan, Lange, & Yarlett, 2020). This is probably because these reefs provide larger

areas covered with turf algae, their preferred feeding microhabitat (Adam et al., 2011; Gilmour, Smith, Heyward, Baird, & Pratchett, 2013; Han, Adam, Schmitt, Brooks, & Holbrook, 2016; Morais et al., 2020; Russ, 2003; Russ, Questel, Rizzari, & Alcala, 2015; Taylor et al., 2019). With increased or maintained parrotfish populations, one may anticipate consistent or increased corallivory, if coral-feeding activity remains a constant, if small, proportion of the diet of these fishes. Instead, we observed clear evidence of decreased predation. This decrease could be attributed to a change in foraging behaviour. Most parrotfishes target turf algae-covered surfaces on the reef (Bonaldo et al., 2014). Thus, the observed overall reduction in scars on massive *Porites* may reflect a shift in the foraging behaviour of parrotfishes driven by an increase in the availability of turf-covered substratum following mass coral mortality (Goatley & Bellwood, 2011). While we cannot confirm that this was the case at Lizard Island, changes in the foraging behaviour of parrotfishes following coral mortality have been documented at other locations (Burkepile, 2012).

It is also worth noting that changes in reef fish populations associated with habitat loss may take some time to become apparent (Graham et al., 2007), and therefore trends in parrotfish abundance need to be interpreted with caution. Nevertheless, parrotfish scars on massive *Porites*, contrary to parrotfish populations, decreased in frequency. Thus, our data suggests that, at least in the short term, changes in the composition and structure of the reef diminished the footprint of parrotfish predation on massive *Porites*. This underscores the importance of incorporating direct measures of ecological function, such as coral predation, in ecological studies assessing responses to reef degradation. Overall, parrotfish abundance at Lizard Island does not appear to be a major factor influencing predation on corals at this time, consistent with studies in other reef systems (Burkepile, 2012; Roff, Ledlie, Ortiz, & Mumby, 2011).

6.5.2. The effect of colony size

In 2008, the highest levels of parrotfish predation were observed on the crest and the reef flat (Bonaldo & Bellwood, 2011). On a mid-shelf reef such as Lizard Island, these shallow reef habitats typically concentrate the largest foraging activity of parrotfishes regardless of whether they graze on turf algae surfaces (Bellwood et al., 2018) or predate on live coral (Bonaldo & Bellwood, 2011). In 2018, after a series of severe disturbances impacted this reef, the crest and reef flat continued to sustain the highest intensity of parrotfish corallivory, albeit with much lower scar densities. These lower densities, despite minor or unclear changes in parrotfish numbers, raise the question of why the pattern of feeding remained but overall rates decreased, especially on the flat and the crest. One possible explanation is coral colony size.

The number of parrotfish scars on massive *Porites* increased with colony size. However, this increase was not proportional and, as a result, the density of scars was substantially higher on small *Porites* colonies than on bigger, older, colonies. This pattern was observed across all reef zones and, thus, appears to be intrinsic to the trophic interaction, rather than influenced by depth or exposure to waves. The higher density of scars on small colonies could be associated with the degree of surface curvature, with more curved small colonies being more prone to parrotfish predation, especially by excavating parrotfishes (Bellwood & Choat, 1990). Alternatively, it may also reflect other properties, such as fewer nematocysts or differences in tissue thickness. Regardless of the cause, focused predation on small colonies could have important ramifications.

The reduction in the overall intensity of parrotfish predation in 2018 coupled with the higher density of scars on small colonies suggest that a shift in the size structure of massive *Porites* may have occurred. It is possible that the series of disturbances this reef experienced over the last decade may have not only driven an increase in the proportion of massive *Porites* cover relative to total coral cover, but it may also have increased the proportion of large massive *Porites* colonies via differential survival of large colonies. This is particularly relevant if we consider the patterns

of coral mortality associated with tropical cyclones. Lower survivorship of juveniles is a generalised feature of corals – and indeed all other animals. In corals, structurally complex morphotypes (e.g., tabular, corymbose corals) become increasingly more vulnerable to hydrodynamic forces as they grow in size (Madin & Connolly, 2006). However, massive corals are disproportionally impacted by intense wave action (such as those generated by cyclones Ita and Nathan) when they are small (Madin et al., 2014). Thus, it is likely that the abundance of juvenile massive *Porites* decreased over the study period due to differential mortality of juveniles following the impact of cyclones Ita, and especially Nathan. This could have contributed to the decline in parrotfish scars.

The comparatively high scar densities we observed on small colonies will impose an energetic burden on this vulnerable life stage, potentially constraining colony growth by diverting resources towards wound healing (Meesters, Noordeloos, & Bak, 1994). Indeed, even when parrotfish corallivory does not cause total or partial colony mortality, persistent parrotfish predation may result in reduced colony growth, lower reproductive output (Ward, 1995), higher exposure to disease (Nicolet, Chong-Seng, Pratchett, Willis, & Hoogenboom, 2018), or a reduced ability to cope with future environmental stress (Rotjan et al., 2006). Given the high scar densities that small *Porites* colonies are exposed to, this appears to be a difficult ontogenetic phase for massive *Porites* corals, with the potential for a size-based escape from the worst effects of predation by parrotfishes.

This is important because although *Porites* colonies can handle high levels of environmental stress, high rates of parrotfish predation on small colonies may represent yet another source of colony mortality; with potential negative effects acting in synergy with other types of disturbances (e.g., storms or heat stress). As massive *Porites* become one of the dominant corals on reefs in the Anthropocene, the need to understand the role of potential natural coral predators in regulating their growth and habitat distributions increases. Our study corroborates

findings from previous studies indicating that parrotfishes are important coral predators on Indo-Pacific reefs that can be responsible for significant colony damage (Bellwood et al., 2003; Bonaldo & Bellwood, 2011; Rotjan & Lewis, 2005, 2008; Welsh et al., 2015). Potential reductions in the abundance of young corals in particular is a concern as this demographic bottleneck may underpin further coral decline (Hughes et al., 2007; Hughes & Tanner, 2000).

6.6. Conclusion

Our findings provide new insights into the effect of parrotfish corallivory on the coral reefs of the Anthropocene. Previous research indicated that parrotfishes may be capable of shaping the survival and distribution of massive *Porites* (Bonaldo & Bellwood, 2011; Rotjan & Lewis, 2005). Here, we showed that the overall density of scars diminished in the aftermath of severe degradation, suggesting that an increase in the relative proportion of massive *Porites* did not stimulate parrotfish corallivory, at least in the short term. Importantly, our findings indicate that the impact of parrotfish predation on massive *Porites* is disproportionately greater on small colonies. Therefore, colony growth may provide escape from predation. Although colony size-specific parrotfish predation on massive *Porites* has not been documented on undisturbed reefs, if the observed pattern of disproportionately higher scar density on small colonies is a persistent pattern, parrotfish corallivory on Indo-Pacific reefs could represent an important factor modulating the dynamics of massive *Porites* populations in the Anthropocene. Given the exceptionally long lifespan of massive *Porites* colonies, however, it is likely that the ecosystem-wide effects of high predation on their early ontogenetic stages may take several decades to emerge.

Parrotfishes have probably scarred corals for millions of years and our results suggest that they will continue to do so in the Anthropocene. Despite little evidence of strong effects of parrotfish predation on reef growth, we show that parrotfish corallivory on small colonies may be

a significant factor shaping the distribution and abundance of this dominant coral on Anthropocene reefs. It is unclear what the long-term effects of coral reef degradation will be and whether parrotfish predation will inflict enough damage to have a significant impact on dwindling coral communities in the future. Nevertheless, the observed patterns and potential for negative impacts warrant a renewed look into the function of reef fishes in regulating coral community dynamics.

Chapter 7

CONCLUDING DISCUSSION

This thesis investigated how labrids cope with the challenges associated with feeding on cnidarians. Combining morphological, histological, and ecological (dietary) data, I explored the role of soft tissues in the feeding ecology of cnidarian-feeding fishes. In this thesis, I contribute three key advances that clarify important aspects of the trophic ecology of these fishes. First, I revealed remarkable oral modifications of the soft tissues that are correlated with a diet mainly comprised of corals and planktonic cnidarians. I also found that the key trait that underpins the specialized feeding modes of these fishes is the secretion of abundant mucus. Lastly, my findings suggested the existence of mucus-based food partitioning amongst planktivorous labrids. Here, I discuss the significance of these findings and I propose future directions to expand the lines of research presented herein.

7.1. The importance of the soft anatomy in fish feeding.

Studies of the role of the soft anatomy in food procurement have been primarily confined to tetrapods (Bramble & Wake, 2013; Kier & Smith, 1983). In fishes, research on soft tissues is primarily restricted to descriptions of their architecture and histochemical properties (e.g., Agrawal & Mittal, 1991, 1992a, 1992b; Drelich, Monteiro, Brookins, & Drelich, 2018; Fishelson, 1974; Mittal & Agrawal, 1994; Mittal & Whitear, 1979; Pinky & Mittal, 2008). Despite the thorough anatomical descriptions, however, the patterns of association between these anatomical traits and the ecology of fishes remained largely unexplored. Indeed, ecomorphological studies of the soft tissues in the feeding apparatus of fishes has only generated marginal interest among fish ecologists, whose research focus has predominantly centered on prominent variations in external features such as those of thick-lipped cichlids (Baumgarten et al., 2015; Colombo et al., 2013).

Remarkably few studies to date have examined reef fishes (but see Schubert et al. 2003; Fishelson and Delarea 2014; Tebbett et al. 2018b).

In an effort to fill this knowledge gap, **chapter 2** took an in-depth look at the structure of the mouth of the tubelip wrasse *Labropsis australis*, identifying a rare type of lip that is a critical component of the feeding mode of this corallivore. These fishes seal the coral surface with their lips to suck and remove the surface layer of mucus secreted by corals. This chapter showed that the feeding mode of this labrid relies on these specialized lips. Furthermore, it led to a broader analysis which established folded, mucus-secreting lips as a shared morphological trait in the Labrichthyinii tribe (**chapter 3**). The folded lips in the one-lined tubelip wrasse *Labrichthys unilineatus*, in particular, represents a key functional innovation (*sensu* Wainwright & Longo, 2017) in the oldest extant lineage of corallivorous fishes. Therefore, the significance of the unusual lips of tubelip wrasses extends far beyond that of an interesting anatomical feature. The emergence of this novel morphology likely sparked the origin of fish corallivory, marking a pivotal shift in the ecological relationship between corals and fishes.

Chapter 2 also showed that tubelip wrasses suck coral mucus by pressing their tube-shaped lips against the coral surface. Therefore, the lip morphology requires a protective mechanism to avoid the coral's nematocysts. Using histology, I found that the key mechanism that enables corallivory in tubelip wrasses is the secretion of a mucous layer on their lips. Mucus has previously been reported to help other reef organisms avoid damage by nematocysts. For example, the mucus secreted by the aeolid nudibranch *Aeolidia papillosa* has been shown to inhibit nematocyst discharge (Greenwood et al., 2004). In another well-known example in reef fishes, anemonefishes (subfamily Amphiprioninae) coat themselves in a thick layer of mucus secreted on their skin (Lubbock, 1980), a strategy that helps them avoid the nematocysts in the tentacles of the anemones in which they find shelter. In addition to protection from the nematocysts, the mucus on the lips of tubelip wrasses may also protect the lip skin from abrasion (Pinky, Mitta,

Ojha, & Mittal, 2002). **Chapters 2-4**, therefore, provided evidence of a positive correlation between the ability of a fish to secrete oral mucus and its ability to exploit a cnidarian-based diet. This suggests that mucus may facilitate feeding on corals and gelatinous zooplankton, in what might be the latest in a long list of functions for mucus (Shephard, 1994).

Previous studies of tubelip wrasses looked at the teeth, jaws, and pharyngeal apparatus – in terms of their myology and osteology. This has produced results that, for tubelip wrasses, are interesting but unremarkable (Wainwright et al., 2004). Only when we look at their soft tissues do the exceptional abilities of tubelip wrasses become visible. Lips represent only a part of the soft anatomy but extraordinary lips in many other wrasses (e.g., *Hemigymnus*) and other families (e.g., the coral-feeding pomacentrid *Cheiloprion labiatus* or large-lipped haemulids) point to a whole new field of feeding ecology waiting to be explored. This thesis highlights the need to look beyond muscles and bones in the feeding ecology of fishes.

7.2. The trophic ecology of planktivorous reef fishes: A change of paradigm.

In the water column, fishes of the genus *Cirrhilabrus* developed another solution to the ingestion of cnidarian tissues and the consumption of gelatinous zooplankton. As in coral-feeding tubelip wrasses, *Cirrhilabrus* also relies on a modified soft anatomy that boosts the secretion of mucus. However, in this case the mucus is secreted in the mucosa that lines the buccal cavity, not in the lips (**chapter 4**). The gut content analyses in **chapter 4** provided the first indication of food partitioning in planktivorous labrids, a trophic separation that may have been driven by the ability of *Cirrhilabrus* to coat its buccal cavity with a layer of mucus. The results of the gut content analyses indicated a limited overlap between the trophic niches of AOM feeders (*Cirrhilabrus*) and crustacean feeders (*Pseudocoris* and *Thalassoma amblycephalum*), a pattern that was strongly supported by the stable isotope analyses (**chapter 5**). **Chapter 5**, therefore, questions the traditional paradigm that, given an even prey size and availability, planktivorous reef fishes exploit

the same pool of particles in the water column (Davis & Birdsong, 1973; Hobson & Chess, 1978). These multiple sources of evidence suggest that pelagic food webs on coral reefs are more complicated than previously thought. This is, again, a pattern that has been first revealed from an analysis of soft tissues (and mucus secretion ability).

7.3. Coral reef reconfigurations in the Anthropocene: a new challenge for coral-feeding fishes?

Although labrids developed innovative morphologies to counter the defences of their cnidarian prey, they may now be facing a new obstacle: a rapid shift in the availability of prey due to climate change. Extensive coral mortality due to multiple mass coral bleaching events since 1998 has placed a new focus on the effect of anthropogenic climate change on coral reefs (Bellwood, Pratchett, et al., 2019; Hughes, Barnes, et al., 2017; Norström et al., 2016). Data collected at Lizard Island prior to and following a series of disturbances showed that severe coral loss had no clear effect on parrotfish abundance –the major predator of *Porites* corals– (**chapter 6**), but did result in a relative increase in massive *Porites* cover. Remarkably, this did not promote coral predation on *Porites*. Instead, coral predation diminished. Overall, **chapter 6** showed that trophic interactions between hard corals and parrotfishes are complex and that subtle effects may go unnoticed unless the activities of fishes, not just their abundance, are quantified.

In all data chapters, a close analysis of fish-cnidarian interaction revealed a need for a fundamental change in our assumptions. From mucus-based feeding in corallivorous tubelips to a previously undocumented major division in planktivores (also based on mucus) and, finally, to unexpected changes in the behaviour of the primary predators on massive corals. All rely on the careful understanding of feeding morphologies and/or behaviours.

7.4. Avenues for future research

This thesis contributes important advances to our understanding of the trophic ecology of reef fishes, but it also opens up a series of intriguing questions. How prevalent are the morphological innovations described? How does the mucus prevent damage? How much mucus do these fishes secrete? With the exception of *Labrichthys unilineatus* (which is an obligate corallivore throughout its entire life), juvenile tubelip wrasses are cleaners and they do not shift their diet to corals until they mature (Cole, 2010). In this thesis, only adult tubelip wrasses were included because the focus was to examine morphological variation of the oral soft tissues and its relationship with the consumption of cnidarians. The cleaner wrasse *Labroides dimidiatus*, a lineage of tubelip wrasses that has transitioned to cleaning (Baliga & Law, 2016), lost the folds and the ability to secrete mucus in its lips (**Chapter 3**). Is it possible, therefore, that juvenile tubelip wrasses also lack mucus-secreting lips and this trait develops ontogenetically as these fishes mature and shift their diet from cleaning to corallivory?

Another interesting aspect that merits further research is the underlying mechanism by which mucus confers protection from nematocysts. The literature presents two alternative views which are not mutually exclusive. Some authors have argued that mucus can inhibit nematocyst discharge, as in the case of aeolid nudibranchs (Greenwood et al., 2004), while others maintain that there is insufficient evidence of chemical inhibition of nematocysts and that mucus may simply prevent injury by providing a protective barrier (Lubbock, 1980; Mebs, 1994). Although these hypotheses were not tested in this thesis, the addition of new fish species to the list of known organisms using mucus to avoid nematocysts indicates that mucus, somehow, acts as an efficient shield. Resolving this mechanism could prove useful for a number of purposes such as the development of novel pharmaceutical products.

The elevated volume of goblet cells observed in the lips of tubelip wrasses and the buccal cavity of fairy wrasses suggests that these fishes can secrete abundant mucus. However, mucus

secretion rates are yet to be quantified. Future experimental studies could empirically quantify rates of mucus secretion and test differences across species, fish size, and evaluate the potential effects of a range of environmental factors, opening the door for a suite of interesting ecological questions.

The main food item recorded in the gut contents of *Cirrhilabrus* was an amorphous organic matter (AOM). **Chapters 4 and 5** explain the difficulties of discriminating between the different food sources that likely contribute to the AOM. AOM is, thus, a vague term that encompasses a diversity of prey items. However, the advent of new technological approaches such as DNA metabarcoding have the potential to start untangling the composition of amorphous organic matter. There are many new avenues to follow. Hopefully, this thesis will provide an impetus for future exciting research.

7.5. Concluding remarks

This thesis highlights the crucial role of the soft anatomy in underpinning the feeding modes of cnidarian-feeding labrids. My findings suggest that the distinctive anatomy of the mouths of tubelip and fairy wrasses, which enabled these fishes to secrete elevated amounts of oral mucus, is associated with a diet dominated by cnidarians, a novel food source for labrids. I also provided evidence of the existence of a trophic separation between AOM-feeding fairy wrasses (genus *Cirrhilabrus*) and other planktivores. This is relevant in the context of coral reef food webs because it provides, to my knowledge, the first direct evidence of sustained food partitioning in planktivorous reef fishes. Lastly, I revealed complex trophic interactions between coral predators and stress-tolerant corals in the face of widespread coral reef reconfiguration. In summary, I showed that the study of soft tissues can uncover functional modifications that support sophisticated feeding modes. This thesis provides new insights that improve our

understanding of the trophic ecology of fishes on coral reefs calls for a renewed look into the

mouths of fishes, one that considers the soft anatomy as an integral part of the feeding apparatus.

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Appendix A

SUPPLEMENTARY MATERIAL TO CHAPTER 2

Lip histology preparation

Fishes were collected from coral reefs on the Great Barrier Reef (GBR), Australia. Specimens were euthanized with clove oil, then immersed in an ice-water slurry. The standard length (SL) was recorded, and the head was removed and fixed in Bouin's fixative for 20-24 h. Samples were rinsed, stored in 70% ethanol, then decalcified for 24-48 hours in Gooding and Stewart's decalcifying fluid. Each head was divided in half dorsoventrally and dehydrated through a graded ethanol series (70% to absolute), cleared in xylene, and paraffin-embedded. Serial sagittal sections (5 μm) were obtained using a MicroTec CUT4060 rotary microtome, mounted on glass slides, and stained using the Masson's trichrome technique. The Masson stain allows for clear differentiation between the epithelium and the underlying connective tissue, making it an optimal stain for morphological analyses of the lip epithelium. Additional sections were stained with the combined Alcian Blue (pH 2.5) - Periodic Acid Schiff (PAS) technique to identify reactive mucopolysaccharides in the lip epithelium (Yamabayashi 1987); a useful stain for detecting mucus in various fish tissues (Fletcher et al. 1976). Photomicrographs were obtained with an Olympus SZ40 stereo microscope equipped with an SZ-CTV adapter and an Olympus DP21 digital camera. Sagittal sections closest to the midline were selected for morphological analyses.

Scanning Electron Microscopy (SEM) imaging

For scanning electron microscopy, the lips from a tubelip (*Labropsis australis*) and a typical wrasse (*Coris gaimard*) were prepared by fixation in Bouin's fixative solution for 24 h, storage in 70% ethanol, soaking in freshwater for 24 hours, rinsed 10 times to remove ethanol, particulates and mucus, then stored in a -80°C freezer overnight. Samples were dried in a freeze drier (Alpha

1-2 LDplus, Martin Christ, Germany) at - 55°C at vacuum for 48 h, mounted on stubs, sputter-coated with gold, and examined in a JEOL JSM- 5410LV scanning electron microscope.

Feeding behaviour

We obtained video sequences from two live *L. australis* to examine the role of lips during feeding. Fish were maintained in individual 70 L tanks and fed live hard corals (*Acropora spp.*). High-speed video footage of feeding events was recorded at either 500 or 1,000 frames s^{-1} using a Sony DSC-RX100 IV (Sony Corp., Tokyo, Japan) digital camera. Feeding strikes were analyzed using Quicktime (v.10.4, Apple Inc. Cupertino, USA). Only clear images in lateral view were used in the analyses, where depression of the hyoid apparatus could be observed.

Overall, we collected data from 85 feeding strikes. We timed three distinct phases in frame-by-frame analysis of the feeding strikes (i.e., jaw protrusion, suction, and jaw retraction). The duration of jaw protrusion was from the initiation of jaw protrusion to the moment of lip contact with the coral surface. Suction duration was marked by hyoid depression (and retraction of the insertion of the pectoral fins). The duration of jaw retraction was from the loss of lip contact with the coral to full jaw retraction. We also measured the time the lips were in contact with the coral surface. Finally, we recorded visual evidence of coral tissue removal.

The feeding behaviour of *L. australis* was characterized by a careful examination of the coral surface, the slow placement of the lips near the coral surface, followed by a short sharp suck (a 'kissing' action), before slowly withdrawing (Supplementary Movies 1.1 and 2.2). The sucking action often resulted in a short audible sound ('tuk'). These short strikes (or 'kisses') involved three distinct phases: the protrusion of the jaws, rapid suction following contact of the lips with the coral surface, and the retraction of the jaws. The protrusion of the jaws took on average 21.4 ± 0.6 msec (mean ± SE). Coral contact was followed by a vertical rotation of the neurocranium and a depression of the hyoid arch that together generated the suction force. The

lips were in contact with the coral surface for 20.9 ± 1.8 msec. The suction phase, lasted 13.1 ± 0.4 msec. After suction, the jaws were retracted (20.5 ± 0.6 msec). We recorded visual evidence of live coral tissue removal in 10 strikes (24.4% of all strikes where the surface could be seen).

The high-speed video image analysis indicated that *L. australis* briefly placed their lips in contact with the coral prior to a powerful suck. The lips did not grab or hold coral material, rather they appear to be used for sealing the lips over a small localized area, presumably to increase suction-feeding efficiency. The relatively small proportion of sucks with visual evidence of coral tissue removal suggests that *Labropsis* might predominantly feed on coral mucus.

Diet of *Labropsis australis*

The gut contents of 14 adult *L. australis* were examined (62-121 mm Total Length). Fishes were collected from reefs on the GBR and placed on ice shortly after capture. The entire intestine was removed, and the content of the anterior intestine quantified by examining 40 point intercepts [collection and analysis details in (Bellwood et al. 2006)]. The gut contents of 14 specimens examined were overwhelmingly dominated by coral organic matter (mucus, tissue, and cellular debris); 97.3 ± 1.6 % (SE) of point intercepts. Coral tissue was identified by the presence of occasional zooxanthellae and nematocysts. The remaining 1.6 ± 1.6% was amorphous organic matter, and 1.1 ± 0.6% calcareous material, presumably coral skeletal fragments or reef sediments.

Food preference of tubelip wrasses

Wrasses (Family Labridae) are a particularly speciose group with a prominent presence in coral reef fish communities (Randall et al. 1997; Myers 1999; Bellwood and Wainwright 2002; Cowman et al. 2009). However, of over 600 species of wrasses (Parenti and Randall 2000), only

18 feed on corals (Cole et al. 2008). In this study, we evaluated the structure of the exceptional feeding apparatus that enables tubelip wrasses to feed on corals. Tubelip wrasses (i.e., wrasses in the genera *Labropsis*, *Labrichthys*, *Diproctacanthus*, and *Larabicus*) include several species of small coral-feeding fishes with tube-shaped fleshy lips (Randall et al. 1997; Myers 1999; Cowman et al. 2009; Parenti and Randall 2000). These fishes (with the exception of the Red Sea endemic *Larabicus*) are widespread across the Indo-Pacific region and can be locally abundant (Cole et al. 2010). Randall (2005) speculated that the fleshy lips of *Labropsis*, *Labrichthys* and *Diproctacanthus* are particularly well suited to a diet of coral polyps. However, tubelip wrasses appear to target damaged areas of coral colonies (McIlwain and Jones 1997; Cole et al. 2009), where abundant mucus is produced (Daumas and Thomassin 1977; Bruckner and Bruckner 2000, 2015). It is therefore likely that at least three (*Labropsis*, *Labrichthys*, and *Diproctacanthus*), but possibly all four, genera of tubelip wrasses feed to a significant extent on coral mucus rather than polyps.

Relationship between lip morphology and diet among tubelip and cleaner wrasses

The lips of *Labrichthys unilineatus* and *Diproctacanthus xanthurus* are similar to the mucus-secreting lips observed in *L. australis* (Randall et al. 1997; Myers 1999; Huertas and Bellwood 2018). Cleaner wrasses (*Labroides sp.*) are also close relatives of *Labropsis* (Cowman et al. 2009; Westneat and Alfaro 2005), but these fishes have a diet based predominantly on fish mucus and parasitic gnathiid isopods (Grutter and Bshary 2003). Interestingly, *Labroides* lacks the extensive mucus-producing structures observed in the lips of coral-feeding tubelip wrasses (Huertas and Bellwood 2018[chapter 3 in this thesis]), although they do have fleshy folds in the internal surface of the lips (Randall 1958). The shared preference for mucus, whether it is produced by fish or coral, may be the result of a shared derived trait that arose in a common ancestor of tubelip and cleaner wrasses. The presence of nematocysts in corals, however, may have resulted in the development of the unusual type of lips that enables tubelip wrasses to exploit coral material.

Functional interpretations

The extreme modifications of the lips of tubelip wrasses are indicative of the importance of mucous secretion in these fishes. Although the exact role of mucus in feeding in tubelip wrasses has not been identified, there are at least three possible functions: a) a protection from nematocysts, b) a sealant during suction, and c) an aid to ingestion of detached material. Most fishes secrete small amounts of mucus throughout their epidermis, that likely aids in reducing drag and facilitating locomotion (Rosen and Cornford 1971; Daniel 1981; Bernadsky et al. 1993). However, protection from damage is by far the most commonly reported role of fish mucus, be it protection from UV (Zamzow and Losey 2002; Zamzow 2007; Eckes et al. 2008), abrasion (Whitear and Mittal 1984), infection (Ingram 1980; Austin and Al-Zahrani 1988; Ebran et al. 1999; Videler et al. 1999; Subramanian et al. 2007; Esteban 2012), or in anemonefishes, the stinging of nematocysts (Fautin 1991; Mebs 1994, 2009). Although the exact mechanism is not well understood, skin mucus is considered to be a physical rather than a chemical barrier. In anemonefishes, the skin mucus inhibits nematocyst discharge and prevents the fish from being penetrated by the anemone's venom (Lubbock 1980). This protective role may also be exploited by other species of fishes that inhabit anemones or other cnidarians (Randall and Fautin 2002). Coral gobies (genus *Gobiodon sp.*), for example, are obligate coral-dwelling fishes that live in close contact with tissues loaded with nematocysts. While the toxic properties of the skin mucus exuded by *Gobiodon* have been shown to deter predators (Lassig 1981; Munday et al. 2003; Schubert et al. 2003; Gratzer et al. 2015), the large amounts of mucus secreted by their scaleless skin (Myers 1999) suggests that coral gobies may also use their skin mucus for protection from the nematocysts of the corals in which they live.

Tubelip wrasses feed on the surface of corals. In the case of *L. australis*, at least, this involves a short sharp suck while in contact with the coral surface. Morphological data suggest that this suction is not particularly powerful (Wainwright et al. 2004), although it is evidently strong enough to remove small patches of coral tissue. Furthermore, suction pressure rapidly

diminishes away from the mouth opening (Longo et al. 2016). One way to increase suction

strength may be to ensure a close contact between the lips and the coral. This could be further

enhanced by having thick mucus on the fishes' lips (acting like grease on an o-ring) and flexible

lips to ensure maximum contact on an uneven or rounded surface (precluding hardened thick

lips). Feeding observations of *L. australis* revealed that they have the capacity to open their mouth

wide. However, during a feeding strike they choose to close their lips forming a tube, and to

press them onto the coral surface. This indicates that tubelip wrasses use an innovative feeding

mode based on highly specialized lips that enable them to consume coral mucus and/or tissues.

Supplementary Movies

Supplementary Movie 1.1. A feeding sequence by *Labropsis australis* on *Acropora* sp. In this
footage the three feeding stages are apparent: jaw protrusion, suction and jaw retraction. The closed
mouth 'kissing' feeding mode is clearly visible (500 frames per second).

Supplementary Movie 1.2. A feeding sequence by *Labropsis australis* on *Acropora* sp. In this footage the three feeding stages are apparent: jaw protrusion, suction and jaw retraction. The closed mouth 'kissing' feeding mode is clearly visible (1000 frames per second).

Supplementary References

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Appendix B

SUPPLEMENTARY MATERIAL TO CHAPTER 3

Supplementary Figures
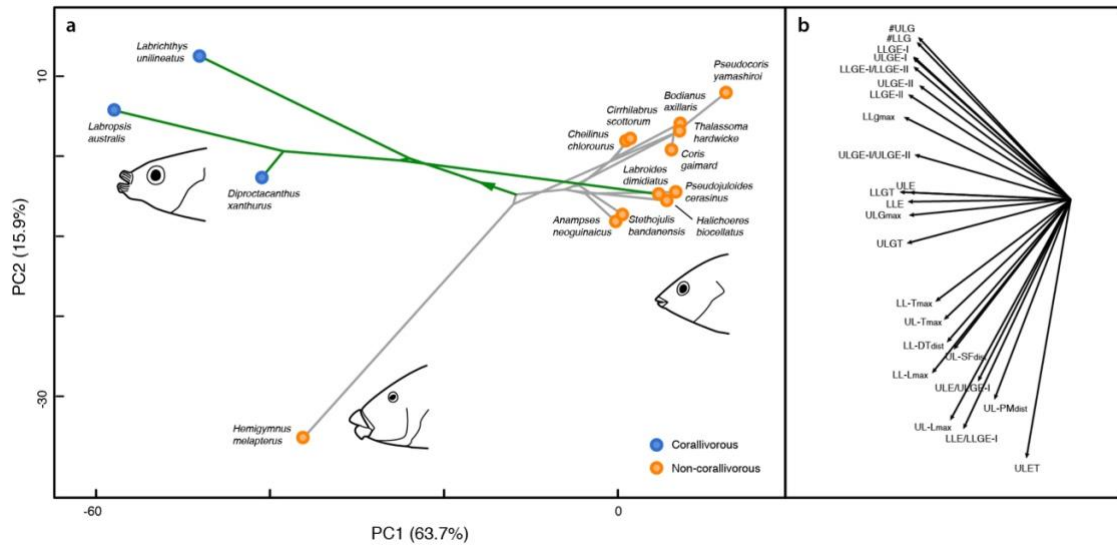
Supplementary Figure 3.1. (A) Phylomorphospace for 15 labrid species based on 27 lip morphological traits with a pruned labrid tree superimposed. Blue circles represent corallivorous tubelip wrasses with a highly folded secretory epithelium; orange circles represent non-corallivorous wrasses with a non-secretory lip. Green lines in the tree connect species within the tribe Labrichtyinii. Arrow indicates the diversion of the most recent common ancestor of the labrichthyines. Note the position of the cleaner wrasse *Labroides dimidiatus* clustered together with the wrasses with a typical lip configuration despite its close phylogenetic association with tubelip wrasses. In the phylogenetic reconstruction for this pPCA we used *Bodianus mesothorax* as a replacement for *B. axillaris* following Floeter et al. (2017). **(B)** Relative contribution of the main vector loadings to the variation. Description of traits in Table 3.1.
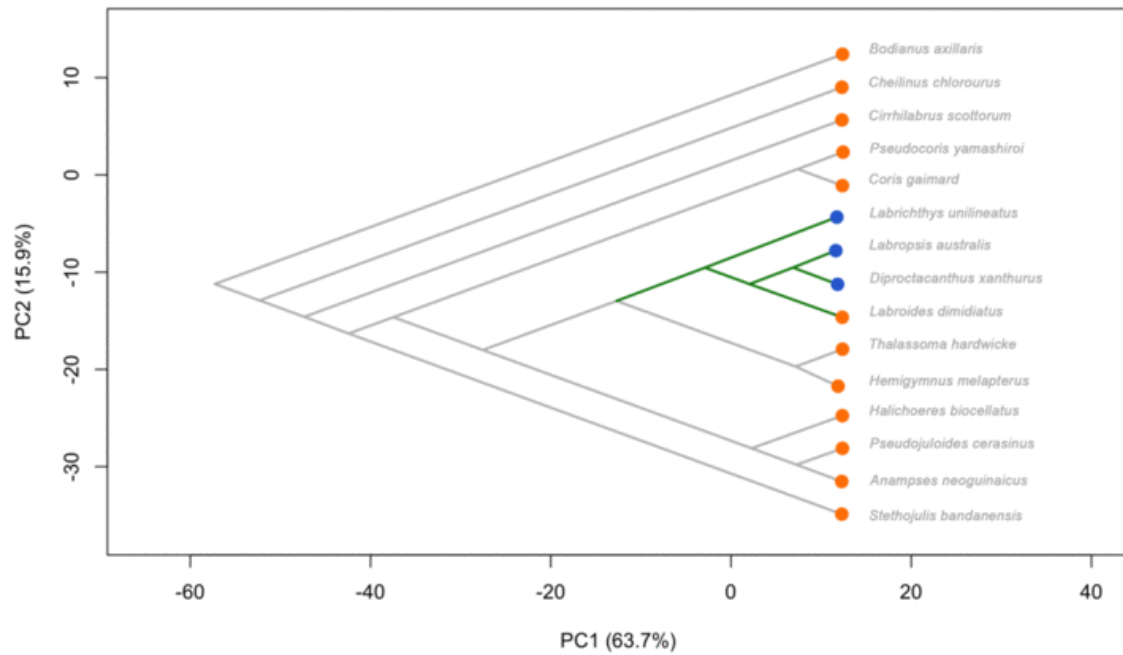
Supplementary Figure 3.2. Animated projection of the tree with 15 labrid species into the phylomorphospace based on 27 lip morphological traits shown in Figure 3.4 and Supplementary Figure 3.1. Blue circles represent corallivorous tubelip wrasses with a highly folded secretory epithelium; orange circles represent non-corallivorous wrasses with a non-secretory lip. Green lines in the tree connect species within the tribe Labrichtyinii. We used *Bodianus mesothorax* as a replacement for *B. axillaris* following Floeter et al. (2018).

Supplementary Tables

Supplementary Table 3.1. List of species included in this study and their respective diet. SL = Standard Length; SE = Standard Error.

Species	n	Mean SL ± SE (mm)	Diet	Refs.
Labropsis australis	4	81.6 ± 4.2	Obligate corallivore	Randall et al. 1997; Randall 2005; Cole et al. 2010
Labrichthys unilineatus	2	93.6 ± 17.0	Obligate corallivore	McIlwain and Jones 1997; Randall et al. 1997; Cole et al. 2010
Diproctacanthus xanthurus	2	51.6 ± 3.7	Obligate corallivore	Randall et al. 1997; Cole et al. 2010
Labroides dimidiatus	2	54.4 ± 5.3	Fish mucus & crustacean ectoparasites	Grutter and Bshary 2003, 2004; Kramer et al. 2015
Coris gaimard	3	98.1 ± 18.0	Invertivore	Hobson 1974; Shibuno et al. 1994; Myers 1999
Thalassoma hardwicke	3	77.7 ± 7.2	Invertivore/Piscivore	Myers 1999
Anampses neoguinaicus	2	74.1 ± 19.3	Invertivore	Kramer et al. 2015
Cirrhilabrus scottorum	2	84.2 ± 2.4	Invertivore (Planktivore)	Myers 1999
Hemigymnus melapterus	2	60.1 ± 11.4	Invertivore	Randall et al. 1997; Myers 1999; Kramer et al. 2015
Pseudojuloides cerasinus	2	70.5 ± 0.4	Invertivore	Kramer et al. 2015
Stethojulis bandanensis	2	88.5 ± 7.4	Invertivore	Myers 1999; Wainwright et al. 2004; Kramer et al. 2015
Bodianus axillaris	1	115.9	Invertivore	Myers 1999
Cheilinus chlorourus	1	117.0	Invertivore	Myers 1999
Halichoeres biocellatus	1	80.8	Invertivore	Kramer et al. 2015
Pseudocoris yamashiroi	1	94.2	Invertivore (Planktivore)	Myers 1999; Wainwright et al. 2004; Kramer et al. 2015

All fishes were collected on coral reefs from the Great Barrier Reef, Australia, except *Diproctacanthus*, which was collected from South-East Asia

Supplementary Table 3.2. Mean values for morphological traits from each species. Values have been standardized by dividing each measurement (in μm) by the standard length of the fish. Trait descriptions in Table 3.1.

Species	Depth RC	ULE	UL-SF$_{dist}$	UL-L$_{max}$	UL-T$_{max}$	UL-PM$_{dist}$	ULG$_{max}$	ULGE-I	ULGE-II	ULE/ ULGE-I	ULGE-I/ ULGE-II	#ULG	ULET	ULGT
Labropsis australis	220.9	3251.4	184.8	491.5	277.7	294.6	87.8	2239.8	642.6	1.5	3.8	24.5	7.5	12.7
Labrichthys unilineatus	346.6	2723.5	211.0	416.8	151.1	168.3	60.1	1880.7	400.3	1.5	4.7	22.0	6.6	9.6
Diproctacanthus xanthurus	216.6	2073.5	168.8	417.9	179.9	287.7	89.8	1479.7	548.3	1.4	3.4	14.5	11.2	12.8
Labroides dimidiatus	132.0	1153.7	89.5	322.2	123.6	265.0	0	0	0	NA	NA	0	5.6	0
Coris gaimard	142.4	1328.8	140.8	296.5	105.6	112.3	0	0	0	NA	NA	0	7.1	0
Thalassoma hardwicke	185.8	1215.4	166.5	254.1	97.2	101.5	0	0	0	NA	NA	0	6.6	0
Anampses neoguinaicus	159.2	1396.9	105.4	395.6	173.6	183.8	0	0	0	NA	NA	0	9.5	0
Cirrhilabrus scottorum	242.1	885.8	171.6	302.0	48.8	73.4	15.2	103.0	16.6	2.8	3.6	2.0	5.7	1.9
Hemigymnus melapterus	186.5	2553.3	348.3	791.7	238.6	284.4	56.1	241.0	46.9	2.9	3.1	1.0	15.1	10.6
Pseudojuloides cerasinus	104.9	1294.6	43.8	260.3	132.5	232.1	0	0	0	NA	NA	0	10.1	0
Stethojulis bandanensis	155.2	1590.1	182.5	251.4	135.7	178.5	0	0	0	NA	NA	0	12.0	0
Bodianus axillaris	204.9	1196.2	87.6	273.7	32.9	98.9	0	0	0	NA	NA	0	8.8	0
Cheilinus chlorourus	214.2	1547.8	226.0	321.4	69.7	83.4	0	0	0	NA	NA	0	8.1	0
Halichoeres biocellatus	92.9	1129.5	89.8	211.7	167.8	102.8	0	0	0	NA	NA	0	12.8	0
Pseudocoris yamashiroi	135.8	1070.8	105.0	177.3	42.7	48.3	0	0	0	NA	NA	0	5.5	0

Supplementary Table 3.2. (continued)

Species	LLE	LL-SF$_{Vdist}$	LL-L$_{max}$	LL-T$_{max}$	LL-DT$_{dist}$	LLG$_{max}$	LLGE-I	LLGE-II	LLE/ LLGE-I	LLGE-I/ LLGE-II	#LLG	LLET	LLGT
Labropsis australis	3134.1	285.1	482.4	331.2	342.4	108.0	2399.9	644.9	1.3	3.8	21.8	10.3	14.2
Labrichthys unilineatus	2878.9	252.5	415.2	186.0	335.0	114.0	2014.1	468.7	1.5	4.3	22.5	10.6	12.5
Diproctacanthus xanthurus	2018.9	122.2	520.2	237.5	266.9	85.8	1444.1	470.2	1.4	3.2	13.5	12.8	12.1
Labroides dimidiatus	1035.6	94.8	280.6	182.1	265.4	0	0	0	NA	NA	0	5.2	0
Coris gaimard	1190.6	183.6	292.0	62.7	122.6	0	0	0	NA	NA	0	8.3	0
Thalassoma hardwicke	1124.2	187.2	272.5	75.2	111.8	0	0	0	NA	NA	0	5.0	0
Anampses neoguinaicus	1546.2	129.8	361.4	172.5	257.0	0	0	0	NA	NA	0	9.8	0
Cirrhilabrus scottorum	872.1	217.3	306.1	68.1	95.1	0	0	0	NA	NA	0	5.0	0
Hemigymnus melapterus	2615.3	181.5	615.7	248.5	366.6	45.0	279.6	132.4	2.6	1.6	1.5	10.9	9.7
Pseudojuloides cerasinus	1257.2	83.3	244.5	110.0	164.3	0	0	0	NA	NA	0	8.3	0
Stethojulis bandanensis	1436.4	147.6	260.1	161.0	204.9	0	0	0	NA	NA	0	12.5	0
Bodianus axillaris	1221.8	223.0	297.1	69.0	46.8	0	0	0	NA	NA	0	9.3	0
Cheilinus chlorourus	1811.4	327.5	298.1	97.4	135.6	0	0	0	NA	NA	0	8.7	0
Halichoeres biocellatus	1410.7	76.8	217.5	148.7	217.7	0	0	0	NA	NA	0	9.8	0
Pseudocoris yamashiroi	826.0	160.9	197.3	45.8	29.5	0	0	0	NA	NA	0	8.0	0

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Shibuno T, Hashimoto H, Gushima K (1994) Changes with growth in feeding habits and gravel turning behavior of the wrasse, *Coris gaimard*. Japanese J Ichthyol 41:301–306

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Appendix C

SUPPLEMENTARY MATERIAL TO CHAPTER 4

Supplementary Methods

When using the photomerge tool, we used the relocation option. In some cases, however, this option did not produce a satisfactory output, and in these cases, we used the perspective option instead. When the perspective option was used, we ensured that no distortion occurred by overlaying the outputs generated on images of the entire head taken with an Olympus SZ40 (Olympus, Hamburg, Germany) stereo microscope. Next, we removed debris in the oral cavity on histological sections. Care was taken not to remove small clusters of epithelial cells that had detached during sample processing. Finally, we adjusted the brightness, contrast, and saturation of the composite images to improve clarity and sharpness for image analysis. Tips for study species for which their phylogenetic position within the tree has not yet been resolved due to insufficient or lack of molecular data available (i.e., *Cirrhilabrus laboutei* and *Cirrhilabrus lineatus*) were added at the node where *C. punctatus* diverged from the *C. exquisitus*/*C. scottorum* clade. This group is therefore represented as a polytomy.

Supplementary Results

Goblet cell density was highest in *Cirrhilabrus scottorum* (27.88 ± 11.58 goblet cells per 100 μm section [±SE]) and lowest in *Pseudojuloides cerasinus* (1.68 ± 0.53 goblet cells per 100 μm section) and *Stethojulis bandanensis* (1.55 goblet cells per 100 μm section). The lining mucosa was thickest in *C. scottorum* (50.09 ± 16.49 μm) and thinnest in *Labroides dimidiatus* (12.33 μm). Highest values of mean goblet cell width were documented in *C. scottorum* (10.05 ± 1.65 μm) and *Cirrhilabrus lineatus* (9.98 ± 0.05 μm) while lowest mean goblet cell width values corresponded

with *S. bandanensis* (2.55 µm) and *Bodianus axillaris* (3.74 µm). Mean goblet cell length ranged

between 24.64 ± 3.03 µm for *C. scottorum* and 2.98 µm for *S. bandanensis.*

Supplementary Figures

Supplementary Figure 4.1. (see next page) Anatomical measurements used to quantify mucus secretion ability throughout the buccal cavity of fishes. Ten equidistant sampling locations (#1 to #10) were spread along the roof of the buccal cavity, between the front end of the neurocranium and the start of the pharynx. Likewise, the remaining ten (#11 to #20) were evenly spread along the base, between the tip of the tongue and the pharynx

Cirrhilabrus exquisitus (AB-PAS)

1 Mucosa thickness

2 # Goblet cells/100 µm

Goblet cells

3 Max. length

4 Max. width

Epithelial tissue

Connective tissue

50 µm
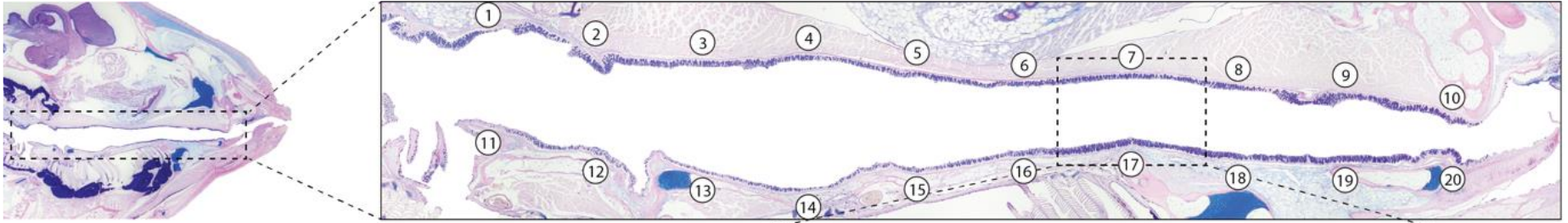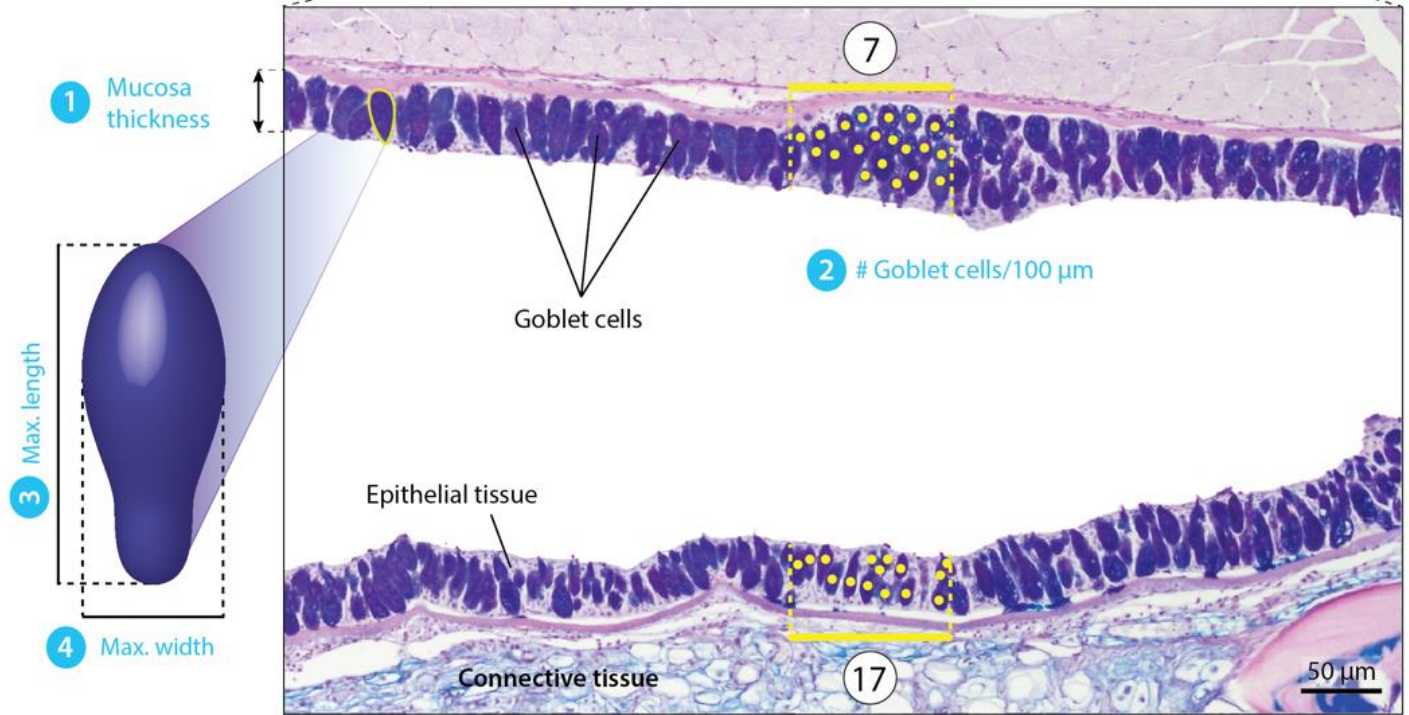
Cirrhilabrus scottorum, planktivore, high AOM content in gut
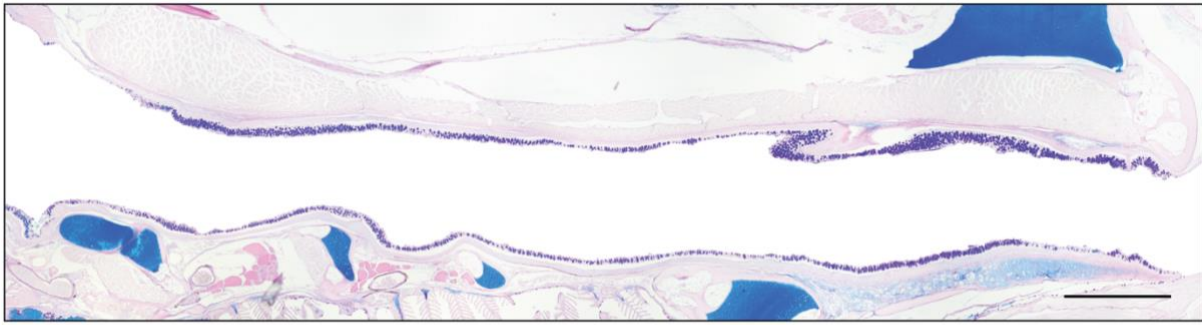
Thalassoma amblycephalum, planktivore, low AOM content in gut
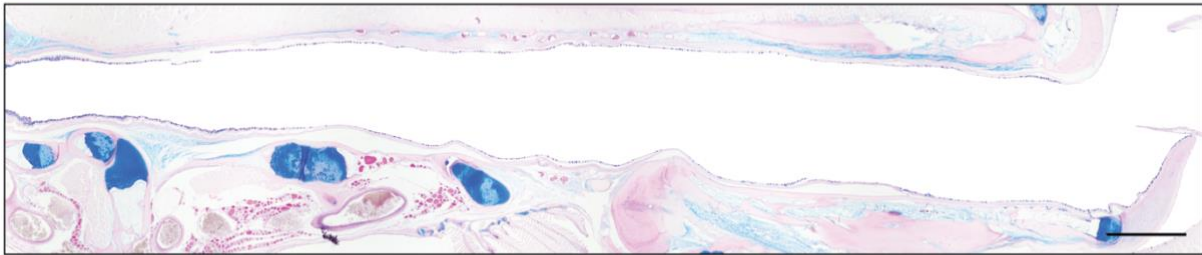
Stethojulis bandanensis, mobile invertebrate feeder, low AOM content in gut
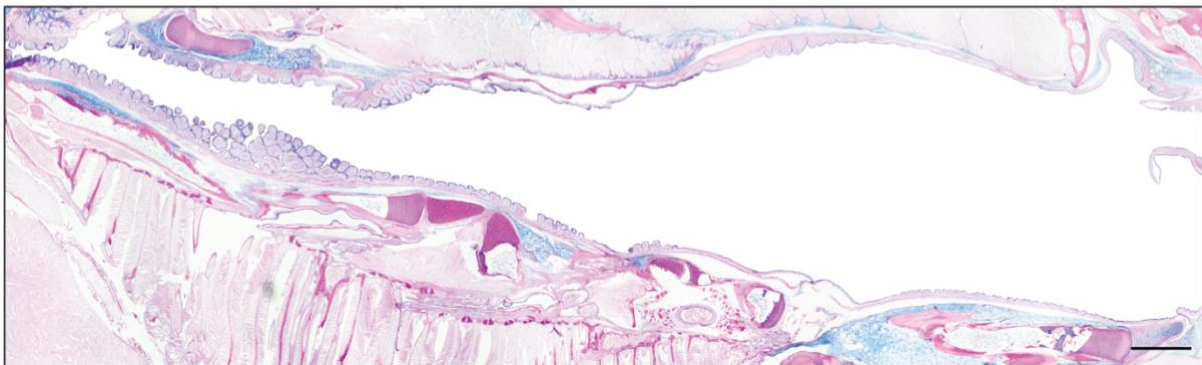
Supplementary Figure 4.2. Region evaluated in the buccal cavities of a Scott's fairy wrasse (*Cirrhilabrus scottorum*), the blunt-headed wrasse (*Thalassoma amblycephalum*), and the redspot wrasse (*Stethojulis bandanensis*). AOM = Amorphous organic material. Scale bars = 500 μm

Supplementary Table

Supplementary Table 4.1. List of species included in this study and their respective trophic guild. All specimens were adult individuals and were predominantly collected on coral reefs from the Great Barrier Reef (Australia). SL = Standard Length; SE = Standard Error

Species	*Morphological data*		*Gut content data*		Trophic guild	References
	Mean SL ± SE (mm)	n	Mean SL ± SE (mm)	n		
Cirrhilabrus exquisitus	80.0 ± 0.3	3	57.2 ± 5.0	28	Planktivore	Kuiter 2002
Cirrhilabrus laboutei	57.3 ± 3.3	3	72.9 ± 2.1	28	Planktivore	Kuiter 2002
Cirrhilabrus lineatus	66.6 ± 0.3	3	79.7 ± 6.5	25	Planktivore	Kuiter 2002
Cirrhilabrus punctatus	89.8	1	64.2 ± 4.7	34	Planktivore	Kuiter 2002
Cirrhilabrus scottorum	80.5 ± 6.0	3	88.2 ± 1.8	37	Planktivore	Myers 1999
Pseudocoris yamashiroi	94.2	1	70.1 ± 3.7	21	Planktivore	Myers 1999; Wainwright et al. 2004; Kramer et al. 2015
Thalassoma amblycephalum	93.4 ± 3.7	3	70.5 ± 3.2	30	Planktivore	Randall et al. 1997
Thalassoma hardwicke	77.7 ± 7.2	3	107.4 ± 5.2	30	Mobile invertebrate feeder	Myers 1999
Anampses neoguinaicus	74.1 ± 19.3	2	108.3 ± 8.0	21	Mobile invertebrate feeder	Kramer et al. 2015
Bodianus axillaris	115.9	1	119.0 ± 2.5	25	Mobile invertebrate feeder	Myers 1999
Coris gaimard	98.1 ± 18.0	3	162.4 ± 12.5	21	Mobile invertebrate feeder	Hobson 1974; Shibuno et al. 1994; Myers 1999
Halichoeres biocellatus	80.8	1	68.7 ± 3.3	24	Mobile invertebrate feeder	Kramer et al. 2015
Hemigymnus melapterus	60.1 ± 11.4	2	161.5 ± 9.0	31	Mobile invertebrate feeder	Randall et al. 1997; Myers 1999; Kramer et al. 2015
Pseudojuloides cerasinus	70.5 ± 0.4	2	69.5 ± 2.4	11	Mobile invertebrate feeder	Kramer et al. 2015
Stethojulis bandanensis	88.5 ± 7.4	2	78.3 ± 2.3	26	Mobile invertebrate feeder	Myers 1999; Wainwright et al. 2004; Kramer et al. 2015
Labroides dimidiatus	54.4 ± 5.3	2	62.7 ± 1.9	31	Cleaner	Grutter and Bshary 2003, 2004; Kramer et al. 2015
Labropsis australis	81.6 ± 4.2	4	62.4 ± 3.5	17	Corallivore	Randall et al. 1997; Cole et al. 2010
Labrichthys unilineatus	93.6 ± 17.0	2	104.2 ± 6.7	24	Corallivore	McIlwain and Jones 1997; Randall et al. 1997; Cole et al. 2010
Diproctacanthus xanthurus	51.6 ± 3.7	2	47.5 ± 2.2	15	Corallivore	Randall et al. 1997; Cole et al. 2010

Supplementary References

Cole AJ, Pratchett MS, Jones GP (2010) Corallivory in tubelip wrasses: diet, feeding and trophic importance. J Fish Biol 76:818–835

Grutter AS, Bshary R (2003) Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. Proc R Soc B 270 Suppl:S242–S244. doi: 10.1098/rsbl.2003.0077

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Kramer MJ, Bellwood O, Fulton CJ, Bellwood DR (2015) Refining the invertivore: diversity and specialisation in fish predation on coral reef crustaceans. Mar Biol 162:1779–1786

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Wainwright PC, Bellwood DR, Westneat MW, Grubich JR, Hoey AS (2004) A functional morphospace for the skull of labrid fishes: Patterns of diversity in a complex biomechanical system. Biol J Linn Soc 82:1–25

Appendix D

SUPPLEMENTARY MATERIAL TO CHAPTER 5

Supplementary Figure
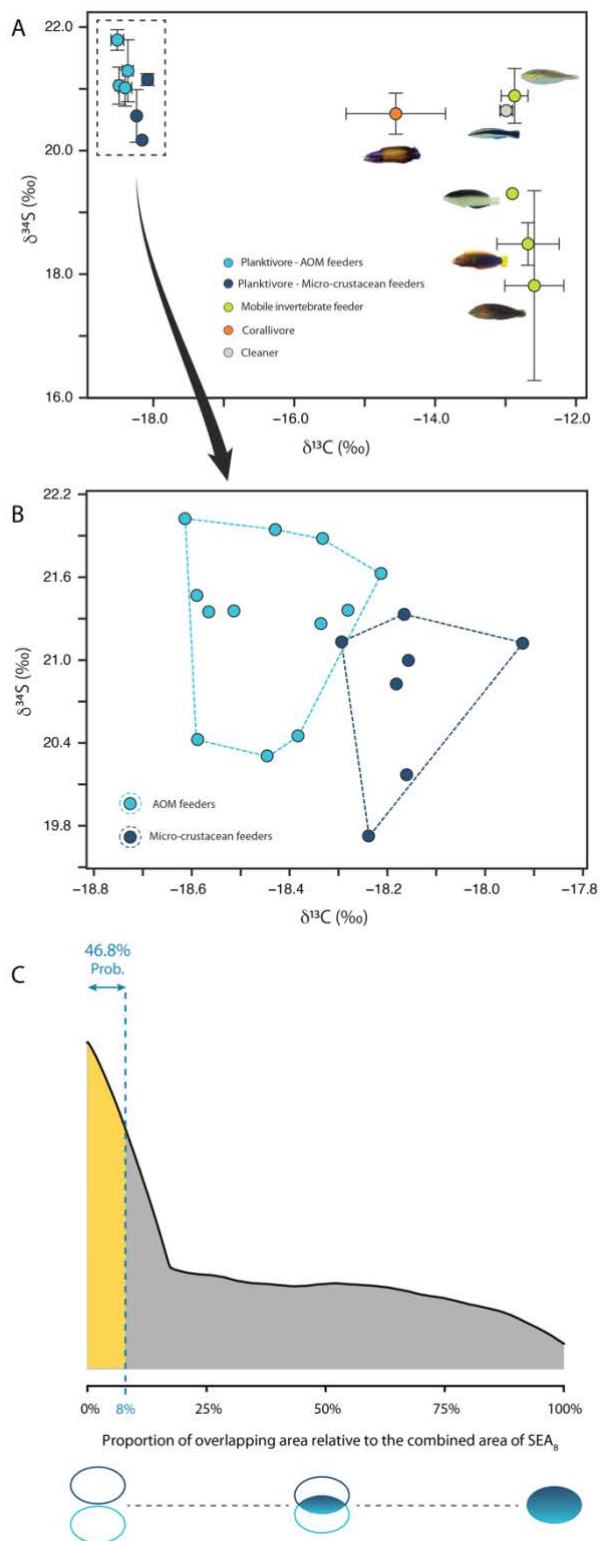
Supplementary Figure 5.1. (see previous page) Isotopic niches of the two groups of planktivorous wrasses identified based on differences in gut contents. (A) Biplot of mean $\delta^{13}C$ and $\delta^{34}S$ of dorsal muscle tissue of reef-associated wrasses. Error bars represent the standard error of the mean. **(B)** Isotopic space occupied by planktivorous wrasses. Points represent raw values for each planktivorous wrasse. Polygons delineate AOM feeders (cyan) and micro-crustacean feeders (blue). Values in supplementary figures 5.1.A and 5.1.B are summarized in Table 5.1. **(C)** Density plot showing the probability of overlap between the 95% prediction Bayesian estimates of standard ellipse areas (SEA_B) of fairy wrasses (AOM feeders) and other planktivorous wrasses (micro-crustacean feeders). The probability is indicated by the proportion of 1,000 draws from the posterior distribution.

Supplementary Table

Supplementary Table 5.1. Sample sizes of the gut contents analyses and the stable isotope analysis, and references to support trophic guild classification. Mob. invert. feeder = mobile invertebrate feeder; AOM = Amorphous organic matter.

		# Fishes included		
Species	**Trophic guild**	**Gut content analyses**	**Stable isotope analyses**	**References**
Cirrhilabrus laboutei	Planktivore (AOM feeder)	15	3	Huertas and Bellwood, 2020
Cirrhilabrus scottorum	Planktivore (AOM feeder)	37	3	Myers 1999; Huertas and Bellwood, 2020
Cirrhilabrus lineatus	Planktivore (AOM feeder)	25	3	Huertas and Bellwood, 2020
Cirrhilabrus exquisitus	Planktivore (AOM feeder)	28	3	Huertas and Bellwood, 2020
Pseudocoris yamashiroi	Planktivore (micro-crustacean feeder)	21	3	Kramer et al. 2015; Myers 1999; Wainwright et al. 2004
Pseudocoris heteroptera	Planktivore (micro-crustacean feeder)	0	1	Randall et al. 2015
Thalassoma amblycephalum	Planktivore (micro-crustacean feeder)	30	3	Myers 1999
Stethojulis bandanensis	Mob. invert. feeder	26	3	Myers 1999; Wainwright et al. 2004; Kramer et al. 2015
Anampses neoguinaicus	Mob. invert. feeder	21	1	Kramer et al. 2015
Halichoeres biocellatus	Mob. invert. feeder	24	3	Kramer et al. 2015
Coris gaimard	Mob. invert. feeder	21	3	Hobson 1974; Shibuno et al. 1994, Myers 1999
Labroides dimidiatus	Cleaner	31	3	Grutter and Bshary 2003, 2004
Labropsis australis	Corallivore	17	3	Randall et al. 1997; Randall 2005, Cole 2010

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Appendix E

SUPPLEMENTARY MATERIAL TO CHAPTER 6

Supplementary Methods

Parrotfish and benthic surveys

To avoid unconscious bias when surveying parrotfish abundance and estimating coral cover, surveyors in 2018 (Victor Huertas and Renato Morais) did not access the data from 2008.

Mapping reef zones

To provide a reef-wide estimate of parrotfish corallivory at the two time periods, we calculated the weighted average number of scars across the study location by mapping the four reef zones (slope, crest, flat, and back reef) using the software Google Earth Pro. The reef slope was identified visually by a darker blue colour due to deeper water. The light, brown-coloured crest was identified as a narrow strip on the exposed side of the reef lining the reef edge adjacent to the slope. The reef flat was identified by a uniform turquoise area behind the crest that was mostly devoid of topographic features. Finally, the back reef was located behind the flat and identified by a darker shade of mottled blue and a noticeable increase in patchiness of the reef area adjacent to the lagoon.

Supplementary Figure

SLOPE

CREST

FLAT

BACK

0.001   0.010   0.100   1.000

Size distribution of massive *Porites colonies* (m²)
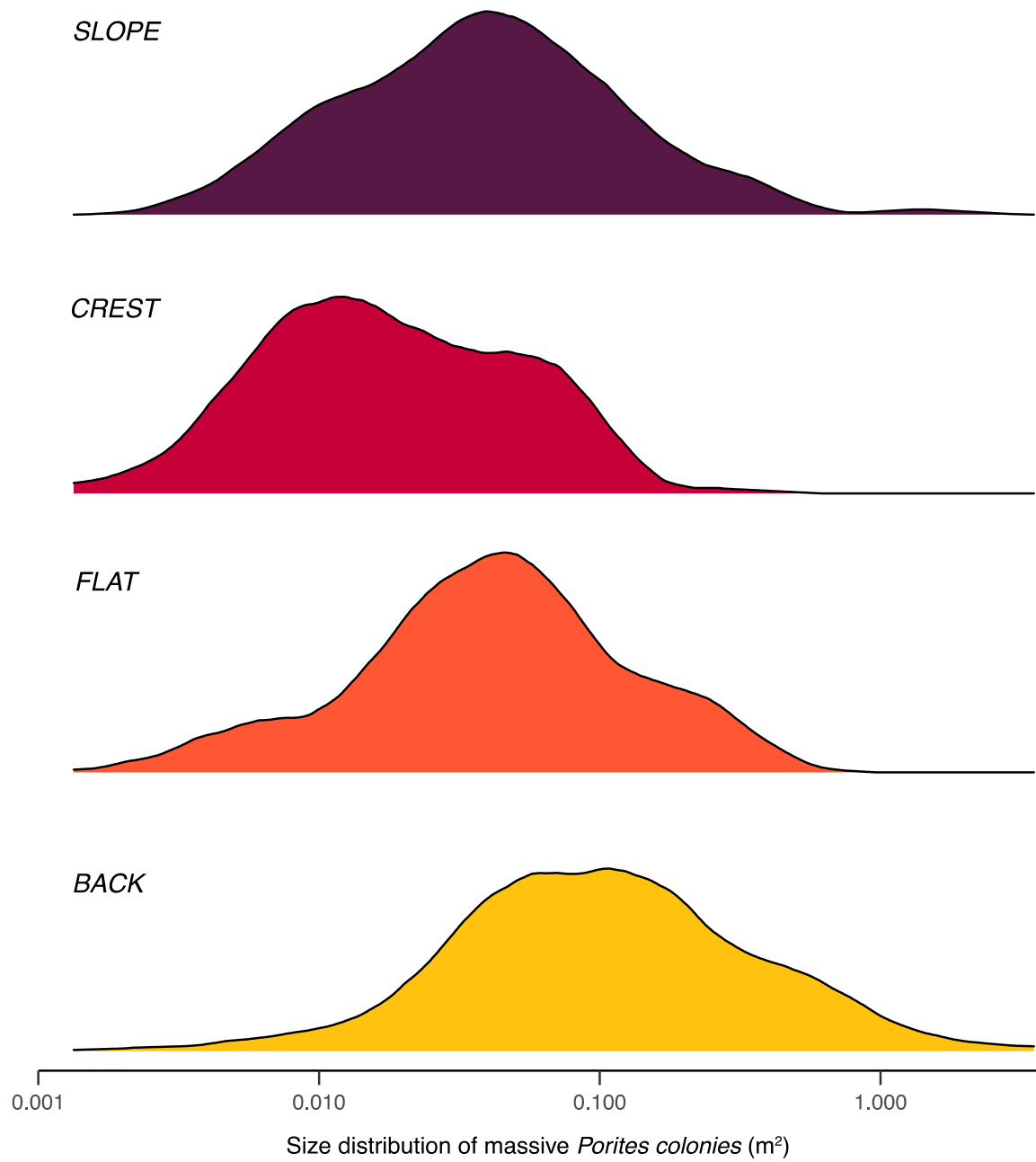
Supplementary Figure 6.1. Density plots showing the size distribution of massive *Porites* corals across the study location in 2018 at Lizard Island, on the Great Barrier Reef. Colony size was estimated based on the planar surface area. Data is presented in the log scale.

Supplementary Tables

Supplementary Table 6.1. Performance of generalized linear models (GLMs) with a negative binomial distribution where parrotfish abundance is the response variable and 'Year' (2008, 2018) and 'Zone' (slope, crest, flat, back) are included as fixed effects. Df: degrees of freedom; LL: maximum loglikelihood of the model; AICc: corrected Akaike Information Criterion; ΔAIC: delta Akaike Information Criterion; wAIC: Akaike Information Criterion weights. wAIC value of 1 indicates 100% confidence that the model is the most parsimonious model for inference, and 0 indicates 0% confidence. Most parsimonious model is shown in bold.

Model	df	LL	AICc	ΔAIC	wAIC
Parrotfish abundance ~ Year x Zone	**9**	**-430.123**	**879.9**	**0**	**1**
Parrotfish abundance ~ Year + Zone	6	-443.143	899.0	19.14	0
Parrotfish abundance ~ Zone	5	-445.489	901.5	21.61	0
Parrotfish abundance ~ Year	3	-451.830	909.9	29.97	0
Parrotfish abundance	2	-455.977	916.1	36.16	0

Supplementary Table 6.2. Tukey's pairwise post-hoc comparisons of parrotfish abundance between surveys conducted in 2008 and 2018 across the four reef zones at Lizard Island. Tests were performed in the log scale. SE: Standard Error of the mean; ns: Non-significant; *: Significant.

2018 vs 2008	Ratio	SE	Z ratio	P value	Significance
Slope	1.633	0.431	1.860	0.063	ns
Crest	1.471	0.375	1.513	0.130	ns
Flat	3.499	0.892	4.911	< 0.001	*
Back	0.496	0.128	-2.716	0.007	*

Supplementary Table 6.3. Parrotfish predation scars on massive *Porites* in 2008 and 2018 at the study site, Lizard Island, on the Great Barrier Reef. SE: Standard Error of the mean.

	Mean scars m^{-2} ± SE	
Zone	2008	2018
Slope	57.5 ± 3.8	141.4 ± 25.7
Crest	670.7 ± 80.8	278.1 ± 51.1
Flat	735.4 ± 129.3	182.4 ± 50.5
Back	58.8 ± 6.9	86.5 ± 14.9

156

Supplementary Table 6.4. Summary of the generalized linear model results used to evaluate the probability of a massive *Porites* colony being bitten at Lizard Island, on the Great Barrier Reef. Habitat, colony area, and their interaction are included as fixed effects. SE: Standard Error of the mean; ns: Non-significant.

Coefficients	Estimate	SE	Z value	P value	Significance
(intercept)	-0.044	0.144	-0.305	0.760	ns
Crest	0.043	0.287	0.149	0.882	ns
Flat	-0.273	0.294	-0.930	0.352	ns
Back	0.341	0.220	1.549	0.121	ns
Colony area	0.593	0.944	0.629	0.529	ns
Crest * colony area	7.657	6.382	1.200	0.230	ns
Flat * colony area	0.642	2.634	0.244	0.807	ns
Back * colony area	-0.037	1.058	-0.035	0.972	ns

Supplementary Table 6.5. Summary of the generalized linear model results used to evaluate the relationship between the density of scars and the area of massive *Porites* colonies at four reef zones at Lizard Island, on the Great Barrier Reef. Habitat, log10(colony area), and their interaction are included as fixed effects. SE: Standard Error of the mean; ns: Non-significant; *: Significant.

Coefficients	Estimate	SE	Z value	P value	Significance
(intercept)	2.848	0.248	11.507	<0.001	*
Crest	0.926	0.501	1.849	0.064	ns
Flat	-0.619	0.533	-1.161	0.246	ns
Back	1.035	0.291	3.554	<0.001	*
$\log_{10}$(colony area)	-1.664	0.163	-10.219	<0.001	*
Crest * $\log_{10}$(colony area)	0.379	0.292	1.299	0.194	ns
Flat * $\log_{10}$(colony area)	-0.597	0.355	-1.682	0.093	ns
Back * $\log_{10}$(colony area)	0.741	0.208	3.573	<0.001	*

Appendix F

PUBLICATIONS DURING CANDIDATURE

Publications arising from thesis chapters

Huertas, V., Bellwood, D.R. (2017) Mucus-secreting lips offer protection to suction-feeding corallivorous fishes. *Current Biology*, 27, R406-R407. doi:10.1016/j.cub.2017.04.056
(CHAPTER 2)

Huertas, V., Bellwood, D.R. (2018) Feeding innovations and the first coral-feeding fishes. *Coral Reefs*, 37, 649-658. doi: 10.1007/s00338-018-1689-7
(CHAPTER 3)

Huertas, V., Bellwood, D. R. (2020) Trophic separation in planktivorous reef fishes: a new role for mucus? *Oecologia*, 192, 813-822. doi: 10.1007/s00442-020-04608-w
(CHAPTER 4)

Huertas, V., Morais, R. A., Bonaldo, R. M., Bellwood, D. R. (2021) Parrotfish corallivory on stress-tolerant corals in the Anthropocene. *PLoS One*, 16(9), e0250725. doi: 10.1371/journal.pone.0250725

(CHAPTER 6)

Manuscript in review

Huertas, V., Radice, V. Z., Morais, R. A., Bellwood, D. R. Food partitioning in planktivorous reef fishes.
(CHAPTER 5)

Other peer-reviewed publications published during candidature

Tebbett, S. B., Goatley, C. H. R., **Huertas, V.**, Mihalitsis, M., Bellwood, D. R. (2018) A functional evaluation of feeding in the surgeonfish *Ctenochaetus striatus*: the role of soft tissues. *Royal Society Open Science*, 5, 171111. doi: 10.1098/rsos.171111

Huertas, V., Byrne, M. (2019) Observation of mass spawning of the sea cucumber *Holothuria coluber* at Lizard Island, Great Barrier Reef, Australia. *SPC Beche-de-mer Information Bulletin*, 39, 79-80. doi: 10.1017/s0025315418000061

Morais, R. A., Depczynski. M., Fulton, C. J., Marnane, M. J., Narvaez, P., **Huertas, V**, Brandl, S. J., Bellwood, D. R. (2020) Severe coral loss shifts energetic dynamics on a coral reef. *Functional Ecology*, 34, 1507-1518. doi: 10.1111/1365-2435.13568