Co-culture applications for enhanced coral production

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Abstract

Coral aquaculture is expanding to meet increasing demands for sustainably produced corals from the ornamental industry and reef restoration operations. Mass production of corals is currently constrained by high costs and bottlenecks in production, including low survival of sexually propagated recruits and poor understanding of many species-specific requirements. Co-culture is the practice of growing two or more organisms in the same or connected systems for benefits including enhanced production, waste remediation, product diversification and biocontrol of fouling and/or parasites. It is currently practiced in various aquaculture industries and could present a cost-effective solution to many of the challenges currently faced by coral aquaculture.

To date there is limited understanding of the potential applications of co-culture to enhance coral aquaculture. To explore the current knowledge in this field, a comprehensive literature review (**Chapter 1**) was undertaken. Control of fouling algae by a range of herbivorous species was identified as a potential application of co-culture, as algae poses as significant threat to corals' survival and growth in the captive environment, particularly in the recruit grow-out phase (up to six months post-settlement). The review also highlighted the potential to incorporate schooling fish into coral aquaculture to provide nutritional enrichment to corals through their waste products, which may address current deficiencies in captive coral nutrition. In addition, biocontrol of pests and parasites was identified, which could utilise natural predators to prevent or control outbreaks within aquarium systems. For this thesis, the areas of algae control around recruits and nutritional enrichment of corals by schooling fish were chosen as topics of interest.

To effectively control algae around coral recruits, it was proposed that a succession of grazers be trialled, to target various algae species during different coral grow-out phases. **Chapter 2** tested the efficacy of microherbivores at controlling algae around six species of early-post settlement coral recruits over a four-month grow-out period. It was found that for all coral species, co-culture with a microherbivore could equal or exceed production of

recruits under manual removal of algae by an aquarist, while significantly reducing husbandry time. In general, larger Acroporid recruits were suited to co-culture with the gastropod grazer *Calthalotia strigata*, whilst smaller non-Acroporids like *Goniastrea retiformis* performed well with juvenile *Tripneustes gratilla* sea urchins, likely due to the combination of low disturbance to the corals by these grazers and their promotion of relatively short algal turf communities.

Chapter 3 investigated the appropriate age and size of coral recruits for introducing larger fish grazers to coral grow-out tanks. Specifically, the study assessed the probability of recruit mortality by exposing week-old, single-polyp and month-old, multi-polyp coral recruits to herbivorous fish with a range of grazing strategies for 24-hours. The "cropping" grazer species were found to have the lowest risk of mortality for all ages/sizes of Acroporid (*A. kenti* and *A. millepora*) and *Goniastrea retiformis* recruits. Once Acroporids had reached the month-old, multi-polyp stage they were resistant to most forms of grazing, with mortality decreasing to negligible levels. Tracking algae removal by the fishes, it was found that the cropper *Acanthurus nigrofuscus* represented the best trade-off in efficiently reducing the cover of turf algae whilst minimising risk to recruits, with the recommendation that fishes are not introduced to recruit culture until at least one-month post-settlement.

Chapter 4 explored the potential for nutritional enrichment of corals by schooling fish. Coral species were cultured under various combinations of co-culture with juvenile *Chromis viridis* and/or a supply of live feeds (*Artemia*, rotifers and mixed microalgae). The selected corals included species with a greater autotrophic (A) or heterotrophic (H) capacity, as well as species that were typically associated with fish in the wild (F) and those that were not (N). It was found that access to dissolved fish wastes, whether the fish were in the tank with the coral or if the corals were simply supplied with filtered wastewater from a fish tank, could enhance growth, protein and symbiont density in *Porites lutea* (AN), *Acropora kenti* (AF) and *Pocillopora verrucosa* (HF). However, the more heterotrophic *P. verrucosa* showed greater enhancement of these factors with access to live feeds. It was likely that the benefits from fish-derived nutrients stemmed from enrichment of the corals' symbionts by nitrogen and

phosphorous in the fishes' dissolved wastes, suggesting that co-culture with schooling fish could enhance production of more autotrophic or fish-associated coral species.

Chapter 5 further explored the underlying physiological and microbial responses of *P. verrucosa* to co-culture with fish and access to live feeds, comparing the status of these corals to field collected samples to assess overarching effects of captivity. It was found that compared to field corals, all captive corals were deficient in the important storage lipid TAG, attributed to the coral catabolising this lipid to fuel recovery after fragmentation. The captive corals displayed an inability to recover TAG lipids from supplied diets, instead relying on translocation from photosymbionts, which were less effective in captivity due to lower light levels. Additionally, the associated microbial communities shifted from field sampled corals to those held within the captive environment. The microbial community associated with the field samples were dominated by the bacterial symbiont *Endozoicomonas,* and across the different fish and diet treatments, only corals in the mixed fish and lived feeds cultures displayed a similar community profile, potentially due to the nutrient balance in these systems more closely matching that found on reefs.

Finally, **Chapter 6** synthesises the information from the literature review of **Chapter 1** and findings from **Chapters 2 – 5** to provide recommendations for appropriate coral-companion pairings in the *ex situ* aquaculture environment. Ideal companions for algae control and nutrient enrichment from post-settlement to adult stages are identified for a variety of coral species, and suggestions for effective pest or parasite biocontrol companions are also provided. Critical considerations for the coral aquaculture industry to efficiently adopt these practices are discussed, as well as key future research and development areas. Overall, this thesis addresses critical knowledge gaps in the appropriate application of co-culture companions for aquaculture-reared corals, providing practitioners a framework to implement these practices across different coral aquaculture ventures.

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CHAPTER 1 - General introduction

1.1 Expanding mass propagation of corals

Anthropogenic impacts, such as the increased frequency of heat-induced mass bleaching, are driving the global decline of coral reefs (Dietzel et al., 2020; Emslie et al., 2024; Henley et al., 2024; Hughes et al., 2019; Pandolfi et al., 2011). These declines have resulted in an increased interest in restoration of degraded reefs and supporting coral resilience through various techniques, including substrate stabilisation, larval enhancement, coral gardening and selective breeding (Bostrom-Einarsson et al., 2020; Quigley et al., 2019). The sustainability of wild harvest of corals for the ornamental trade has also come under scrutiny as consumer awareness grows (Harriott, 2003). Consequently, there is a growing coral aquaculture industry aimed at supplying corals for reef restoration activities and providing an alternate to wild harvested corals in the ornamental trade (Barton et al., 2017).

The majority of corals are colonial species, with colonies consisting of multiple identical polyps as a repeating unit, an attribute which lends itself to asexual reproduction via fragmentation (Highsmith, 1982). Methods for asexual propagation of corals are well established and typically involve fragmenting larger adult colonies into smaller units, which are then grown out in *in situ* nurseries or *ex situ* tank systems until they reach the desired size (Barton et al., 2017). However, sexual production of coral is becoming more prevalent due to its several advantages: it avoids destruction of broodstock colonies (Bostrom-Einarsson et al., 2020; Edwards et al., 2010), maintains higher genetic diversity (Baums, 2008), allows selective breeding for traits such as heat resistance (Anthony et al., 2017; Quigley et al., 2019; van Oppen et al., 2015), and yields higher numbers of propagules than asexual fragmentation (Edwards et al., 2010; Guest et al., 2013).

To date, sexual propagation of corals has been conducted at relatively small-scales (Hughes et al., 2023). However, as restoration programs around the world seek to expand their out-planting efforts, techniques for mass production of corals are being developed (Bostrom-

Einarsson et al., 2020; Randall et al., 2020; Severati et al., 2024). Randall et al. (2020) reviewed the current state of sexual propagation techniques for corals and identified critical bottlenecks in production, including low post-settlement survival of recruits and poorly understood nutritional requirements (particularly heterotrophic) of all life stages of corals which can impact survival, growth and general health. Current strategies to address these issues, such as extensive removal of deleterious algae or fouling in grow-out systems or around outplants to increase coral survival, can be challenging to scale and often require significant husbandry effort, increasing production costs (Nakamura et al., 2011; Omori & Iwao, 2014).

At present, both asexual and sexual coral propagation remain prohibitively expensive for mass production, despite the economics of coral production being recognised as an important factor in upscaling the industry (Edwards et al., 2010). The estimated investment required to restore just one hectare of degraded reef with outplants range between $50,000 and over $1 million USD, due to factors such as high labour costs for skilled workers, extensive equipment requirements, and use of consumables like electricity (Edwards et al., 2010; Guest et al., 2013; Hughes et al., 2023). Despite these high initial costs, studies have found that the cumulative survival and growth of sexual outplants was higher than asexual, supporting the continued pursuit of sexually propagated coral (Baria-Rodriguez et al., 2019; Guest et al., 2023). Nevertheless, the combination of high production costs and low survival rates of sexually produced corals currently constrains the scaling up of coral aquaculture for reef restoration or commercial operations. This has sparked interest in exploring potentially effective, low-cost solutions to address existing production bottlenecks.

1.2 Co-culture in aquaculture

Co-culture is the practice of rearing two or more species in the same or connected systems, to achieve various benefits. In aquaculture, co-culture can be employed to maximise the output of a culture system (Sharma et al., 1999; Wang et al., 1998), remediate waste (Hannah et al., 2013; Sun et al., 2020), and control pest and parasite species (Hung et al., 2013; Vaughan et al., 2018). Co-culture systems can also benefit from perceived higher

sustainability, for example reduced use of chemicals, compared to traditional monoculture aquaculture operations, which can increase the public approval and potentially attract a price premium to such products (Barrington et al., 2008; van Osch et al., 2019).

Successful examples of co-culture aimed at increasing system yield include aquaculture farms which stock multiple species that occupy different ecological niches within a culture environment, such as black and grass carp (Sharma et al., 1999) or tilapia and shrimp (Wang et al., 1998). These systems typically yield higher profits compared to similar monocultures. For example, a scallop and kelp polyculture system in Sungo Bay was found to generate profits up to 1.2 times higher than a scallop only culture system (Fang et al., 1996), while high density Nile *Tilapia* and African sharptooth catfish cultures demonstrated a net profit return of USD 110.70, compared to a loss of USD 89.30 in a tilapia monoculture (Shoko et al., 2016). Secondary cultured organisms may also utilise wastes produced by the primary target species, reducing reliance on feeds and remediating nutrient contamination from farms. For instance, benthic sea cucumbers can be cultivated under fin-fish cages or in wastewater, increasing yield while reducing its environmental impact (Hannah et al., 2013; Sun et al., 2020; Yokoyama, 2013). Similarly, extractive species such as seaweed are used to remove wastes and oxygenate farmed fish water (Kang et al., 2020; Neori et al., 1996).

The benefits of co-culture extend beyond the potential for producing additional species for sale within the same system, but also can offer a reduction in required husbandry or chemical use. The costs associated with controlling biofouling on mariculture cages or tanks can be significant, with conservative estimated ranging from 5 – 10% of production costs (Fitridge et al., 2012). Natural methods of controlling fouling, such as introducing grazers or predators, can replace more expensive methods like manual removal or chemical treatments. For example, in bivalve aquaculture systems, sea urchins have been deployed on small-scales to control the growth of ascidians and algae (Epelbaum et al., 2009; Lodeiros & García, 2004; Ross et al., 2004).

Biological control of parasites is also useful. In Atlantic salmon sea-cage aquaculture, parasitic copepods can reduce growth, cause external damage to the fish, and spread diseases, leading to an estimated loss of US$436 million in 2011 in the Norwegian salmon industry (Abolofia et al., 2017; Bowers et al., 2000; Pike, 1989). Various treatments have been employed to treat or prevent infections, including chemical baths (Ernst et al., 2001), food additives (Grave et al., 2004) and changes to pen design (Geitung et al., 2019). However, since the 1980s, co-culture of salmon with cleaner-fish that actively prey on the sea lice has also been used to help control outbreaks (Bjordal, 1991; Powell et al., 2018). Cleaner fish can delay the need for chemical treatments until later in the season and reduce louse population growth, offering a more sustainable approach to parasite control (Barrett et al., 2020; Blanco Gonzalez, 2019).

Given that co-culture holds the potential to increase farm yield, reduce environment costs and raise profits, it is being promoted globally as a sustainable method to enhance aquaculture across a broad range of species (Alexander et al., 2016; Carras et al., 2019; Knowler et al., 2020). Coral aquaculture naturally lends itself to co-culture applications, due to a long history of ornamental mixed mesocosms in public and private aquariums providing the groundwork for successful coral-companion pairings, and extensive ecological studies highlighting the importance of different species interactions in maintaining coral populations on wild reefs. Co-culture offers a potentially cost-effective solution to address current bottlenecks in coral production while reducing man-hours and/or equipment and consumable use in coral propagation systems (Henry et al., 2019; Ladd & Shantz, 2020; Shaver & Silliman, 2017). Additionally, co-culture allows coral aquaculture operations to produce additional products within the same system. For example, sea urchins such as *Tripneustes gratilla* could be cultivated alongside corals, serving both as a method of algae control, and to be out-planted alongside corals, or sold to ornamental aquarists (Barrows et al., 2023; Cano et al., 2021; Craggs et al., 2019). This review explores the evidence for the use of co-culture in *ex situ* coral culture systems as a method to control fouling, supply nutrition to corals through trophic linkages, and provide biological control of coral pest and parasite species.

1.3 Interactions between corals, algae and herbivores

Interactions between corals, algae and herbivores throughout corals' life cycles have been extensively studied, and are noted to be both complex and dynamic (as reviewed in Inagaki and Longo (2024)). In the early stages of a coral's lifecycle, algae can both induce and inhibit larval settlement. Coral settlement has been found to be induced most commonly by species of live crustose coralline algae (CCA) (Baird & Morse, 2004; Davies et al., 2014; Heyward & Negri, 1999), as well as bacteria or microbial biofilms (Erwin et al., 2009; Negri et al., 2001; Petersen et al., 2021; Webster et al., 2004) and related chemical extracts from CCA, bacteria and cnidarians (Erwin & Szmant, 2010; Gomez-Lemos et al., 2018; Sneed et al., 2014; Whitman et al., 2020). However, species of macroalgae (Birrell et al., 2008b; Diaz-Pulido et al., 2010; Vermeij et al., 2009), some algal turfs (Birrell et al., 2005) and other species of CCA (Harrington et al., 2004; Whitman et al., 2020) have been found to inhibit coral settlement. Algae can also pre-empt space, forming a physical settlement barrier to corals (Kuffner et al., 2006; Leong et al., 2018; Littler & Littler, 1999). Abiotic factors such as light (Ricardo et al., 2021; Strader et al., 2015), sedimentation (Ricardo et al., 2021) and the availability of clear substrate (Elmer et al., 2018) further influence the behaviour and site selection of settling coral larvae.

Once corals settle and metamorphose they compete with various algae, including CCA, for space. Both corals and algae have developed defences and strategies to maintain their position on the reef benthos; an extensive review of these competitive mechanisms can be found in McCook et al. (2001). In summary,algae can cause coral tissue loss through direct contact or allopathy (Littler & Littler, 1997; Nugues et al., 2004a; Rasher & Hay, 2010), serve as reservoirs for opportunistic coral pathogens (Nugues et al., 2004b), and algal growth can facilitate sediment accumulation thereby smothering corals (Leong et al., 2018; Nugues & Roberts, 2003). Corals and algae may also overgrow each other, either directly over tissue or by shading their competitor (Box & Mumby, 2007; Diaz-Pulido et al., 2009; Eckrich & Engel, 2012). Even species of crustose coralline algae, a settlement cue for many coral species, can overgrow recruits and kill them (Craggs et al., 2019; Jorissen et al., 2020). These interactions

are complex, and outcomes vary depending on the environmental conditions (Burkepile et al., 2013; McCook et al., 2001; Sotka & Hay, 2009), the fitness of individuals (Tanner, 1995), the age of the competitors (Olsen et al., 2014) and other external factors. In general, however, an increase in algae correlates with decreased coral health, survival and growth.

Herbivores play a crucial role in maintaining coral reef health by controlling algae growth (Adam et al., 2015; Jompa & McCook, 2002), opening up space for coral recruitment and growth (Ceccarelli et al., 2005). The loss of herbivores often leads to algal dominance on reefs, with fast-growing species such as *Sargassum* quickly overgrowing corals in the absence of grazing pressure (Bellwood et al., 2006; Cheal et al., 2010; Hughes et al., 1987). Large herbivorous fish such as Scarids, Acanthurids or Siganids are common grazers (Burkepile & Hay, 2008; Lirman, 2001), though in degraded areas where fish have been removed, invertebrate herbivores such as sea urchins become responsible for algae control (Bodmer et al., 2015; Coma et al., 2011; Kuempel & Altieri, 2017). A diverse assemblage of grazers with different feeding strategies is essential for controlling various functional groups or species of algae (Burkepile & Hay, 2008, 2010; Carpenter, 1986; Tebbett et al., 2017b).

While herbivores are vital for top-down control of algae growth on reefs, they can also pose a threat to corals through over-grazing. Small post-settlement recruits are particularly vulnerable to mortality from direct interactions with fish or invertebrate grazers, whereas larger corals are more resilient and can survive the same grazing pressure that would impact newly settled recruits (Brandl et al., 2013; Christiansen et al., 2009; O'Leary et al., 2013; Penin et al., 2011; Whitman et al., 2024). Excluding larger herbivorous fish by caging has been shown to improve the survival of wild coral spat, such as *Acropora* sp., despite the increased algae biomass inside the cages (Baria et al., 2010; Trapon et al., 2013). In a similar manner, the deliberate feeding on corals by some omnivores, such as parrotfish, can negate the beneficial effects of their algae removal (Miller & Hay, 1998). These observations underscore the delicate balance needed for corals to thrive alongside herbivores: the herbivore assemblage must remain at sufficient densities and species composition to control

the growth of algae but must not reach population levels that depress coral growth through their feeding activities.

Effective control of algae in coral aquaculture is fundamental for success (Yap & Molina, 2003). There is a plethora of anecdotal evidence in the grey literature from ornamental aquarists and hobbyists regarding the use of herbivores for algae control (https://www.reef2reef.com/forums/). In the established scientific literature, coral aquaculture studies also often refer to the species of grazers deployed in tanks or recruited near coral farms that control algae proliferation as a part of their methodology. For example, Nakamura et al. (2011) used gastropods (*Trochus niloticus* and *Lunella granulate)*, and herbivorous fish (*Acanthurus triostegus* and *Siganus spinus)* to manage fouling algae in *ex situ* raceway grow-out of juvenile *Acropora tenuis.* Shafir et al. (2006), developed a resident school of *Siganus rivulatus* fishes and sea urchins *Diadema setosum* in their floating *in situ* coral nursery. Indeed, the recruitment of a diverse assemblage of herbivorous fish and grazing invertebrates has been shown to significantly reduce fouling in reef-based nurseries, potentially eliminating the need for manual cleaning of farm structures and outweighing the potential losses from corallivory (Knoester et al., 2024; Knoester et al., 2019; Knoester et al., 2023).

Ex situ coral tanks volumes impose limits on the size and number of grazers that can be supported, making research into the comparative efficacy and dietary preference of some common aquarium grazers valuable for building an effective grazing population. For example, Ng et al. (2014) examined biofilm development and dietary preferences in single and multi-choice experiments with the gastropod *Trochus maculataus* and the sea urchin *Salmacis sphaeroides*. They found that while the urchin promoted assemblages dominated by turf algae and bare tile, the gastropod primarily targeted filamentous green algae, resulting in an even distribution of other functional groups. *S. sphaeroides* also displayed different feeding preferences when offered common fouling algae in single or multi-choice experiments, influencing its suitability as a grazer depending on the existing algal composition in a coral production system. Although Watson et al. (2018) did not investigate

feeding selectivity, they did compare feeding amount by weight and survival for three gastropod species (*Turbo bruneus, Tectus fenestratus* and *Tegula eiseni*) in aquarium systems, identifying *T. bruneus* as most effective cleaner on the basis of higher survival and grazing activity. Factors such as these are crucial when building a grazing population for *ex situ* grow-out systems, alongside the potential effects of grazers on the corals themselves.

Although evidence for the effectiveness of co-culture of adult corals and herbivorous grazers in *ex situ* systems is largely anecdotal, the culture of coral recruits with microherbivores has been studied more rigorously. Omori (2005) first noted that corals favourably recruited to and exhibited high survival in *Trochus niloticus* aquaculture enclosures compared to the surrounding reef in the shallow waters of Miyako Island. This was attributed to the sheltered, low-sediment, high-food environment created by the design of the boxes, and the grazing by the juvenile gastropods controlling the development of competitive algae (Omori et al., 2007). Other species of grazers have been trialled in a variety of coral-aquaculture systems as a method of algae control. Juvenile sea urchins *Salmacis sphaeroides* (Toh et al., 2013a), *Lytechinus vareigatus* (Serafy et al., 2013) *, Mespilia globulus* (Craggs et al., 2019) and *Tripneustes gratilla* (Barrows et al., 2023), as well as herbivorous gastropods *Trochus niloticus* (Villanueva et al., 2013)*, Trochus maculatus* (Toh et al., 2013a), *Calthalotia strigata* (Neil et al., 2021)*, Turbo haynesi* (Neil et al., 2021)*, Lithopoma americanum* and *Batillaria minima* (Henry et al., 2019) have all been tested as potential co-culture species with coral recruits or juveniles. These studies consistently reported that co-culturing corals with grazers *ex situ* improved survival rates, increased growth rates, and enhanced overall health metrics of corals compared to no-grazer controls (Craggs et al., 2019; Henry et al., 2019; Serafy et al., 2013; Toh et al., 2013a; Villanueva et al., 2013).

While Serafy et al. (2013) found that under human-cleaning , growth of *Acropora cervicornis* was higher than in the urchin co-culture, they noted that the reduced labour costs associated with the use of grazers provided a cost-effective trade-off. Similarly, Henry et al. (2019) found that the time required to clean tanks with snails was less than half the time it

took to clean tanks maintained by an aquarist alone. These findings provide strong evidence that co-culture is a cost-effective method for increasing the yield of *ex situ* coral aquaculture.

1.4 Nutrition for corals

Many Scleractinian corals depend on their endosymbiotic algae (Symbiodiniaceae) for much of their energy and nutrition, primarily through the transfer of photosynthates from the symbiont to the host, as well as corals feeding directly off excess algal cells (Wiedenmann et al., 2023). However, corals are mixotrophs, and heterotrophic feeding is also central to their energy budgets (Davies, 1984; Goreau et al., 1971; Houlbreque & Ferrier-Pages, 2009). In their native reef ecosystems, corals readily feed on available plankton (Johannes et al., 1970; Lewis & Price, 1975; Palardy et al., 2006), bacteria (Kushmaro & Kramarsky-Winter, 2004; Sorokin, 1973), detritus and fine particulate matter (Anthony, 2000; Roff et al., 2009). In contrast, in captivity corals can be fed formulated artificial diets in the form of powders or pellets (Conlan et al., 2019; Forsman et al., 2011), cultured and enriched live feeds such as *Artemia salina* nauplii (Hii et al., 2008; Tagliafico et al., 2018), or wild harvested sources such as copepods (Ferrier-Pages et al., 2003; Palardy et al., 2006).

Providing corals with heterotrophic food sources has been shown to enhance survival and growth (Ferrier-Pages et al., 2003; Forsman et al., 2011), improve tissue condition (Houlbreque et al., 2004), and boost photosynthetic rates (Borell et al., 2008; Houlbreque et al., 2004). However, the results of feeding are complex and species dependent. For instance, feeding with rotifers was found to reduce the fecundity of *Pocillopora verrucosa* in captivity, though oocytes were larger in fed colonies (Séré et al., 2010). Meanwhile, *Galaxea fascicularis* showed no significant difference in survival and growth when fed with *Artemia* or starved (van Os et al., 2012). Similarly, *Acropora millepora* and *Pocillopora acuta* responded differently to the same diets; growth was highest in *A. millepora* provided unfiltered seawater (constituting a natural source of dissolved nutrients, suspended particulate matter and microbiota) and in *P. acuta* when provided a novel, micro-bound diet (Conlan et al., 2018a).

Interestingly, responses to diets appear to be relatively consistent within genus. Conlan et al. (2017a) found that unfiltered seawater led to highest survival and growth in five species of *Acropora* recruits when compared to a novel particulate diet or enriched rotifers. Although there have been efforts to develop more balanced diets for captive Acroporids, coral in captivity often display lipid and fatty acid profiles that indicate depleted energy reserves (Conlan et al., 2018a; Conlan et al., 2019). This is likely in part due to inadequate heterotrophic diets when compared directly to wild sourced corals (Conlan et al., 2018a). Developing diets that more closely resemble the composition of what is available on reefs could help reduce these deficits. However, while commercial or formulated diets may be less effective at promoting coral growth, they are often more cost effective due to the reduction in labour compared to collecting or culturing live-feeds (Forsman et al., 2011).

Both field and aquarium-based studies have investigated the nutritional benefits corals derive from companion organisms, providing evidence of a net positive effect on coral health (Ladd & Shantz, 2020; Shaver & Silliman, 2017). Waste products such as ammonia can be taken up, retained and recycled by corals, or may be excreted to their symbiotic Symbiodiniaceae (Muscatine & D'Elia, 1978; Rahav et al., 1989). Mokady et al. (1998) suggested that the boring bivalve, *Lithophage simplex,* normally considered a parasite, could supply a significant proportion of the ammonium requirements of its coral host *Astreopora myriophthalma*, through its nitrogenous wastes. Similarly, nutrients from excreted wastes of schooling grunts (*Haemulon flavolineatum* and *H. plumieri*) have been shown to significantly increase the growth of the corals they aggregated over during the day (Meyer & Schultz, 1985a, 1985b; Meyer et al., 1983). Fish-associated *Acropora palmata* exhibited elevated nitrogen (N) and phosphorous (P) levels in their tissues compared to corals without fish aggregates (Meyer & Schultz, 1985b), with estimates suggesting that fish could supply 30 – 75% of the maximum nitrogen fixation rate of the corals (Meyer et al., 1983). On Florida reefs, grunt aggregations created nutrient 'hotspots' where N and P delivery was respectively 10 and 7 times higher than in the surrounding reef areas, leading to a 1.5-fold increase in growth rates of *Acropora cervicornis* (Shantz et al., 2015). Interestingly, when comparing fish-derived nutrient enrichment to manipulative or anthropogenic enrichment,

fish-derived enrichment had a positive effect on coral calcification, while other types of enrichment generally had a negative effect, with the exception of manipulative phosphate enrichment, which showed a slight positive impact (Shantz & Burkepile, 2014).

Mutualistic fish that reside within coral branches can supply similar nutritional benefits to their host corals by supplying nutrient rich wastes, as well as increasing oxygenation through the diffusive boundary layer of corals via swimming or fanning their fins (Garcia-Herrera et al., 2017; Pinnegar & Polunin, 2005). *Pocillopora* species hosting *Dascyllus* spp. exhibit elevated symbiont and protein concentrations compared to non-hosting colonies, as well as improved photosynthetic efficiency during and after bleaching events (Chase & Hoogenboom, 2019; Shantz et al., 2022). *Dascyllus marginatus* damselfish positively impacted their *Stylophora pistillata* hosts by increasing the water flow between the coral's inner branches with their swimming behaviour, which leads to increased photosynthetic rates (Berenshtein et al., 2015; Garcia-Herrera et al., 2017), and also increasing growth rates of the coral (Liberman et al., 1995). Laboratory studies suggest the coral's uptake of the wastes of their resident fish, which, combined with the water movement provided by the fish fanning, may explain the benefits of hosting fish (Berenshtein et al., 2015; Garcia-Herrera et al., 2017; Holbrook et al., 2008).

However, the net effect of hosting fish is influenced by local environmental conditions. For example, in low light conditions, resident fish can enhance coral growth, whereas in high-flow areas with abundant nitrogen supply, fish presence may reduce coral growth (Chase et al., 2014). In calm, low-nutrient areas, aggregating grunts (Haemulidae) can double the amount of ammonia available to corals, making these conditions ideal for mutualistic fish-coral interactions (Meyer & Schultz, 1985a). Additionally, colony morphology and the number and size of resident fish also influence waste nutrient retention. Larger fish produce more wastes, and more open coral colonies experience faster nutrient flushing (Holbrook et al., 2008). For captive corals, where nutrients can be limited and colony size and morphology more restricted, co-culturing with schooling fish could provide valuable source of nutrition, while minimising the husbandry requirements for aquarists.

1.5 Coral pest and parasite control

Numerous predators and parasites prey on corals, ranging from well-known macro-organisms such as *Acanthaster* seastars, which can devour entire colonies in less than a day (Pratchett et al., 2014), to much smaller organisms like flatworms (Rawlinson et al., 2011) or copepods (Cheng & Dai, 2009) that are only millimetres in length. Aquaculture environments offer the ideal conditions for pests to thrive, as these conditions are typically high-density monocultures of the prey organisms, with stable environmental conditions. These settings offer a ready supply of nutrients from the feeding of the cultured prey organisms, and a lack of natural predators prevents top-down population control of the pest species. While it is simple to exclude larger pests or predators from *ex situ* environments, smaller cryptic species can often be missed in initial screenings and go on to rapidly propagate in the culture systems (Barton et al., 2020a; Barton et al., 2021).

There have been many cases of accidental predator or parasite infections in coral cultures or experimental systems. For example, Forsman et al. (2006) reported an outbreak of the corallivorous nudibranch *Phestilla sibogae* on *Porites compressa,* which significantly impacted the survival and growth of coral fragments. Adult *P. sibogae* (2-3cm) were accidentally collected and introduced with the *Porites* colonies, and though the adult nudibranchs were swiftly removed, hidden eggs masses were not discovered until a month later, resulting in a continued infection with juveniles. Even with weekly manual removal their population persisted, demonstrating the difficulty in manually controlling outbreaks in aquaculture systems (Forsman et al., 2011).

Another common coral parasite problematic in aquaria is *Prosthiostomum acroporae*, the *Acropora*-eating flatworm (Barton et al., 2019; Rawlinson et al., 2011). High-density infections of this parasite can kill adult corals, and even when infection does not result in mortality, it can severely depress the overall health of the corals (Barton et al., 2019; Hume et al., 2013). Like *P. sibogae*, this flatworm is highly camouflaged on its coral host, lays its eggs cryptically, and is very fecund, making it easy for it to infect and spread through

aquaculture systems. Although manual removal and chemical treatments to kill or control these species are possible, such methods are expensive, time-consuming and may be ineffective if not done regularly or effectively (Barton et al., 2021; Forsman et al., 2006).

Some pests, while not directly feeding on corals, can still have deleterious effects and pose significant challenges for aquarists. Vermetid gastropods, commonly called “worm snails”, are sessile invertebrates that form coiled shells and feed using a mucus net (Shima et al., 2010; Shima et al., 2013). These pests are common in aquarium systems and can reduce coral survival and cause deformed growth when in close proximity, likely due to an as-yet described mechanism of their mucus nets (Shima et al., 2010; Zvuloni et al., 2008). Another well-known pest is the *Aiptasia* anemone (<3cm diameter) which can rapidly colonise and proliferate through aquarium systems (Chen et al., 2008; Patoka et al., 2020). These anemones are problematic because they capture food intended for cultured animals and can sting other organisms or aquarists (Patoka et al., 2020). Once established, *Aiptasia* are notoriously difficult to eradicate, with ongoing manual removal through scraping, siphoning or chemical treatments possible but often time-consuming and ineffective.

Cost effective methods of prevention and treatment of both pests and parasites are essential, ideally minimising the time and materials required for husbandry. One potential low-cost method is to use biological controls, organisms that prey on pests but not on the host organism, such as the previously mentioned use of cleaner fish in salmon sea-cage culture (Bjordal, 1991; Powell et al., 2018). In the context of coral aquaculture, *Aiptasia pallida* anemones can potentially be controlled by species like Chaetodontids (e.g. *Chaetodon kleinii*) (Nakamura et al., 2011) or cleaner shrimp from the *Lysmata* genus (Calado & Narciso, 2005; Rhyne et al., 2004). While the efficacy of Chaetodontids in controlling these pests have not been thoroughly tested outside ornamental hobbyist settings, *Lysmata* shrimp have been shown to be an effective predator of *Aiptasia* in aquarium trials, both as individuals and in groups (Calado & Narciso, 2005; Rhyne et al., 2004). Although the shrimp’s efficacy in mixed systems (with species other than the shrimp and the anemones) has not been tested, there are indications that *Lysmata* shrimp will

continue to consume *Aiptasia* even when offered alternate food sources (Calado & Narciso, 2005). Additionally, larger shrimp preferred in mixed systems to reduce the risk of predation by other organisms, consume more anemones than smaller individuals (Rhyne et al., 2004). Mutualistic organisms can also help alleviate the effects of pests; for example, *Trapizia* crabs are common on branching *Pocillopora* corals and have been found to counteract the effect of Vermetid snails on coral growth (Stier et al., 2010).

Biological control of coral parasites has shown promise in aquaculture settings. Barton et al. (2020b) found that both *Pseudocheilinus hexataenia* (six-line wrasse) and *Lysmata vittata* (peppermint shrimp) can be effective predators of the Acropora-eating flatworm, although each species had limitations. *P. hexataenia* was found to consume 100% of adult flatworms on corals but not feed on egg capsules, while *L. vittata* fed on both eggs and adults though displayed more variable success rates. The coral feeding nudibranch, *Phestilla sibogae,* has been problematic in laboratory systems at the Hawaii Institute of Marine Biology, yet is rare on the surrounding reefs (Gochfeld & Aeby, 1997). This discrepancy is attributed to the absence of the nudibranch's natural predators, small crustaceans, wrasse and butterfly fish, in the laboratory tanks. When these predators were added to the tanks, they reduced survival of the pests by up to 57.3% (Gochfeld & Aeby, 1997). However, the efficacy of any control species must be carefully investigated before widespread use as it is unlikely that biological control on its own will be effective enough to completely control pests. These approaches should be as part of a multifaceted risk management strategy, alongside other control methods, such as pre-emptively treating and isolating corals in a quarantine area for a set period of time prior to their introduction into the main aquarium systems (Barton et al., 2021).

1.6 Summary and knowledge gaps

Whilst co-culture practices are widespread in coral production and holding systems, there is limited standardisation across different facilities or culturists, with many co-culture partners being selected on an ad-hoc basis or simply from what organisms are available at the time. Furthermore, there is often limited testing and/or understanding of the actual impact or

effectiveness of such pairings. A similar lack of rigour can be highlighted in the Norwegian salmon aquaculture industry, where the use of cleaner fish to control sea-lice outbreaks has been criticised in recent years as being inefficient and not producing the outcomes desired, requiring more consideration in how cleaner fish are utilised (Barrett et al., 2020; Lennox et al., 2022). As the coral aquaculture industry is in its infancy, improving our understanding of suitable conditions to maximise health of corals and improve production efficiencies is paramount. Testing the potential benefits and costs of co-culture practices is central to developing a sustainable and cost-effective coral aquaculture industry. As such, three main co-culture areas have been identified as requiring more extensive research and development to successfully integrate them into coral aquaculture.

Firstly, the interactions between algae, corals and herbivores in aquaria need to be quantified and tested in large-scale systems. This requires a comprehensive understanding of feeding preferences, survival and population dynamics of grazers within aquaria, as well as an assessment of potential impacts to the corals and the grazers themselves. An as yet unexplored question is the effect of using a succession of grazers with different dietary preferences or ecological niches to control a wider range of algae, particularly as corals grow and become more resistant to harsher forms of grazing. Ng et al. (2014) and Watson et al. (2018) have highlighted several key criteria for selecting suitable grazers for coral co-culture: (1) high survival of grazers under a range of conditions, (2) high grazing activity, and (3) consideration of dietary preferences to ensure effective control of correct algae species. Additional factors to consider are behaviour of the grazers; such as whether they spend any time out of the water (Watson et al., 2018), and the availability of the grazers near the culture site (Watson et al., 2012).

Investigating a wider range of potential grazers would also be valuable for both identifying the most cost-effective species to use and the possibility of creating two useful products from one production space. Caribbean reefs experienced losses of coral and the proliferation of macro-algae after disease outbreaks severely impacted the population of the grazing sea urchin *Diadema antillarum* (Hughes, 1994). *Diadema* cultures are typically monocultures,

thus co-culture production of corals and sea urchins could mean that both these groups can be out-planted to reefs at the same time, saving time and money for restoration efforts (Butler et al., 2024; Pilnick et al., 2021).

Secondly, the effects of schooling fish as an alternate source of nutrition for captive corals need to be thoroughly quantified. While field studies have shown increased growth rates in corals with resident fish, the long-term effects of fish-waste enrichment in captivity have not been studied; the longest aquaria study to date was performed by Chase et al. (2018) which ran for 66 days. It would be valuable to quantify differences in survival, growth and tissue composition of corals maintained in co-culture with fish compared to those fed using more traditional methods, such as *Artemia* and Rotifer live-feeds or commercially available pellets or powders. A greater variety of coral species should also be tested, as previous studies have primarily focused on branching Acroporids or Pocilloporids. The potential impact of fish on other aspects of coral health such as their microbial community should also be explored, as these topics have been relatively unstudied to date but encompass an important aspect of coral's fitness and status (Bourne et al., 2016; Krediet et al., 2013).

Finally, co-culture as a method of pest or parasite control has been used at large-scales in the salmon industry, offering valuable insights into potential challenges and trials that need to be conducted before this approach is scaled-up for coral aquaculture (Overton et al., 2020; Powell et al., 2018). The efficiency of parasite removal must be tested in replicated mixed culture systems representative of real, large-scale coral culture facilities, prior to widespread implementation of cleaner organisms. Similar to salmon aquaculture, some level of manual removal or chemical treatments will likely still be required, so studies should investigate the most effective combination of techniques (Barton et al., 2021).

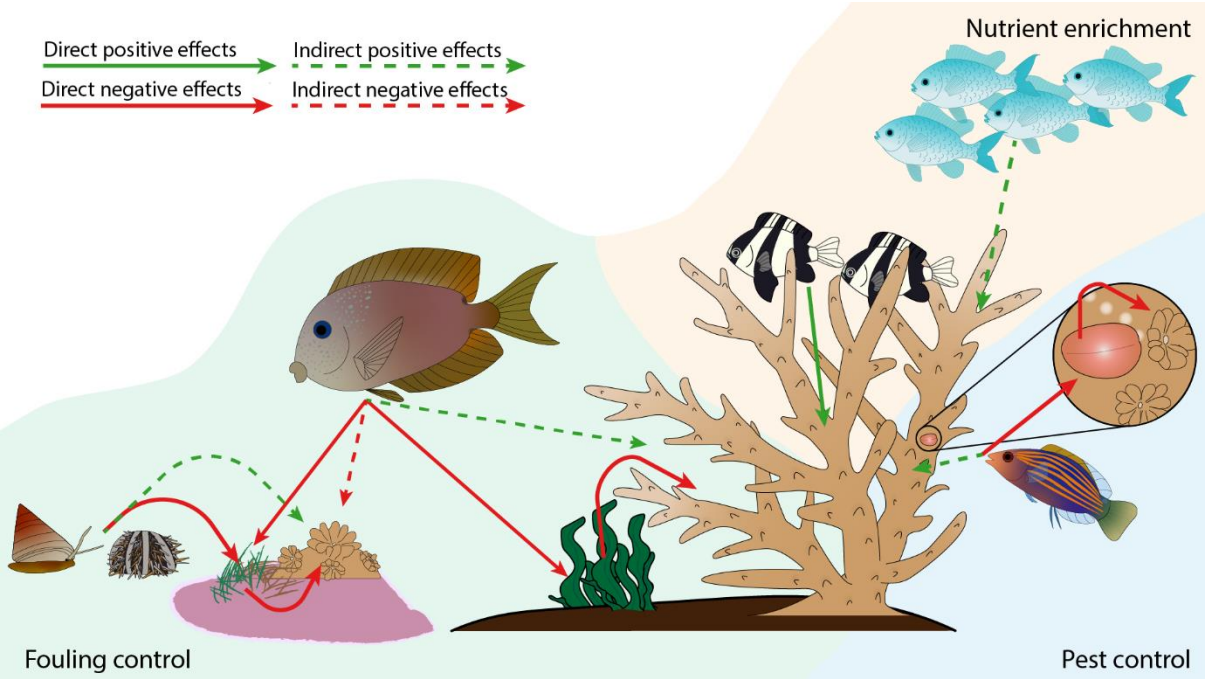

Figure 1.1: Summary of the three major potential co-culture applications in coral aquaculture. **Fouling control**: Negative impacts of algae can be mitigated by herbivores, leading to indirect positive effects on juvenile and adult corals, but larger herbivores may create indirect negative effects on younger corals by overgrazing. **Nutrient enrichment**: Schooling fish around adult colonies may have indirect or direct positive effects via nutrient enrichment and increased water flow, depending on how closely they associate with the colonies. **Pest control**: Natural predators of coral parasites can have indirect positive effects on colonies by predating on the pests in the system.

1.7 Thesis aims and objectives

Co-culture in coral aquaculture offers many areas of interest, but for this thesis, two key areas were selected: further investigating the control of fouling around coral recruits using different species of grazers at different stages of coral grow-out and exploring the potential nutrient enrichment of coral by schooling fish. These investigations aim to develop a timeline of effective co-culture companion species for different coral species and purposes.

Chapter 2 investigates the effectiveness of several types of microherbivores, including juvenile sea urchins, gastropods and hermit crabs, in comparison to human-controlled algae management across different species of coral recruits. This chapter aims to identify the most suitable coral-grazer combinations to promote recruit survival and growth, while also

evaluating the cost-effectiveness of grazer versus aquarist algae management. **Chapter 3** focuses on identifying the age and/or size at which different coral species become resistant to different types of fish grazing. It also assesses the types and quantities of algae removed by fish to help identify suitable candidates for fish and coral recruit co-culture. The findings of these first two chapters contribute to creating a detailed timeline for introducing new species of grazers into coral recruit culture to manage different types of fouling algae during grow-out.

To explore the potential for nutrient enrichment of corals by schooling fish, **Chapter 4** assesses the effects of co-culturing four coral species, which vary in their levels of heterotrophy and natural associations with fish, alongside a school of juvenile *Chromis viridis*. The corals response metrics assessed included growth characteristics and the response of the endosymbionts. These metrics were correlated to the availability of the different nutrient sources derived from both the feeds and water quality parameters, influenced by the fish (particularly nitrogen in the form of ammonia). **Chapter 5** identifies the underlying biochemical responses of *Pocillopora verrucosa* to fish co-culture, examining their proximate composition, lipid and fatty acid class composition, and associated microbial community responses to dissolved and particulate nutrients and the live feeds. Finally, **Chapter 6** synthesises the findings of this comprehensive study, integrating insights from this literature review with the data generated in the thesis chapters to construct a timeline of appropriate companions for different coral species. Chapter 6 also discusses considerations for producers, such as potential mixed species out-planting.

1.8 Thesis structure

This thesis is presented as a series of research papers formatted for journal publication. Where chapters have already been formally published, the relevant citation is provided. Due to this format, some duplication of information between chapters may occur. Supplementary materials for each chapter are included as appendices.

CHAPTER 2 - Size matters: microherbivores make a big impact in coral aquaculture

2.1 Abstract

Reef restoration activities and the ornamental trade are increasing the demand for sexually propagated corals. One challenge faced in scaling up the aquaculture production of corals is high mortality as a result of fouling organisms overgrowing coral spat, with manual removal of algae and other fouling organisms being costly and time consuming. Here I test the use of microherbivore grazers as a potential biocontrol method for fouling in coral aquaculture and compare their effectiveness to manual cleaning by an aquarist. Recruits of six coral species (*Acropora millepora, Acropora kenti, Goniastrea retiformis, Porites lobata, Dipsastraea speciosa* and *Lobophyllia corymbosa*) were reared for 112 days with aquarist cleaning, or co-cultured with gastropods (*Calthalotia strigata* or *Turbo haynesi*), sea urchins (*Tripneustes gratilla* or *Echinomentra mathaei)*, the hermit crab *Clibanarius* cf. *taeniatus* or under a control treatment with no grazers. Corals grown in the aquarist cleaning treatment displayed high survival and growth, though similar responses were observed for most coral species grown with *C. strigata* or *T. gratilla*, likely due to minimal damage via overgrazing and the promotion of relatively short turf algal communities in these treatments. However, effort required, measured as average cleaning time, was 2–3 times greater in the aquarist treatment compared to *C. strigata* or *T. gratilla* treatments. Survival of coral recruits housed with *C.* cf. *taeniatus*, *E. mathaei* or *T. haynesi* were variable, likely due to the dominance of long, filamentous turf algae in tanks with *E. mathaei,* and physical disturbance to recruits by *C.* cf. *taeniatus* and *T. haynesi*. These results demonstrate microherbivores have potential for application in aquaculture to promote production, while also reducing labour costs.

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Thesis chapter has some differences from the published manuscript in response to thesis examiner's comments.

2.2 Introduction

Sexually propagated reef building corals are in increasingly high demand for coral reef restoration and the ornamental industry (Barton et al., 2017; Leal et al., 2014). If aquaculture is to meet this demand, a broad range of species must be produced at scales comparable to those observed on healthy reef systems, in the order of $10^5 – 10^7$ juveniles/ha (Fisk & Harriott, 1990; Hughes et al., 1999; Jonker et al., 2019). To achieve mass culture at this scale, coral propagation methods need further refinement to enhance survival and reduce the costs of production (Randall et al., 2020).

A key bottleneck in scaling up captive sexual propagation of corals is high mortality of individual recruits <5-10mm diameter, within ~100 days post-settlement (Babcock et al., 2003; Doropoulos et al., 2016; Doropoulos et al., 2012; Guest et al., 2013; Randall et al., 2020). The causes of this mortality on natural reefs are varied and attributed to competition, space allocation, predation and anthropogenic impacts among others (Baird et al., 2006; Edmunds et al., 1998; Piniak et al., 2005). In aquaculture settings, the causes of early post-settlement mortality can be more readily detected, which presents opportunities to test novel approaches to improve husbandry protocols leading to increased survivorship and growth of new coral recruits. Given the Type 3 survival exhibited by many corals (highest mortality in early life stages), the potential for gains in production is likely greatest in the first 100 days after recruits settle (Doropoulos et al., 2016; Randall et al., 2020).

Competition between coral recruits and algae on settlement substrates is thought to be a key cause of mortality in coral aquaculture (Craggs et al., 2019). Biofouling algae are ubiquitous and deleteriously affect coral survival and growth via direct overgrowth, contact causing abrasion, shading, disease transfer and allelopathy (Jompa & McCook, 2003; McCook et al., 2001; Nugues & Roberts, 2003; Paul et al., 2011; Webster et al., 2015). After ~100 days post-settlement or once corals reach > 1 mm in diameter, they become resistant to many of the negative effects of fouling algae, and mortality plateaus (Doropoulos et al., 2012; Johns et al., 2018). Control of algae in recruit grow-out systems allows corals to reach a size at which the algae has minimal negative impacts on recruits and is vital to improve production of corals at scale.

Control of fouling is essential across almost all aquaculture systems, with a range of mitigation measures implemented (Fitridge et al., 2012). Manual cleaning is standard practice in coral aquaculture but is time consuming and expensive (Baria-Rodriguez et al., 2019). One way to reduce cleaning costs is to co-culture desired animals with species that graze on fouling organisms; for example, sea urchins and shrimp have been trialled as biocontrol agents in suspended scallop cultures and found to be successful at reducing fouling and potentially enhancing growth of the scallops (Dumont et al., 2009; Lodeiros & García, 2004; Zhanhui et al., 2014). Co-culture of adult corals and herbivores is a well-established method to control algal proliferation in grow-out systems (Barton et al., 2017; Forsman et al., 2006), however, large herbivores have been observed to have deleterious effects on recruits through overgrazing (Christiansen et al., 2009; Penin et al., 2010). Small herbivores, however, may be an alternative to control algae growth whilst minimising disturbance to corals recruits (Petersen & Tollrian, 2001). Under *in situ* conditions, small invertebrate herbivores have demonstrated significant grazing potential, even playing a crucial role in preventing phase-shifts in over-exploited reefs by providing functional redundancy for fished species (Altman-Kurosaki et al., 2018; Kuempel & Altieri, 2017). Therefore, these small-bodied invertebrate 'microherbivores' (Altman-Kurosaki et al., 2018), <5 cm in body length, represent potential ideal candidates for the control of fouling algae in coral aquaculture systems (Ladd & Shantz, 2020; Neil et al., 2021).

Previous studies, both *in situ* and *ex situ* nursery-based, have investigated co-culture with a range of gastropod (Henry et al., 2019; Neil et al., 2021; Omori, 2005; Toh et al., 2013a; Villanueva et al., 2013), juvenile sea urchin (Barrows et al., 2023; Craggs et al., 2019; Serafy et al., 2013; Toh et al., 2013a) and seastar (Neil et al., 2021) species as potential grazers. These studies demonstrated that microherbivores can improve the survival and growth of branching coral recruits. While the grazers tested across these studies were diverse, gastropod species primarily fed upon filamentous algae or biofilms, whilst the echinoderms preferred crustose coralline algae (CCA) or macroalgae (Ng et al., 2014). Previous studies predominantly focused on only one type of grazer (Barrows et al., 2023; Craggs et al., 2019; Henry et al., 2019; Omori, 2005; Villanueva et al., 2013), used ramets instead of coral recruits (Serafy et al., 2013) or lacked a comparison to manual control by an aquarist (Barrows et al., 2023; Craggs et al., 2019; Henry et al., 2019; Neil et al., 2021; Toh et al.,

2013a; Villanueva et al., 2013). By including multiple grazers and evaluating their effects on coral recruits relative to manual control of fouling organisms, we can derive a more comprehensive and effective assessment of the utility of microherbivores.

Cost minimisation becomes critical when supplying coral recruits and juveniles for reef restoration efforts, and for the upscaling of sexual coral production for the ornamental trade in the face of increasing restrictions on wild harvest. Minimising labour is key, as this can account for up to 56% of total hatchery costs (Baria-Rodriguez et al., 2019). This is relevant both for growing the corals themselves and supplying the microherbivores for algae control, as grazers with high requirements for their culture and care may be prohibitively expensive to apply at larger scales. In addition, it would be beneficial if herbivorous co-culture organisms could be sold as a secondary product (Craggs et al., 2019; Toh et al., 2013a).

Many species of small herbivorous gastropods such as *Calthalotia strigata* and *Turbo haynesi* are commonly found in marine aquaculture systems and are capable of reproducing with minimal husbandry, making them attractive prospects for readily available grazing companions (Watson et al., 2018). Small hermit crabs species such as *Clibanarius* sp. can remove significant amounts of algal biomass and are also relatively common in the marine ornamental trade (Altman-Kurosaki et al., 2018; Francis et al., 2019). Juveniles of sea urchins have also been suggested as potential grazers that could provide a secondary source of income, both as potential edible species such as *Tripneustes gratilla* and ones commonly sold in the ornamental industry such as *Echinometra* sp. (Craggs et al., 2019). In this study, I assessed growth and survival of recruits of six coral species in the presence of gastropod, hermit crab and sea urchin grazers compared to corals grown under manual removal and no-grazer control treatments, to better understand which microherbivores are likely to be beneficial for controlling biofouling in coral aquaculture systems.

2.3 Material and Methods

2.3.1 Grazers

Sea urchin *Echinometra mathaei* juveniles were cultured in the Australian Institute of Marine Science's (AIMS) National Sea Simulator ("SeaSim") as per the methodology presented in the Appendix B. In brief, adult urchins were spawned using 1*M* KCl injections, then larvae raised

in a roller bottle system with regular water changes, fed a mix of *Chaetoceros muelleri* (CS-176) and *Tisochrysis lutea* (CS-177) microalgae (from the CSIRO Australian National Algae Culture Collection (ANACC)). Settlement was induced at 15 days post fertilisation, using mixed CCA communities. Urchins were then reared on CCA, cultured biofilms and benthic diatoms for ~1 month, until they reached ~0.5 mm in test diameter. Juvenile *Tripneustes gratilla* sea urchins, 2 – 3 weeks post settlement ~0.3 mm in diameter, were sourced from Southern Cross University, produced following the methods described in Mos et al. (2011). Small hermit crabs (~23 mm L × 14 mm W) of the species *Clibanarius cf. taenaitus* were purchased from marine ornamental wholesaler Cairns Marine. Gastropods *Calthalotia strigata* (5 – 9 mm diameter) and *Turbo haynesi* (5 – 9 mm diameter) were collected from established populations in coral holding tanks in the SeaSim. All grazers were cleaned and checked for potential fouling on shells or spines prior to introduction to experimental tanks.

2.3.2 Coral spawning

Adult broodstock corals were collected from Falcon (-18.766483, 146.5355), Davies (-18.825622, 147.626881) and Esk (-18.763967, 146.519617) reefs in the central region of the Great Barrier Reef. They were then transported, with flow-through seawater, to the National Sea Simulator Facility where they were held in flow-through, temperature-controlled tanks, under natural lighting until spawning commenced (see Appendix B Table 1 for details of spawning).

Prior to sunset, individual coral colonies were isolated in 60 L tanks, where spawning occurred. Released gametes (egg-sperm bundles) were collected and then bundles were broken up with gentle agitation. Eggs and sperm were separated by means of a 150 µm plankton mesh and the eggs washed three times with sperm-free filtered seawater (FSW, filtered to 0.1 µm). Eggs and sperm were then combined to allow fertilisation. After fertilisation, coral larvae were cultured in 65 L conical tanks with aeration and continuous flow-through temperature controlled FSW. After ~5 days, competency assays were undertaken according to Heyward and Negri (1999). Competent coral larvae were introduced to clean 50 L tanks with trays of aragonite coral plugs (OceanWonders, 22 mm diameter). To ensure sufficient CCA coverage to induce settlement, plugs had been conditioned for ~8 weeks in established SeaSim tanks with a mix of *Trochus*, *Turbo* and

Stomatella gastropods providing algae control. Tanks were left with low water levels (~10 L) for a 2–4 h period post larvae introduction, then returned to flow-through with FSW at ~0.4 L min^{-1}. Settlement was assessed 2 days after introduction of larvae, then fragments of adult broodstock colonies were introduced to begin Symbiodiniaceae infection in recruits. These fragments remained with the recruits for 10 days, after which the adult fragments were removed and the experiment began. During the acclimation period tanks were kept in the same conditions (temperature, water flow, lighting etc.) as in the experiment, detailed below.

2.3.3 Experimental set-up

Twenty-eight 50 L tanks (60 × 30 × 33 cm) were assigned to one of seven grazing treatments: no grazers (Control), 30 *Turbo haynesi* (*Turbo*), 30 *Calthalotia strigata* (*Calthalotia*), 30 *Echinometra mathaei* (*Echinometra*), 30 *Tripneustes gratilla* (*Tripneustes*), 15 *Clibanarius* cf. *taeniatus* (*Clibanarius*), and cleaning by an aquarist (Aquarist), with four replicate tanks per treatment. Tanks were randomly placed in groups of four into 250 L water baths to help maintain temperature, then supplied FSW at 27.5°C at 0.8 L min^{-1}, providing ~1 turnover per hour (see Appendix B Table 2 for a summary of water quality). Tank outlets in the *Turbo*, *Calthalotia*, *Echinometra* and *Tripneustes* treatments were fitted with a 200 μm mesh to prevent grazers from escaping through the drain. Tanks were each fitted with one Tunze® Turbelle nanostream® 6015 to provide water circulation. Light was supplied by 28 AquaIllumination® Hydra LEDS, with an even mix of blue and white light at 100 μmol cm^{-2} s^{-1}, from 0800 – 1600 with 1-hour ramps.

Plugs with recruits of the six coral species (*Acropora millepora, Acropora kenti, Goniastrea retiformis, Porites lobata, Dipsastraea speciosa* and *Lobophyllia corymbosa*) were placed in a randomised array in replicate 77-plug trays. Due to the low number of *Lobophyllia corymbosa* recruits, two arrays were used. Control, *Calthalotia* and *Tripneustes* treatments had 17 *Acropora millepora, Acropora kenti* (previously *Acropora tenuis*, recently delineated in Bridge et al. (2023)) and *Goniastrea retiformis*, 9 *Porites lobata*, 6 *Dipsastraea speciosa*, 4 *Lobophyllia corymbosa* and 7 blank plugs (plugs conditioned in the same manner, but with no recruits settled onto them). Aquarist, *Turbo*, *Echinometra* and *Clibanarius* treatments had 17 *Acropora millepora, Acropora kenti* and *Goniastrea retiformis*, 9 *Porites lobata*, 6

Dipsastraea speciosa and 11 blank plugs (Appendix B Figure 1). One 77-plug tray of the corresponding arrangement for the treatment was then assigned to each replicate tank.

Trays with plugs and grazers were introduced to the 50 L tanks ~2 weeks post-settlement of the corals. The experiment ran for 112 days, during which tanks were fed with microalgae mix (*Nannochloropsis oceania* (CS-702)*, Chaetoceros muelleri* (CS-176), *Tisochrysis lutea* (CS-177), *Dunaliella* sp. (CS-353) *Proteomonas sulcata* (CS-412) (ANACC)) at a rate of 2000 cells mL^{-1} and HUFA enriched *Artemia* nauplii and rotifers at a rate of 0.5 individuals mL^{-1}, added as a daily pulse at 3:00pm. Cleaning was conducted twice weekly for all treatments with the walls and bottom of the tank around the tray scrubbed, then walls cleaned with a scraper to remove any encrusting algae. Care was taken to ensure the tray and grazers were not disturbed during the cleaning. Excess sediment or wastes were removed by siphoning. For the aquarist treatment, tanks were cleaned in the same manner, but the experimental trays were then removed and placed in a shallow bath containing 27.5°C FSW where the trays were then cleaned by hand using small brushes, scalpels and tweezers to remove algae on and around the plugs. Trays were then returned to their experimental tanks.

2.3.4 Data collection

High resolution images of submerged plugs and recruits were taken every 14 days using a Nikon® DSLR D810 and four Ikelite® DS161 strobes on a computer-controlled camera cart. From these photos, survival counts of corals were taken every fortnight. Coral growth was measured as the relative change in the benthic surface area of recruits ($[Area_{day\ 112} – Area_{day\ 0}]/Area_{day\ 0}$) using ImageJ software (Rasband, 2015), and was measured from the beginning (D0) to the end (D112) of the experiment. For a measure of productivity for each tank, for each coral species the mean final recruit was multiplied by the survival rate for that replicate tank. This result was then multiplied by 100, to estimate what surface area of coral would be produced if the tank started with 100 recruits, then averaged across the replicate tanks for each treatment.

To assess algae assemblages under the different grazing treatments, high resolution photographs of recruit-free “blank” plugs were analysed using Coral Point Count with Excel Extensions (CPCe) software (Kohler & Gill, 2006). Twenty random points were assigned to

each plug, and each point was then categorised as either a broad functional group: "Crustose coralline algae", "Filamentous algae", "Turf algae" or "Green endolithic algae", or more specifically where possible as: "*Dictyota* sp.", "*Bryopsis* sp.", "*Lobophora* sp." or "*Derbesia* sp.". A few non-algae fouling species were observed on the plugs, categorised as either "Sponge" or "Vermetid worms", and non-fouled areas were assigned either "Sediment" if there was sedimentation build-up or "Bare plug" (See Appendix B Figure 2 for example photos of common fouling types). These points were then converted into percentage coverage estimates for the different categories. Seven blank plugs from each tank were assessed for coverage at Day 0, 56 and 112.

Counts of living grazers were conducted monthly, while any deceased grazers were removed and recorded during bi-weekly cleaning. The total time taken to clean each tank per week was recorded to the nearest minute.

2.3.5 Data analyses

All data analyses were conducted in R (R Core Team, 2020) and RStudio v1.3.1073 (RStudioTeam, 2020). Survival data was analysed using cox mixed effects models (package: coxme, (Therneau, 2020)), with treatment as a fixed effect and replicate tank and plug as random effects, with plug nested within tank. Akaike information criterion (AIC) was used to select models with the best fit, then estimated marginal means (package: emmeans, (Lenth, 2020)) with a Tukey adjustment used for pairwise comparison of treatments. Growth, productivity, cleaning time and overall grazer survival data were similarly analysed, using a linear mixed effects model (package: lme4, (Bates et al., 2015)) in the place of a cox model, and a cube-root or log data transformation to improve model fit based on residual and Q-Q plots.

Fouling assemblages in treatments were visualised using NMDS plots, then overall comparisons at each time point performed via the ANOSIM function with 999 permutations using bray distances (package: vegan, (Oksanen et al., 2020)). Where differences were found, indicator species were identified using multi-level pattern analysis with a group equalised point biserial correlation (r.g species-site group association function) (package: indicspecies, (De Caceres & Legendre, 2009)), and pairwise comparisons made with permutational

multivariate analysis of variance (PERMANOVA) using distance matrices (adonis) with a Bonferroni correction.

2.3.6 Ethics statement

All research was conducted in accordance with the Great Barrier Reef Marine Park Authority permit (G21/38062.1).

2.4 Results

2.4.1 Grazer survival and cleaning

Initially thirty grazers per tank were added, but by the end of the experiment the densities had been reduced to 25.5 ± 1.6 *E. mathaei*, 23.6 ± 2.1 *T. haynesi*, 21.5 ± 12.6 *C. strigata* and 20.7 ± 1.1 *T. gratilla* per tank (mean ± SEM). There was no evidence of statistical differences among these densities (pairwise emmeans, $p > 0.05$, df = 12), though high variation in *C. strigata* population numbers between different tanks was observed (between 4 and 58 individuals) due to differential mortality and breeding of these gastropods. The exception was *Clibanarius* cf. *taeniatus* which was initially stocked with 15 animals per tank and subsequently declined to much lower grazer densities than the other treatments at 2.5 ± 0.5 individuals per tank at the end of the experiment (pairwise emmeans, $p < 0.05$, df = 12), though some of the replicates for *C. strigata* did have similar densities to these hermit tanks (pairwise emmeans, $p = 0.0562$, df = 12, t ratio = 3.118). By day 112, *E. mathaei* and *T. gratilla* had reached an average test diameter of 7.4 ± 0.2 mm and 10.3 ± 0.3 mm (after starting at ~0.5 mm and ~0.3 mm respectively). *T. haynesi* and *C. strigata* averaged 8.5 ± 0.1 mm (4.6 – 11.8 mm diameter) and 4.8 ± 0.1 mm (2 – 10.5 mm diameter; *C. strigata* had smaller maximum sizes due to the presence of juveniles), while the size of *C.* cf *taeniatus* were unchanged from the start of the experiment.

Cleaning time was significantly higher in the Aquarist tanks compared to all other treatments (pairwise emmeans, $p < 0.001$, df = 384), averaging 28.0 ± 1.5 mins for total cleaning required each week (Fig. 2.1). The control treatment averaged 7.6 ± 0.4 mins and only involved minimal cleaning of the walls and bottom of tanks. *Echinometra*, *Tripneustes* and *Turbo* treatments required 11.2 ± 0.4 mins, 9.8 ± 0.3 mins and 8.9 ± 0.3 mins of cleaning time respectively, which was significantly longer than the control treatments (pairwise

emmeans, $p < 0.05$, df = 384). Cleaning times for the *Calthalotia* (8.5 ± 0.3 mins) and *Clibanarius* (8.2 ± 0.3 mins) treatments were not significantly longer than the control treatment (pairwise emmeans, $p > 0.05$, df = 384). Longer cleaning times in *Echinometra*, *Tripneustes* and *Turbo* tanks is attributed to the extra effort required to avoid or move these grazers during the cleaning. *E. mathaei* and *T. gratilla* were vulnerable to accidental damage from aquarists and *T. haynesi* aggregate in corners or edges of tanks, requiring them to be moved before cleaning in that area could commence. Cleaning time in Control treatment tanks was similar to *Calthalotia* and *Clibanarius* since the majority of algal growth in tanks was concentrated on the plugs and trays, where algae had already been established, and the ease of avoiding these species when cleaning.

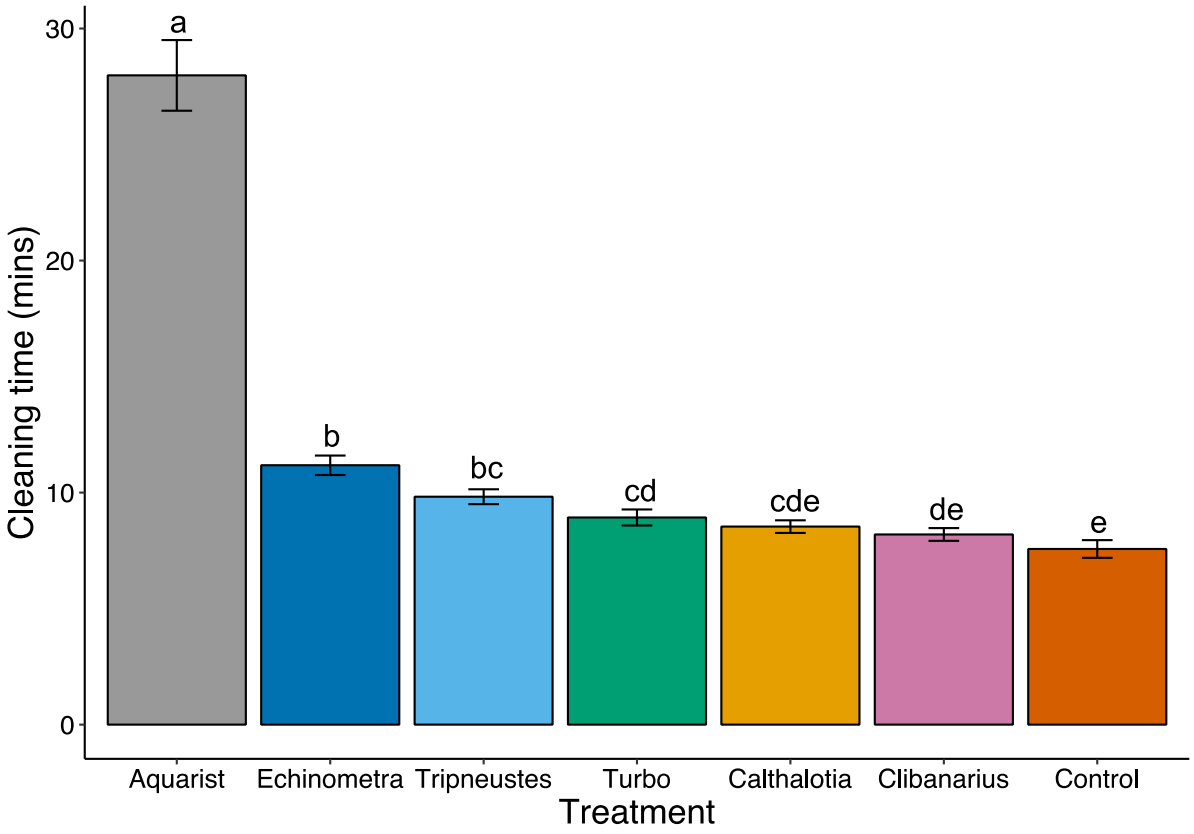

Figure 2.1: Time (minutes) required to manually clean replicate tanks of fouling organisms in different grazing treatments. Letters represent significance levels based on results from pairwise comparison of estimated marginal means of linear mixed effects model. Data are means ± SEM, n = 4.

2.4.2 Coral recruit survival and growth

Acropora millepora averaged 37.2 ± 2.34%, 36.5 ± 5.78% and 30.0 ± 9.62% (mean ± SEM) survival at 112 days under *Calthalotia*, Aquarist and *Clibanarius* treatments respectively,

which was significantly higher (pairwise emmeans, $p < 0.05$) when compared to all other grazing treatments (Fig. 2.2A). *Echinometra* produced significantly lower survival (pairwise emmeans, $p < 0.05$) for *A. millepora* than all other treatments, while there was no evidence for differences in survival (pairwise emmeans, $p > 0.05$) among Control, *Turbo* and *Tripneustes* treatments. *A. millepora* also experienced its highest growth in the Aquarist tanks compared to all other treatments (Fig. 2.3A), with a final average size of 1.5 ± 0.1 mm^2 (mean ± SEM; Table 2.1) (pairwise emmeans, df = 486, $p < 0.01$). *Clibanarius* depressed growth of *A. millepora* (pairwise emmeans, df = 486, $p < 0.01$) compared to *Calthalotia*, *Tripneustes* and *Turbo* treatments, but there was no evidence for differences in growth between the Control and any other grazing treatment (pairwise emmeans, df = 486, $p > 0.05$). The estimated surface area produced per tank (assuming starting with 100 recruits) was highest in *Calthalotia* (41.2 ± 6.7 mm^2 per 100 recruits) and Aquarist (53.5 ± 10.7 mm^2 per 100 recruits), though there was no evidence these were a significant increase compared to the other treatments (pairwise emmeans, $p > 0.05$).

Table 2.1: Basal surface area of coral recruits (mean ± se) at the start of the experiment (day 0) and after 112 days in grazing treatments.

	Acropora millepora	*Acropora kenti*	*Goniastrea retiformis*	*Porites lobata*	*Dipsastraea speciosa*	*Lobophyllia corymbosa*
Day 0 (mm^2) (pooled from all treatments)	0.915 ± 0.004 n = 2267	0.916 ± 0.005 n = 1833	0.199 ± 0.002 n = 1018	0.153 ± 0.001 n = 579	0.302 ± 0.004 n = 388	0.630 ± 0.034 n = 61
Day 112 (mm^2)						
Aquarist	1.474 ± 0.070 n = 114	3.907 ± 0.316 n = 73	11.130 ± 1.164 n = 84	1.142 ± 0.193 n = 32	11.368 ± 0.992 n = 33	na
Calthalotia	1.102 ± 0.058 n = 109	4.075 ± 0.602 n = 54	24.301 ± 2.415 n = 3	na	13.431 ± 7.533 n = 4	50.597 ± 8.974 n = 9
Clibanarius	0.781 ± 0.030 n = 85	0.802 ± 0.057 n = 49	na	na	na	na
Control	0.860 ± 0.035 n = 65	1.866 ± 0.159 n = 62	5.467 ± 0.787 n = 38	0.228 ± 0.034 n = 18	3.288 ± 0.524 n = 14	3.841 ± 0.856 n = 8
Echinometra	0.722 ± 0.047 n = 6	1.308 ± 0.241 n = 18	6.185 ± 1.426 n = 41	na	6.815 ± 1.501 n = 36	na
Tripneustes	1.089 ± 0.054 n = 59	3.064 ± 0.430 n = 38	39.764 ± 5.437 n = 66	1.110 ± 0.333 n = 10	33.147 ± 7.505 n = 23	83.192 ± 11.931 n = 7
Turbo	1.259 ± 0.143 n = 55	2.247 ± 0.279 n = 64	13.248 ± 2.681 n = 25	2.746 ± 0.654 n = 5	36.270 ± 9.189 n = 22	na

After 112 days, *Acropora kenti* experienced highest survival in the Aquarist treatment (36.2 ± 3.48%), which was significantly higher than in all other treatments (pairwise emmeans, $p < 0.05$) except *Calthalotia* (23.0± 1.78%) and Control (26.0 ± 4.64%) (pairwise emmeans, $p > 0.05$). *Calthalotia* and Control, however, only represented a moderate increase in survival compared to the *Echinometra* and *Clibanarius* treatments (pairwise emmeans, $p < 0.05$) (Fig. 2.2B). Relative growth of *A. kenti* across treatments was highest in the Aquarist treatment, though this was not significantly higher than growth observed in the *Calthalotia* (pairwise emmeans, $p = 0.6289$, df = 346, t ratio = 1.682) or *Tripneustes* (pairwise emmeans, $p = 0.3491$, df = 348. T ratio = 2.110) treatments, with final mean sizes of 3.9 ± 0.3 mm^2, 4.1 ± 0.6 mm^2 and 3.1 ± 0.4 mm^2 respectively (Table 2.1; Fig. 2.3B). Growth in these treatments was higher than in the Control, *Echinometra* and *Turbo* treatments (pairwise emmeans, $p < 0.05$), though growth in the *Tripneustes* treatment was not significantly different from the *Turbo* tanks (pairwise emmeans, $p = 0.0506$, df = 348, t ratio = 2.962). Similar to *A. millepora*, productivity was greatest in Aquarist (137.6 ± 21.4 mm^2 per 100 recruits), which was significantly higher than all other treatments except *Calthalotia* (92.6 ± 31.5) (pairwise emmeans, $p = 0.5659$, df = 18, t ratio = 1.797).

The highest survival of *Goniastrea retiformis* recruits was observed in the *Tripneustes* treatment (48.3 ± 15.3% at 112 days), which was greater (pairwise emmeans, $p < 0.001$) than survival in all other treatments except the Aquarist (43.0 ± 12.9%; Fig. 2.2C). No *G. retiformis* recruits survived to 112 days under the *Clibanarius* treatment. *Tripneustes* also produced the highest average growth of *G. retiformis* recruits (39.8 ± 5.4 mm^2 final size; Table 2.1), significantly higher (pairwise emmeans, $p < 0.0001$) than all other treatments, with the exception of recruits subjected to *Calthalotia* grazing (24.3 ± 2.4 mm^2, pairwise emmeans, $p = 0.9994$, df = 231, t ratio = -0.334). However, growth in the *Calthalotia* treatment was not significantly different to growth in the other treatments (pairwise emmeans, $p > 0.05$) (Fig. 2.3C). Productivity was also highest in *Tripneustes* (1,868.1 ± 1,036.4 mm^2 per 100 recruits), though there was no evidence this was significantly higher than in the other treatments with surviving recruits (pairwise emmeans, $p > 0.05$).

Porites lobata survival at 112 days was significantly higher in the Aquarist treatment (42.2 ± 7.27%) compared to all treatments with grazers (pairwise emmeans, $p < 0.05$). Though average survival was also higher in Aquarist than the Control (14.3 ± 10.1%), there was no significant difference between these two treatments (pairwise emmeans, $p = 0.2669$, z ratio = -2.254) (Fig. 2.2D). *Calthalotia* and *Tripneustes* treatments had similar survival curves to the Control (pairwise emmeans, $p > 0.05$), while *Clibanarius*, *Echinometra* and *Turbo* all had significantly lower survival than the Control treatment (pairwise emmeans, $p < 0.05$). Though the rate of mortality did vary, *Calthalotia*, *Clibanarius* and *Echinometra* all ended the experiment (112 days) with no surviving *P. lobata* recruits. All treatments with surviving recruits where algae were removed (Aquarist, *Tripneustes* and *Turbo* treatments) displayed higher growth compared to the Control (pairwise emmeans, $p < 0.05$), though there were no differences among the grazed treatments themselves (pairwise emmeans, $p > .05$) (Fig. 2.3D), with final respective basal surface areas of 1.1 ± 0.2 mm^2, 1.1 ± 0.3 mm^2 and 2.8 ± 0.67 mm^2 (Table 2.1). Productivity was also higher in Aquarist (46.4 ± 19.2 mm^2 per 100 recruits), *Turbo* (45.8 ± 0 mm^2 per 100 recruits) and *Tripnesutes* (34.3 ± 13.2mm^2 per 100 recruits) than Control (4.4 ± 2.4 mm^2 per 100 recruits), though there was no evidence for differences between the treatments (pairwise emmeans, $p > 0.05$).

For *Dipsastraea speciosa,* the Aquarist treatment displayed the highest average survival at day 112 (62.0 ± 13.4%), though this was not significantly higher compared to treatments *Echinometra* (57.2 ± 19.8%; pairwise emmeans, $p = 0.9999$, z ratio = -0.310), *Tripneustes* (54.5 ± 18.6%; pairwise emmeans, $p = 0.9998$, z ratio = -0.378) or *Turbo* (41.9 ± 21.2%; pairwise emmeans, $p = 0.0570$, z ratio = -2.903) (Fig. 2.2E). However, all these treatments, except *Turbo*, did display significantly higher survival compared to the Control (36.9 ± 22.1%) (pairwise emmeans, $p < 0.05$). No *D. speciosa* recruits survived to day 112 under the *Clibanarius* treatment. *D. speciosa* growth was significantly higher (pairwise emmeans, $p < 0.05$) in the Aquarist (final mean size 11.4 ± 1.0 mm^2), *Tripneustes* (33.1 ± 7.5 mm^2) and *Turbo* (36.3 ± 9.2 mm^2) treatments compared to *Echinometra* and Control (Table 2.1). However, growth was not statistically different among Aquarist, *Calthalotia*, *Tripneustes* or *Turbo* treatments (pairwise emmeans, $p > 0.05$) (Fig. 2.3E). Similarly, though there was no evidence for statistical differences (pairwise emmeans, $p > 0.05$), productivity was highest in

Tripneustes (3011.8 ± 1270.8 mm^2 per 100 recruits) and *Turbo* (2353.4 ± 1938.9 mm^2 per 100 recruits).

Survival of *Lobophyllia corymbosa* was highest in *Tripneustes* (40.0 ± 17.0%) and *Calthalotia* (39.4 ± 14.2%) treatments and lowest in the Control treatment (10.0 ± 5.77%) though differences were not statistically significant (pairwise emmeans, $p > 0.05$)(Fig. 2.2F). *L. corymbosa* growth in *Tripneustes* and *Calthalotia* treatments was significantly greater compared to the Control treatment (pairwise emmeans, $p < 0.05$), though not statistically significant between the individual grazer treatments (pairwise emmeans, $p = 0.0702$, df = 18.7, t ratio = -2.375), with corals in *Tripneustes* and *Calthalotia* treatments growing to 83.2 ± 11.9 mm^2 and 50.6 ± 9.0 mm^2 respectively (Table 2.1; Fig. 2.3F). *Tripneustes* productivity (4518.8 ± 815.5 mm^2 per 100 recruits) was also significantly higher than the Control (pairwise emmeans, $p = 0.0486$, df = 3.91, t ratio = -3.636), though not than *Calthalotia* (2891.4 ± 943.2 mm^2 per 100 recruits; pairwise emmeans, $p = 0.4644$, df = 3.91, t ratio = -1.285).

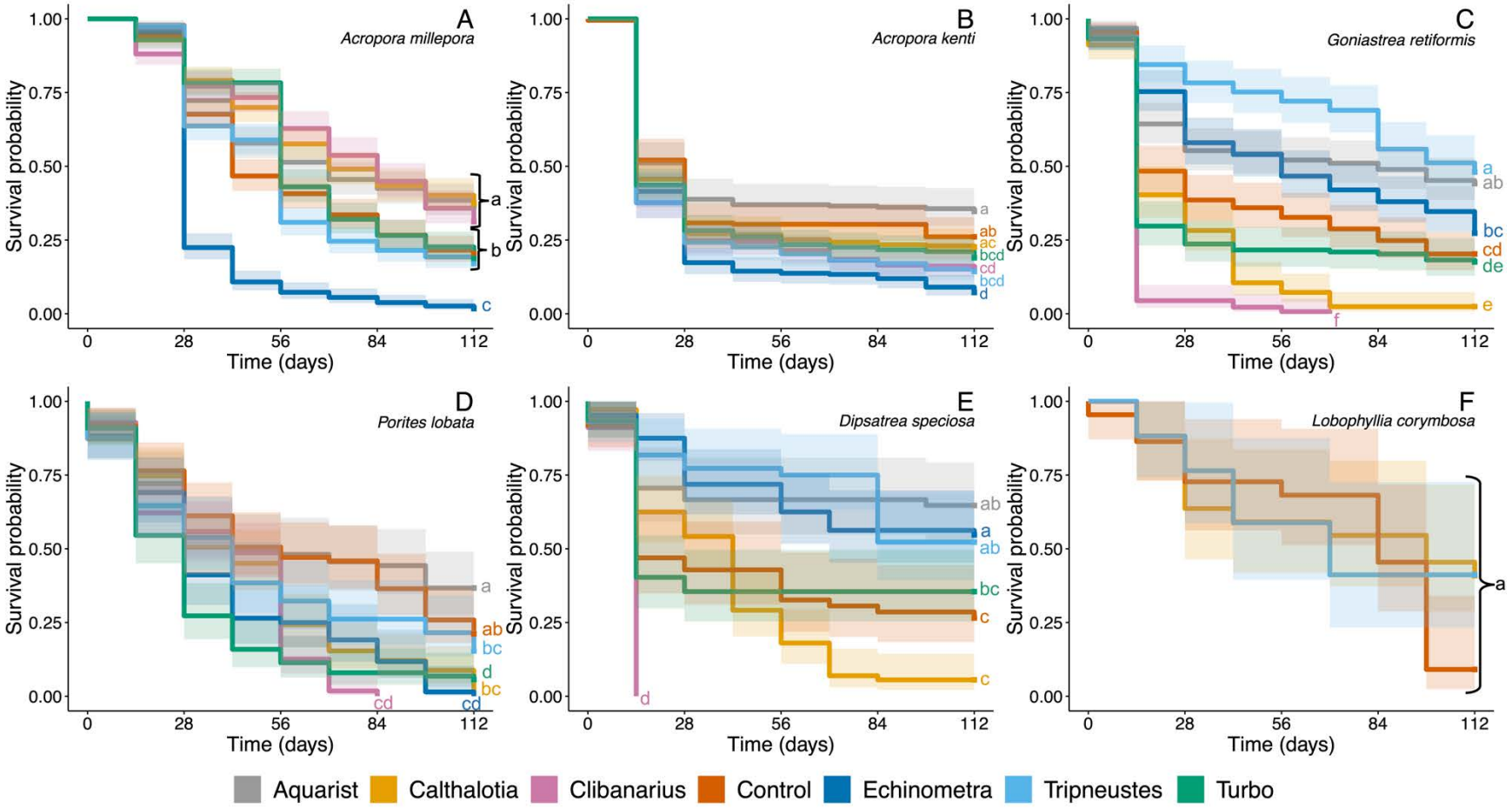

Figure 2.2: Kaplan-Meier survival plot with 95% confidence intervals of coral recruits under different grazing treatments from day 0 – 112; A) *Acropora millepora*, B) *Acropora kenti*, C) *Goniastrea retiformis*, D) *Porites lobata*, E) *Dipsastraea speciosa* and F) *Lobophyllia corymbosa*. Annotations indicate significance levels from pairwise comparison of estimated marginal means of cox mixed effects models.

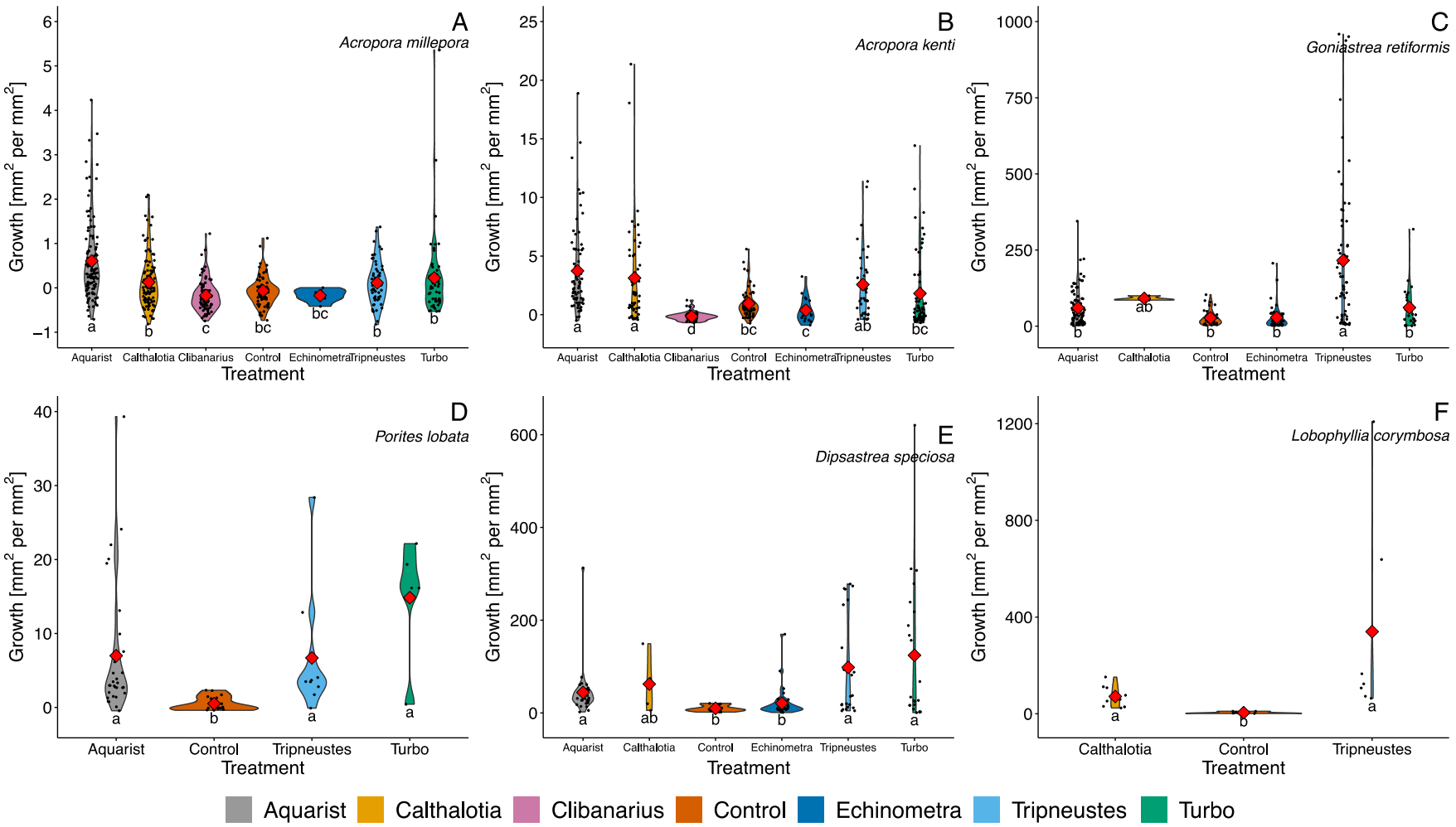

Figure 2.3: Violin plots of relative growth of coral recruits under different grazing treatments from day 0 to 112. A) *Acropora millepora*, B) *Acropora kenti*, C) *Goniastrea retiformis*, D) *Porites lobata*, E) *Dipsastraea speciosa* and F) *Lobophyllia corymbosa.* Growth is measured as relative change in the benthic surface area of recruits ($[Area_{day\ 112} - Area_{day\ 0}]/Area_{day\ 0}$) with mean growth indicated by the red diamond. Annotations indicate significance levels from pairwise comparison of estimated marginal means of linear mixed effects models.

2.4.3 Fouling assemblages

Algae coverage did not vary among treatments at the start of the experiment (ANOSIM statistic R -0.01453, sig 0.9583), with plugs dominated by CCA communities (64.6 ± 2.75%), primarily *Mesophyllum* sp. with some *Lithophyllum* sp. also present. However, by day 56, differences were observed among the grazing treatments (ANOSIM statistic R 0.2543, sig 0.0001) (Fig. 2.4). CCA was abundant in all treatments (>30% surface area) and plug surfaces that were relatively bare but impregnated with endolytic green algae were also common (>10%). Algal turf communities were apparent in all treatments except *Calthalotia* and *Clibanarius*, containing a wide variety of genera (e.g., *Chaetomorpha*, *Audouninella*), but typically short in height (<0.5 cm). Filamentous algae and bare tile surface were also observed across all treatments, though Control, *Tripneustes*, *Echinometra* and *Turbo* treatment tanks had higher densities of filamentous algae (~9 – 18%) compared to the other treatments (multipatt p = 0.0002). Communities in *Calthalotia* and *Clibanarius* were similar, due to high coverage of CCA (66.1 ± 3.3% and 71.4 ± 3.0%, multipatt p = 0.0001) in these treatments. Aquarist, Control and *Echinometra* had similar fouling assemblages (pairwise adonis adjusted p-value > 0.05), driven by high levels of algal turfs (>20%, multipatt p = 0.0001). Finally, *Echinometra* tanks were found to have similar levels of bare plug compared to the *Tripneustes* treatment (12.8 ± 3.0% and 22.0 ± 2.8%, multipatt p = 0.0001), which was likely responsible for driving their similarity (pairwise adonis adjusted p-value > 0.05).

At day 112, differences in fouling assemblages among treatments were observed (ANOSIM statistic R 0.2736, sig 0.0001) and the overall composition had changed (Fig. 2.4). CCA was abundant in all treatments (>30%), though had significantly higher levels in *Clibanarius* and *Turbo* tanks (62.9 ± 3.4% and 64.1 ± 2.8%) compared to other treatments (multipatt p = 0.0001). Areas with endolithic green algae were present in all treatments, covering ~10 – 25% of plug surfaces. The only treatments with significant coverage of algal turfs were the Aquarist (39.6 ± 6.2%) and Control (24.1 ± 6.6%) treatments (multipatt p = 0.0001), though turfs in the Control treatment would be characterised as longer, denser turfs (long sediment-laden algae turfs: LSAT), whilst Aquarist turfs were shorter, less dense communities (short productive algal turfs: SPAT) (Goatley et al., 2016; Tebbett & Bellwood, 2019). *Echinometra* and *Tripneustes* (pairwise adonis adjusted p-value = 0.294) treatments maintained higher areas of bare plug compared to other treatments (28.8 ± 2.5% and 27.3 ± 2.7%, multipatt p =

0.0001), similar to observations at day 56. Three of the *Calthalotia* tanks also had high levels of CCA coverage (61.9 ± 3.9%), however, one replicate in this treatment experienced an outbreak of the brown macroalgae *Dictyota* sp., which resulted in dissimilarity from the other CCA dominated tanks (multipatt p = 0.0001). When this tank was removed from the analysis, fouling assemblages from *Calthalotia* were similar to the *Turbo* and *Clibanarius* treatments (pairwise adonis adjusted p-value > 0.05).

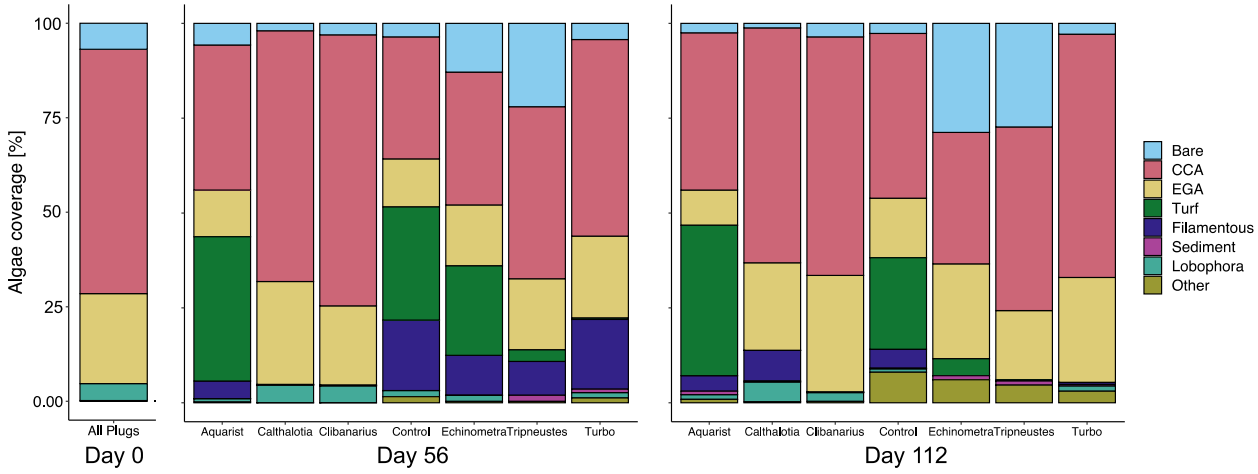

Figure 2.4: Stacked barplots summarising coverage of algae assemblages on blank tiles housed in the seven grazing treatments at day 0, day 56 and day 112. Day 0 summarises all plugs, as there were no differences detected between the different treatments at this timepoint. For day 112, points from *Calthalotia* tank 1 have been removed due to *Dictyota* sp. outbreak. Fouling categories "Bare" = bare tile, "CCA" = Crustose coralline algae and "EGA" = Endolithic green algae. Note, fouling categories "*Dictyota* sp.", "*Bryopsis* sp.", "Sponge" and "*Vermetid* sp." have been grouped into "Other" due to their low coverage.

2.5 Discussion

2.5.1 Drivers of coral recruit mortality

Control of algae by regular physical removal has been demonstrated to increase survival and growth of corals in culture (Serafy et al., 2013). In this study, coral recruits faced potential negative interactions with macroalgae, turf-algal complexes, or encrusting calcareous algae, with the highest survival and growth across coral species most frequently recorded where the aquarist controlled the competing biofilm communities. Importantly, a number of other grazing treatments also demonstrated a marked influence on the algal competitors, with improved coral survival and growth relative to ungrazed controls. Overall, larger Acroporids performed well under *C. strigata* grazing, whilst the smaller *G. retiformis* and *D. speciosa*

recruits did better under *T. gratilla.* Mid-size *L. corymbosa* recruits experienced high survival and growth with both *C. strigata* and *T. gratilla* grazer species. *P. lobata*, which were the smallest recruits, displayed lower survival in the grazer treatments compared to the Control and Aquarist treatments, though still high growth under aquarist, *T. haynesi* and *T. gratilla*. The effectiveness of the different grazing treatments on the growth and survival of the various coral species is likely attributed to several factors, including the influence of grazers in structuring the algae assemblages which compete with the coral recruits, the vulnerability of corals to disturbance from the grazers themselves, and how size of the coral recruits interacts with both these factors.

2.5.2 Role of grazers in structuring algal assemblages

Experimental treatments *Calthalotia*, *Tripneustes* and Aquarist generally displayed high survival and growth of recruits of all the coral species, though each treatment resulted in distinct algal communities on the associated settlement plugs. *Calthalotia* tanks were typically dominated by the CCA *Mesophyllum* sp. and *Lithophyllum* sp.. Though CCA is capable of overgrowing and killing coral recruits (Buenau et al., 2012; Craggs et al., 2019), field studies have shown that some species including *Lithophyllum* sp. can promote coral survival by alleviating competition with turf algae (Jorissen et al., 2020). *Tripneustes* grazing produced substrates with large areas of bare plug, which similarly reduces competition with fouling organisms, promoting coral survival and growth (Knoester et al., 2019; Serafy et al., 2013). Though Aquarist tanks were dominated by algal turfs, these were shorter and less dense than turfs known to have a negative impact on coral success (Birrell et al., 2005; Goatley et al., 2016; Tebbett & Bellwood, 2019). Generally therefore, the *Calthalotia*, *Tripneustes* and Aquarist treatments created algal assemblages that likely minimised negative interactions that impact coral performance, instead promoting the growth of short algal turfs with few filaments that could abrade, overshadow or overgrow the recruits (McCook et al., 2001; Titlyanov et al., 2007).

2.5.3 Size escape thresholds critical for coral recruit survival

Size-escape thresholds likely played a role in structuring recruits' responses to the algae and grazing pressures in the current study. Previous research has shown that as coral recruits grow larger, the likelihood of algae causing mortality lessens (Doropoulos et al., 2012; Johns

et al., 2018). For example, *Lobophora* seaweeds inhibit settlement and survival of early recruits, though have little effect on coral survival once recruits are >1 mm in size (Johns et al., 2018). In this study, mortality of the recruits was highest in the first 30 days of culture, but once past this threshold, relatively stable survival rates were observed for all treatments. This suggests that in the early stages of culture, particularly for the smaller recruits such as *P. lobata*, interaction with grazers was likely more important as a driver of survival rather than the algae assemblage. The lack of effect of algae in the early stages of the experiment may be a result of the relatively clean surfaces at the start of the experiment (dominated by CCA and EGA) and the relatively high density of macroinvertebrate grazers within the tanks (initially ~40 m^2) compared to natural grazing conditions on the GBR (e.g. 5.2 m^2 from Klumpp and Pulfrich (1989)).

Grazers can have deleterious effects on coral recruit survival and growth due to damage associated with their grazing behaviour (i.e. bites), which is often directly related to body size of the grazers and the recruits (Christiansen et al., 2009; Do Hung Dang et al., 2020; Doropoulos et al., 2012; Korzen et al., 2011; O'Leary et al., 2013). The smallest recruits in this experiment (*G. retiformis*, *D. speciosa* and *P. lobata*) all experienced low survival under the *Clibanarius* treatment, despite the relatively low number of *C.* cf. *taeniatus* surviving to the end of the experiment (~2.5 per tank). *C.* cf. *taeniatus* were the largest herbivores used here and are particularly voracious grazers (Altman-Kurosaki et al., 2018). This potentially explains their negative effect on survival of the smaller recruits, as they produced algal assemblages similar to *C. strigata* tanks (CCA dominated), but recruits experienced more abrupt, earlier mortality in the *C.* cf. *taeniatus* tanks. Inversely, of the microherbivores tested here, one of the two smallest grazers, *Tripneustes gratilla* sea urchins, produced high survival for the smaller coral recruits. The smaller size of these urchins and therefore more limited disturbance to the recruits, could have contributed to this increased survival. Indeed, *P. lobata*, the smallest recruits in this experiment, had highest survival in the two treatments with minimal direct disturbance to the recruits, i.e. the Aquarist and Control treatments. These two treatments had different algae assemblages; Control being dominated by long, more sediment and filamentous algae laden turfs whilst Aquarist had shorter, less dense ones, suggesting that the primary driver of survival in the smaller *P. lobata* recruits was disturbance from grazers, not algae.

Though Control and Aquarist treatments typically had higher survival for smaller recruits, growth from day 0 to day 112 in these treatments was equalled or exceeded by growth under a grazing species. Thus, for smaller coral species, overgrazing pressures may pose a significant threat in the first 1–2 months post-settlement, but beyond this point the presence of grazers appears to have growth benefits for any surviving corals. For coral grow-out facilities, identifying the appropriate time to add grazers will be vital to minimise husbandry costs while maximising growth.

Coral recruits that are characterised by larger early life-stage size displayed higher resistance to grazing pressure. For example, *A. millepora* displayed similar survival rates between the Aquarist treatment and treatments with an increasing potential for disturbance from grazers, *Calthalotia* and *Clibanarius*, from the early stages of the experiment. These larger recruits instead seemed more vulnerable to algae in their earlier life stages since *A. millepora* had low survival by day 56 under both the Control and *Echinometra* treatment, both of which were characterised by a high prevalence of long, sediment laden turfs and filamentous algae (Birrell et al., 2005; McCook et al., 2001). This is similar to previous studies, that have found that until recruits reach certain sizes, they remain vulnerable to deleterious algae (Buenau et al., 2012; Johns et al., 2018). After this point, though survival was not impacted, growth was still promoted under the treatments Aquarist and *Calthaltoia,* that facilitated shorter, less deleterious algal communities (Goatley et al., 2016; Jorissen et al., 2020; Tebbett & Bellwood, 2019). Therefore, the application of grazers to control fouling is beneficial for larger (>0.6 mm^2) recruits to promote early survival and remains beneficial for growth during grow-out.

2.5.4 Factors to consider for up-scaling biocontrol with microherbivores

Combining microherbivore grazing with low-level aquarist cleaning is a model proposed by Serafy et al. (2013) to facilitate large-scale culturing of corals. Though aquarist-only cleaning can produce higher survival and growth of coral recruits, associated labour costs would be a significant burden for large-scale culturing. In this experiment, aquarist cleaning required 2–3 times greater time investment compared to grazed treatments, but did not result in a notably higher culture efficiency. For example, *A. millepora* recruits had similar average

survival under aquarist cleaning and *C. strigata* grazing treatments, and only a marginal increase in final recruit size (1.47 vs 1.10 mm^2). Aquarist tanks however required 28 minutes of cleaning per week, compared to 8.5 minutes for *C. strigata*'s tanks. Based on minimum hourly wage of $14.59 USD[2], aquarist tanks require an additional weekly investment of $4.74 USD per 50L tank to achieve similar results to *C. strigata* grazing. This equates to an additional $247 USD per 50 L tank per year: this would quickly balloon to a significant cost to producers if coral aquaculture is expanded to large-scale production. For example, the upcoming KAUST Coral Restoration Initiative aims to produce 40,000 corals annually in a 1,000 m^2 pilot facility (King Abdullah University of Science and Technology, 2023). Our 50 L tanks were ~0.8 m^2, thus applying aquarist cleaning across a 1000 m^2 facility would equate to an annual investment of ~300,000 USD just for algae control. Though *C. strigata* culture is relatively easy to incorporate into coral culture systems due to low maintenance costs and ease of reproducing in captivity, it will nevertheless require some husbandry resources that need to be factored into costs of the system. In addition, if using multiple species in parallel or at different life-stages of culture, the benefits need to be further calculated in large-scale systems with financial viability always being central to incorporating grazers in large-scale coral aquaculture systems.

An additional consideration for the use of microherbivores is the type of fouling species they can control, with a single species being unable to control all types of fouling algae. For example, there was an outbreak of *Dictyota* sp. in one of the *C. strigata* tanks, demonstrating the inability of this gastropod to control this noxious brown macroalgae (Paul et al., 2011). Potentially deleterious vermetid snails (Shima et al., 2010; Shima et al., 2013) and the brown algae *Lobophora* sp. (Vieira, 2020) were also observed in this study, though not at problematic levels. As such, increased targeting of potentially uncontrolled pest species in co-culture by either an aquarist or the introduction of a second grazing species that feeds upon it should be considered. While previous studies have reported that mixed grazing communities can be less effective (Lodeiros & García, 2004; Neil et al., 2021) or have little additional benefits to single species (Atalah et al., 2016), sequential application of

[2] based on Australian Aquaculture industry award rate of $22.61 AUD for full time, ≥ 20 year old, level 1, 16th August 2022 https://calculate.fairwork.gov.au/

different types of grazers may be a better solution. In this experiment, I observed that smaller recruits had higher survival under treatments with low disturbance (i.e. aquarist or urchins, the smallest grazers) in the early stages, while still showing improved growth by day 112 under treatments with larger grazers (e.g. *T. haynesi*). In a sequential grazer application, algae control in the first 1 – 2 months post-settlement could be provided by an aquarist or juvenile sea urchin such as *T. gratilla* to increase survival, then larger grazers such as *T. haynesi* introduced post 2-months to assist with more vigorous algae control, once the recruits have grown large enough that over-grazing becomes a less substantial threat.

For scaling-up of grazer use in coral-aquaculture, we must consider what coral species would be produced, as these results indicate grazer applications must be tailored to different species. Results from the present study suggest co-culture benefits from the gastropod *Calthalotia strigata* for Acroporid species, sea urchin *Tripneustes gratilla* for *G. retiformis,* and both sea urchins and gastropod *Turbo haynesi* for *D. speciosa*. In restoration operations, production typically focuses on fast-growing branching species like Acroporids to mass produce large numbers of propagules to plant on reefs (Bostrom-Einarsson et al., 2020; Randall et al., 2020). As such, the application of a gastropod like *Calthalotia strigata* would be ideal, as I have shown that it can enhance survival and growth of Acroporid recruits and is easy to maintain in tanks as it is self-sustaining. This species also stays relatively small (<1 cm shell length), thus could be reused in different grow-out systems. In the ornamental industry, where production is typically focussed on smaller-scale output of high-value pieces (i.e. attractive colour morphs, species with restricted harvesting) like *Scolymia* sp., a higher level of investment into grazing could be justified due to the higher potential return from individual corals and potentially from grazers themselves (Barton et al., 2017). For example, juvenile sea urchins require more labour to produce than snails, but demonstrate greater potential to increase recruit survival and growth of smaller species like *G. retiformis* and could be sold as a secondary product on the ornamental market.

The density at which grazers are applied, thus how many would need to be produced, should also be a consideration. Our initial density of ~40 grazers per m^2 of tank space serves as a good starting point for culture facilities, but the final density of grazers within tanks had dropped to varying levels by the end of the experiment. Self-sustaining populations of

grazers like *C. strigata* would likely reach an equilibrium point during the grow-out period, but species such as *T. gratilla* may require regular 'top-ups' of their population to maintain sufficient grazer density.

Altogether, when deciding how to apply co-culture to coral recruit production we must consider (1) which species of coral will be cultured, (2) at what scale will production occur (thus how many grazers), (3) the trade-off between enhanced survival of corals and potential costs of grazer production and (4) potential secondary markets or re-use of grazers. Overall, these results indicate co-culture is a scalable, cost-effective method to improve survival rates of aquacultured corals in their early life stages.

CHAPTER 3 - Let the fish do the cropping: identifying fish grazers to improve coral aquaculture

3.1 Abstract

Controlling the growth of fouling organisms in coral aquaculture is a recognised approach to enhance survival during grow-out of recruits. Herbivorous fish can reduce algae growth, though indiscriminate grazing by the fish pose a risk to the early life stages of corals. To identify a suitable age or size to introduce fish to coral recruit culture, settlement tiles with one-week-old, single polyp and one-month-old, multi-polyp *Acropora millepora, Acropora kenti* and *Goniastrea retiformis* were exposed to "brusher", "cropper" and "concealed-cropper" fish grazers for 24 hours. In general, Acroporid recruits displayed lower mortality than *Goniastrea* recruits across all types of grazing, and younger, smaller recruits were more vulnerable to grazing, with the "brusher" fish functional group more likely to cause mortality. Grazing by the "brusher" *Ctenochaetus binotatus* resulted in the highest mortality across all treatments with week-old, single-polyp recruits experiencing 2.5% and 8.6% mortality for *A. millepora* and *A. kenti* respectively, and as high as 88.9% mortality for *G. retiformis*. In contrast, month-old Acroporids that were 2 - 7 polyps in size, displayed <1% probability of mortality when exposed to the same *C. binotatus* grazing. Grazing intensity of the fish also played a role, as fish belonging to the same functional group with higher bite rates caused higher recruit mortality. Overall, "cropper" *Acanthurus nigrofuscus* represented the best trade-off between minimising recruit mortality whilst reducing algae coverage on the settlement tiles. Based on these results, coral recruit grow-out operations would gain the most benefit by introducing fish grazers once corals reach the multi-polyp stage at >1 month old for Acroporids and other fast-growing species, and later (likely > 2 months) for slower growing species such as *G. retiformis*.

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3.2 Introduction

The demand for sustainably produced sexually propagated coral recruits is growing, due to a burgeoning ornamental trade and the need to supply reef restoration efforts globally (Barton et al., 2017; Ferse et al., 2021). Sexual production and nursery rearing of corals requires a higher initial investment but has been shown to be more cost-effective due to increased survival rates compared to asexual transplants (Baria-Rodriguez et al., 2019; Guest et al., 2023). However, the production of corals remains hampered by critical bottlenecks, including high mortality in the post-settlement grow-out phase (Randall et al., 2020). Growth of fouling organisms around recruits can be problematic in the early stages of culture, interfering with growth and increasing mortality (Birrell et al., 2008a; Jompa & McCook, 2003; Jorissen et al., 2020; Tebben et al., 2014). Crustose coralline algae (CCA), a common settlement inducer, can quickly overgrow recruits (Buenau et al., 2012; Craggs et al., 2019; Jorissen et al., 2020), and filamentous algae can colonise aquaria systems, causing reductions in coral settlement and potential overgrowth of recruits (Birkeland, 1977; Birrell, 2003). Other more persistent fouling algae species such as macroalgae *Bryopsis* sp. or *Dictyota* sp. can have allelopathic effects on different coral life stages, and potentially cause tissue loss or reduced growth via abrasion or shading (Chapter 2; (Fong et al., 2019; McCook et al., 2001; Paul et al., 2011)). Control of fouling can be achieved via labour intensive approaches such as coating plugs in anti-fouling (Roepke et al., 2022; Tebben et al., 2014) or removal by aquarists (Serafy et al., 2013), or by less labour-intensive biological solutions such as co-culture of corals with herbivores (Ladd & Shantz, 2020). In the early stages of grow-out, microherbivores have been shown to be effective at controlling successional filamentous algae and CCA, however other algae species often still require manual removal (Chapter 2; (Guest et al., 2023)).

Herbivorous fish can control algal species that are resistant to smaller grazers, both in natural and aquaria systems (Brawley & Adey, 1981; Burkepile & Hay, 2008; Hughes et al., 2007). For example, in mid-water nurseries, coral fragments benefited from the recruitment of herbivorous fish, which reduce cleaning requirements (Frias-Torres et al., 2015; Frias-Torres & van de Geer, 2015; Knoester et al., 2023). Importantly however, incidental predation by herbivores can also be a driver of mortality in young recruits (Baria et al., 2010; Penin et al., 2010; Trapon et al., 2013; Whitman et al., 2024). Guest et al. (2013) noted that

caging of *in situ* nurseries was beneficial as it excluded herbivorous fish in the early stages of grow-out, but once a suitable size was reached, cages could be removed to allow fish to feed on deleterious fouling algae. This size-escape threshold is critical, with Doropoulos et al. (2012) demonstrating that the survival of 2-month *Acropora millepora* corals subjected to grazing was inversely to proportional size. Similarly one-week-old, single polyp recruits of *Pocillopora damicornis* experienced high mortality when exposed to grazing by S*alarias fasciatus* (combtoothed blenny), however, once they reached five weeks of age with multiple polyps, recruit survival was high with only minor damage observed (Christiansen et al., 2009).

Size-escape thresholds for coral recruits can be reached relatively quickly for many species, though these thresholds can vary depending on the feeding method of grazers. Herbivorous fish common in aquaria typically belong to defined functional groups including “brushers”, “croppers” or “concealed croppers” (Tebbett et al., 2022). Brushers, sometimes called “combers”, target detritus in the algal matrix by using long, comb-like teeth to brush though algal filaments and the substrate (Purcell & Bellwood, 1993; Wilson, 2000; Wilson et al., 2003). Brushers are considered more nominal herbivores since their gut-contents are dominated by detritus material and sediment, while any algae removal is likely incidental (Christiansen et al., 2010; Marshell & Mumby, 2012; Tebbett et al., 2017b). Croppers primarily target turf algae by using their shorter teeth to nip off strands above the substrate (Purcell & Bellwood, 1993; Tebbett et al., 2017b). Concealed croppers function in a similar fashion to croppers, but their long narrow snouts allow them to harvest algae from small crevices unavailable to other grazers (Tebbett et al., 2022). Survival has been demonstrated to be higher for coral recruits subjected to *Salarias fasciatus* and *Acanthurus nigrofuscus* grazing, which use brushing and cropping grazing methods respectively, but decreased when exposed to juvenile parrot fish grazing (*Scarus* spp.), which employ a “scraping” feeding method that effectively scrapes and removes the top layer of material from the substratum (Bellwood & Choat, 1990; Christiansen et al., 2010; Doropoulos et al., 2012; Tebbett et al., 2017b). It is worth noting however, that *Scarus* spp. are not typically used in aquaria as they are known to consume coral (Doropoulos et al., 2012).

Whilst it has been established that grazers with scraping feeding strategies pose a threat to coral recruit, questions remain around how grazers with less disruptive feeding methods will affect corals of different species and ages. In *ex situ* coral culture, control of fouling growth in grow-out-systems with grazing fish is a low-effort alternative to time-consuming manual cleaning, thus identifying size-escape thresholds of coral recruits under various feeding strategies could offer practitioners a guideline for when fish grazers may be introduced to culture. To this end, this study compared the survival of *Acropora millepora*, *Acropora kenti* and *Goniastrea retiformis* recruits at one-week and one-month post-settlement when exposed to a variety (brushing, cropping and concealed croppers) of different fish grazing strategies, and the algae removal by each of the fish species during the exposure.

3.3 Materials and Methods

3.3.1 Coral and fish preparation

Fifty 20 x 20 cm (400 cm^2) concrete tiles were conditioned prior to coral spawning in established aquaria at the Australian Institute of Marine Science's National Sea Simulator to develop a ~10 % coverage of crustose coralline algae (CCA). Tiles were then frozen and stored at -20 °C to kill CCA and biofilm to prevent competition between the coral recruits and the encrusting algae.

Gravid colonies of *Acropora millepora*, *Acropora kenti* (formerly *A. tenuis*, see Bridge et al. (2023)) and *Goniastrea retiformis* were collected from Falcon Reef (-18.767116, 146.534248) and Esk Reef (-18.766080, 146.520822) in the Great Barrier Reef Marine Park in November and December 2023, then returned to the Australian Institute of Marine Science's National Sea Simulator. On spawning nights colonies were monitored and gametes collected, fertilised, then cultured until competent to settle as per Severati et al. (2024). Competent larvae were settled onto the pre-conditioned concrete settlement tiles at a concentration of 1000 larvae per tile. During settlement, larvae were provided with Symbiodiniaceae (*Cladocopium* sp. C1) at a concentration of 10,000 cells mL^{-1} to initiate symbiont inoculation. Tiles were first settled during November, then corals grown-out for one month in mesocosm systems to allow the settlement tiles to develop a fouling community. These tiles were then settled again with new recruits from the December spawning, to ensure all tiles contained both month-old (November) and week-old (December) recruits. The exception was the *G.*

retiformis settlement tiles, which had been left to develop a fouling community the same as the other tiles, but were only settled in December due to a lack of gravid broodstock in November. The settlement tiles were then fragmented into smaller 5 x 5 cm (25 cm^2) sections and left for a further five days in the mesocosm tanks, until the younger recruits were approximately one week old.

Fish species commonly used in coral aquaculture and representing a range of grazing strategies as identified in Tebbett et al. (2022) and Wilson et al. (2003) were selected, including *Ctenochaetus binotatus* (brusher), *Acanthurus nigrofuscus* (cropper), *Zebrasoma scopas* (concealed cropped) and *Salarias fasciatus* (brusher). Fish were sourced from ornamental suppliers and introduced to clean 60L fibre-glass tanks four days prior to the start of the experiment for acclimation, with one fish per tank and four replicate tanks per fish species. Tanks contained shelters of appropriate size for the different fish species and were covered with a shade-cloth to help reduce stress and prevent jumping by fish. Each tank was supplied with 0.875 L min^{-1} of 0.1µm filtered seawater (FSW) at 27°C and sufficient light across a daily photoperiod of 12 hours, with light intensity (photosynthetically active radiation) being 100 µmol cm^{-2} s^{-1} at the bottom of the tank with the shade-cloth on.

3.3.2 Data collection

Fish were starved overnight prior to the introduction of tiles with coral recruits to encourage feeding off the tile's algal communities. Each 25 cm^2 tile section was photographed using a high-resolution camera (Nikon® DSLR D810) then randomly assigned to tanks, with four *A. millepora,* three *A. kenti* and one *G. retiformis* tile per tank. Alongside the fish, two control treatments were conducted; an uncleaned control where tiles were simply placed in the tanks then left undisturbed, and a manual cleaning control, where algae was cleaned from the tiles by an aquarist using a small brush. Tiles were left in the tanks for 24 hours, during which fishes were filmed using GoPros to monitor their activity. After 24 hours, tiles were removed and rephotographed.

At the conclusion of the experiment, fish where anaesthetised with Aqui-S® (540 mg mL^{-1} isoeugenol) then photographed, and body and snout width measured using callipers. Other body measurements were taken from photographs, with snout length measured as per

Brandl and Bellwood (2013) (Fig. 3.1). Bite-scar measurements were taken where possible by photographing visible scars with a scale-bar and their total surface area determined via ImageJ (Schneider et al., 2012). Only bite-scars with distinct edges, separate from other scars, were used for analysis, and for *S. fasicatus* the area of the top and bottom of the scar were summed for a total bite-scar measurement. Fish were then transferred to larger coral-holding tanks to live-out their natural lifespan.

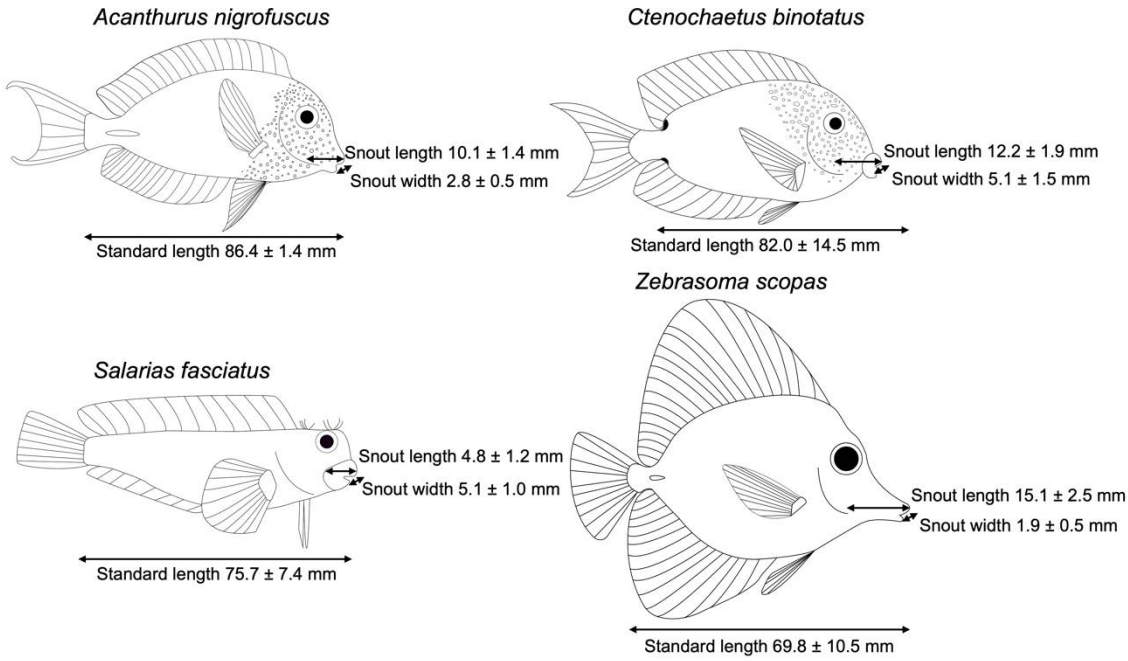

Figure 3.1: Summary of standard length, snout length and snout width measurements for each fish species, presented as mean ± standard deviation.

For each fish, six random five-minute video segments were analysed to determine bite rate and substrate of bite-scar (tank walls or outside of shelters vs coral tiles) during the coral exposure, for a total of 30 mins per fish. Videos were selected using a random number generator, and before inclusion in the analysis were assessed for any signs of disturbance of the fish (e.g. by aquarists conducting checks). If a fish appeared disturbed (e.g. retreating to hides for > 1 minute), the video was excluded from the analysis and a new clip randomly selected. All the fish species included in the experiment are diurnal feeders who are active throughout daylight hours, when the videos were taken. Bite rate was converted to bites per minute, which was used as a measurement of grazing pressure for the different fish. ImageJ was used to measure the size of recruits and track their survival post-exposure. Coral Point

Count with CPE (Kohler & Gill, 2006) was used to estimate coverage of different types of algae and other organisms (bare tile, algal turf, filamentous algae, detritus, dead and alive coral recruits and fouling worms) pre and post-exposure.

3.3.3 Data analysis

Data were analysed using *R* version 4.3.1 (R Core Team, 2023) and *RStudio* version 2023.06.1 (Posit team, 2023), using the *brms* package (Bürkner, 2021) and *rstan* (Stan Development Team, 2024). Bayesian hierarchical models were used to compare mortality of corals under different grazing pressures, bite rates of fish and coverage of fouling, using a range of binomial, negative binomial and zero-inflated negative binomial models with tank ID as a blocking factor. For all Bayesian models weakly informative priors were used, and MCMC sampling diagnostics were performed by checking for well-mixed trace-plots, r-hat (<1.01), autocorrelation (<0.25) and effective sample size (>1000). Density plots of the posterior and DHARMA residuals were used to check goodness of fit. Bayesian probabilities were then calculated to determine likelihood of true differences between treatments. Code with details of priors and models can be found on GitHub, and an overview of the model parameters including priors and outputs (median and 95% CI) are included in the Appendix C.

3.3.4 Ethics statement

All research was conducted in accordance with the Great Barrier Reef Marine Park Authority permits (G23/49085.1, G21/45348.1 and G21/38062.1) and James Cook University Animal Ethics Permit (A2920).

3.4 Results

3.4.1 Fish grazing behaviour and changes to algae coverage

All fish had similar standard lengths (Fig. 3.1), though their jaw widths varied from ~2 mm in *Zebrasoma scopas* to ~5 mm in *Ctenochaetus binotatus* and *Salarias fasciatus*. The area of bite-scar produced by the fish was measured in *C. binotatus, S. fasciatus* and from one individual of *Acanthurus nigrofuscus*, though bite scars in the *Z. scopas* and remaining *A. nigrofuscus* were not observed, likely due to their small mouth size. *C. binotatus* and *S. fasicatus* both had similar mean snout widths (~5.1 mm), though the mean bite-scar area of *C. binotatus* was much smaller (31.4 ± 11.4 mm^2) than that of *S. fasicatus* (73.2 ± 18.7 mm^2)

(mean ± sd). For both species, individuals with larger fish snout-width had a larger bite-scar area. The single *A. nigrofuscus* that was able to be measured had much smaller bite-scars than either of the other species, at 1.0 ± 0.4 mm^2.

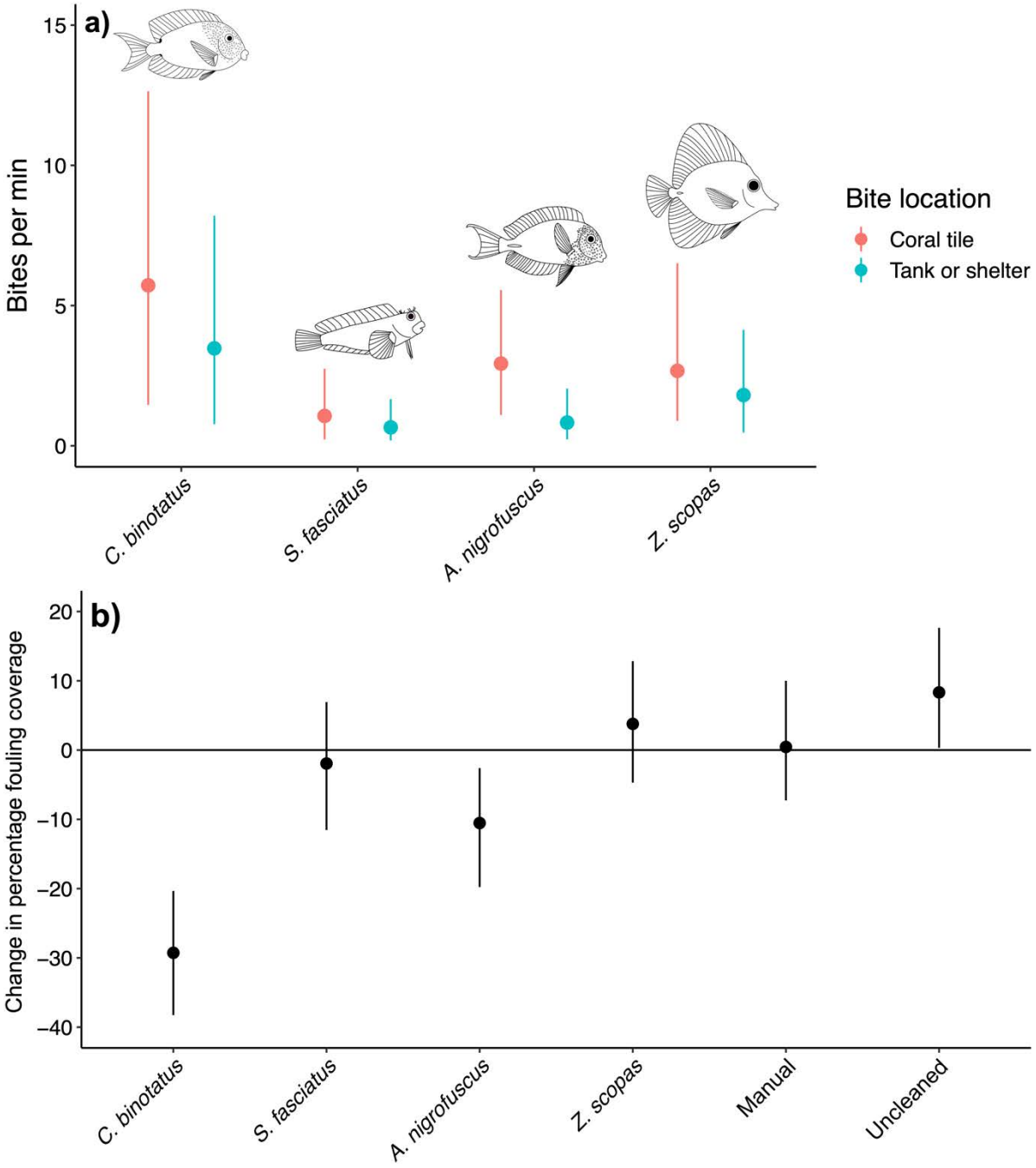

Figure 3.2: a) Grazing intensity of the different fish species, measured as number of bites per minute. b) Absolute change in percentage fouling coverage on tiles after exposure to the different treatments. Dots and bars represent modelled medians and 95% credibility intervals (calculated as highest posterior density interval).

Overall median grazing intensity (measured as the sum of bites per minute on coral tiles, tank walls and shelters) varied between fish species (Fig. 3.2a), with *C. binotatus* having the highest (Bayesian P > 89%) and *S. fasciatus* the lowest (P > 86%) bite rates. *C. binotatus* also had a greater grazing intensity on coral tiles (measured as bites per minute on just the coral tiles) than all other species (P > 87%) and *S. fasciatus* had a lower rate than all others (P > 89%). Though all fish species had higher rates of grazing on the coral tiles than the tank walls or shelter, only *A. nigrofuscus* showed evidence for a significantly higher bite rate on the tiles than on the shelters or walls of the tank (P = 99.5%). In the videos all fish were observed grazing adjacent or directly over recruits whilst biting coral tiles.

Median percentage coverage of fouling (i.e., the sum of turf algae, filamentous algae and detritus) ranged from ~27% - 42% on the tiles prior to the start of the experiment. After the 24-hour exposure to fish, only *A. nigrofuscus* and *C. binotatus* tanks saw reductions in total fouling coverage, of 10.5% and 29.3% respectively (P >99%, Fig. 3.2b), which resulted in median fouling coverages of 31.5% and 6.5%. The uncleaned tanks saw a median increase of 8.3% in fouling (P = 97.6%), whilst the *Z. scopas, S. fasciatus* and manual tanks had no evidence for a change in fouling coverage (Fig. 3.2b). The average time required for an aquarist clean all the tiles in a manual cleaning tank was 9.1 ± 1.5 minutes (mean ± sd). In *C. binotatus* and the uncleaned tanks the change in fouling could likely be attributed to changes in filamentous algae coverage, which dropped from a median of 27.5% to 6.1% coverage in *C. binotatus* tanks and grew from 17.5% to 30.5% in the uncleaned tanks pre- and post-fish exposure. *A. nigrofuscus* tanks, however, saw a significant decrease in turf algae coverage from 10.8% to 5.1%.

3.4.2 Coral mortality across species and age

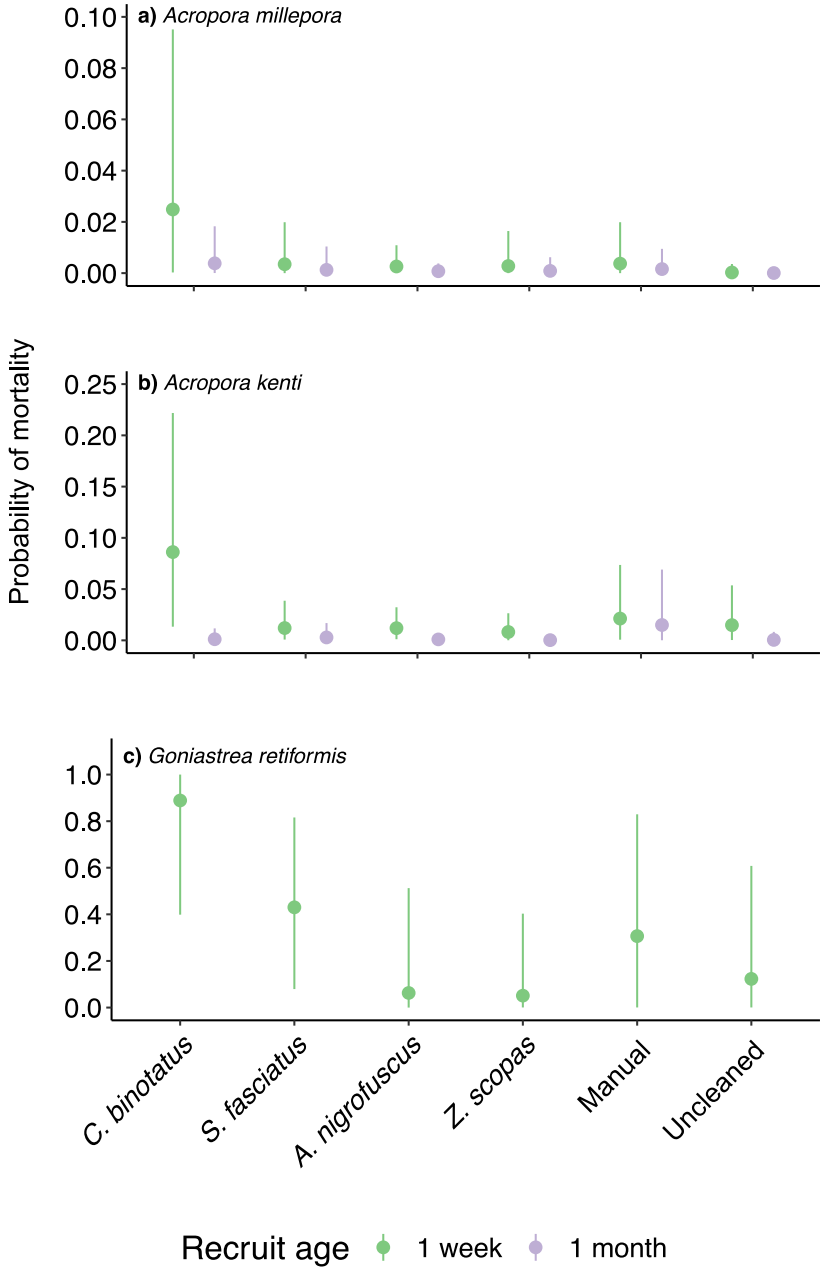

Figure 3.3: Probability of mortality of coral recruits from different grazing pressures at 1 week (single polyp recruits) and > 1 month (multi-polyp recruits) post settlement for a) *Acropora millepora*, b) *Acropora kenti* and c) *Goniastrea retiformis*. Dots and bars represent modelled medians and 95% credibility intervals (calculated as highest posterior density interval).

Mean *Acropora millepora* recruit density was similar for week old, single polyp and month old multi polyp recruits, though density varied between different tiles, at 2.1 ± 0.2 per cm^2 of settlement tile (mean ± se) for both. *A. kenti* density was also consistent between week-old and month-old recruits, though lower on average, at 1.1 ± 0.1 recruits per cm^2. Week old,

single-polyp *A. millepora* and *A. kenti* recruits were similar in size, with non-chimera individuals having mean basal areas of 1.43 ± 0.31 mm^2 and 1.45 ± 0.30 mm^2 respectively (mean ± sd). One month old *A. millepora* recruits displayed a small increase in mean basal area to 1.49 ± 0.30 mm^2, whilst *A. kenti* recruits had a smaller area at 1.32 ± 0.26 mm^2 (likely due to growth being directed upwards rather than basally), with both having ~2 – 7 polyps per recruit. Both *A. millepora* and *A. kenti* recruits had low mortality rates under all the tested grazing pressures (Fig. 3.3a, b), with the highest probability of mortality occurring in single-polyp, one-week-old recruits under the *Ctenochaetus binotatus* treatment (*A. millepora*: 2.5% median chance of mortality, P > 92%; *A. kenti*: 8.6% median chance of mortality, P > 91%). In general, single-polyp recruits were more likely to die than their month-old counterparts when exposed to grazing pressure. Uncleaned recruits (both week-old, single and month-old, multi-polyp) had the lowest mortality for *A. millepora* (P > 87%). *A. kenti* saw its lowest modelled mortality under *Z. scopas* grazing, though similar mortality rates were found in the other treatments (barring *C. binotatus* for the single polyp recruits and manual cleaning for the multi-polyp). The majority of Acroporid recruits that experienced mortality had a skeleton remaining, though one-week-old, single polyp recruits under *C. binotatus* grazing had similar numbers of recruits that experienced mortality that were completely removed (i.e. no skeleton left).

Week old, single-polyp *G. retiformis* recruits had a much lower settlement density than the Acroprids, at 0.13 ± 0.04 recruits per cm^2. These recruits were much smaller than their Acroporid counterparts, at 0.30 ± 0.09 mm^2, and experienced much higher mortality than the Acroporid recruits of the same age (Fig. 3.3c). Under *C. binotatus* grazing, median modelled probability of mortality was 88.9%, with >92% certainty that this mortality was higher than all other treatments. *S. fasciatus* and the manual cleaning treatment also had relatively high modelled mortality rates, at 43.0% and 30.7% respectively, though only *S. fasciatus* had evidence this represented an increase compared to the remaining three treatments (P > 84%). Lowest modelled mortality was found in the *A. nigrofuscus* and *Z. scopas* treatments (6.3% and 5.1% respectively), though these mortality rates were still higher than those observed in *A. millepora* and *A. kenti*. All but one of the *G. retiformis* recruits that died in the experiment were completely removed.

3.5 Discussion

Overall, the two Acroporid species displayed much lower mortality than the smaller *G. retiformis* recruits, though all corals experienced highest mortality under *Ctenochaetus binotatus* grazing. Month-old, multi-polyp *A. millepora* and *A. kenti* had lower mortality rates than their younger, single-polyp counterparts, and of those that experience mortality, younger recruits were more likely to be completely removed than month-old ones. Given the short duration of the experiment it is unlikely that competition with algae was a major contributor to mortality, as typical algal competitive mechanisms such as overgrowth and abrasion take longer to have significant effects, thus mortality can be attributed to natural attrition rates of recruits and disturbance from fish grazing or aquarist cleaning (Jorissen et al., 2020; McCook et al., 2001). The reduction in mortality in the month-old, multi-polyp recruits is consistent with previous research which has shown once recruits reach the 6 – 8 polyp stage they are much less likely to die from over-grazing pressure (Christiansen et al., 2009; Doropoulos et al., 2012), possibly due to the one-week-old recruits not having completely developed skeletal structures (Babcock et al., 2003; Yuan et al., 2018). *G. retiformis* also had much higher rates of mortality, and of the recruits that experienced mortality the majority were completely removed. The Acroporid recruits were far larger than *G. retiformis*, at ~1.4 mm^2 in surface area compared to ~0.3 mm^2, which follows the principle that smaller recruits are more vulnerable to over-grazing.

The different feeding strategies of the fishes incorporated in this study (i.e., “brushers”, “croppers” and “concealed croppers” (Tebbett et al., 2022)) is the likely explanation for why coral recruit mortality rates varied between fish species. Fishes classified as brushers target detritus and sediment typically at the base of the algae matrix (Tebbett et al., 2017a), whilst croppers typically nip at algae above the substratum, with little detritus or sediment found in their gut (Purcell & Bellwood, 1993; Tebbett et al., 2017b; Tebbett et al., 2022). The brusher species used here, *C. binotatus* and *S. fasciatus*, had wider jaws than the cropper (*A. nigrofuscus*) or concealed cropper (*Z. scopas*) species, and *A. nigrofuscus* has been recorded as having a much smaller jaw opening (113°) than other brusher species like *C. striatus* (180°) (Purcell & Bellwood, 1993). The smooth concrete tiles used to settle the corals also meant recruits had no access to microhabitats which can provide refugia from fish grazing

(Brandl et al., 2013; Trapon et al., 2013). Their feeding methods coupled with smaller jaw-sizes in both *A. nigrofuscus* and *Z. scopas* may indicate these fish rarely came into direct contact with the coral recruits in this experiment even when grazing near them, resulting in the lower mortality rates than either *C. binotatus* and *S. fasciatus*.

However, feeding strategy alone is not solely responsible for coral mortality patterns, as both *C. binotatus* and *S. fasciatus* are classified as "brushers" yet had markedly different impacts on recruit survival. Different feeding rates between the species likely influence recruit mortality, as a higher feeding rate means greater likelihood of the fish coming into contact with the corals. Though there was variation among the individual fish, *C. binotatus* took significantly more bites on the coral tiles than *S. fasciatus* in the recorded period. This is consistent with previous studies who estimated wild *S. fasciatus* take ~140 bites per hour, whilst wild *Ctenochaetus striatus* (another brusher Acanthurid) have ~1800 bites per hour (Choat et al., 2004). In this study *C. binotatus* also had a greater reduction in fouling coverage than *S. fasciatus*, further suggesting that grazing was either lower or at least less effective in the latter.

Interestingly, whilst the individuals in this experiment had similar jaw widths, *C. binotatus* had a lower mean bite-scar area, at around half the size of *S. fasciatus*. Potentially, this greater bite size of *S. fasciatus* may explain their lower grazing rate, as per bite they can harvest a greater mass of algae. Coupled with their smaller body size, this means they consume a higher relative percentage of their body mass than *C. binotatus* with each bite. Indeed, roughly averaging the mean decrease in fouling coverage on the coral tiles by the total number of bites by the fish in that tank shows each bite of *C. binotatus* removed ~0.25% of fouling coverage compared to ~0.31% removed by *S. fasciatus*. While both brushers, these fish also function in slightly different manners. As per Christiansen et al. (2010) and observations of feeding from this experiment's videos, *S. fasciatus* apply both premaxillary and dentary jaws to the substrate, then pull the jaws towards each other while pulling backwards with their bodies, using their angled teeth to comb through algal filaments for food. In contrast, *Ctenochaetus* brushers have long, comb-like teeth which they use in a 'dustpan and brush' method, where the dentary jaw is used to scrape the substrate and collect detritus against a collection plate in the premaxillary jaw (Fishelson & Delarea,

2013; Tebbett et al., 2018). It may be that *S. fasciatus's* feeding method has a lower impact on the coral recruits, despite covering a larger average area. This, coupled with a lower feeding rate, likely contributes to the lower mortality rates observed under *S. fasciatus* grazing.

Only *C. binotatus* and *A. nigrofuscus* reduced fouling coverage over the 24-hour exposure, with coverage increasing in the uncleaned treatment due to the fast growth of filamentous algae. Whilst the other treatments did not reduce fouling coverage, they did prevent it from increasing as observed in the uncleaned tanks. Growth of fouling on surfaces during coral grow-out will be dependent on what fouling community is initially present, which will vary depending on the conditioning practices of the individual producers. However, even on relatively clean settlement surfaces, fouling by filamentous algae develops in the weeks post-settlement (Guest et al., 2013). Here I froze tiles to reduce competition between algae and recruits, resulting in an early-successional environment for the settled corals. Tebbett et al. (2017b) reported that *C. striatus* is more adept at clearing early successional (< 6 weeks old), loosely attached algal turfs compared to *A. nigrofuscus*, who were better at cropping older, well-attached turfs (Marshell & Mumby, 2012; Tebbett et al., 2017b), consistent with observations of this study.

The fouling community present on settlement/grow-out surfaces may also influence the grazing behaviour of any fish companions utilised in culture systems. However, many coral aquaculture facilities are moving toward utilising frozen or similarly 'clean' tiles to induce settlement of corals whilst minimising initial algae competition. Thus, the relatively early successional algal communities observed in this study are likely representative of the kinds of communities young recruits will be exposed to during grow-out. The development of the later successional algae community will then be primarily influence by the system the settlement surfaces are cultured in, what fouling communities are present in the systems and the application of grazers or husbandry to control algae growth.

From this study, *A. nigrofuscus* represented the best trade-off between removing a significant amount of fouling from the tiles, and minimising mortality of Acroporid recruits recruits. However, the short duration (24-hours) of the experiment should be kept in mind

when making generalisations; while the information from this experiment is valuable, larger scale-trials over a longer duration would be ideal to confirm recruits have reached size-escape threshold in response to this species. Whilst the uncleaned tanks had low mortality for all recruit species, uncontrolled fouling growth in the first 6 months post-settlement can lead to increased mortality and reduced growth of coral recruits, particularly if fouling species resistant to smaller, less disruptive grazers such as *Dictyota* sp. algae propagate (Chapter 2). Therefore, practitioners must strike the balance of introducing fish grazers early enough that they can effectively control fouling species whilst minimising impact on the recruits. From the data presented in this study, the ideal introduction point is after the recruits have reached the multi-polyp stage at around one month post settlement for fast growing coral species. However, as past work has shown that small invertebrate herbivores are sufficient to control early-successional algae growth such as filamentous algae whilst minimising impact to corals in the early stages (<2 months) of grow-out, the introduction of fish could be safely delayed until even later to further reduce impact on the corals (Chapter 2; (Neil et al., 2021)). Indeed, *A. nigrofuscus* was found to mostly remove algae turf, which complements the filamentous algae control that can be provided by invertebrate herbivores. *G. retiformis* also experienced lower mortality in the *A. nigrofuscus* treatment, though it was generally more vulnerable to grazing than the Acroporids, thus it would likely be best for practitioners to wait until the recruits reach the multi-polyp stage before introducing this fish. If culturing coral recruits with invertebrate herbivores and fish in succession, practitioners should wait to introduce fish until after two months post-settlement, or even later if culturing slower growing species that have still not reached the multi-polyp stage.

Overall, these results show that fish grazers can reduce the coverage of fouling algae in coral culture, and mortality of coral recruits is minimised when exposed to cropper or concealed cropper grazers as opposed to brushers. Co-culturing coral recruits with a less disruptive species like the cropper *A. nigrofusucus* provides an effective way to reduce algae growth whilst minimising disturbance to corals. Delaying the introduction of fishes until after one-month post-settlement can significantly reduce coral mortality in response to grazing pressure. The outcomes of this work provide a valuable guideline for introducing fish into recruit grow-out for coral aquaculture practitioners.

CHAPTER 4 - Improving coral grow-out through an integrated aquaculture approach

4.1 Abstract

Some coral species in natural reef systems derive benefits from fish which live in close association with them. To test potential benefits of incorporating fish in *ex situ* coral culture, corals that typically have fish associations (*Acropora kenti* and *Pocillopora verrucosa*) and those that do not (*Porites lutea* and *Platygyra daedalea*), were grown in aquaria under different fish-associated treatments for three months. Physiological performance of the corals, including growth, protein content, symbiont density and photosynthetic efficiency were assessed in the different treatments where corals were 1) kept with a school of *Chromis viridis* fed a pelleted diet, 2) supplied filtered water from a tank housing *C. viridis*, 3) fed live feeds whilst maintained with *C. viridis*, 4) supplied only with live feeds, 5) supplied with a pelleted fish diet without *C. viridis*, and 6) not supplied feeds and without *C. viridis*. Whilst the responses of the corals varied among species, generally exposure to fish or fish-water increased the protein and/or symbiont density within coral tissue. *A. kenti* and *P. lutea,* which derive a higher proportion of their energy requirement from autotrophy, displayed improved growth in the fish treatments, whilst the more heterotrophic *P. verrucosa* grew fastest when supplied with live feeds. The more heterotrophic, slow-growing *P. daedalea* did not show significant improvements in growth under any of the treatments, and there were no major differences in photosynthetic efficiency between treatments in any of the corals. These results indicate that incorporating fish into coral culture could provide an accessible source of nitrogen and phosphorous enrichment via the dissolved portion of the fish's wastes, and, in-turn, enhance the growth of corals more reliant on autotrophy, like Acroporids. The results point to potential efficiency gains for coral husbandry practices, with the aim of satisfying the growing demands of reef restoration and ornamental aquaculture.

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4.2 Introduction

Integrated multi-trophic aquaculture is the practice of raising two or more types of organisms in proximity to each other, which may utilise the waste products of one species to act as nutritional input to the other (Knowler et al., 2020). Typically, a primary heterotrophic species such as a finfish is grown on a formulated diet, then wastes and/or waste-water from this primary species are transferred to an extractive secondary species such as seaweed or a filter-feeding organism (Yokoyama, 2013; Zamora et al., 2018). Taking advantage of such relationships can allow farms to maximise production of both the primary and secondary species (Hargrave et al., 2022), while acquiring other benefits such as waste-water remediation (Ansari et al., 2017; Chavez-Crooker & Obreque-Contreras, 2010; Custódio et al., 2017), or an added price premium to integrated aquaculture products due to sustainability credentials or certifications (Carras et al., 2019; Knowler et al., 2020). Other services, such as parasite control (Imsland et al., 2020; Powell et al., 2018; Vaughan et al., 2018) or mediation of bacterial communities (Ying et al., 2018) may also be provided by the organisms cultured alongside the primary product.

Some coral species derive physiological effects from fish associations. For example, schooling juvenile *Haemulon* that reside over *Porites furcata* corals during the day resulted in increased coral growth rates compared to colonies of the same species with no fish present (Meyer & Schultz, 1985b; Meyer et al., 1983). Branching corals such as Pocilloporids often host damselfish (Pomacentridae) of various species (Chase & Hoogenboom, 2019), and may have faster growth rates (Chase et al., 2014; Holbrook et al., 2008; Liberman et al., 1995), increased photosynthetic activity (Garcia-Herrera et al., 2017) and an increased resistance to bleaching (Chase et al., 2018; Shantz et al., 2022) compared to corals without fish present. Associated fish may also alleviate stressors caused by sedimentation on their hosts (Chase et al., 2020). Such benefits may come from increased water movement resulting in higher oxygen and CO_2 transport within the colony structure due to the physical behaviour of the fish, including fanning (Berenshtein et al., 2015; Chase et al., 2018; Garcia-Herrera et al., 2017; Goldshmid et al., 2004; Shantz et al., 2022) or from nutrient enrichment in the form of both dissolved and particulate wastes from the fishes metabolic and digestive processes (Holbrook et al., 2008; Liberman et al., 1995; Meyer & Schultz, 1985a; Meyer et al., 1983; Shantz et al., 2022; Shantz et al., 2015).

Most scleractinian coral species are thought to be mixotrophic but vary in their capacity and requirement for heterotrophic nutrient sources. One method to determine the relative rates of heterotrophic vs autotrophic energy acquisition in coral is to examine the overlap of δ^{13} C and δ^{15} N stable isotopes ratios in the coral host and their algal symbionts, with higher overlap indicating a higher reliance on autotrophic (i.e. symbiont derived) nutrition (Maier et al., 2010). Using this method, and supplementing with evidence such as fatty acid biomarkers, it has been determined that genera such as *Platygyra* and *Pocillopora* are more heterotopic, *Porites* mixotrophic and *Acropora* more autotrophic. (Conti-Jerpe et al., 2020; Radice et al., 2019a; Radice et al., 2019b).

Production of corals in captive systems must increase if it is to meet the growing demand of the ornamental trade in addition to the emerging requirement of healthy individuals for out-planting to rehabilitate reefs. Consumer derived nutrients (i.e. uptake of wastes from higher-trophic-order species by lower-order species) offer a potential mechanism to improve coral growth for restoration purposes, as demonstrated for *in situ* nurseries or at out-plant sites (Ladd & Shantz, 2020; Shaver & Silliman, 2017). The nutrition of corals kept in captivity for research or propagation remains an obstacle to long-term holding, as current commercial feeds are considered suboptimal for enhancing growth and are expensive to feed at large-scales (Conlan et al., 2018a). Diets and feeding regimes should ideally be species specific, yet a lack of research has hindered their widespread suitability (Conlan et al., 2019; Forsman et al., 2011; Tagliafico et al., 2018). Integrating fish into captive coral culture may therefore contribute to a more holistic nutrition for corals, either from uptake of dissolved wastes to support symbiont populations (Burris, 1983; Wiedenmann et al., 2023) or heterotrophic feeding on egested particulate wastes (Anthony, 1999; Houlbreque & Ferrier-Pages, 2009). If reef restoration and ornamental coral aquaculture facilities aim to keep large coral colonies long-term as broodstock, the fanning of hosting damselfish may also be beneficial at preventing stagnant zones from forming in the deep inner branches where flow is reduced (Chamberlain & Graus, 1975). As such, this study aims to further elucidate such mechanisms of beneficial fish-coral interaction by evaluating coral performance either in association with schooling damselfish, the dissolved or particulate waste isolated from damselfish, or conventional coral aquaculture diets, i.e. mass produced live feeds (Osinga et al., 2011;

Petersen et al., 2008). Importantly, a range of coral species has been selected to span the autotrophic-heterotrophic energy acquisition spectrum, and those with, and without, close fish associations.

4.3 Methodology

4.3.1 Experimental set-up

Five punitive genotypes (nominally based on the distance between the colonies when collected) each of *Porites lutea, Platygyra daedalea, Pocillopora verrucosa,* and *Acropora kenti* (formerly *Acropora tenuis* (Bridge et al., 2023)) were collected from 2 - 7 m depth on Davies Reef (-18.825622, 147.626881) on the Great Barrier Reef, QLD, Australia. Corals were then brought to the Australian Institute of Marine Science's National Sea Simulator (SeaSim) in Cape Cleveland, QLD, Australia, where they were fragmented into ~10 g nubbins, and allowed to heal for one month prior to the beginning of the experiment. During this period, corals were acclimated to experimental conditions including water flow, water temperature and light intensity (details below). Though different coral species have slightly different ideal environmental conditions, the parameters were all within acceptable culture conditions for these species, and represented a reasonable approximation of the conditions corals may be kept in commercial aquaculture facilities. Ten randomly selected fragments from each colony were also collected and frozen at -20 ℃ when the corals first arrived at the facility (deemed "Field" samples) and after the one-month acclimation period (deemed "Post-acclimation" samples) for subsequent analysis.

Twenty-eight 50 L experimental tanks, contained in water jackets to help control temperature, were set-up and were randomly assigned one of the six experimental treatments, with four replicate tanks per treatment. The six treatments were; "Fish" corals kept in co-culture with a school of ten *Chromis viridis,* "Dissolved" corals supplied water filtered to 50 μm from a tank hosting a school of ten *C. viridis*, "LiveFeeds + Fish" corals kept with ten *C. viridis* while also given a supply of live feeds, "LiveFeeds" corals supplied only live feeds, "Pellets" corals supplied only the pelleted diet fed to the fish, and "Control" corals kept with no fish and supplied no feeds. All *C. viridis* were juveniles (<5 cm total length) and were sourced from a commercial supplier, then 10 fish randomly assigned to each of the Fish, LiveFeeds + Fish and Dissolved treatment tanks, with a fish biomass of 13.8 ± 1.9 g

(mean ± sd) per tank. Schools of *C. viridis* were allowed one week to acclimate to the experimental conditions prior to the start of the experiment, during which they were fed in the same manner as during the experiment (detailed below). A biomass of ~14g per tank was chosen as this is similar to observed populations of damselfish that aggregate on wild coral colonies, thus providing a likely physiological response to the coral, in addition to being a reasonable holding density to ensure the welfare of the fish (Chase & Hoogenboom, 2019).

Ten fragments of each coral species were weighed using buoyant weight and placed into a randomised array into each of the replicate tanks, with a roughly even number of each genotype per tank. Replicate tanks were then subjected to one of the six treatments for three months. Each tank was supplied with 0. 1 µm filtered seawater (FSW) at 28 ± 0.1 °C, at a flow-through rate of 0.8 L min^{-1}, resulting in approximately one complete exchange of tank water per hour. Tanks were each fitted with a circulation pump (Turbelle® nanostream® 6015, Tunze Aquarientechnik, Penzberg, Germany) to enhance water circulation within the tank. Light was supplied by one LED light (Hydra, AquaIllumination, Bethlehem USA) per tank, at 150 µmol cm^{-2} s^{-1} from 0830 – 1630 with an even mix of blue and white light, with one-hour ramps at the beginning and end of the light period each day.

4.3.2 Feeds and maintenance

Tanks containing *C. viridis* and the Pellet treatment tanks were fed twice daily at 0900 and 1530 with 0.12 g per tank of AF Tiny Fish Feed (Aquaforest, Brzesko, Poland) which had been processed briefly in a blender to reduce pellet size (≤1 mm). Live feeds for the LiveFeeds and LiveFeeds + Fish treatments consisted of Polyunsaturated fatty acid enriched *Artemia* nauplii, SELCO® S.parkle enriched rotifers and a mix of microalgae (*Nannochloropsis oceania, Tisochrysis lutea., Chaetoceros muelleri, Dunaliella* sp., *Proteromonas sulcata*) fed to tanks at a rate of 0.5 nauplii mL^{-1}, 0.5 rotifers mL^{-1} and 2000 cells mL^{-1}. LiveFeeds and LiveFeeds + Fish feeding occurred as a daily pulse of food at 1500, after which fish were fed their afternoon pellet allotment. Tanks were cleaned and siphoned twice weekly, and trays holding coral fragments were swapped for clean trays once a week, to minimise the growth of algae around the corals. All care was taken to minimise harm to the experimental animals, following animal ethics approvals.

4.3.3 Data collection

Growth was measured via buoyant weight (to 0.001 g), with proportional growth of coral fragments calculated by taking the raw buoyant weight of each fragment at the end of the experiment and dividing by the buoyant weight prior to the start of the experiment. Photosynthetic efficiency was measured as dark-adapted maximum quantum yield (Fv/Fm), by taking measurements in the morning just prior to the beginning of the light ramp (Walz Mini-PAM, Heinz Walz, Effeltrich, Germany).

Water samples were collected weekly from tanks, at the same time each day prior to morning fish feeding. Each experimental tank was sampled, including both the fish holding tank and coral tank for the Dissolved treatment. These samples were analysed for dissolved organic carbon (DOC; Shimadzu TOC-L), for dissolved inorganic nutrients (NH_4, PO_4, NO_2 + NO_3, NO_2; SEAL Analytical AutoAnalyser) and particulate nitrogen and carbon (Shimadzu TOC-L with TNM-L and SSM).

At the end of the experiment fish were anaesthetised using AQUI-S® (isoeugenol, 540mg L^{-1} AQUI-S New Zealand Ltd) then weighed. After the final buoyant weight and photosynthetic efficiency measurements were taken, two fragments of each species from each replicate tank were randomly selected and preserved at -20 °C. The tissue from these fragments was then removed using FSW and high-pressure air and the resulting tissue blastate homogenised, ensuring to keep the fragments and blastate on ice. Tissue blasted coral skeletons were dried at 60 °C and wax dipped (Stimson & Kinzie, 1991) to determine surface area, which was used to standardise Symbiodiniaceae counts and protein content.

A Pierce™ BCA Protein Assay Kit was used to measure protein content within the coral blastate (detailed methodology provided in Appendix D). A 2 mL aliquot of the homogenised tissue blastate was also taken, filtered through a 20 µm cell strainer using FSW, then fixed using 10% formalin. Subsamples of the fixed blastate were analysed in duplicate on a BD Acurri™ C6 Plus CSampler Flow Cytometer to determine Symbiodiniaceae density, with a density plot of forward scatter and side scatter and a manually placed ellipse gate used to count symbiont cells. Accuracy of the flow cytometer symbiont counts was assessed by initially comparing the flow cytometer counts to samples manually counted using a

haemocytometer under a light microscope. No significant variation was found between the two methods, thus the remainer of the samples were analysed using the flow cytometer.

4.3.4 Data analysis

All data analysis was conducted using RStudio version 2023.6.1.524 (Posit team, 2023) and R version 4.3.1 (R Core Team, 2023). Relationships between treatments and the response variables were examined using Bayesian hierarchical models using *brms (Bürkner, 2021)* and *rstan* (Stan Development Team, 2024). Model distribution and link function varied between different predictors: details are available in Appendix D Table 1. In general, treatment was the effect, with tank replicate, genotype and/or sample as blocking factors. In all cases, weakly informative priors were used, calculated from the median and absolute median deviation of the reference group for the model. For all models, Markov chain Monte Carlo sample diagnostic outputs were checked for well-mixed traceplots, chain convergence (R-hat < 1.01), autocorrelation and effective sample size. Plots of posterior predictive checks and DHARMa residuals were then examined to determine goodness of fit for each model. Median and 95% credibility intervals (calculated as 95% Highest Posterior Density Interval) were calculated to visualise differences in responses between treatments. The Bayesian probability that the response of one treatment was greater than the response of another treatment was calculated for pairwise analysis of treatments, and is presented as $P_{x>y}$ (i.e. probability that response of treatment 'x' is greater than treatment 'y').

4.3.5 Ethics statement

This experiment was conducted in compliance with the Great Barrier Reef Marine Park Authority Collection permit (G12/35236.1) and James Cook University Animal Ethics permit (A2787).

4.4 Results

4.4.1 Water quality

A summary of the modelled median and 95% credibility intervals for each water quality parameter is presented in Table 4.1. Ammonium (NH_4) concentrations were 2.64 times higher (modelled median) in tanks with access to fish wastes than those without. Higher NH_4 was recorded in the LiveFeeds + Fish treatment (0.449 µmol L^{-1}) compared to Fish (0.351

µmol L^{-1}), indicating some additional NH_4 from the live feeds, though there was no difference in the concentration of NH_4 between the Control (0.112 µmol $L^{-1}$and LiveFeeds (0.114 µmol L^{-1}) treatment tanks. Water from the Pellets treatment, however, contained slightly higher NH_4 concentrations (0.173 µmol L^{-1}) than the Control tanks. The Dissolved fish holding tanks also had a slightly higher concentration of NH_4 (0.385 µmol L^{-1}, 0.348 – 0.417) compared to the Dissolved coral tanks (0.229 µmol L^{-1}, 0.211 – 0.252) that it was supplying.

N:P ratio was highest in LiveFeeds + Fish (10.3), followed by Fish (8.9) and the Dissolved fish tank (9.1), and was lowest in Control (6.1). Generally, NO_2, NO_3 and PO_4 were highest in LiveFeeds + Fish tanks, followed by Fish and the Dissolved fish tanks. LiveFeeds and Pellets varied, but typically had slightly higher concentrations than the Control and Dissolved coral tanks, which had the lowest concentrations of these dissolved nutrients. LiveFeeds + Fish had the lowest DOC concentration, whilst the Dissolved fish tanks had the highest, followed by the Dissolved coral tanks. DOC was relatively similar in all other treatments. Particulate C and N was likely influenced by filamentous algae observed growing in the tanks (and was visibly observed on filters during PC/N sampling), but was typically highest in the Dissolved corals tanks, lowest in Live Feeds, and relatively similar in all other tanks.

Table 4.2: Water quality from treatment tanks during experiment (median and 95% CI (HPDI) from Bayesian hierarchical models)

Treatment	NH_4 ($\mu mol\ L^{-1}$)	NO_2 ($\mu mol\ L^{-1}$)	NO_3 ($\mu mol\ L^{-1}$)	PO_4 ($\mu mol\ L^{-1}$)	DOC ($mg\ L^{-1}$)	PC ($\mu g\ L^{-1}$)	PN ($\mu g\ L^{-1}$)	N:P
Control	0.112 0.102 – 0.122	0.049 0.044 – 0.052	0.917 0.824 – 1.028	0.169 0.161 - 0.176	1.13 1.10 – 1.16	54.29 49.64 – 58.69	11.55 10.53 – 12.66	6.1 5.8 – 6.5
Pellets	0.173 0.157 – 0.189	0.057 0.052 – 0.063	1.153 1.023 – 1.284	0.171 0.164 – 0.179	1.11 1.09 – 1.14	57.31 52.64 – 62.55	12.76 11.63 – 14.07	7.8 7.3 – 8.3
LiveFeeds	0.114 0.103 – 0.124	0.053 0.048 – 0.058	1.047 0.927 – 1.165	0.178 0.170 – 0.186	1.13 1.10 – 1.16	45.47 42.09 – 48.88	9.67 8.77 – 10.61	6.6 6.2 – 7.0
LiveFeeds + Fish	0.449 0.410 – 0.491	0.058 0.052 – 0.063	1.419 1.265 – 1.580	0.184 0.176 – 0.192	1.07 1.04 – 1.10	52.95 49.52 – 57.37	10.57 9.53 – 11.56	10.3 9.7 - 10.9
Fish	0.351 0.322 – 0.383	0.056 0.051 – 0.061	1.223 1.084 – 1.358	0.179 0.170 – 0.187	1.13 1.10 – 1.17	55.68 50.58 – 60.68	11.60 10.48 – 12.68	8.9 8.4 – 9.5
Dissolved Coral tank	0.229 0.211 – 0.252	0.050 0.045 – 0.054	0.849 0.764 – 0.955	0.141 0.133 – 0.149	1.16 1.13 – 1.20	64.01 57.69 – 70.28	13.75 12.50 – 15.15	7.5 7.1 – 8.0
Dissolved Fish tank	0.385 0.348 – 0.417	0.051 0.046 – 0.056	1.229 1.107 – 1.379	0.175 0.166 – 0.182	1.22 1.18 – 1.26	58.59 52.83 – 64.20	11.74 10.61 – 12.83	9.1 8.6 – 9.7

4.4.2 Growth performance, nutritional composition and symbiont density

4.4.2.1 Pocillopora verrucosa

There was no difference in the proportional growth of *Pocillopora verrucosa* between the LiveFeeds + Fish (1.078 x greater buoyant weight than initial weight three months after the start of experiment), Fish (1.081 x greater) or Dissolved (1.080 x greater) treatments ($P <$ 85%), though all treatments exhibited higher growth compared to the Control (1.063 x greater) ($P_{LF,F,D>C} > 90\%$). *P. verrucosa* fragments exhibited highest growth overall in the LiveFeeds treatment (1.109 x greater), with evidence this was a higher growth than the Control, Pellets, Dissolved, Fish and LiveFeeds + Fish ($P_{L>C,P,D,F,LF} = 92\%$) treatments (Fig. 4.1a). *P. verrucosa* fragments in the Pellets treatment had much lower growth (1.049 x greater) compared to all other treatments ($P_{D,F,L,LF>P} > 95\%$) except for Control ($P_{C>P} = 87\%$).

Tissue protein content was highest in *P. verrucosa* fragments from the LiveFeeds treatment (427.5 µg cm^{-2}), with very strong evidence this represented an increase over the corals in the other treatments ($P_{L>C,D,F,LF,P} > 99\%$) (Fig. 4.1b). Control (327.9 µg cm^{-2}) and LiveFeeds + Fish (297.4 µg cm^{-2}) corals had the next highest protein concentrations, but the evidence suggested only Control represented a significant increase compared to the Dissolved (270.6 µg cm^{-2}), Pellets (282.8 µg cm^{-2}) and Fish (258.0 µg cm^{-2}) treatments ($P_{C>D,P,F} > 91\%$). Field fragments also had relatively high protein (327.1 µg cm^{-2}), and this was higher than the Post-acclimation fragments (266.2 µg cm^{-2}) ($P_{Fie>Pa} = 96\%$).

After three months, there were few differences in photosynthetic efficiency between the *P. verrucosa* fragments among the treatments (Fig. 4.1c), though Fish treatment corals tended to exhibit a lower photosynthetic efficiency compared to all other treatments (0.627 Fv/Fm) ($P_{C,D,L,LF,P>F} > 90\%$). Symbiont densities were higher in the LiveFeeds fragments compared to all other treatments (1.48 x10^6 cm^{-2}, $P_{L>C,F,D,LF,P} > 97\%$) (Fig. 4.1d). Only Dissolved *P. verrucosa* (1.23 x10^6 cm^{-2}) were also found to have higher symbiont concentrations than the Control ($P_{P>C} = 92\%$), whilst Fish (1.19 x10^6 cm^{-2}), LiveFeeds + Fish (1.16 x10^6 cm^{-2}) and Pellets (1.16 x10^6 cm^{-2}) all had similar concentrations to the Control fragments (1.04 x10^6 cm^{-2}). All fragments subjected to the experimental treatments had higher densities than the Field (0.77 x10^6 cm^{-2}, 0.53 – 0.99) and Post-acclimation corals (0.86 x10^6 cm^{-2}, 0.63 – 1.08) ($P_{>Fie,S} > 90\%$).

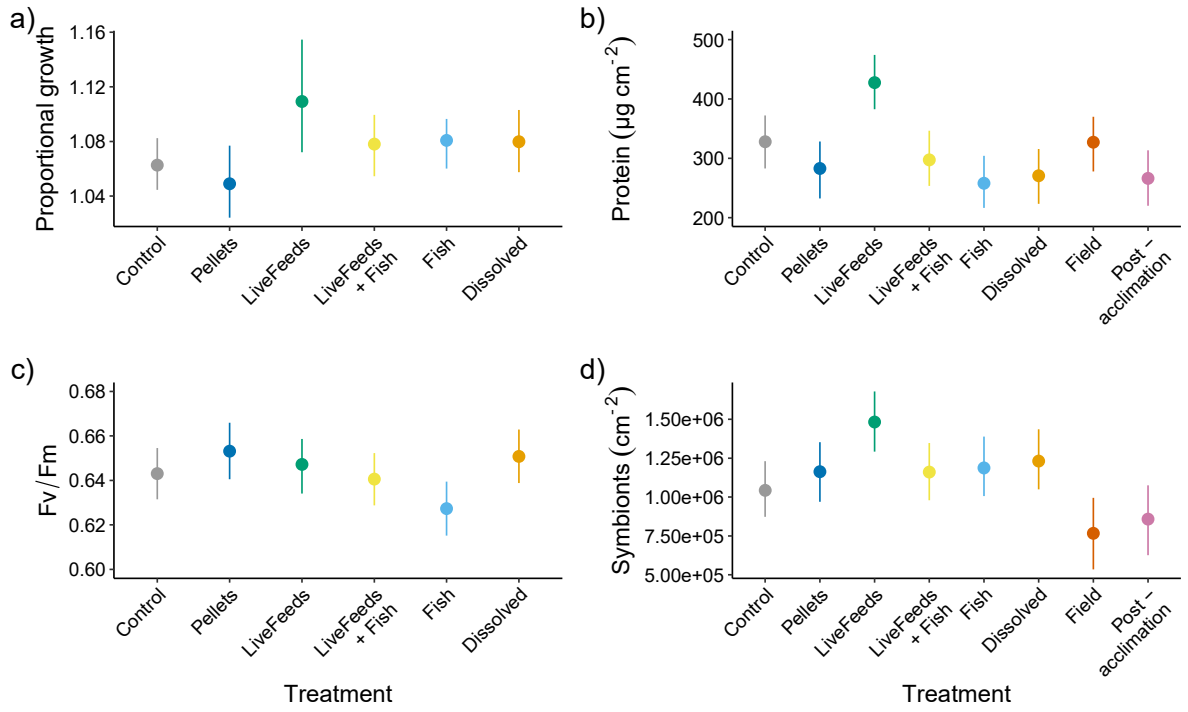

Figure 4.1: *Pocillopora verrucosa* a) Proportional growth (Buoyant weight after three months subjected to different dietary treatments ÷ buoyant weight at start of the experiment), b) Tissue protein content, c) Photosynthetic efficiency measured as Fv/Fm after three months exposure to treatments and d) Concentration of Symbiodiniaceae in tissue. Coloured points and bars represent modelled median and 95% credibility intervals.

4.4.2.2 Acropora kenti

Acropora kenti was the only species to experience significant mortality during the experiment; Dissolved treatment tanks had the highest overall survival (62.5 % ± 8.5 %; mean ± SE) whilst Control had the lowest (30.0 % ± 5.8 %), and the remaining treatments all had relatively similar survival levels (40 – 53%). Growth was also highest in Dissolved (1.036 x greater), with evidence this growth was higher than all other treatments ($P_{D>F,L,LF,P}$ > 90%) except the Control ($P_{D>C}$ = 83%) (Fig. 4.2a). There was no evidence for a difference in growth between Fish, LiveFeeds + Fish, LiveFeeds or Pellets, or between these treatments and the Control (P < 85%).

Despite having the highest growth, fragments in the Dissolved treatment (256.4 µg cm^{-2}) had similar protein concentrations to corals subjected to the Pellets (269.7 µg cm^{-2}) and LiveFeeds + Fish (256.7 µg cm^{-2}) treatments ($P_{D<P,LF}$ < 85%). Control (235.6 µg cm^{-2}), Fish (226.8 µg cm^{-2}) and LiveFeeds (228.2 µg cm^{-2}) all had slightly lower protein content

compared to the other treatments. In contrast, protein was highest in Field samples of *A. kenti* compared to Post-acclimation and all fragments from treatments (Fig. 4.2b), and Post-acclimation fragments also had higher protein than any of the treatment corals (P > 85%).

Similar to *P. verrucosa*, there were few differences in photosynthetic efficiency between the treatments, though there was strong evidence that Fv/Fm was lower in LiveFeeds + Fish and higher in LiveFeeds ($P_{L>LF}$ = 98%; Fig. 4.2c). Symbiont densities were greatest in Dissolved (6.07 x10^5 cm^{-2}) and lowest in Control fragments (3.58 x10^5 cm^{-2}), whilst the other treatment corals had relatively similar symbiont concentrations (4.47 – 5.51 x10^5 cm^{-2}) (Fig. 4.2d). Post-acclimation (8.67 x10^5 cm^{-2}) fragments had higher median symbiont densities than the treatment and Field corals (P > 98%), though Field corals had similar symbiont densities (6.42 x10^5 cm^{-2}) to both Dissolved and Pellets fragments ($P_{F<D,P}$ < 85%).

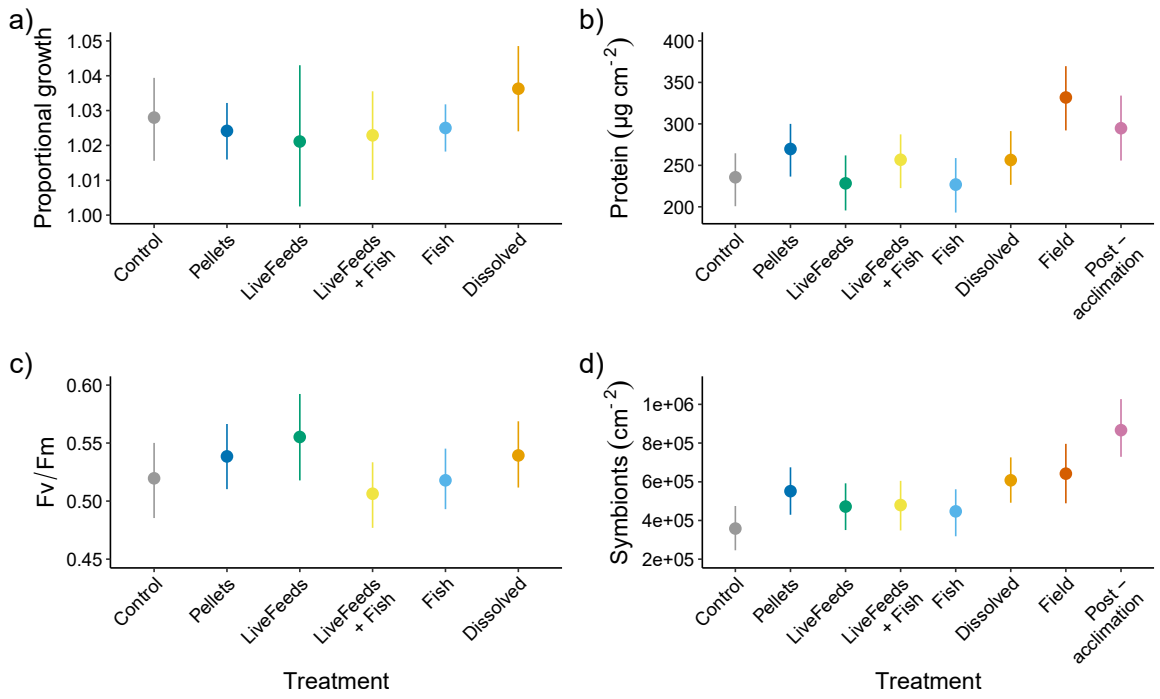

Figure 4.2: *Acropora kenti* a) Proportional growth (Buoyant weight after three months subjected to different dietary treatments ÷ buoyant weight at start of the experiment), b) Tissue protein content, c) Photosynthetic efficiency measured as Fv/Fm after three months exposure to treatments and d) Concentration of Symbiodiniaceae in tissue. Coloured points and bars represent modelled median and 95% credibility intervals.

4.4.2.3 Porites lutea

Porites lutea fragments exhibited highest growth in Fish (1.099 x greater), Dissolved (1.098 x greater) and LiveFeeds + Fish (1.092 x greater) treatments. Pellets had lower growth

compared to all other treatments (1.067 x greater; $P_{>P} > 98\%$), and Control (1.089 x greater) and LiveFeeds (1.089 x greater) had lower growth than fragments in the Fish treatment ($P_{F>C,L} > 86\%$; Fig. 4.3a).

P. lutea fragments from the LiveFeeds + Fish (1145.3 µg cm^{-2}) treatment had the highest protein concentration of the treatment corals ($P_{LF> > 92\%)}$, though the Fish fragments (969.9 µg cm-2), and Field fragments of *P.lutea* (1368.4 µg $cm^{-2.}$) also had similar protein content $P_{LF>Fie,F} < 85\%$). All remaining treatment fragments had median concentrations of 706 – 757 µg cm^{-2} (Fig. 4.3b). Post-acclimation fragments also had higher protein content than the majority of the treatments (1086.0 µg cm^{-2}), though was not dissimilar to that observed in the LiveFeeds + Fish and Fish treatment ($P_{S<LF,F} < 85\%$).

Photosynthetic efficiency was higher in the fish treatments (Dissolved, Fish and LiveFeeds + Fish) compared to the Control ($P_{F,LF,D,>C} > 98\%$). LiveFeeds also had lower photosynthetic efficiency relative to these treatments ($P_{F,M,D>L} > 90\%$), while fragments in Pellets had similar levels to the three fish treatments (Fig. 4.3c). Symbiont concentrations were elevated in the three fish treatment (2.25 – 2.40 x10^6 cm^{-2}) compared to both Control (1.92 x10^6 cm^{-2}) and LiveFeeds (1.73 x10^6 cm^{-2}) ($P_{D,F,LF>C,L} > 87\%$). Field (1.71 x10^6 cm^{-2}) and SeaSim (1.96 x10^6 cm^{-2}) had lower concentrations compared both Pellets and the three fish treatments (Fig. 4.3d), though only Field corals had evidence of being significantly lower ($P_{Fie<D,F,LF,P} > 93\%$).

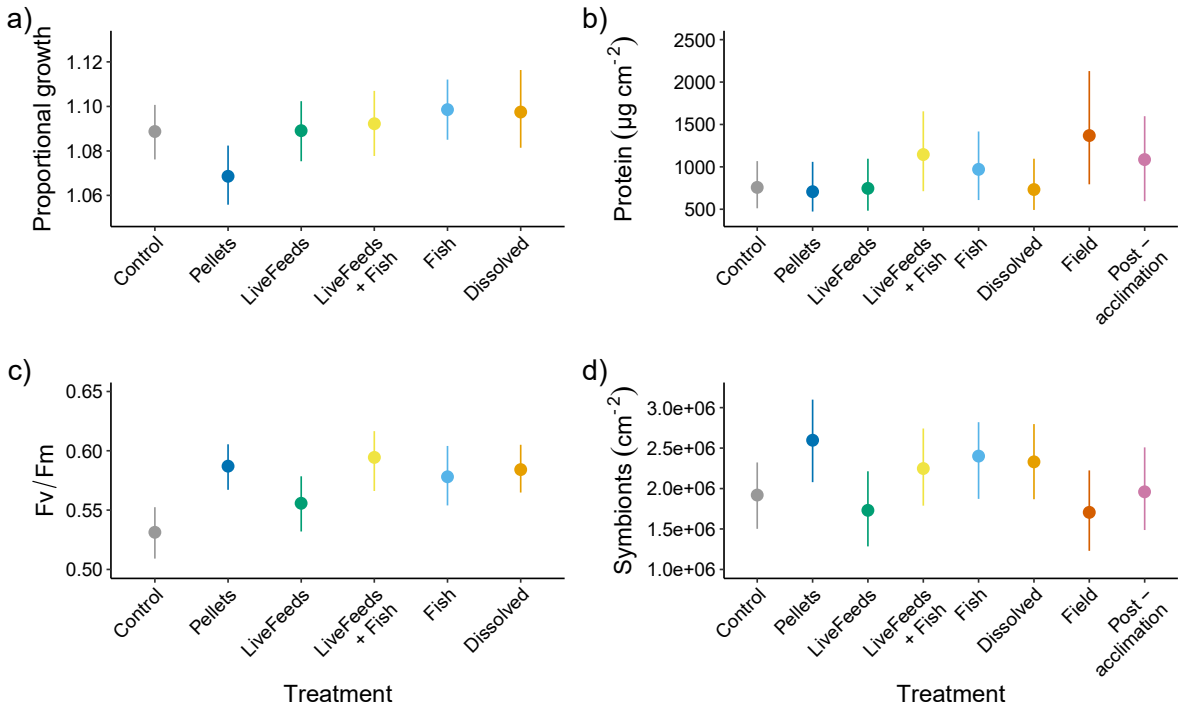

Figure 4.3: *Porites lutea* a) Proportional growth (Buoyant weight after three months subjected to different dietary treatments ÷ buoyant weight at start of the experiment), b) Tissue protein content, c) Photosynthetic efficiency measured as Fv/Fm after three months exposure to treatments and d) Concentration of Symbiodiniaceae in tissue. Coloured points and bars represent modelled median and 95% credibility intervals.

4.4.2.4 Platygyra daedalea

The highest growth in *Platygyra daedalea* fragments was observed in the LiveFeeds treatment (1.042 x greater). Though this was not significantly higher compared to the Control (1.042 x greater; $P_{L>C}$ = 53%) there was evidence this was an improvement over the LiveFeeds + Fish (1.017 x greater), Fish (1.023 x greater) and Pellets (1.025 x greater) treatments ($P_{L>LF,F,P}$ > 95%; Fig. 4.4a) .

Protein content was highest in Pellets (591.3 µg cm^{-2}) and Dissolved (590.2 µg cm^{-2}) though this did not represent a significant increase compared to Control fragments (512.8 µg cm^{-2}; $P_{D,P>C} \approx$ 84%). Fish (530.0 µg cm^{-2}) and LiveFeeds + Fish (493.0 µg cm^{-2}) also had similar protein levels to the Control, whilst LiveFeeds (464.3 µg cm^{-2}) had the lowest concentration of all the treatment fragments (Fig. 4.4b). Field *P. daedalea* samples had much higher protein content than any of the treatment or Post-acclimation corals ($P_{Fie>}$ > 97%), though Post-acclimation fragments had similar levels to both Dissolved and Pellets (570.6 µg cm^{-2}).

Conversely, while photosynthetic efficiency was similar in Pellets and Dissolved, it was lower than the other treatments ($P_{C,F,LF,L>P,D}$ > 90%). The other treatments had similar efficiency levels, though corals in the Fish treatment displayed the highest overall photosynthetic efficiency (Fig. 4.4c). Symbiont concentrations were also highest in the Fish (1.28 x10^6 cm^{-2}) and LiveFeeds + Fish (1.19 x10^6 cm^{-2}) fragments (Fig. 4.4d), though only Fish corals had consistent evidence of concentrations being higher than other treatment corals ($P_{F>C,D,L,P}$ > 87%). Dissolved (1.12 x10^6 cm^{-2}), Control (1.09 x10^6 cm^{-2}) and LiveFeeds (1.07 x10^6 cm^{-2}) all had slightly lower concentrations compared to these treatments, with the Pellets (0.95 x10^6 cm^{-2}) having the lowest of all the treatment corals. Field and Post-acclimation also had lower symbiont concentrations compared to all the *P. daedalea* fragments in the treatments ($P_{>Fie,S}$ > 85%).

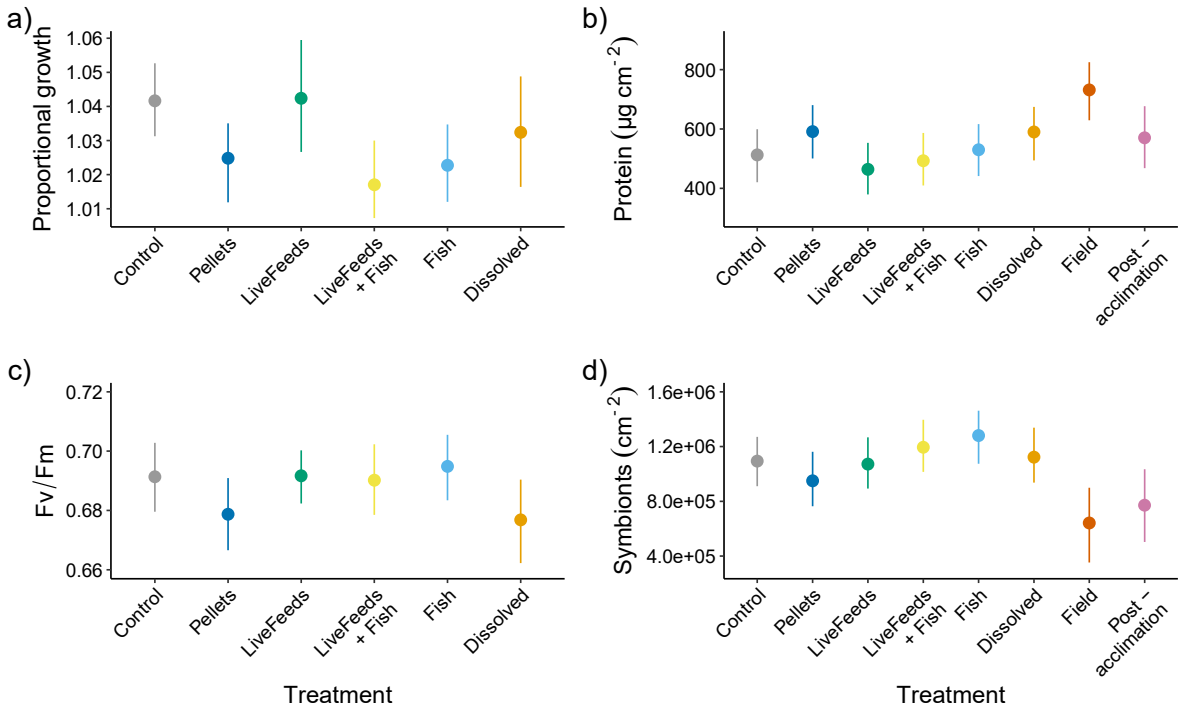

Figure 4.4: *Platygyra daedalea* a) Proportional growth (Buoyant weight after three months subjected to different dietary treatments ÷ buoyant weight at start of the experiment), b) Tissue protein content, c) Photosynthetic efficiency measured as Fv/Fm after three months exposure to treatments and d) Concentration of Symbiodiniaceae in tissue. Coloured points and bars represent modelled median and 95% credibility intervals.

4.5 Discussion

4.5.1 Coral species varied responses to treatments

In this study corals were exposed to various isolated or combined dissolved and particulate wastes from schooling damselfish. While the response of the different coral species varied, all species displayed some positive responses to the presence of fish wastes. For example, tissue protein and symbiont densities were higher in *A. kenti* and *P. lutea* with access to dissolved fish wastes, with growth also slightly enhanced in *P. lutea* under these treatments. While live feeds resulted in the highest growth and protein content for the more heterotrophic *Pocillopora verrucosa*, the presence of fish or dissolved fish wastes could increase growth and symbiont concentrations in this species compared to unfed controls. *Platygyra daedalea* had a more mixed response but did also have higher symbiont and protein densities in treatments with dissolved fish wastes.

Coral fragments performed well in both LiveFeeds + Fish and Fish treatments (where fish were kept with the corals) and the Dissolved treatment (where corals only had access to dissolved fish wastes), suggesting that benefits were primarily from the dissolved portion of fish waste. Though there was little evidence corals directly fed on fish faeces, it has been suggested that leeching from faecal pellets could still be an important source of nitrogen or phosphorous for corals (Meyer et al., 1983; Schiettekatte et al., 2023). Higher protein and symbiont concentrations in corals due to association with fish is in line with previous studies on fish-coral interactions (Chase et al., 2018; Chase et al., 2020), as is the increased growth observed in *P. lutea* and *P. verrucosa* in the present study (Holbrook et al., 2008; Liberman et al., 1995; Meyer & Schultz, 1985b).

4.5.2 Effects of nutrient enrichment by fish

Treatments with access to fish waste were associated with increased nitrogen and phosphorous availability (particularly NH_4), which likely influenced the responses observed in the present study, particularly relating to changes in photobionts concomitant with moderate nitrogen levels (Grover, 2002; Marubini & Davies, 1996; Morris et al., 2019; Muller-Parker et al., 1994; Rosset et al., 2015; Shantz & Burkepile, 2014). Carbon-rich photosynthates from algal symbionts can often meet the energy demands and sustain

growth of corals, and excess symbiont cells can be digested by coral hosts in order to uptake important nutrients like nitrogen and phosphorous (Falkowski et al., 1984; Wiedenmann et al., 2023). High nitrogen availability with phosphorous limitation can disturb the coral-algae symbiosis, leading to increased susceptibility to bleaching (Rosset et al., 2017; Wiedenmann et al., 2012), though high NH_4 enrichment with simultaneous high PO_4 enrichment can increase the density of symbionts whilst also decreasing the translocation of fixed carbon from symbionts to the coral host (Ezzat et al., 2015; Morris et al., 2019). Enrichment of both nitrogen and phosphorous at moderate levels has shown to increase carbohydrate and nitrogen content of coral tissue (Blanckaert et al., 2023; Wiedenmann et al., 2023), with a N:P ratio of 4 – 7 considered normal for Great Barrier Reef waters (Rosset et al., 2017).

In this experiment, N:P ratio was influenced by the presence of fish; being higher in treatments with fish (e.g. 10.3 in LiveFeeds + Fish vs 6.9 in Control), though there was evidence for PO_4^{3-} enrichment from both LiveFeeds and the treatments with fish. As such, it is likely most coral in the experiment experienced moderate N and P enrichment, with *P. verrucosa* and *P. lutea* demonstrating higher growth and symbiont densities in treatments with high N:P ratios (Dissolved, Fish, LiveFeeds + Fish) than Control corals. Photosynthetic rates were also observed to be within normal ranges for all corals (Kuanui et al., 2020). Taken together this suggests that the supply of fixed carbon from symbionts was not adversely affected by N and P enrichment, and that the higher symbiont densities may represent an increased food supply for the corals as they may 'farm' and digest the excess algal cells (Wiedenmann et al., 2023).

However, high symbiont density does not necessarily correspond to ubiquitous high growth. For example, *P. daedalea* subjected to treatments with fish displayed the highest symbiont concentrations yet recorded its lowest growth. Similarly, *A. kenti* demonstrated higher symbiont densities in the Dissolved treatment, but rates of growth remained similar to the low-symbiont density Control corals. The observed N:P levels were potentially optimal for *P. lutea* and *P. verrucosa*, though for *P. daedalea* and *A. kenti* they potentially compromised carbon exchange with the symbionts, demonstrating the importance of considering species specific responses of corals to nutrient availability (Blanckaert et al., 2023; Morris et al., 2019). Similarly, *A. kenti* was the only coral with significant mortality during the experiment.

The cause of this mortality is difficult to determine, though given a relatively high level (> 30%) was observed in all treatments, potentially the culture conditions were not ideal for this species.

Interestingly, there were few differences in photosynthetic efficiency (F_v/F_m) between the treatments for any of the coral species; in contrast to previous studies which found that under ambient conditions fish could induce higher photosynthetic rates in their coral hosts (Chase et al., 2018; Garcia-Herrera et al., 2017; Shantz et al., 2022). However, these previous published experiments were done under lower flow conditions, and in the case of Garcia-Herrera et al. (2017) with small colonies, whilst the present experiment used fragments and circulation pumps to remove the possible effects of oxygenation from fish fanning (Berenshtein et al., 2015; Goldshmid et al., 2004). This could account for the limited response of photosynthetic efficiency to the fish, as previous research has shown increased flow can drive increases in coral photosynthesis, thus the higher flow may have dampened any influence from the fish (Mass et al., 2010).

All coral species in the present experiment recorded different protein and symbiont densities compared to those from the field and those acclimated to the facility prior to the commencement of the experiment, however it should be noted that wild corals do experience natural, seasonal fluctuations in various physiological parameters including their nutritional composition (Conlan et al., 2020; Fitt et al., 2000). General increases in symbiont density between the field to the captive-acclimated corals were likely driven by acclimation to the lower light conditions compared to the field (Leletkin, 2000; Titlyanov, 1991), increased nitrogen (Marubini & Davies, 1996; Muller-Parker et al., 1994; Stambler, 1998) and food availability (Houlbreque et al., 2004). Protein was also higher in field corals compared to captive corals, apart from *P. verrucosa* which had a significantly higher protein concentration in the LiveFeeds treatment. Protein is typically higher at sites of growth in corals (Conlan et al., 2018b), though higher growth rates are not always associated with elevated protein concentrations (Conlan et al., 2018a), and concentrations may be reduced if the dietary protein supply is inadequate, where protein may be transported from less vital areas of the coral and subsequently catabolised to maintain physiological function (Wilson, 2002). Nevertheless, it is likely that the higher protein, for example in *P. verrucosa* fragments

in the LiveFeeds treatment, was due to the higher growth of in these corals, whilst the lower concentration of the other corals compared to the Field samples may be a result of protein depletion due to a sub-optimal supply of dietary protein.

4.5.3 The influence of heterotrophic feeding

While the presence of fish did enhance the growth of *P. verrucosa* fragments, the highest growth for this species was observed in the LiveFeeds treatment. This genus has been identified as comparatively more heterotrophic than *Acropora* or *Porites* (Radice et al., 2019a), which would explain the greater growth, protein and symbiont concentration observed when given access to live feeds. Past research has shown that even in high light environments, more heterotrophic corals with access to heterotrophic food sources will grow faster and have higher symbiont and protein concentrations compared to unfed colonies (Conlan et al., 2018a; Ferrier-Pages et al., 2003; Forsman et al., 2011; Houlbreque et al., 2004). Interestingly, there was no significant difference observed between the LiveFeeds + Fish treatment (which also had access to live feeds) and the Fish or Dissolved treatment. A likely explanation for this is the *C. viridis* in the tank were opportunistically feeding on the supplied live feeds before the corals were able to capture them, resulting in a lower food availability for the corals. *P. daedalea* have also been identified as more heterotrophic than other coral species (Conti-Jerpe et al., 2020), but did not have evidence for a difference in growth rates between the Control and LiveFeeds treatments. *Platygyra* corals have been noted to be tolerant to a wide range of conditions (Chui & Ang, 2017; Conti-Jerpe et al., 2020; Kuanui et al., 2020) and exhibit relatively slow growth (Weber & White, 1974); thereby, it may be that the experimental conditions where not sufficient to create a significant different in growth rates for this species.

Across all coral species in the present experiment, the feeding of fish pellets to coral fragments directly resulted in reduced growth compared to the other feed treatments and the unfed control. Some tissue loss was also observed in fragments from this treatment, consistent with the Osinga et al. (2012) observation that batch feeding with dry food could potentially harm corals. The use of batch feeds to provide optimal nutrition for captive corals remains a vexing topic for researchers and aquarists, though it is obvious that at least some

level of heterotrophic feeding or nutrient enrichment is necessary to enhance production performance (Forsman et al., 2011).

4.5.4 Considerations for integrating fish into large-scale coral aquaculture

Long-term impacts of nutrient enrichment on corals should be considered when considering how to implement fish into coral aquaculture. This experiment demonstrated some benefits from moderate N:P enrichment, though chronic nutrient enrichment can have both negative and positive effects, depending on the level of enrichment (Becker et al., 2021; Fox et al., 2021; Vega Thurber et al., 2014). Such responses are complex and often species specific, even within closely related coral species, thus commercial coral propagation must carefully consider what nutrient profiles are most appropriate to support the range of species they wish to culture. Similarly, the effectiveness of the nutrient enrichment of the fish will depend on how the water in the system is retained or treated. Here we utilised a relatively high turnover flow-through system, but the rate of retention or removal of NH_4 would likely be lower in recirculating systems that use biological and chemical filters to control excess nutrients. As such, coral aquaculture facilities need longer-term observations to determine how effective nutrient enrichment by companion fish would be their unique systems.

As a potential source of enrichment, NH_4 is the symbionts' preferred form of inorganic nitrogen, whilst NO_3 from anthropogenic sources is less favoured (Morris et al., 2019), thus incorporating fish into coral culture may be a convenient method of provisioning coral with a more balanced nutrient profile (N/P) to maintain holobiont health. While NH_4 and PO_4 can also be dosed using readily available inorganic liquid fertilisers, research has shown corals generally respond better to natural sources of these nutrients, such as fish, than artificial ones (Shantz & Burkepile, 2014). Additionally, if necessary, fish nutrients and artificial dosing could be used in conjunction to achieve a desired nutrient profile, similar to the paired application of chemical treatments and cleaner fish to control parasitic copepods in salmon aquaculture (Barrett et al., 2020; Blanco Gonzalez, 2019).

Though the direct effects of fish co-culture on coral growth performance and symbiont concentration may vary depending on species and husbandry conditions, the application of fish in coral aquaculture can have other benefits. Fish such as *Pseudocheilinus hexataenia*

have been shown to be potential biological controls for coral parasites like *Prosthiostomum* flatworms (Barton et al., 2020b). Similarly, herbivorous species such as Acanthurids are also used as algae controllers both in *ex situ* facilities where they are deliberately added to aquaria (Leal et al., 2014; Nakamura et al., 2011) or *in situ* nurseries where they may be released or naturally recruit (Frias-Torres et al., 2015; Omori & Iwao, 2014; Shafir et al., 2006). As such, it is worth considering the potential roles fish could play in co-culture scenarios, both as pest and/or algae controllers, and as a source of nutrient enrichment. Based on this experiment's results, 14 g of fish biomass per 50 L with twice daily feeds for the fish could be suggested as an initial baseline density of fish in coral culture systems, with subsequent adjustment depending on which species of fish is used.

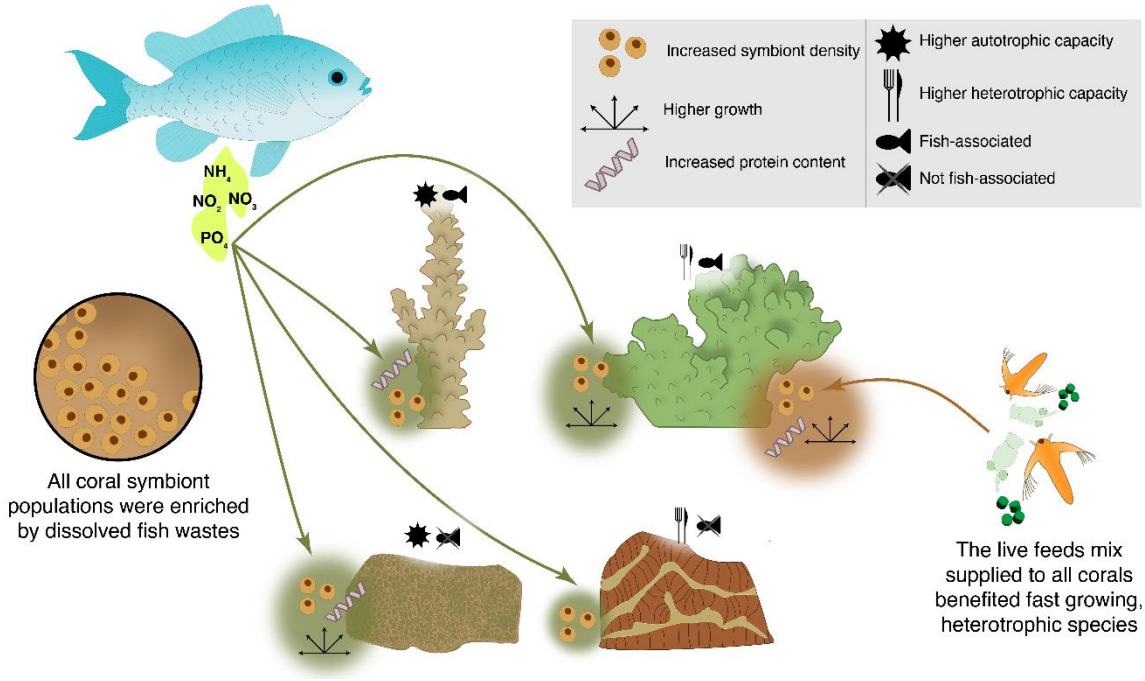

Figure 4.5: Visual summary of the observed benefits from fish co-culture on the various coral species.

Overall, our study has demonstrated that integrated fish-coral culture has the potential to enhance physiological traits of cultured corals by supplying a source of moderate nutrient enrichment. However, the response of corals to fish co-culture varies markedly between genera, thus producers must carefully consider the nutrient requirements of their targeted coral species before integrating fish into culture (Fig. 4.5). The baseline of 14 g of fish biomass per 50 L of tank water provides a preliminary target for producers, though needs to

be tailored to different coral's requirements, and to the variable nutrient output of different fish species if introducing fish companions to serve other functions, e.g. algae removal. Ultimately, integrating fish into coral culture represents a potential efficient, minimal-husbandry method to provide enrichment to cultured corals, which will improve productivity to meet the growing demands of both reef restoration and the ornamental industry.

CHAPTER 5 - Nutritional and microbial responses of *Pocillopora verrucosa* to co-culture with *Chromis viridis* damselfish

5.1 Abstract

Close associations between fish and corals have been demonstrated to produce benefits such as increased growth and thermal tolerance in coral. To identify potential underlying microbial and nutritional responses of corals to such associations, and whether they could be applicable to enhancing coral aquaculture, wild-collected *Pocillopora verrucosa* were cultured with various combinations of live feeds and schools of juvenile *Chromis viridis* damselfish over a three-month period. Relative to unfed controls, total lipid concentrations were elevated in captive corals with access to dissolved fish wastes and at moderate levels in those supplied live feeds, likely due to preferential direction of energy to growth in corals with access to the heterotrophic nutrients of the live feeds. Protein content was also enhanced in corals with access to live feeds and/or dissolved fish wastes, though the coral supplied with only live feeds displayed the highest protein concentration of all treatments. However, all captive corals demonstrated a significant reduction in storage lipid concentrations compared to field corals. Fatty acid analysis indicated these shifts were likely the result of higher light levels in the field supporting Symbiodiniaceae photosynthesis, and potentially higher heterotrophic nutrient acquisition through feeding on wild zooplankton populations. Microbial communities associated with coral tissues were influenced by the presence of different feeds and fish, with the combination of live feeds and *C. viridis* in the tanks contributing to a bacterial community most like wild *P. verrucosa,* dominated by *Endozoicomonas* affiliated taxa. Integrating fish into captive coral culture alongside feeding with appropriate live feeds therefore represents a potential simple approach to enhance growth, symbiont, total lipid and protein content, and maintain field-like microbial communities in captive corals.

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5.2 Introduction

Sustainable production of coral in aquaculture systems is important to supply reef restoration endeavours and the burgeoning ornamental industry (Barton et al., 2017; Randall et al., 2020). Enhancing survival and growth of sexually and asexually produced coral propagules is critical to meet production demands, with captive environmental conditions such as light, water quality, fouling and heterotrophic feeding all vital factors for successful aquaculture (Ferrier-Pages et al., 2003; Forsman et al., 2011; Houlbrèque et al., 2003; Neil et al., 2021). Nutrition is a critical parameter for captive production of any animal, though in corals, up to 90% of their nutritional needs may be met by translocated photosynthates and direct feeding on their algal endosymbiont partner, Symbiodiniaceae (Falkowski et al., 1984; Wiedenmann et al., 2023). However heterotrophic feeding has been shown to have additional benefits such as enhanced coral survival, growth and increased resistance to environmental stressors (Ferrier-Pagès et al., 2010; Ferrier-Pages et al., 2003; Toh et al., 2014). Studies on coral nutrition have demonstrated the need to tailor composition and delivery of feeds to suit individual species to maximise uptake while minimising cost (Conlan et al., 2018a; Conlan et al., 2019; Ding et al., 2021; Kuanui et al., 2016; Osinga et al., 2011; Tagliafico et al., 2018; Toh et al., 2014). Most systems that maintain captive corals use mass produced feeds such as enriched *Artemia* or rotifers, microalgae and/or commercial diets (Borell et al., 2008; Petersen et al., 2008; Séré et al., 2010; Toh et al., 2014), though more natural or mixed diets have been shown to have improved effects on coral survival and growth (Conlan et al., 2018a; Conlan et al., 2017a). As such, the implementation of more complex diets may be beneficial in future coral aquaculture efforts.

Alongside nutritional development, culture practices are also an important consideration in the implementation of new aquaculture ventures. Methodologies such as integrated multi-trophic systems which involved growing species in linked or mixed mesocosm systems rather than in monoculture, are gaining prominence (Barrington et al., 2008; Knowler et al., 2020; Ridler et al., 2007). The successful integration of multiple species within the same system has a positive impact on the economic and environmental feasibility of the culture system (Hala et al., 2024). On reef systems, corals that have close associations with fish have been observed to have higher growth (Holbrook et al., 2008; Liberman et al., 1995; Meyer &

Schultz, 1985b) and photosynthetic rates (Garcia-Herrera et al., 2017), in addition to better responses to stressors such as high temperatures (Chase et al., 2018; Shantz et al., 2022) or sedimentation (Chase et al., 2020). These benefits have been attributed to different mechanisms; firstly, fish activity promotes water circulation throughout the coral colony, which reduces the diffusive boundary layer and in turn increases oxygen and nutrient availability and photosynthesis (Garcia-Herrera et al., 2017; Goldshmid et al., 2004; Liberman et al., 1995). Secondly, fish excrete dissolved nitrogen and phosphorous, which are limiting nutrients for coral endosymbiont growth, in forms that are readily assimilated by corals and/or their symbionts (e.g. ammonia)(Holbrook et al., 2008; Meyer & Schultz, 1985a; Shantz & Burkepile, 2014; Shantz et al., 2015). Some have also suggested that corals may actively feed upon the particulate organic matter (POM) provided by fish faeces (Meyer et al., 1983; Shantz et al., 2022). As such, integrating fish into coral aquaculture could present a relatively simple method to improve coral production via improved physiological performance and nutritional status.

When testing the effects of different nutrient sources or culture conditions on corals, it is important to consider several aspects of the coral holobiont response. Whilst survival and growth are typically the primary metrics producers look to optimise, factors such as nutritional status, including energy stores, and microbiome composition should be considered. These factors can help assess coral fitness for restoration out-planting or commercial export, both of which represent physiologically challenging events (Boch et al., 2019; Delbeek, 2008; Gantt et al., 2023; Lirman, 2000). The two most energy dense macronutrients, namely, lipid and protein, represent important energy sources for coral growth and reproduction, and are utilised to help the animal survive during periods of stress such as bleaching (Grottoli & Rodrigues, 2011; Houlbreque & Ferrier-Pages, 2009). Changes to coral microbial communities can be indicative of stress, though also occur in response to external factors such as environmental conditions, and may have physiological implications for the coral host (Bourne et al., 2016; Voolstra et al., 2024).

The results presented in Chapter 4 demonstrated that fish-associated branching coral *Pocillopora verrucosa* had increased growth and symbiont density when supplied with live feeds, or dissolved fish wastes from juvenile *Chromis viridis,* compared to unfed control

corals. To identify the underlying drivers of these changes and derive potential metrics for coral fitness for long-term holding or out-planting and export, these *Pocillopora verrucosa* fragments were further assessed for proximate, lipid class and fatty acid composition, in addition to microbial community composition. Importantly, the status of cultured corals was compared to their field state before they had been brought into the facility, to assess any changes due to acclimation to the captive environments.

5.3 Methodology

5.3.1 Experimental design

Pocillopora verrucosa nubbins were collected and cultured in experimental conditions as per Chapter 4. Briefly, *Pocillopora verrucosa* were collected from Davies Reef (-18.825622, 147.626881) on the Great Barrier Reef, then brought to the Australian Institute of Marine Science's National Sea Simulator (SeaSim) and fragmented into ~10 g nubbins. Corals were allowed 1.5 months to recover and acclimate to captive conditions, before being assigned to one of twenty-four experimental tanks (four replicates per treatment) and subjected to one of six treatments for a three month grow-out; "LiveFeeds" supplied with a mix live feeds, "Fish" co-cultured with ten juvenile *Chromis viridis* damselfish being fed a pellet diet, "Dissolved" supplied with water from a tank of ten juvenile *C. viridis* passed through a 50 μm filter, "LiveFeeds + Fish" co-cultured with ten juvenile *C. viridis* while also given a supply of live feeds, "Pellets" supplied only with the pellet diet fed to the fish, and "Control" kept with no fish and supplied no feeds. Live feeds for the LiveFeeds and LiveFeeds + Mix treatments consisted of polyunsaturated fatty acids (PUFA) enriched *Artemia* nauplii, rotifers and a mix of microalgae (*Nannochloropsis oceania, Tisochrysis lutea, Chaetoceros muelleri, Dunaliella* sp., *Proteomonas sulcata*) at a rate of 0.5 nauplii mL^{-1}, 0.5 rotifers mL^{-1} and 2000 algae cells mL^{-1} and fed once a day as a pulse of food, whilst fish schools were fed twice daily with 0.12 g of Aquaforest® AF Tiny Fish Feed. For an assessment of nutrient digestibility, an inert, indigestible marker Yttrium Oxide (Y_2O_3) was incorporated into the fish feed (0.1 %) by top coating pellets. Tanks were supplied with seawater filtered to 0.1 μm at 28 ± 0.1 °C, at a rate of 0.8 L min^{-1}, resulting in approximately one turnover per hour with one circulation pump per tank (Turbelle® nanostream® 6015, Tunze Aquarientechnik, Penzberg, Germany). Light

was supplied by one LED light (Hydra, AquaIllumination, Bethlehem, USA) for each tank from 0830 – 1630 at 150 μmol cm^{-2} s^{-1}, with one-hour ramps at sunset and sunrise.

5.3.2 Data collection

Pocillopora verrucosa fragments were sampled for biochemical and microbial analysis at three timepoints: i) when the corals arrived at the SeaSim after collection from the wild ("Field"), ii) after fragmentation and the 1.5 months acclimation period, just prior to the start of the experiment ("Post-acclimation") and iii) at the end of the experiment after three months subjected to the experimental treatments ("Treatment"). For microbial analyses, snips of branch tips (which included skeleton, tissue and mucus) were taken then stored in salt-saturated pH 8.0 DMSO-EDTA at -20 °C. Water samples for microbial analysis were also taken at these times, by collecting 50 mL of water in a sterile Falcon tube, filtering this water through a cell strainer into a sterile 50 mL syringe, then filtering again through a 0.22 μm membrane. Membranes were then frozen and stored at -80 °C until DNA extractions commenced. Water samples were taken in triplicate from holding tanks for the field and post-acclimation time-points, while one sample from each replicate tank for each treatment taken at the end of the experiment, for a total of four water samples for each treatment. Once nubbins had been sampled for microbial analysis, they were patted with paper towel to remove excess water, wrapped in aluminium foil and secured in an airtight bag before being stored at -20 °C in preparation for analysis of proximate, lipid class and fatty acid composition. *Artemia*, rotifer and microalgae samples were also collected by centrifuging aliquots from larger feed batches to concentrate the plankton, then removing excess water and freezing at -20 °C.

5.3.3 Microbiome analyses

DNA was extracted from *P. verrucosa* snips using a QIAGEN Blood and Tissue kit, with the Proteinase K digestion replaced with a bead beating step, using 4 x 1 mm stainless steel beads and 0.25 mL 0.7 & 0.15 mm garnet mix beat at 5.5 m s^{-1} for 30s on a FastPrep-24™ 5G. DNA from water samples (0.22 μm membranes) was also extracted using a QIAGEN Blood and Tissue kit, using the protocol supplied by the manufacture. All extracted DNA samples were then cleaned using a Promega Wizard® DNA Clean-Up System. Samples were

sequenced by the Ramacoitti Centre for Genomics (University of New South Wales, Sydney, Australia), using Illumina MiSeq 2x300 bp paired-end sequencing targeting the 16S rRNA V3-4 region (341 – 805).

Bioinformatic processing of microbial data was performed via QIIME2 version 2023.9 (Bolyen et al., 2019) using the DADA2 pipeline (Callahan et al., 2016). Forward sequences were truncated to 255 bp and reverse to 240, and the first 11 and 6 bp were trimmed from forward and reverse sequences respectively. Representative ASVs were classified via a Naïve Bayes classifier trained using the V3-4 region from the SILVA database (version 138.1, 99). All further data analysis was conducted using R v4.3.1 (R Core Team, 2023) and RStudio v2023.06.1+524 (Posit team, 2023), where phyloseq was used to import and filter data (phyloseq (McMurdie & Holmes, 2013)). Eukaryote, mitochondria and chloroplast sequences were removed from the feature table, then samples with less than 1000 reads removed and amplicon sequence variants (ASVs) filtered to keep only taxa with $\geq$ 0.001% relative abundance in at least one sample. Samples were rarefied to 1026 reads, then observed richness and Shannon evenness calculated (microbiome (Lahti & Shetty, 2012-2019)) and compared using a Kruskal-Wallis rank sum test followed by pairwise comparisons of estimated marginal means (emmeans (Lenth, 2020)) from generalised linear mixed effects models (glmmTMB (Brooks et al., 2017)). PERMANOVA (vegan (Oksanen et al., 2024)) and indicator species analysis (indicspecies (De Caceres & Legendre, 2009)) was used to compare community composition of corals within the different treatments, and ordination plots using redundancy analysis with ASV data standardised using the Hellinger method were used to visualise similarity in community structure.

5.3.4 Biochemical analyses

Corals and diets were processed and analysed as per Conlan et al. (2014). In brief, frozen samples were freeze-dried for 48 hours then coral nubbins crushed *in toto* at 70 kN with a stainless-steel hydraulic press. Ash content was measured by incineration in a muffle furnace for 18 hrs at 450 °C, and protein content (Kjeldahl nitrogen; N x 6.25) was determined via the Kjeldahl method (AOAC, 2000) using an automated Kjeltech™ 8400 (FOSS, Analytical Co. Ltd., Sweden). Lipids were cold extracted from 2 g of crushed sample with

dichloromethane:methanol (2:1) for total lipid quantification (Folch et al., 1957), then an aliquot taken for lipid class analysis using an Iatroscan MK 5s thin layer chromatography-flame ionisation detector. Resulting chromatograms were then manually integrated using eDAQ PowerChrom v2.7.9 software to identify and quantify individual lipid classes. The remaining lipid aliquot underwent acid-catalysed methylation to esterify fatty acids into methyl esters (FAME), with 100 µL of 23:00 (0.75 mg mL^{-1}) added as an internal standard. FAME were subsequently analysed with gas chromatography—flame ionisation detector (GC-FID) (Agilent Technologies 7890A, USA) equipped with a DB23 capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness; SGE Analytical Science, Ringwood, Victoria, Australia), a flame ionisation detector (FID), an Agilent Technologies 7693 autosampler injector and a split injection system (splitless injection) following methods presented in (Francis & Turchini, 2017). Individual fatty acid peak areas were corrected by theoretical relative FID response factors (Ackman, 2002), and identified and quantified relative to external standards (Sigma-Aldrich, Inc., St. Louis, USA and NuChek Prep Inc., Elysian, USA) using GC ChemStation software (Agilent Technologies, USA). To test for the presence of Y_2O_3 in corals, fish feed and faeces, samples were dissolved in HNO_3, then Y_2O_3 concentration determined via Inductively Coupled Plasma Optical Emission spectroscopy at the James Cook University Advanced Analytical Centre.

5.3.5 Data analysis

Data analysis was conducted using R v4.3.1 (R Core Team, 2023) and RStudio v2023.06.1+524 (Posit team, 2023). Bayesian generalised linear mixed effects models were used to model proximate composition, lipid class and fatty acid class content of *P. verrucosa* fragments. Bayesian models were run via the brms package (Bürkner, 2021) with Stan (Stan Development Team, 2023). While the link function and formula of models varied, in general treatment was treated as a fixed effect, with genotype, replicate tank and/or sample treated as blocking factor. All model samplers were checked for well-mixed traceplots, autocorrelation, chain convergence (R-hat < 1.01) and effective sample size. Model fit was assessed using posterior probability checks and DHARMa residuals. Median and 95% credibility intervals (CI: calculated as 95% highest posterior density intervals) were then calculated, and Bayesian probability that the response of one treatment was greater than another also calculated. For fatty acid data, analysis of similarity using Bray-Curtis distances,

pairwise PERMANOVA and indicator species analysis was used to identity differences between treatments, and principal components analysis was used to create ordination plots (factoextra (Kassambara & Mundt, 2020)).

5.3.6 Ethics statement

All research was conducted in accordance with the Great Barrier Reef Marine Park Authority permit (G12/35236.1) and James Cook University Animal Ethics Permit (A2787).

5.4 Results

5.4.1 Faeces uptake by corals

The Y_2O_3 marker was detected in both the pellets (0.722 ± 0.031 g kg ^{1}, (mean ± sd)) and fish faeces (0.523 ± 0.0004 g kg^{-1}), though it was not detected in any of the coral nubbins (limit of detection being <0.00025 g kg^{-1}).

5.4.2 Diet lipid and fatty acid composition

The supplied diets, including *Artemia,* rotifers, microalgae and fish pellets, different in their fatty acid (FA) and lipid class composition. *Artemia* FA composition was characterised by higher 18:1n-9t, 18:3n-3, 18:1n-7 and 16:3n-4 concentrations (as % of total fatty acids) than the other diets (Fig. 5.1a). *Artemia* and the fish pellets also had a much higher proportion of TAG compared to the rotifers and mixed algae (Fig. 5.1b). Higher relative concentrations of AMPL characterised the mixed microalgae samples, and both microalgae and rotifers had higher proportions of phospholipids than the *Artemia* and fish pellets.

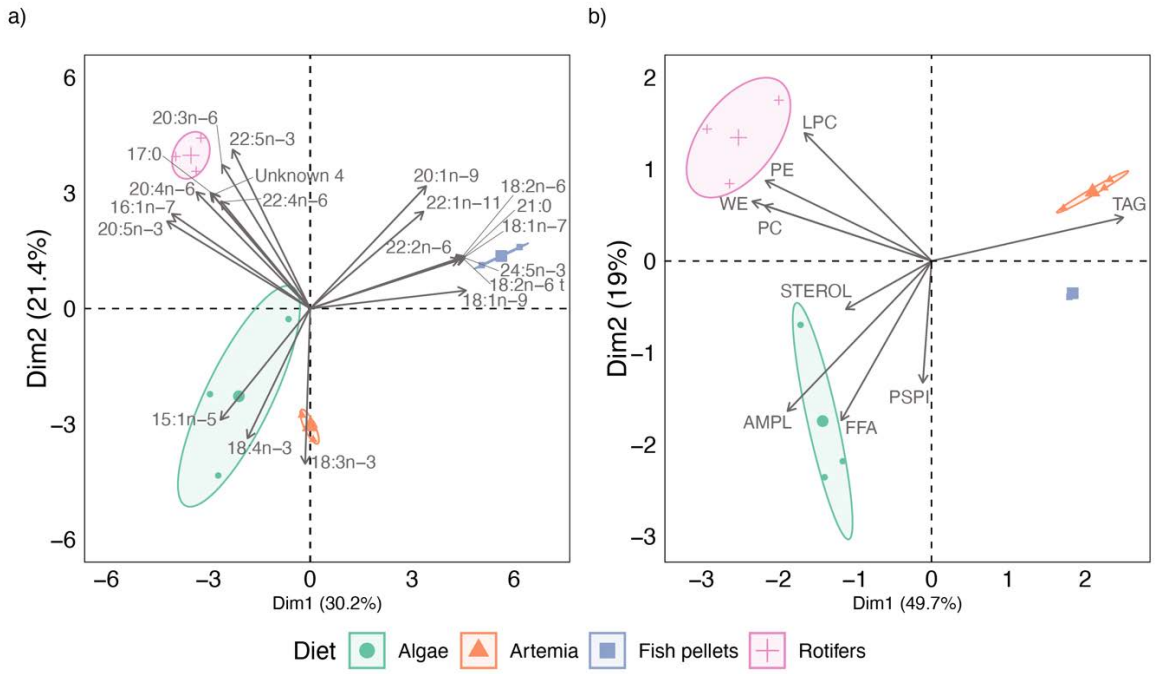

Figure 5.1: Principal Components Analysis of a) fatty acid composition and b) lipid class data for the different diets fed to the corals, showing ellipses of 95% confidence intervals and the twenty most influential fatty acids.

LCP = Lysophosphatidylchloline, PC = Phosphatidylcholine, PSPI = Phosphatidylserine and Phosphatidylinositol, PE = Phosphatidylethanolamine, AMPL = Acetone mobile polar lipids, Sterol = Sterol, FFA = Free fatty acids, TAG = Triacylglyceride, WE = Wax esters

5.4.3 Coral proximate composition

Proximate composition of the *Pocillopora verrucosa* fragments varied between the different treatments and the Field and Post-acclimation samples. Ash content associated with coral fragments was relatively consistent across all samples. *P. verrucosa* had slightly higher ash in treatments where fish were present (Fish, Dissolved, and LiveFeeds + Fish) and Pellets (~944 – 950 mg g^{-1} DW) compared to Control (939 mg g^{-1} DW; P > 86%; Fig. 5.2a), whilst LiveFeeds corals had middling values compared to other treatments (943 mg g^{-1} DW). Field samples had slightly lower ash content, though this was similar to the concentrations of Control and LiveFeeds corals (P < 85%).

Protein concentrations of coral fragments was highest in the LiveFeeds treatments (128.9 mg g^{-1} AFDW; P > 94%), but also elevated in Dissolved, Fish and LiveFeeds + Fish (~100 – 108 mg g^{-1} AFDW) compared to corals grown in the Control or Pellets treatments (~59 – 71 mg g^{-1}

AFDW) (P > 99%; Fig. 5.2b). Field samples had relatively low protein (75.2 mg g^{-1} AFDW), and though Post-acclimation samples showed a slightly higher concentration (89.7 mg g^{-1} AFDW) there was no evidence this represented a significant increase compared to the Field samples (P = 81%).

Control corals had the lowest total lipid content (95.4 mg g^{-1} AFDW) out of all the treatment samples (P > 91%; Fig. 5.2c). Lipid concentration was highest in Dissolved and Pellets (~162 – 177 mg g^{-1} AFDW), and both treatments where corals had access to live feeds (LiveFeeds and LiveFeeds + Fish) had similar total lipid content (120.9 and 114.8 mg g^{-1} AFDW respectively). Field samples had lower total lipid concentrations (100.7 mg $g^{=1}$ AFDW) than Post-acclimation corals (140.0 mg $g^{=1}$ AFDW) (P = 94.5 %).

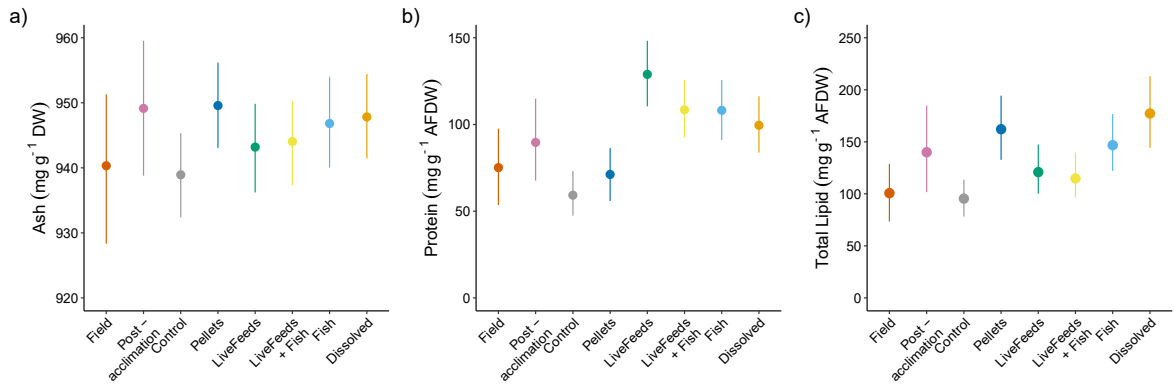

Fig. 5.2: Proximate composition of sampled *Pocillopora verrucosa* fragments, including a) dry-weight ash content, b) ash-free dry weight protein content, c) ash-free dry weight total lipid content. Points and bars represent modelled median and 95% credibility intervals.

5.4.4 Coral lipid class composition

There was a higher proportion of total storage lipids (sum of WE (wax esters), TAG (triacylglycerols), FFA (free fatty acids) and 1, 2 DAG (diacylglycerols)) within Control corals compared to other the treatments (P > 85%; Fig. 5.3) except LiveFeeds + Fish (P = 77.2%). This can be attributed predominately to higher WE and TAG in Control corals (105.9 and 63.3 mg g^{-1} lipid respectively), while LiveFeeds + Fish had elevated FFA (58.7 mg g^{-1} lipid) compared to the other treatments. While WE and TAG remained relatively low in LiveFeeds corals, these samples had similar FFA concentrations to LiveFeeds + Fish fragments (58.6 mg g^{-1} lipid) and had the highest concentration of 1,2 DAG (93.2 mg g^{-1} lipid). Due to lower

storage lipids, corals subjected to the LiveFeeds, Dissolved, Fish and Pellets treatments had a higher proportion of lipids that can perform structural roles (Sterol, AMPL (acetone mobile polar lipid), PE (phosphatidylethanolamine), PSPI (phosphatidylserine/phosphatidylinositol), PC (phosphatidylcholine) and LPC (lysophosphatidylcholine)) lipids compared to Control corals (P > 85%). For corals supplied with LiveFeeds, AMPL remained low (222.9 mg g^{-1} lipid), with a higher concentration of phospholipids (PE, PSPI, PC, LPC) contributing to the overall increase in structural lipids.

Field samples were characterised by significantly higher storage lipid content (394.3 mg g^{-1} lipid) in comparison to any of the captive corals (treatment corals and Post-acclimation samples), driven primarily by a much higher concentration of TAG (252.0 mg g^{-1} lipid). Even when accounting for lower total lipids in Field samples compared to some treatments (Fig. 5.2c), TAG was significantly higher in Field samples than all other corals, with a modelled median of 24.7 mg g^{-1} AFDW of TAG compared to ~3 – 6 mg g^{-1} AFDW in the Post-acclimation and treatment corals (P = 100%). Field corals also had a comparatively low proportion of AMPL (145.4 mg g^{-1} lipid) compared to the captive corals (~220 – 420 mg g^{-1} lipid). Post-acclimation corals had the lowest storage lipid content (180.3 mg g^{-1} lipid) compared to all other samples (P = 100%), with lower WE (52.6 mg g^{-1} lipid) and TAG (23.8 mg g^{-1} lipid) in comparison to other corals (~75 - 111 and ~32 – 252 mg g^{-1} lipid).

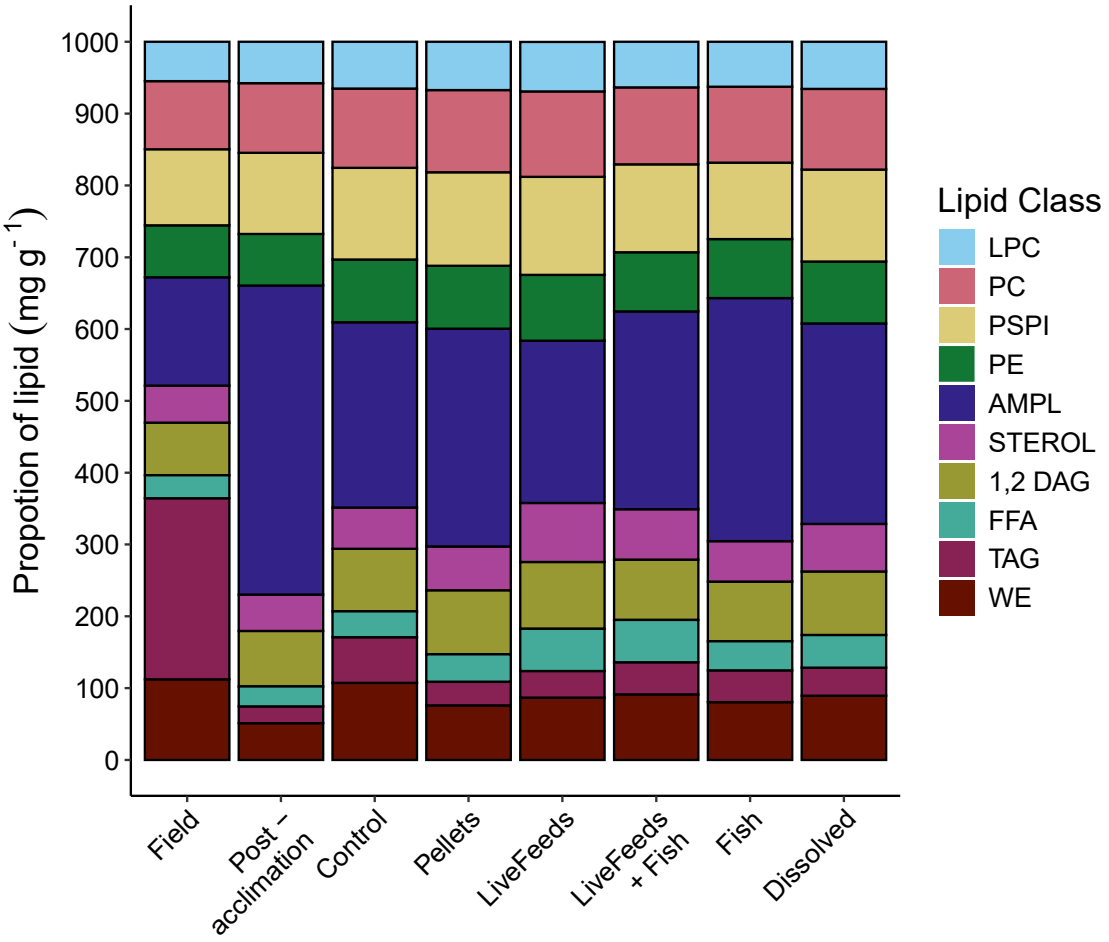

Figure 5.3: Relative proportions of different lipid classes within the total lipid fraction of *Pocillopora verrucosa* fragments subjected to the experimental treatments or collected from the field or post-acclimation to the experimental system.

LCP = Lysophosphatidylchloline, PC = Phosphatidylcholine, PSPI = Phosphatidylserine and Phosphatidylinositol, PE = Phosphatidylethanolamine, AMPL = Acetone mobile polar lipids, Sterol = Sterol, FFA = Free fatty acids, DAG = 1,2-Diacylglycerol, TAG = Triacylglyceride, WE = Wax esters

5.4.5 Coral fatty acid class composition

Fatty acid (FA) composition of corals varied between Field, Post-acclimation and treatment groups (Fig. 5.4a; PERMANOVA with 10,000 permutations: F = 20.112, p-value < 0.001), as did dispersion (PERMDISP with 10,000 permutations: F = 2.9989, p-value = 0.0052). Dissolved, Fish and Pellet treatment corals had similar FA composition, as did coral in the Fish and LiveFeeds + Fish treatments (adjusted pairwise PERMANOVA p valves > 0.05). Polyunsaturated fatty acids (PUFA; including all individual fatty acids with two, or more, double bonds between carbon atoms in the fatty acid molecule) as percentage of total fatty

acids were elevated in all captive corals (treatments and Post-acclimation) compared to Field samples (P = 100%), whilst monounsaturated fatty acids (MUFA) were higher in the Field and Post-acclimation samples compared to treatment corals (P > 99%). Saturated fatty acids (SFA) were higher in Field corals (P > 99%), and LiveFeeds had significantly lower SFA levels compared to all other treatment corals (P > 89%). LiveFeeds and LiveFeeds + Fish nubbins had higher PUFA concentrations compared to all other captive corals (P > 92%), though the Dissolved, Fish and Pellets treatments all had higher PUFA than the Control nubbins (P > 98%).

Indicator species analysis showed that LiveFeeds corals were associated with increased levels of 20:3n-3 (Fig. 5.4b), though 18:3n-3 was associated with and at higher concentration in both the LiveFeeds and Post-acclimation samples. 18:1n-7 and 15:1n-5 were associated with and higher in LiveFeeds, LiveFeeds + Fish and Post-acclimation (all corals with access to live feeds). Field samples' dissimilarity to captive coral was driven by 20:1n-9, 18:1n-9, 16:0, 20:3n-6, 20:0 and 18:1n-9t, whilst Post-acclimation samples had increased concentrations of 22:1n-11, 24:0, 10:0, 18:0. All captive corals were associated with increased concentrations of 18:4n-3, 20:5n-3 (EPA), 20:4n-6 (ARA) and 16:3n-4, and treatment corals were also associated with 22:6n-3 (DHA). Field samples and Post-acclimation corals had a lower PUFA n-3:n-6 ratio (1.19 and 1.32 respectively) than any of the treatment corals (1.55 – 1.80, P > 96%). Field corals also had a slightly lower LC-PUFA n-3:n-6 ratio (1.48) compared to all treatment corals (~1.65 – 1.80; P > 89%) except Pellets, though Post-acclimation corals had the lowest LC n-3:n-6 of all at 1.22 (P > 97%).

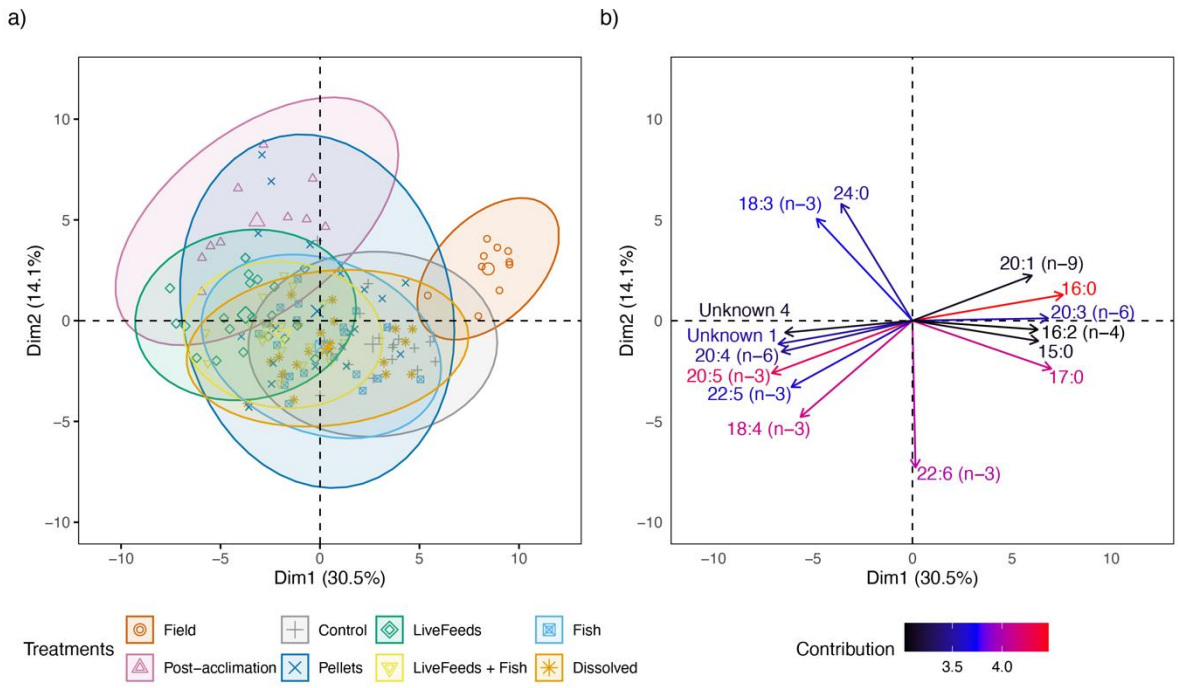

Figure 5.4: Principal Components Analysis of fatty acid classes from *Pocillopora verrucosa* fragments, showing a) ellipses of 95% confidence intervals for the different treatments and b) the fifteen most influential individual fatty acids and their contribution to the overall variance.

5.4.6 Microbial communities

A total of 9,044,994 high-quality 16S rRNA amplicon reads were recovered from 140 samples and following trimming and quality filtering 27, 598 ASVs were identified. Assessing diversity metrics across all treatments, there was significant differences in richness (Kruskal-Wallis H(7) = 28.636, p – value = <0.001; Fig. 5.5a) and evenness (Kruskal-Wallis H(7) = 20.706, p – value = 0.0042; Fig. 5.5b) in the coral associated coral microbial communities. Specifically, post-hoc tests revealed richness (i.e. observed number of ASVs) was significantly lower for corals from the LiveFeeds + Fish treatment compared to all other samples except the Control (Fig. 5.5a; p-values < 0.05). Field samples had a significantly lower Shannon Index than all the other corals samples within the captive environment treatments, indicating a less even distribution of species in these Field samples (Fig. 5.5b; p-values < 0.05).

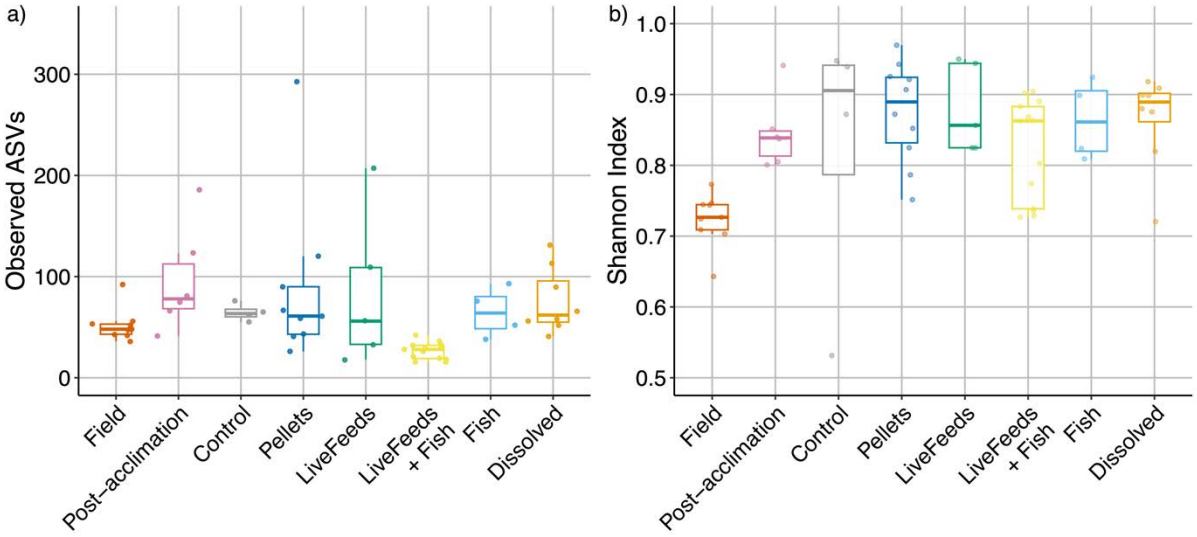

Figure 5.5: Boxplots of a) species richness as observed ASVs and b) evenness measured using Shannon Index.

The microbial communities of all coral samples were dominated by ASVs associated with Gammaproteobacteria, ranging from a mean of ~31% of retrieved reads in the Dissolved treatment samples to ~97% in the field corals. Alphaproteobacteria and Bacteroidia were the next most common classes, though their relative abundance varied between treatments, accounting <1% to ~25% and ~1% to ~21% of reads retrieved respectively. ASVs associated with other bacterial classes remained at <5% mean relative abundance in coral samples, with the exception of Clostridia (10.2% ± 20.5%) and Bacilli (7.2% ± 5.7%) in the Dissolved treatment samples.

Field corals communities were dominated by ASVs identified as belong to the Family *Endozoicomonadaceae* (96.0% ± 2.5%), whilst all other identified bacterial families remained at ≤ 0.7% mean relative abundance. The Post-acclimation samples showed a shift in coral microbial community composition, with the relative abundance of *Endozoicomonadaceae* affiliated reads lower at 51.9% ± 20.8% (Fig. 5.6). Higher ASV richness was observed in these Post-acclimation samples (Fig. 5.5a), with a comparative increase in the relative abundance of ASVs affiliated with the families *Beijerinckiaceae* (7.3% ± 11.8%) and *Psuedomonadaceae* (4.4% ± 5.5%), and a general increase in mean relative abundance of other bacterial families, including *Amoebophilaceae, Peptostreptococcales-Tissierellales* and *Cyclobacteriaceae,* to ~1 – 2.7%.

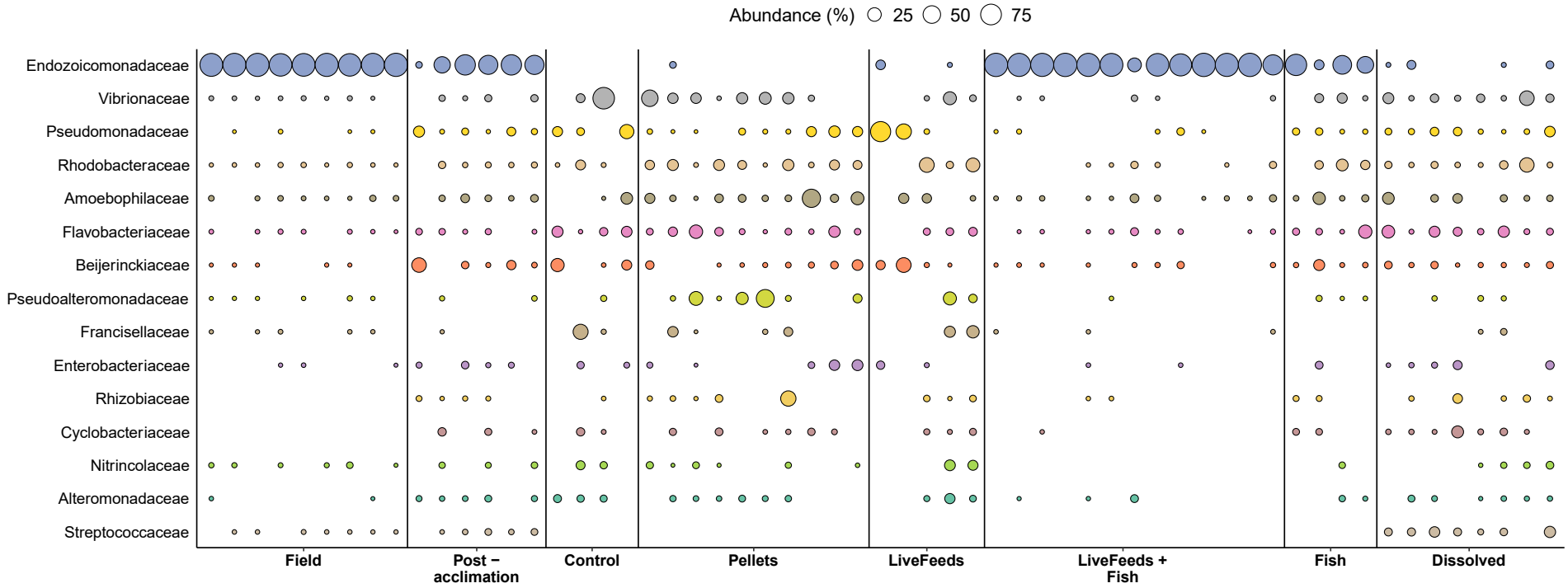

Figure 5.6: Sample by sample breakdown of the top 15 bacterial Families in microbial communities, as bubble plots of relative abundance.

After three months in the captive aquarium environment, there were no retrieved sequences affiliated with *Endozoicomonadaceae* in the Control coral samples (Fig. 5.6). Additionally, corals from the LiveFeeds, Pellets and Dissolved treatments displayed low mean relative abundance of *Endozoicomonadaceae* affiliated reads (0.2% – 1.9%). Only the LiveFeeds + Fish and Fish corals demonstrated *Endozoicomonadaceae* dominated microbial communities, with respective means of 89.5% ± 20.7% and 48.0% ± 29.8% of identified ASVs being associated with this family (Fig. 5.6). This *Endozoicomonadaceae* dominance was relatively consistent across all samples from the LiveFeeds + Fish, though the relative concentration of these ASVs varied between individual Fish treatment samples (Fig. 5.6).

Control coral samples demonstrated a higher relative abundance of *Vibrios* (22.4% ± 40.2%) than other treatments, though this was driven by one replicate sample (Fig. 5.6). Across all other treatments *Vibrios* were present, ranging from mean relative abundance of 11.6% to 0.2%, and were at their lowest in LiveFeeds + Fish. LiveFeeds had a higher mean abundance of *Rhodobacteraceae* affiliated reads than other corals, identified by indicator species analysis (12.5% ± 15.6%; p-value = 0.0450), whilst *Pseudoalteromonadaceae* were at higher abundance in both Pellets and LiveFeeds samples (10.7% ± 17.2% and 5.66% ± 9.69%; p = 0.0472). Dissolved corals where characterised by higher abundance of reads affiliated with *Streptococcaceae* (5.83% ± 5.13%; p-value = 0.0007).

In general, coral associated bacterial communities were different from those found in their surrounding water (PERMANOVA with 10,000 permutations: F = 15.9934, p-value <0.001; Fig. 5.7), and distinct bacterial communities were present between the corals from different treatments, field and post-acclimation samples (PERMANOVA with 10,000 permutations: F = 4.3865, p-value <0.001), Dispersion also varied between the coral samples from the different treatments (PERMDISP with 10,000 permutations: F = 24.251, p-value < 0.001). Field, post-acclimation, Fish and LiveFeeds + Fish samples showed grouping in the redundancy analysis, driven by *Endozoicomonas* abundance, and within this group the field and Fish + LiveFeeds samples were tightly clustered (Fig. 5.7). The remaining coral samples from treatments Control, Pellets, Dissolved and LiveFeeds displayed a second grouping, driven by *Vibrionaceae, Rhodobacteraceae, Flavobacteriaceae* and *Pseudomonadacea* (Fig. 5.7).

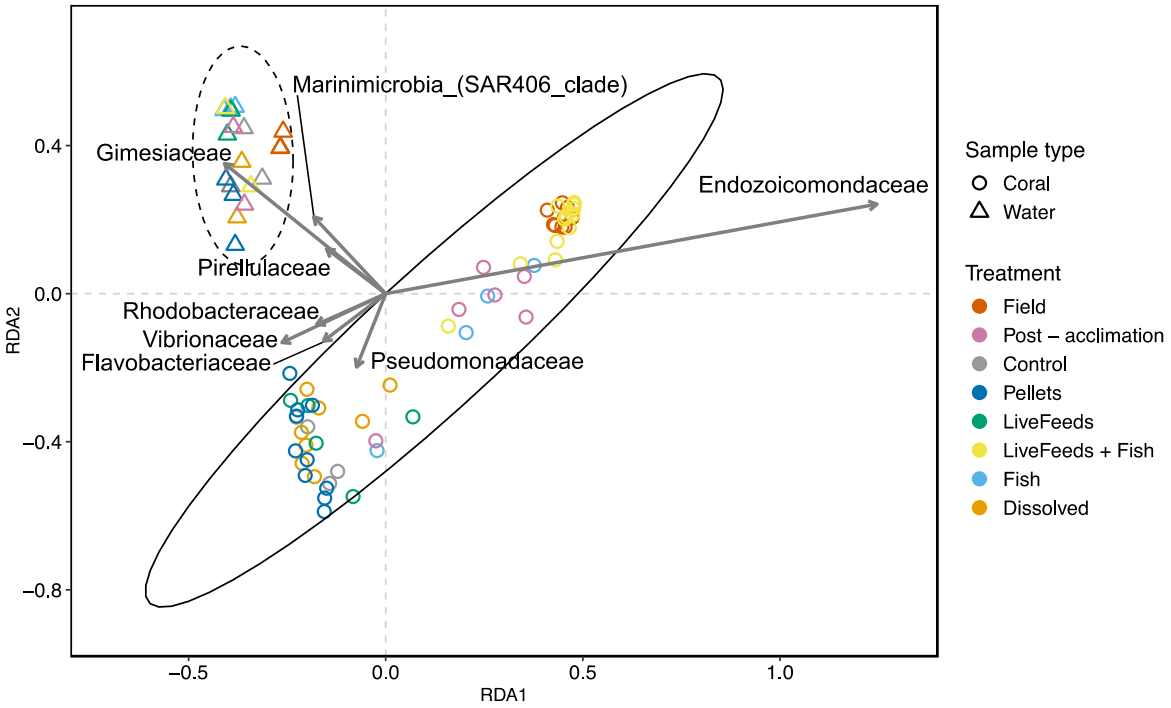

Figure 5.7: Redundancy analysis (RDA) ordination plot of coral and water bacterial community, with the key driving bacterial Family ASVs and 95% confidence intervals drawn as ellipses.

5.5 Discussion

5.5.1 Proximate composition of captive Pocillopora verrucosa

As noted in Chapter 4, *P. verrucosa* with access to live feeds and/or fish wastes have higher growth than unfed corals, likely due to the heterotrophic nutrients supplied by the live feeds (Conlan et al., 2018a; Ferrier-Pages et al., 2003; Houlbreque & Ferrier-Pages, 2009; Huang et al., 2020; Radice et al., 2019a; Toh et al., 2014) and enrichment of corals symbionts from the nitrogen and phosphorous in the fishes wastes (Ezzat et al., 2015; Grover, 2002; Muller-Parker et al., 1994; Wiedenmann et al., 2023). In the present study, the lack of detection of the inert dietary marker in the corals suggests that corals were either not directly feeding on the fishes' faeces, or feeding on them at such a low rate it is unlikely to be a significant source of nutrition. This is supported by a pilot study (Appendix E) that demonstrated that yttrium was detectable in coral tissues after directly providing yttrium-enriched feed, and even after a 1-day period of starvation post-feeding. Therefore, the absence of yttrium is considered evidence that yttrium was not ingested by corals.

Analysis of the proximate composition of coral samples revealed that protein concentrations in corals subjected to the various treatments matched closely with the patterns of growth provided in Chapter 4. Specifically, protein concentrations were highest in LiveFeeds, followed by fragments with access to fish wastes (i.e. Fish, Dissolved and LiveFeeds + Fish), and were lowest in Control and Pellets. The present study, therefore, is consistent with previous research showing that high protein was characteristic of actively growing regions within *Acropora millepora* (Conlan et al., 2018b). Access to an artificial PUFA diet rich in animal protein was found to increase *Goniopora columna* protein content and specific growth, which may also explain why LiveFeeds corals had the highest protein, as they had access to PUFA and protein rich *Artemia* nauplii (Aragão et al., 2004; Ding et al., 2021).

However, increased availability of inorganic nitrogen has been suggested to lead to changes in protein in coral's *Symbiodiniaceae*, which may contribute at least in-part, to the increased protein density in corals with similarly high symbiont concentrations, such as those from the LiveFeeds treatment (Oakley et al., 2023). Normalising mean protein content to the mean symbiont density (Chapter 4) for each treatment provided support for this contributing to higher protein content in the coral samples, showing a higher protein content per unit of symbionts in the LiveFeeds, LiveFeeds + Fish, Fish and Dissolved treatments (Appendix E Figure 1a). During periods of stress or low dietary protein supply, coral tissue protein content can be also reduced as it is directed to maintain physiological function in vital tissues, which may in-part explain the lower protein concentrations in the Control (where corals were unfed) and Pellets (where corals were fed an inappropriate diet, as batch feeding with dry fish pellets have been shown to be poor feeds for corals) (Chase et al., 2020; Conlan et al., 2018a; Houlbreque et al., 2004; Osinga et al., 2012; Porter et al., 1989). Tissue biomass of wild corals varies seasonally and is typically lower in the summer-autumn period when these samples were collected (Conlan et al., 2020; Fitt et al., 2000), which may explain the moderate protein concentrations observed in the Field corals compared to the majority of the captive corals.

The observed total lipid concentrations of ~90 – 200 mg g^{-1} AFDW are similar to previous observations, which have reported values ranging from ~60 – 200 mg g^{-1} (Conlan et al., 2017b; Harland et al., 1993; Rodríguez-Troncoso et al., 2014; Rodríguez-Troncoso et al.,

2016). Comparatively, within the treatment corals, the unfed Control nubbins had the lowest total lipid content, in line with previous findings that at relatively low light (as in this experiment, where light was limited to 150 µmol m^{-2} s^{-1}) calcification may be sustained whilst tissue mass and lipid content decrease (Anthony & Fabricius, 2000). Treignier et al. (2008) found that at low light (100 µmol m^{-2} s^{-1}) feeding could increase storage lipid concentrations and calcification of *Turbinaria reniformis*, whilst in higher light (300 µmol m^{-2} s^{-1}) energy is directed to growth and symbiont proliferation. The presence of live feeds (LiveFeeds and LiveFeeds + Fish) resulted in corals with lower total lipid levels than corals without live feeds (Dissolved, Fish or Pellets treatments). Chapter 4 showed that *P. verrucosa* from the LiveFeeds treatment had higher growth than other corals, suggesting that dietary energy from heterotrophic feeding was directed to growth rather than being retained in coral tissue as lipid (Ferrier-Pages et al., 2003). Furthermore, enrichment of coral symbionts via ammonium provision has been linked with increasing translocation of carbon-rich photosynthates to the coral, which may be stored in tissues as lipids (Dellisanti et al., 2023; Ezzat et al., 2015; Patton & Burris, 1983). This association with ammonium provision and lipid storage may partly explain the increased total lipid content observed in the Fish and Dissolved treatments. It should also be noted that changes in lipid content within the symbiont themselves may have contribute to the observed differences (Zhang et al., 2023). Normalising mean total lipid content to the mean concentration of symbionts in the different treatments (Appendix E Figure 1b) demonstrated that there were differences between treatments, which likely contributed at least in part to changes in lipid content in the samples.

5.5.2 Lipid and fatty acid composition of captive *Pocillopora verrucosa*

Coral total lipid concentrations and composition are known to vary seasonally, with increases in both total lipid and storage lipid content typically reported in corals leading up to spawning events, and subsequently decreasing after spawning (Cirino et al., 2021; Conlan et al., 2020; Oku et al., 2003; Stimson, 1987). While total lipid content within Field samples was similar to some of the captive treatments, all Field corals had significantly higher storage lipid content, particularly TAG. Normalising mean TAG concentrations to symbiont densities from Chapter 4 (Appendix E Figure 1c), showed that TAG content per number of symbionts was far higher in the Field corals than in any of the captive fragments, indicating these

differences are likely not simply due to different symbiont densities. An important storage lipid that can make up to 37% of lipid content in *P. verrucossa*, TAG is readily catabolised for energy in corals, and low levels can be indicative of stress (Benson et al., 1978; Harland et al., 1993; Rodrigues et al., 2008). Fragmentation is known to be physiologically challenging for corals, and smaller corals are known to have higher proportional growth than larger colonies, thus low levels of TAG in Post-acclimation corals could be attributed to corals catabolising TAG to fuel their recovery and regrowth after being fragmented into experimental nubbins (Dornelas et al., 2017; Lirman, 2000; Madin et al., 2020).

However, despite sustained growth, after three months of being subjected to the treatments, TAG concentration remained relativity low in all corals (3.6 – 6.3 % of total lipids), with increased proportions of phospholipids seen in most of the treatment corals. Multiple explanations exist for this, including energy being preferentially directed towards growth or increased provision of lipids towards cellular membrane synthesis to regulate cell membrane fluidity or permeability rather than the proliferation of TAG stores (Lin et al., 2013; Parrish, 2013). While it is difficult to ascertain the exactly what response drove these changes in the corals, we can draw some comparisons to past studies.

TAG concentrations have been found to be positively correlated with light levels, due to both the activity of symbiotic photobionts and (in the field) blooms of phytoplankton serving as heterotrophic food sources (Conlan et al., 2020; Harland et al., 1992; Zhukova, 2007). Indeed in some coral species photosynthesis alone is sufficient to drive recovery of lipids post-stress events (Grottoli & Rodrigues, 2011), though when light is suboptimal some coral species can also utilise heterotrophic feeding to help supplement recovery (Palardy et al., 2008). Here, corals were kept under relatively low light, which may have limited the amount of TAG that could be supplied by the symbionts. Furthermore, while live feeds were supplied to the corals at rates similar to observed densities of wild zooplankton and were sufficient to support enhanced growth, the method of pulse feeding employed in the present study may have reduced the availability of heterotrophic nutrients to a small time window (Palardy et al., 2006).

The higher concentration of 18:3n-3 and 18:1n-7 in corals that were supplied live feeds (LiveFeeds, LiveFeeds + Fish and Post-acclimation) was reflective of their high concentration in *Artemia*, providing evidence that the corals were actively feeding on the *Artemia*. Though *Artemia* nauplii fed to the corals were high in TAG content due to enrichment, wild *Pocillopora* typically feed on zooplankton assemblages abundant in copepods, which themselves typically contain a high concentration of phospholipids (Brett et al., 2009; Kattner & Hagen, 2009; Radice et al., 2019a). The supplied rotifers in the live feeds mixes were high in phospholipids (~45% of total lipids), yet based on the fatty acid compositional data, there was little indication that the corals where actively feeding on the rotifers or the supplied phytoplankton. The lack of feeding on the rotifers and phytoplankton may be attributed to them being smaller (~140 and ~2 – 25 µm respectively) than the 200 – 400 µm size class of plankton *Pocillpora* will readily feed upon (Palardy et al., 2005, 2006). Despite *Artemia* being larger (~650 µm) than this preferred size class, past research has shown *Pocillopora* will readily catch and consume them (Conlan et al., 2018a; Kuanui et al., 2016; Toh et al., 2013b). Artemia therefore offer a potentially readily available source of TAG and their constituent fatty acids for metabolic energy in corals. However, the relatively low dietary energy requirement for corals, which are in part supplied from their symbionts, may have rendered the heterotrophic TAG supply from *Artemia* in excess of their physiological requirement and thus explain why their deposition into coral tissue was found to be limited. Instead, the corals may have preferentially incorporated available phospholipids and directed them towards growth or to maintain membrane function during physiologically stressful culture periods, preferring to source TAG and/or its constitute fatty acids from their symbionts (Conlan et al., 2019; Harland et al., 1992).

The only treatment for which corals were found to significantly increase the TAG proportion of their total lipids where those from the Control treatment. Harland et al. (1992) found that starved anemones would increase their proportion of storage lipid, however it was suggested that this was due to a decline in phospholipids or structural lipids due to cellular catabolism. In this study lipid class data is proportional, thus a decline in structural lipids may at least in part explain the increase in the proportion TAG despite the lower total lipid content observed in the Control fragments. Though under nitrogen limitation TAG has also been shown to increase in density within symbiont cells (La Motta et al., 2024),

standardising the TAG concentration in the corals to the density of their symbionts (Appendix E Figure 1c) showed little variation in the concentration of TAG per symbiont, with Control corals in fact having lower TAG per symbiont than most treatments. These Control corals were kept under the same light conditions as the other treatments, thus could not have had enhanced photosynthesis due to available light levels and had no access to enhanced nitrogen, phosphorous or heterotrophic food sources, thus could not have increased their TAG through these pathways.

In terms of fatty acid composition, the separation between Field corals and captive fragments was driven primarily by 20:1n-9, 18:1n-9, 16:0, 20:3n-6, 20:0 and 18:1n-9t. Both 18:1n-9 and 20:1n-9 have previously been identified as indicative of consumption of zooplankton such as copepods (Radice et al., 2019a). This suggests that the Field and captive corals were able to utilise different sources of heterotrophic food. Palmitic acid (16:0) is the major saturated fatty acid constitute of TAG sourced from coral symbionts, although dietary sources can also contribute to coral 16:0 levels (Figueiredo et al., 2012; Kim et al., 2021; Latyshev et al., 1991; Papina et al., 2003; Zhukova, 2007). The increased 16:0 in field samples compared to captive corals supports the notion that lower light levels in this experiment may have contributed to the low concentration of TAG stores compared to field counterparts (Harland et al., 1993; Yamashiro et al., 1999). 20:5n-3 (EPA), a key long-chain polyunsaturated fatty acid in the omega-3 series, contributed to the separation of captive corals and field corals in the present experiment. Concentrations of EPA are typically higher in symbionts than in their hosts, thus the elevated EPA in captive corals may simply be due to the higher symbiont concentrations (Lim et al., 2017; Papina et al., 2003; Treignier et al., 2008). Furthermore, PUFA were in general present in higher concentrations in captive corals compared to the field, and it has previously been suggested higher PUFA may be associated with optimising the functioning of Symbiodiniaceae under lower light conditions (Rocker et al., 2019; Zhukova, 2007).

Much of the data supports the idea that *P. verrucosa* lipid and fatty acid composition was primarily driven by the activity of their symbionts, but heterotrophic sources could still play an important role. Indeed symbionts have previously been identified as a primary source for many of the lipids incorporated and metabolised by corals, however heterotrophic feeding

can be a significant source of essential omega-3 fatty acids and energy sources such as TAG (Teece et al., 2011). It has been suggested that a higher relative n-3 LC PUFA to n-6 LC PUFA ratio can be an indicator of coral health, and may be linked to increased growth and stress resilience (Bachok et al., 2006; Rocker et al., 2019). In general, Field corals had a lower n3:n6 ratios than captive corals, and LiveFeeds had higher relative n-3 LC PUFA than other treatments. Rocker et al. (2019) suggested that these ratios could be maintained either though higher heterotrophic feeding or a higher relative contribution of photosynthesis if corals are at sites with good water quality. Thus, live feeds could be a potential avenue to improve coral growth and stress resilience via supplementation of n-3 LC PUFA. It should be noted however that factors such as seasonal variation and Symbiodiniaceae density and clade can also influence the relative levels of these fatty acids, thus in these corals this many simply be a function of the higher symbiont densities sustained in the captive corals.

5.5.3 Microbiome shifts of corals from field to captive environments

In this study I observed significant changes in the *P. verrucosa* microbiome depending on the culture conditions in which they were kept. The microbiomes associated with Field corals were dominated by *Endozoicomonadaceae* affiliated taxa, whilst Post-acclimation fragments saw a reduction in the relative abundance of *Endozoicomonadaceae* and higher relative abundances of other bacterial families such as *Beijerinckiaceae* and *Pseudomonadaceae*. These post-acclimation changes, sampled 1.5 months after Field collection, are attributed to the combined physiological challenges of fragmenting the colonies into experimental nubbins then acclimation to the new environment of captivity. Past research has similarly shown that coral microbial communities can be altered after translocations into captive environments. For example, the microbiome of *Siderastrea sidereal* were shown to shift then stabilise into a new composition after 28 days in captivity (Pratte et al., 2015), and the microbiome of the deep-sea coral *Eguchipsammia fistula* changed significantly after one year in captivity, attributed to the differences in environmental conditions in captivity compared to the *in situ* samples (Röthig et al., 2017). *Pocillopora verrucosa*'s microbiome has been shown previously to be relatively stable when subjected to environmental stress (Damjanovic et al., 2020a; Hochart et al., 2023; Pogoreutz et al., 2018; Strudwick et al., 2022; Ziegler et al., 2019), however fragmentation and transfer to the new environmental

conditions in the aquaria are stressful and appear sufficient to overcome the typical robustness of *P. verrucosa*'s microbiome (Puntin et al., 2024; Strudwick et al., 2022).

Few sequences affiliated with *Endozoicomonadaceae* were recovered from coral fragments subjected to the experimental treatments Control, Pellets, LiveFeeds and Dissolved. In addition the microbiome profiles of corals in these treatments displayed an increase in species richness, a response indictive of stress or opportunistic colonisation of pathogenic or non-mutualistic bacteria (McDevitt-Irwin et al., 2017; Meyer et al., 2014; Neave et al., 2016; Voolstra et al., 2024). This was also supported by higher relative abundance of *Vibrionoaceae, Rhodobacteraceae, Pseudoalteromonadaceae, and Streptococcaceae* affiliated taxa. Previous studies have reported *Vibrio* bacteria as pathogenic, which may opportunistically colonise corals and/or increase in abundance when corals are stressed (Tout et al., 2015; Vidal-Dupiol et al., 2011). *Rhodobacteraceae* also commonly increase in compromised corals (Mouchka et al., 2010; Pollock et al., 2017). These corals had been exposed to their respective treatments for 3 months, for a total of 4.5 months in captivity, thus it is likely that the observed microbial community patterns were a response to the environmental conditions in which the corals were kept. This may indicate these captive conditions are significantly different when compared to the coral's natural environment (e.g. different nutrient profiles) (Rosset et al., 2017).

Interestingly, the corals cultured in aquaria with fish displayed higher relative abundance of *Endozoicomonadaceae.* For example, *Endozoicomonadaceae* in the Fish only treatment had a similar relative abundance to the Post-acclimation fragments, while the LiveFeeds + Fish samples were dominated by *Endozoicomonadaceae,* and were the only captive corals to have similar observed abundance of *Endozoicomonadaceae* compared to the field corals. *Endozoicomonas* bacteria have been widely reported as dominant in corals' microbiomes (Pogoreutz & Ziegler, 2024). Across *Pocillopora* species, these bacteria can make up a significant (>75%) proportion of their microbial community, and are suggested to be part of a healthy microbiome (Damjanovic et al., 2020b; Hochart et al., 2023; Neave et al., 2016). They form coral-associated microbial aggregates (CAMAs), predominantly located in the gastrodermis in *P. verrucosa* (Neave et al., 2017b), and are proposed to assist their host through nutrient cycling, amino acid synthesis, and carbohydrate and protein provisioning

(Maire et al., 2024; Neave et al., 2017a; Pogoreutz et al., 2022). However, across different coral species *Endozoicomonas* bacteria have also been detected to reside in mucus, surrounding seawater and gastric cavity regions of the host (Hochart et al., 2023; Lee et al., 2015; Pogoreutz & Ziegler, 2024). Whilst coral microbial communities vary between compartments (e.g. skeleton, tissue, gastric cavity, mucus), branch tip snips were taken for this study, and this should have effectively sampled from all areas to enable an overview of the corals' microbial populations (Bourne & Munn, 2005; Li et al., 2014; Sweet et al., 2010). The exact drivers of coral-*Endozoicomonas* associations remain unclear, though this experiment demonstrated that direct fish-coral association appears to play a role, as only the treatments with fish present in the tank with the corals retained high *Endozoicomonas* abundance (Pogoreutz et al., 2022; Pogoreutz & Ziegler, 2024).

Whilst there have been no published studies looking at the effects of coral-dwelling damselfish on their host's microbiome, past studies have explored the relationship between clownfish and their anemone hosts (Pratte et al., 2018; Roux et al., 2019). These studies have found that the skin/mucus microbiome of both is influenced by the presence of their partner, and if fishes separate from their host anemones their microbiome will shift to match that of non-hosting fish (Pratte et al., 2018; Roux et al., 2019). However, these studies have not been able to determine the mechanisms behind these microbiome changes, outside of the obvious transmission of bacteria from the fish/anemone coming into contact with each other. Whilst in this experiment the *C. viridis* needed to be housed within the same tank as the *P. verrucosa* to maintain *Endozoicomonas* dominance, this is unlikely to be from direct contact, as *C. viridis* does not come into contact with their coral hosts as frequently as anemone fish (Parris et al., 2016). In addition, within native reef environments, adult *Chromis* biomass varies greatly between corals hosts, thus is unlikely to be the principal factor supporting high abundance of *Endozoicomonas* across numerous coral species (Chase & Hoogenboom, 2019). Potentially, these shifts are instead related to the different nutrient balance observed (i.e. carbon:nitrogen:phosphorous) when fish are present in the tank instead of simply supplying their waste water, as observed in Chapter 4.

Unlike direct fish associations, there is evidence that nutrient balances and available nutrients from heterotrophic feeding can influence a coral's microbiome. Galand et al.

(2020) found that the deep-water coral *Madrepora oculata* was able to maintain high *Endozoicomonas* abundance in aquaria when they were supplied with a diatom diet, and hypothesised that this may be due to the diatom diet being most similar to this coral's diet in the wild. Nutrient enrichment, particularly nitrogen, can alter a coral's microbiome, though past studies have found that *Endozoicomonas* can persist through short-term changes in dissolved nutrient availability (Deignan & McDougald, 2022; Pogoreutz et al., 2018; Rice et al., 2019). Chapter 4 showed that there were differences in the nutrient profile of corals tanks that were supplied fish water, tanks that had fish in the tank with the corals and tanks that had fish and the addition of live feeds. In particular, C:N:P ratio in the LiveFeeds + Fish tanks (calculated as 484:10:1, see Appendix E for details) was closest to the Redfield ratio (106:16:1; the consistent ratio of these elements throughout marine phytoplankton and similar to the ratio of these dissolved nutrients in seawater (Redfield, 1958)) out of any of the treatments, and also maintained the highest abundance of *Endozoicomonas*. Potentially, the combination of the live feeds and the fish-derived-nutrients contributed to forming a nutrient balance to sustain *Endozoicomonas* populations, as it has been suggested the maintenance of the coral-*Endozoicomonas* association may require specific nutrient conditions (Pogoreutz & Ziegler, 2024). Further work is needed to identify what factors influence the stability of the corals' microbiome and promotes the high abundance of *Endozoicomonas* taxa across many coral species. Nevertheless, the maintenance of a more "field-like microbial community" in tanks with fish highlights a previously unknown potential benefit from co-culture of corals and fish.

5.5.4 Conclusions

This study aimed to characterise the microbial and nutritional responses of *Pocillopora verrucosa* to co-culture with schooling *Chromis virids* and supply of live feeds, in the context of improving coral physiology in captive culture for long-term holding or prior to outplant or export. Total lipid concentrations varied between captive *P. verrucosa*, but all Post-acclimation corals were deficient in the primary storage lipid class, namely TAG, which was unable to be restored to pre-fragmentation and acclimation levels in any treatment. TAG and individual fatty acids were likely predominantly derived from and influenced by Symbiodiniaceae functioning, and differences between corals in treatments may therefore be attributed to their respective symbiont populations. Yet, there was some evidence that

heterotrophic feeding of Field corals and those with access to live feeds also played a role. Interestingly, coral microbial communities were closest to their field composition in the LiveFeeds + Fish treatment; the closest approximation to wild conditions. Taken together, this presents a complex view of whether fish-coral co-culture may be beneficial for coral aquaculture. Whilst Chapter 4's observation of improved growth is a beneficial outcome for producers (both for restoration and commercial trade), as is the similarity of a captively held coral's microbiome to their field counterparts, the lack of recovery of important energy stores in the form of TAG requires further attention. Future work could test the application of different live feeds varieties, feeds with fatty acid and lipid class compositions tailored to the species-specific feeding and digestive capacity of a variety of cultured corals species. Furthermore, the optimisation of husbandry conditions, including light intensity should be further investigated.

Overall, this study attests that co-culturing corals with fish does not produce any negative effects in the cultured corals, and moreover is capable of enhancing growth, symbiont densities and protein content. Furthermore, the addition of live feeds is capable of maintaining captive coral holobionts in a physiological state closer to that of their wild counterparts. The effect of the live feeds in the LiveFeeds + Fish treatment was likely supressed by the fish feeding on them, reducing availability to the corals, yet elevated levels of coral growth were still maintained. Thus, co-culturing fish and coral with proportionally higher live feed feeding rates holds the potential to improve coral growth and physiology and maintain wild-coral microbial communities prior to outplanting or export.

CHAPTER 6 – General Discussion

6.1 Overview of findings

The application of co-culture in coral aquaculture presents a promising opportunity, offering many avenues to enhance coral production (Ladd & Shantz, 2020). This thesis addresses key knowledge gaps that currently limit the effective implementation of co-culture in *ex situ* coral aquaculture. The outcomes of this work contribute to building a comprehensive knowledge base to inform aquaculture practitioners in selecting co-culture partners for corals to provide functions that enhance coral health and improve production efficiency.

Different species of coral recruit have been shown to be better suited to co-culture with various species of grazers for effective algae control during grow-out. Specifically, Acroporids performed well when co-cultured with the gastropod *Calthalotia strigata*, whilst smaller, non-Acroporid corals performed well alongside juvenile sea urchins *Tripneustes gratilla* (Chapter 2). Fish grazers can be introduced approximately one-month post-settlement for Acroporid corals, with "cropping" species such as *Acanthurus nigrofuscus* (Tebbett et al., 2017b) minimising the risk of mortality from accidental overgrazing in both Acroporid and non-Acroporid recruits, whilst also effectively removing problematic turf algae (Chapter 3). Since microherbivores were shown to be inefficient for controlling turf algae growth, sequential application of first using microherbivores followed by fish grazers during coral grow-out could be an effective method to control algae growth with minimal input from aquarists.

Co-culture with schools of *Chromis viridis* damselfish has the potential to enhance adult coral growth, protein content and symbiont density, particularly in more autotrophic or fish-associated coral species (Chapter 4). This improvement is hypothesised to result from enrichment of the coral's symbionts from increased nitrogen and phosphorus (particularly NH_4) from the fishes' wastes. Further exploration of the physiological and microbial responses of *Pocillopora verrucosa* to fish co-culture revealed that the presence of fish in the tank with the corals maintained an associated microbial community similar to their field-

state, dominated by *Endozoicomonas*, though supply of live feeds was also necessary to fully maintain this association (Chapter 5). Biochemical analysis showed that access to live feeds led to preferential direction of energy towards growth rather than lipid accumulation, which was noted to occur in the corals with enhanced nutrients (N/P) from fish wastes but no access to live feeds. All captive corals were deficient in the important storage lipid TAG, relative to the field controls, likely due to the stress of fragmentation and lower light conditions in the experiment compared to their natural environments.

6.2 Cost-effectiveness considerations

It is well established that, despite advances in knowledge and technology, coral aquaculture and restoration remain extremely costly endeavours (Hughes et al., 2023). Labour and equipment requirements are major contributors to these costs, especially in countries like Australia where wages are high (Baria-Rodriguez et al., 2019). Techniques that reduce labour demands are therefore essential to minimise costs (Severati et al., 2024). Co-culture offers the potential to minimise labour costs by replacing services typically provided by aquarists with similar services provided by the companion organisms (Knoester et al., 2024).

Chapter 2 demonstrated that incorporating microherbivores into recruit grow-out systems effectively minimised husbandry requirements whilst maintaining high survival and growth in recruits. This approach could improve the cost-effectiveness of coral culture by reducing labour costs. Chapter 4 showed that simply supplying corals with dissolved fish wastes enhanced coral growth rates, particularly for more autotrophic or fish-associated species. Feeding the fish that supplemented the corals with their wastes was relatively simple compared to feeding the corals with live feeds. In this study, the *Chromis viridis* were fed with a readily available commercial pellet (Aqua Forest Tiny Fish Feed), priced at $27.35 AUD (2022) per 120 g tub. Each 50 L tank with coral and a school of ten *C. viridis* was fed 0.24 g of feed per day, meaning a tub of pellets could feed a similar tank for approximately 500 days at a price of $0.06 AUD per day. This method of supplementing corals is potentially more cost effective than relying on live feeds, which can be a significant cost to aquaculture ventures due factors such as to the equipment and labour requirements to culture them, inadequate supply of cysts or starter cultures, culture crashes and inconsistent nutritional

quality (Callan et al., 2003). Considering these factors, co-culture represents a potential cost-effective strategy to enhance coral aquaculture. However, to further improve the cost-effectiveness of co-culture, companions to coral should be either readily available or relatively easy to culture, suitable for use throughout their entire lifespan in coral aquaculture tanks or have a secondary market or use to justify the extra costs of culturing or acquiring them.

Companion species that are inexpensive and easily available may be more suitable for use in mass coral propagation than less readily available and more difficult to maintain species, despite potential better performance in aiding coral production. For instance, the use of *Chromis viridis* in Chapters 4 and 5 was driven by several considerations: they naturally associate with coral colonies, their relatively friendly demeanour poses less risk to aquarists or tank companions and they are inexpensive and readily available in the ornamental market. While *Dascyllus* damselfish are more common in coral-fish association studies (Chase et al., 2018; Chase et al., 2020; Garcia-Herrera et al., 2017), they are less suitable for high density culture due to their more aggressive nature (Katzir, 1981). In contrast *C. viridis* can be kept in high densities and are available at relatively low prices; in Chapter 4/5 the 120 fish in the experiment were supplied at a rate of $5.00 AUD (2022) per fish. This price makes it relatively affordable for a coral propagation facility to acquire enough individuals to meet the density recommended by this study - one fish per five litres of tank water (Chapter 4). Similarly, the recommendation to use the gastropod *Calthalotia strigata* for algae around coral recruits was based in part on its ability to self-propagate within coral grow-out systems making it easier to maintain a sufficient population density for effective algae control (Chapter 2; (D'Angelo & Wiedenmann, 2011)). Systems within coral aquaculture facilities could also be dedicated to maintaining a population of these gastropods, or similar self-propagating species, which have less demanding culture conditions compared to other grazers such as sea urchins. These gastropods also do not require extensive husbandry or specialised equipment, further reducing the costs and labour associated with their maintenance.

Companion species that can be utilised in a coral production facility for their entire lifespan are also preferable. For example, *C. viridis* represents a good candidate as these fish typically

do not exceed a maximum total length of ~9 cm (Rekha et al., 2024). They can be housed in a wide variety of tank sizes without growing to a size that might pose a threat to corals or aquarists. Grazing fish, such as Acanthurids, are often sequentially transferred to different types of tanks throughout their life span. Younger, smaller fish can be utilised to control algae growth in coral recruit tanks (Chapter 3), and as they grow larger, and begin to pose more of a risk to recruits and require more space, they can be transitioned to larger tanks with adult corals (Alwany & Sarhan, 2012; Chapman & Kramer, 2000; Doropoulos et al., 2012). Similarly, juvenile sea urchins, which may be used for algae control around coral recruits, can be moved to tanks holding older coral as they outgrow their use in recruit systems (Chapter 2; (Craggs et al., 2019)). Some companion species may only be acquired in their adult stages and kept in specific systems throughout their time in the facility. For example, butterfly fish like *Chaetodon kleinii* are used to control outbreaks of pest anemones such as *Aiptasia* (Nakamura et al., 2011), however should not be kept with younger or smaller corals due to the risk of predation of the corals (Pratchett et al., 2013). Therefore, they would be introduced with the specific purpose of controlling pests in adult holding systems and kept in in these specific systems for their entire lifespan.

One way to offset production costs and justify the culture of companion organisms that may not be utilised for the entire production cycle, is to find secondary uses for these species. One approach may be to focus on the restoration of multiple species, where companion species are not only used to support coral culture but are also targeted for restoration themselves (Sievers et al., 2022). For example, in the Caribbean there is increasing interest in out-planting urchins or other herbivores alongside corals to help combat macroalgae phase shifts on degraded reefs (Butler et al., 2024; Cano et al., 2021; Williams, 2021). Juvenile urchins could be produced and cultured alongside coral recruits, helping to improve coral survival and growth by feeding on fouling algae in the tanks, which also serves as a cost-effective food source for the urchins. Once both urchins and corals reach the desired size they would then be out-planted to the reef together (Barrows et al., 2023). Similarly, ornamental facilities could sell companion species to consumers. Shrimp like *Lysmata* sp. are popular in ornamental hobbyist tanks for their aesthetic appearance and ability to control pest and parasite populations (Calado & Narciso, 2005; Rhyne et al., 2004). A coral

production facility could therefore culture these shrimps for use in their own coral grow-out tanks as pest control, and then sell any excess to consumers.

While current evidence suggests that co-culture could be a cost-effective method to enhance coral aquaculture, a more comprehensive cost analysis of the use of companion species across different stages of the coral production cycle is still needed. The business case and cost-benefit analysis will depend on the specific requirements of each aquaculture endeavour; these analyses are outside the scope of this research project but are highlighted as critical for the success of any future co-culture coral aquaculture projects (Bayraktarov et al., 2016). However, several key considerations can still be identified. For example, while Chapter 2 and other studies such as Serafy et al. (2013) have demonstrated that co-culture can reduce cleaning-related husbandry costs associated with producing juvenile corals, many cost analyses do not account for the additional time and materials required to produce and maintain companion species. A full cost analysis should consider factors such as: 1) the cost of procuring companion species, 2) on-going costs of including them in systems (e.g. specific feeds etc.), 3) potential secondary uses or markets for them that may generate revenue or justify expenses, and 4) the overall benefit they supply to the corals. Such an analysis should be conducted before incorporating companion species into coral culture. However, evidence from this thesis supports the proposition that co-culture is a viable method to reduce costs while benefiting coral production.

6.5 Companion compatibility considerations

6.3.1 Aggression, competition and general behaviour

Producers and aquarists ideally should deploy multiple companion species, each tailored to deliver different benefits in each aquarium system. However, not all species are compatible with each other, so caution must be taken when designing species mixes. *Dascyllus* damselfish, although known for their close associations with corals and the benefits they provide, can be territorial and aggressive towards other fish in the same system (Chase & Hoogenboom, 2019; Katzir, 1981). Similarly, Acanthurids are known to display both intra- and interspecific aggression to other Acanthurids, leading to most facilities avoiding holding more than one individual of the same species in a single system and to exercise caution when introducing individuals of different species (Schober & Ditrich, 1992). The six-line

wrasse (*Pseudocheilinus hexataenia*), though effective at removing parasites such as *Prosthiostomum* sp. flatworms, is territorial and it is not recommended to have more than one individual in a system smaller than 1000 litres (Barton et al., 2020b). Some species may even prey upon others; for example, herbivorous or detritovore hermit crabs are known to occasionally feed upon gastropods (Ribeiro et al., 2017). Understanding the behaviour of companion species is therefore vital to ensuring compatibility. Often, scientific literature may be lacking in this regard, so producers may turn to other sources of information in the "grey literature," such as hobbyist aquarist forums, to find data on species compatibility. Alternatively, they might trial companion mixes on a small scale prior to introducing them across larger tank systems.

Utilising multiple species to perform similar functions within the same grow-out system may sometimes result in each species being less effective than if they were used in isolation. Neil et al. (2021) found that a mix of gastropod and sea star species led to lower survival and growth of coral recruits compared to single species cultures. This reduction in effectiveness may be due to interspecific competition for resources and behavioural interactions that disrupt the grazing activities of their companions. To achieve effective species mixes, it may be beneficial to limit each tank system to one 'type' of companion while critically assessing the function that each species provides. For instance, having only one species of gastropod grazer per tank system could minimise interspecific competition. However, several different species of herbivorous fish (e.g. a cropping species, a concealed cropper species and a brusher species) might be used together to target different kinds of algae effectively. Similarly, Barton et al. (2020b) observed that the potential parasite biocontrol species *Lysmata vittata* and *Pseudocheilinus hexataenia* preyed on different life-stages of the *Acropora* eating flatworm. Therefore, employing both species in the same system might be necessary to ensure effective control of these pests.

There are other behavioural considerations as to whether a species is an appropriate co-culture companion, such as how suited a species is to being held within tank systems. Some gastropod species such as *Turbo* sp. and *Tectus* sp. have a habit of resting at the edge of tanks out of the water (Watson et al., 2018). This can be problematic, as snails can be accidentally knocked out of tanks, causing them to desiccate and die, reducing the overall

population of grazers. Similarly, some fish such as Blendiidae species like *Salarias fasciatus* are prone to jumping, necessitating them being kept in either deeper tanks or the addition of some kind of cover over the tank to prevent their escape and subsequent death (Martin, 1995). Prior knowledge of such behaviour is vital to ensure appropriate allocation and preparation of tanks for different species.

6.3.2 Potential for introduction or transmission of invasive species and diseases

If the end-goal of a coral aquaculture project is to outplant recruits or adults for restoration, co-culture companions should ideally be sourced from the same bioregion the corals came from or are to be out-planted to. This practice helps mitigate the risk of introduction of potentially invasive species, including any potential parasites or diseases they may carry (Henry et al., 2019). While it is unlikely that larger companion species such as fish would be accidentally introduced alongside corals, smaller species like gastropods may be overlooked during out-planting screenings, resulting in their unintended translocated with the corals. Additionally, populations of small organisms, including but not limited to amphipods, isopods and brittle stars (Ophiuroidea), naturally occur in most coral grow-out systems, and may inadvertently be introduced when corals or live rock are brought in from the wild, and propagated to other tanks through movement of animals or tools between systems (Brawley & Adey, 1981; D'Angelo & Wiedenmann, 2011). Parasites and disease may also be inadvertently brought into facilities through inadequate quarantine measures and can be transferred between corals from different locations due to mixed coral cultures or poor sterile practices (Barton et al., 2020a; Gochfeld & Aeby, 1997). As such, ensuring both companion species and corals are sourced from, and destined for the same source bioregion is vital to help prevent the accidental introduction of unwanted organisms to natural ecosystems.

Most countries with active coral aquaculture industries are likely involved in the import and export of ornamental marine species, with the potential for transfers of different organisms between different facilities. Conducting a survey of current companion species in use in coral grow-out systems within a country would be highly useful, with identification of where companion species are sourced from to determine any potential risk of transmission of unwanted organisms. For example, in Australia, the movement of marine animals in the

ornamental trade is relatively unregulated once animals are in the country (Erickson, 2017; Morrisey et al., 2011). This lack of regulation means that organisms can be imported from other countries and then introduced to tanks with locally harvested corals. These corals could subsequently to transferred to other operations or even out-planted to the reef. The ornamental marine trade has previously been identified as a vector of invasive species in several high-profile cases, such as the introduction of lionfish (*Pterois* sp.) to reefs in Florida, where they have become a significant ecological problem (Hixon et al., 2016). Therefore, even ornamental coral producers should take care to ensure they are not exposing their locally grown coral to any potential foreign species. Extreme care must be exercised, particularly in facilities that engage in both aquaculture and import/export activities.

6.4 Effective pairings of corals and companions

Utilising the results of this thesis within the context of prior research, it is possible to develop a set of recommendations for appropriate co-culture companions to supply various services for different corals species across varying life stages.

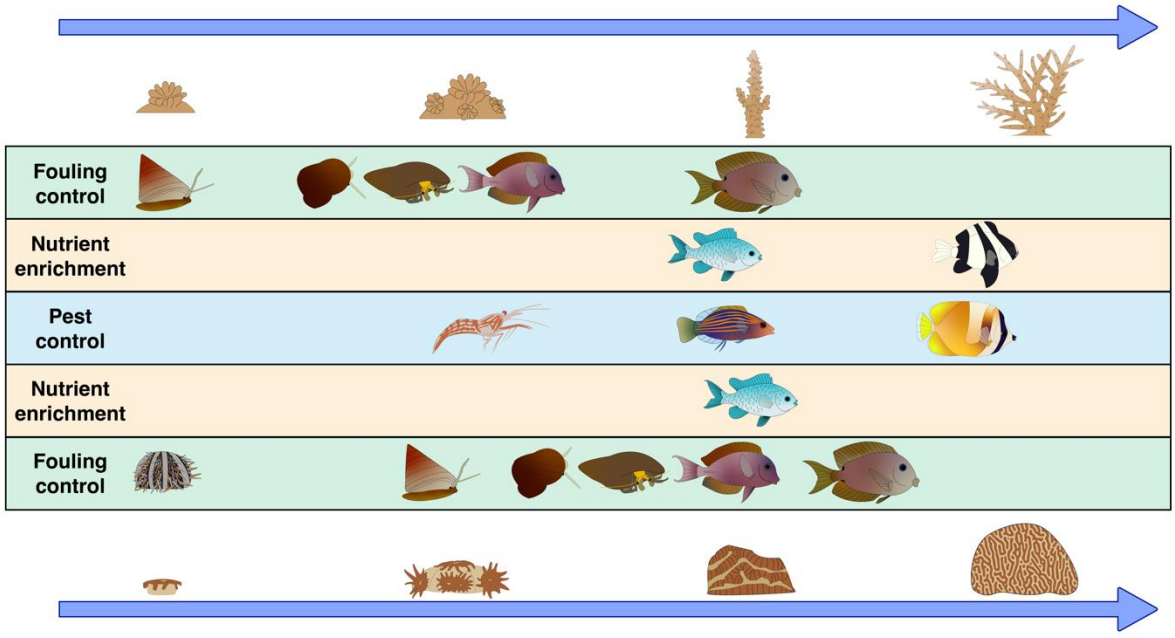

Figure 6.1: Summary timeline of generalised suggested companion groups for branching and massive corals at various stages of grow-out. Nutritional enrichment for branching vs massive also corresponds with the suggestions for autotrophic vs heterotrophic corals.

6.4.1 Fouling control

Young recruits are particularly vulnerable to overgrazing by herbivorous species, as well as the negative effects of algae and fouling (Christiansen et al., 2009; Jorissen et al., 2020; Whitman et al., 2024). To effectively manage algae while minimising harm to the corals, the use of microherbivores in recruit culture is recommended. For larger recruit species (> 0.5 mm^2 surface area) such as Acroporids, pairing them with small grazing gastropods like *Calthalotia strigata* has proven effective. Smaller recruits (< 0.5 mm^2 surface area) perform better with smaller grazing species, such as the juveniles of short-spined urchin *Tripneustes gratilla* (Chapter 2; (Barrows et al., 2023; Henry et al., 2019)).

Recruits can be paired with microherbivores until they reach the muti-polyp stage, which occurs at approximately one month post settlement for faster growing species like Acroporids. At this stage, herbivorous fish can begin to be introduced to coral culture systems to help control algae species that microherbivores may struggle with (Chapter 3). Initial introduction of fish should involve “cropping” grazers such as *Acanthurus nigrofuscus,* to minimise the risk of injury to the growing corals. “Brushing” grazing species should not be introduced until much later in the grow-out cycle when corals have reached a pre-determined escape threshold (Chapter 3). Similarly, other potentially disruptive grazing species, such as hermit crabs, should be avoided until the corals have reached at least the multi-polyp stage.

Slower growing coral species such as *Porites* sp., which often begin with smaller recruit sizes, will take longer to reach size-escape thresholds necessary for introducing more disruptive grazers. The growth dynamics of different coral species should be carefully considered when designing culture systems (Chapter 3; (Doropoulos et al., 2012)). Once corals reach the sub-adult stage, where they begin to resemble their adult morphology, they typically become resistant to most types of grazing, at least from species that can be safely cultured within *ex situ* tanks due to size constraints. At this point, combinations of grazers can be tailored to match the fouling communities that develop within tank systems (Frias-Torres & van de Geer, 2015; Ng et al., 2014; Watson et al., 2018).

When selecting grazing partners for corals, it is important to consider the specific algae or fouling species commonly found within the facility's systems. For example, sea urchins are often recommended for controlling the growth of crustose coralline algae (CCA) within marine mesocosms (Craggs et al., 2019; Ng et al., 2014). CCA is often present in coral tanks, particularly on settlement substrates, as it is used to induce settlement (Abdul Wahab et al., 2023; Jorissen et al., 2021). However, some facilities have begun to freeze CCA covered substrates prior to settlement or use extracts rather than live CCA to eliminate it as a competitor before recruits are undergo metamorphoses (Whitman et al., 2020). If recruits are then kept in a sterile system, the need for a grazing species to control CCA growth is effectively removed. On the other hand, there is an argument for maintaining grazing populations that do not eliminate all algae but instead promote fouling communities that are manageable for aquarists. Whilst CCA can be problematic in recruit culture, it can be beneficial in adult coral holding tanks by preventing colonisation of other, less desirable algae on tank surfaces (Littler & Littler, 2013). In such cases, it may be advantageous to select grazers that target other species of algae in these tanks.

6.4.2 Nutrient enrichment

To date, no studies have specifically examined the effect of nutrient enrichment from fishes on coral recruits. However, more general studies on the addition of nutrients to recruits have shown mixed effects, often dependent on environmental conditions (Humanes et al., 2017; Humanes et al., 2016). The addition of schooling fish was shown to benefit coral fragments of at least ~10 g in size (Chapter 4; Chapter 5), and previous research has demonstrated similar positive responses in larger coral colonies (Holbrook et al., 2008; Meyer & Schultz, 1985b; Shantz et al., 2015). Given these findings, coral-associated schooling fish such as *Chromis viridis* could be introduced to coral culture to provide nutrient enrichment once the corals have reached the sub-adult stage or to asexually propagated coral fragments.

Co-culture with schooling fish is particularly suitable for coral species that depend heavily on autotrophic energy acquisition, such as *Porites lutea,* or those naturally associated with fish, like *Pocillopora verrucosa.* Current evidence suggests that such associations at a minimum increase growth rate of such corals (Holbrook et al., 2008; Liberman et al., 1995). Furthermore, results from Chapter 4 demonstrated that co-culture with fish did not

negatively affect more heterotrophic, non-fish-associated species like *Platygyra daedalea*. This finding indicates that it would be possible to maintain mixed cultures with corals species that benefit from the fish derived nutrient, without adversely affecting other coral species.

Supply of dissolved fish wastes in the absence of live feeds could contribute to lipid proliferation in corals, though such responses still need to be tested across a greater variety of culture conditions and species, particularly under higher light (Chapter 5). Unexpected benefits can be derived from fish co-culture. For example, in Chapter 5 it was found that co-culture with *Chromis viridis,* and simultaneous supply of live feeds, maintained the microbial associated communities of *Pocillopora verrucosa* in their *Endozoicomonas* dominated field state, which may be beneficial for out-planting coral colonies (Strudwick et al., 2022).

Using host-faithful species such as *Dascyllus* may be most appropriate for the culture of larger colonies of branching coral species. Larger branching colonies often suffer from stagnant areas within their innermost branches when cultured in tanks, where low water flow result in food and oxygen deficient areas (Chamberlain & Graus, 1975). The presence of fish in and amongst branches could help mitigate these stagnant zones while simultaneously providing nutrient enrichment (Garcia-Herrera et al., 2017; Goldshmid et al., 2004). Since *Dascyllus* species are also known to be territorial, keeping them in the larger tank systems required for large, adult branching colonies reduces the likelihood of aggression between them and other companion species (Katzir, 1981; Sale, 1971).

6.4.3 Parasite and pest control

Although this thesis did not specifically investigate the use of co-culture for parasite and pest control, some inferences can still be made based on existing research and current understanding of co-culture practices. Reports of coral recruit cultures suffering from the effects of parasites and/or diseases are rare (Barton et al., 2020a). Given that younger and smaller recruits are more vulnerable to disturbance by grazers (Chapter 2; Chapter 3), it is advisable to avoid introducing pest control species into coral cultures until the recruits have at least reached the multi-polyp stage. For smaller, more vulnerable recruit species, delaying the introduction of pest control species until even later stage might be necessary.

Small biocontrol species, such as *Lysmata* cleaner shrimp, could be introduced initially to help prevent parasite outbreaks in recruit cultures. Larger species, like *Pseudocheilinus hexataenia,* can be introduced once the corals reach the sub-adult stage (Barton et al., 2020b; Calado & Narciso, 2005). Butterflyfish, such as *Chaetodon kleinii,* and filefish, such as *Acreichthys tomentosus,* are commonly used to controls of pest species like *Aiptaisa* anemones and parasites such as planaria flatworms and *Phestilla* nudibranchs. However, these species should only be introduced to systems with adult corals due to the risk that these species will feed upon the corals themselves (Gochfeld & Aeby, 1997; Nakamura et al., 2011; Pratchett, 2007). Ensuring that facilities adhere to quarantine procedures, such as screening corals prior to introduction to tanks and minimising cross-contamination between systems, is vital to prevent outbreaks (Barton et al., 2021).

6.5 Future directions

Many avenues remain for future study of co-culture in coral aquaculture. Facilities should explore the use of different local species to act as companions throughout the coral production cycle or ensure individuals of species with cosmopolitan distributions are sourced from local stock. While protocols for using grazers and other species for fouling control are established, further investigations into the succession of different grazers in coral grow-out would be valuable. This would help determine the optimal timing for introducing larger grazers to a wider variety of coral species (Chapter 2; Chapter 3).

For nutrient enrichment, investigating whether coral recruits benefit from fish wastes would be beneficial to help answer questions around appropriate nutrition of recruits and efficacy of different diets for restoration or ornamental production (Chapter 4; (Conlan et al., 2017a)). Additionally, further research into the microbial impacts of co-culture is warranted. Chapter 5 found that the presence of fish in the tank with the coral was crucial for maintaining the dominance of *Endozoicomonas* taxa associated with *Pocillopora verrucosa*, a previously unobserved effect of fish-coral associations. Investigating whether such effects occur in other fish-associated corals species and identifying the underlying mechanisms (nutrient enrichment, direct transfer of microbes etc.) is essential to understand the implications for the coral holobiont (Voolstra et al., 2024).

Finally, larger-scale co-culture trials and comprehensive cost analyses are still necessary to evaluate the effectiveness of companion species throughout the entire coral production cycle (Hughes et al., 2023). These larger-scale trials should be performed through collaboration with coral aquaculture producers, bringing together the results of this thesis with the specific needs of different producers in the coral aquaculture industry.

6.6 Final remarks

Overall, this thesis provides a guideline for practitioners for effective application of co-culture companions in coral aquaculture, shows co-culture offers cost-effective methods to enhance coral production to support the growing reef restoration and ornamental industries. The results of this thesis demonstrate that integrating grazers and schooling fish are effective methods to improve the survival and growth of corals species, providing recommendations for effective parings of corals and companion species. Future research should explore larger-scale parings of corals and companions, with a focus on utilising local species to support burgeoning ornamental aquaculture and coral restoration activities.

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Appendix A Data and Code Availability

Chapter 2 - Size matters: Microherbivores make a big impact in coral aquaculture

Data are available via the AIMS Data Centre https://doi.org/10.25845/RPRZ-BT30

Chapter 3 – Let the fish do the cropping: identifying fish grazers to improve coral aquaculture

Data and code are available via https://github.com/blue-bio/fish_recruit_public

Data are also available via the AIMS Data Centre https://doi.org/10.25845/Z1RD-9R72

Chapter 4 – Improving coral grow-out through an integrated aquaculture approach

Data and code are available via https://github.com/blue-bio/coral_fish_nutrition_public
Data is also available via the AIMS Data Centre https://doi.org/10.25845/2SWS-G533

Chapter 5 – Nutritional and microbial responses of *Pocillopora verrucosa* to co-culture with *Chromis viridis* damselfish

Data and code are available via
https://github.com/blue-bio/pocillopora_fish_nutrition_public
Data is also available via the AIMS Data Centre https://doi.org/10.25845/V8MN-TQ79
The 16S amplicon sequencing data are available in the NCBI Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under the BioProject accession number PRJNA1155229.

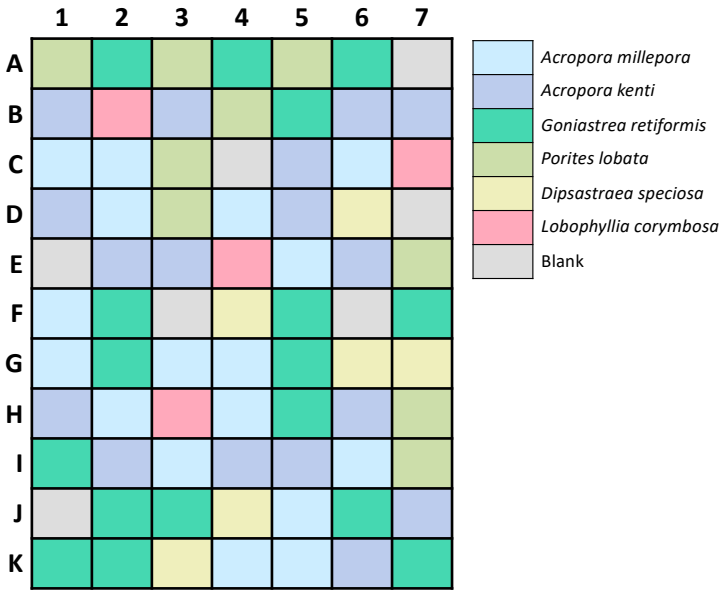
Appendix B Supplementary Information for Chapter 2

Echinometra mathaei culture methods

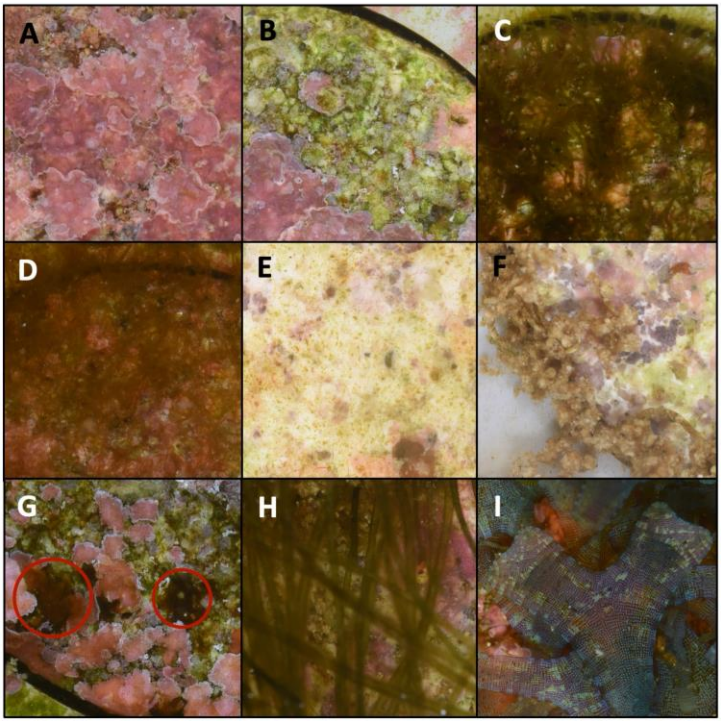
Echinometra mathaei broodstock were spawned using 1mL injections of 1M KCL. Eggs and sperm were collected, then pooled in separate containers. Sperm was added to the eggs at a density of ~100 sperm per egg. Eggs were left to fertilise undisturbed for 1 hour, then added to 27°C filtered seawater (FSW) in static, conical 65L tanks with low aeration. Larvae were left undisturbed in the tanks until the prism stage (~24 hours post-fertilisation), then moved into 1L FSW in Schott bottles on a 'sausage roller' table (See Karelitz et al. (2020) for details). Larvae were kept in suspension at densities of 1 individual per mL, and fed daily with a mix of T. *Isochrysis galbana* and *Chaetoceros muelleri* at 4000 cells mL^{-1}. Every second day a 100% water change was performed by filtering larvae through a 106μm screen, then moving them to a new, clean Schott bottle with FSW. Larvae were cultured until competent to settle at ≥15 days, then were introduced to small settlement jars with 750mL of FSW, low aeration and chips of mixed crustose coralline algae (CCA) communities (primarily *Mesophyllum* sp. and *Lithophyllum* sp.). Once settled and fully metamorphosed, recruits were moved to small 5L tanks with flow-through FSW and a 200μm banjo filter on the outlet. Juvenile urchins were then fed with CCA chips, benthic diatoms (*Nitzchia* and *Amphora* sp.) and cultured biofilms for 1 month, until the grazing experiment began.

Supplementary Table 1: Spawning and settlement information for corals							
Coral Species	**Source Reef**	**Date Spawned**	**Settled** Date, # larvae per 50L tank x # tanks	**Resettled #1**	**Resettled #2**	**Donor colonies added**	**Final plug numbers**
Acropora millepora	Falcon	25/11/2021	30/11/2021 1500 x 4	na	na	2/12/2021	708
Acropora kenti	Davies	23/11/2021	30/11/2021 1500 x 4	3/12/2021 1000 x 4	5/12/2021 2500 x Tank 3 1000 x Tank 4	5/12/2021 Tanks 1, 2 7/12/2021 Tanks 3, 4	499
Porites lobata	Esk and Davies	21/11/2021	30/11/2021 2000 x 4	3/12/2021 1000 x 4	5/12/2021 2500 x 4	Na – mother transfers symbionts	258
Goniastrea retiformis	Falcon	23/11/2021	30/11/2021 2000 x 4	4/12/2021 1000 x 4	na	5/12/2021	554
Dipsastrea speciosa	Davies	23/11/2021	28/11/2021 2000 x 4	1/12/2021 2400 x 4	na	3/12/2021	179
Lobophyllia corymbosa	Esk and Falcon	25/11/2021	3/12/2021 1050 x 4	6/12/2021 2000 x 4	8/12/2021 2000 x Tank 1	8/12/2021 T2, 3 & 4 9/12/2021 T1	54

Supplementary Table 2: Summary of water quality in SeaSim	
Parameter	**Mean ± sd**
pH	8.12 ± 0.104
NH_4 (μmol L^{-1})	0.163 ± 0.072
NO_3 (μmol L^{-1})	1.92 ± 0.350
PO_4 (μmol L^{-1})	0.158 ± 0.049
Alkalinity (μmol kg^{-1})	2300 ± 21.4
DIC (μmol kg^{-1})	2023 ± 24.7
Salinity (ppt)	35.1 ± 0.337



Supplementary Figure 1: Schematic of the randomised plug trays used to hold settled coral recruits across the different experimental treatments. For Control, *Calthalotia* and *Tripneustes* treatments the above array was used; for Aquarist, *Clibanarius*, *Echinometra* and *Turbo* treatments, *Lobophyllia corymbosa* plugs were replaced with Blank plugs. Note: one tray per replicate tank.



Supplementary Figure 2: Example photos of common fouling groups in the experiment. A) Crustose coralline algae (B) Endolithic green algae (C) Algal turf (D) Filamentous algae (E) Bare plug (F) Sediment (G) *Lobophora* sp. (circled areas) (H) *Bryopsis* sp. (I) *Dictyota* sp.

Appendix C Supplementary Information for Chapter 3

Supplementary Table 1: Bayesian model parameters						
Response variable	**Model form**	**Distribution**	**Link**	**Priors**	**Iterations (warmup)**	**Thinning**
Fish bite rate	bites_t ~ treatment*tile_status + (1|treament_rep_r)	Negative Binomial	log	prior(normal(1.8, 2.2), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 3), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	10,000 (5,000)	10
***Acropora kenti* mortality**	dead_post | trials(1) ~ treatment*recruit_age + (1|treament_rep_r)	Binomial	logit	prior(normal(-4.6, 2.5), class = 'Intercept') + prior(normal(0, 2.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd')	10,000 (2,500)	5
***Acropora millepora* mortality**	dead_post | trials(1) ~ treatment*recruit_age + (1|treament_rep_r)	Binomial	logit	prior(normal(-4.6, 2.5), class = 'Intercept') + prior(normal(0, 2.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd')	10,000 (2,500)	5
***Goniastrea retiformis* mortality**	dead_post | trials(1) ~ treatment + (1|treament_rep_r)	Binomial	logit	prior(normal(0, 1), class = 'Intercept') + prior(normal(0, 2.5), class = 'b') + prior(student_t(3, 0, 2.5), class = 'sd')	10,000 (2,500)	5
Detritus percentage cover	detritus ~ treatment*exposure + (1|treatment_rep) zi ~ treatment*exposure	Zero-inflated negative binomial	log	prior(normal(1.9, 1.5), class = 'Intercept') + prior(normal(0, 1.5), class = 'b') + prior(student_t(3, 0, 1.5), class = 'sd') + prior(logistic(0, 1), class = 'Intercept', dpar = 'zi') + prior(normal(0, 1), class = 'b', dpar = 'zi') + prior(gamma(0.01, 0.01), class = 'shape')	5,000 (2,500)	5
Filamentous algae percentage cover	filamentous_algae ~ treatment*exposure + (1|treatment_rep) zi ~ treatment*exposure	Zero-inflated negative binomial	log	prior(normal(3.2, 1.5), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 1.5), class = 'sd') + prior(logistic(0, 1), class = 'Intercept', dpar = 'zi') + prior(normal(0, 1), class = 'b', dpar = 'zi') + prior(gamma(0.01, 0.01), class = 'shape')	5,000 (2,500)	5

Turf algae percentage cover	turf_algae ~ treatment*exposure + (1|treatment_rep) zi ~ treatment*exposure	Zero-inflated negative binomial	log	prior(normal(1.9, 2), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 2), class = 'sd') + prior(logistic(0, 1), class = 'Intercept', dpar = 'zi') + prior(normal(0, 1), class = 'b', dpar = 'zi') + prior(gamma(0.01, 0.01), class = 'shape')	5,000 (2,500)	5
Fouling percentage cover **Fouling = sum of filamentous, turf and detritus percentage cover**	fouling ~ treatment*exposure + (1|treatment_rep) zi ~ treatment*exposure	Zero-inflated negative binomial	log	prior(normal(3.7, 1), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(logistic(0, 1), class = 'Intercept', dpar = 'zi') + prior(normal(0, 1), class = 'b', dpar = 'zi') + prior(gamma(0.01, 0.01), class = 'shape')	5,000 (2,500)	5
Bare tile percentage cover	bare_tile ~ treatment*exposure + (1|treatment_rep)	Gaussian	identity	prior(normal(43, 20), class = 'Intercept') + prior(normal(0, 50), class = 'b') + prior(student_t(3, 0, 20), class = 'sd')	5,000 (2,500)	5

Supplementary Table 2: Results from Bayesian models

		Modelled median (lower – upper 95% credibility interval (highest posterior density interval))					
	Response	***Acanthurus nigrofuscus***	***Ctenochaetus binotatus***	***Salarias fasciatus***	***Zebrasoma scopas***	**Manual**	**Uncleaned**
***Acropora kenti* mortality (probability)**	**1-week-old, single polyp**	0.0119 (0.0011 – 0.0323)	0.0861 (0.0133 – 0.2218)	0.0120 (0.0007 – 0.0386)	0.0082 (0.0001 – 0.0264)	0.0213 (0.0007 – 0.0736)	0.0149 (0.0004 – 0.0536)
	1-month-old, multi-polyp	0.0009 (0.0000 – 0.0059)	0.0011 (0.0000 – 0.0116)	0.0029 (0.000 – 0.0168)	0.00002 (<0.000 – 0.0037)	0.0150 (0.0002 – 0.0690)	0.0004 (0.000 – 0.0083)

***Acropora millepora* mortality (probability)**	**1-week-old, single polyp**	0.0026 (0.000 – 0.0109)	0.0249 (0.0002 – 0.0951)	0.0035 (0.0000 – 0.0199)	0.0028 (0.0000 – 0.0164)	0.0037 (0.000 – 0.0199)	0.0003 (0.0000 – 0.0036)
	1-month-old, multi-polyp	0.0007 (0.0000 – 0.0038)	0.0038 (0.0000 – 0.0183)	0.0013 (0.0000 – 0.0104)	0.0009 (0.0000 – 0.0062)	0.0016 (0.0000 – 0.0095)	0.0000 (0.0000 – 0.0014)
***Goniastrea retiformis* mortality (probability)**	**1-week-old, single polyp**	0.0626 (0.0000 – 0.5122)	0.8888 (0.3989 – 1.0000)	0.4301 (0.0791 – 0.8156)	0.0510 (0.0002 – 0.4029)	0.3066 (0.0003 – 0.8296)	0.1231 (0.0003 – 0.6080)
Bite-rate (bites per min)	**Coral tiles**	2.9 (1.1 – 5.6)	5.7 (1.5 – 12.6)	1.1 (0.2 – 2.7)	2.6 (0.9 – 6.5)	NA	NA
	Tank walls or shelters	0.8 (0.2 – 2.7)	3.4 (0.7 – 8.2)	0.7 (0.2 – 1.7)	1.8 (0.5 – 4.1)		
Detritus cover (%)	**Pre-exposure**	10.43 (7.11 - 14.86)	7.43 (4.32 – 14.8)	13.77 (9.20 – 19.56)	14.21 (9.99 – 20.33)	8.82 (5.34 – 12.89)	10.75 (7.10 – 15.33)
	Post-exposure	6.58 (4.07 – 10.19)	1.54 (0.42 – 3.74)	7.52 (4.75 – 11.17)	11.21 (7.52 – 15.78)	3.63 (2.11 – 5.76)	6.06 (3.83 – 8.67)
Filamentous algae cover (%)	**Pre-exposure**	24.96 (19.45 – 31.58)	27.48 (20.90 – 35.25)	17.99 (13.44 – 23.36)	16.30 (12.05 – 21.34)	21.77 (16.14 – 28.47)	17.46 (12.89 – 22.39)
	Post-exposure	24.99 (19.03 – 32.36)	6.12 (4.06 – 8.36)	23.52 (16.88 – 30.59)	23.14 (17.05 – 30.34)	28.65 (21.88 – 38.14)	30.50 (23.15 – 39.53)
Turf algae cover (%)	**Pre-exposure**	10.78 (3.34 – 23.48)	8.94 (1.62 – 24.76)	5.94 (1.36 – 14.47)	7.47 (0.60 – 32.39)	4.01 (0.68 – 10.92)	5.77 (0.07 – 47.92)
	Post-exposure	5.09 (0.82 – 12.40)	2.83 (0.03 – 28.34)	7.06 (1.03 – 17.54)	7.97 (0.80 – 23.91)	1.61 (0.24 – 6.15)	2.07 (0.00 – 36.88)

Fouling cover (%)	**Pre-exposure**	42.21 (31.53 – 53.05)	35.83 (25.99 – 45.40)	34.00 (25.84 – 44.42)	30.06 (22.47 – 38.51)	30.10 (22.75 – 39.08)	27.07 (20.25 – 35.45)
	Post-exposure	31.51 (23.51 – 40.63)	6.51 (4.44 – 8.94)	32.10 (23.79 – 41.64)	33.84 (26.28 – 45.73)	30.56 (22.00 – 39.86)	35.27 (26.77 – 46.03)
Bare tile cover (%)	**Pre-exposure**	44.04 (36.00 – 51.92)	50.48 (41.93 – 58.07)	55.83 (47.44 – 63.82)	57.06 (49.66 – 65.77)	61.52 (52.38 – 68.70)	60.39 (52.10 – 68.53)
	Post-exposure	58.72 (50.37 – 66.75)	79.85 (70.89 – 87.53)	58.63 (50.79 – 66.73)	53.69 (45.49 – 62.35)	59.99 (52.34 – 68.78)	52.24 (44.10 – 60.13)

Appendix D Supplementary Information for Chapter 4

BCA Protein Assay method adapted for coral samples

A Pierce™ BCA Protein Assay Kit was used to determine protein content within the coral tissue. 500 µL of homogenised tissue blastate was taken and mixed with 500 µL 0.5M NaOH, then incubated at 60 °C for 5 hours. In duplicate, 25 µL of incubated sample and 200 µL working reagent from the kit was then added to a well of 96-well plate, agitated horizontally, then incubated in darkness at 37 °C for 30 mins. The plates were allowed to cool in darkness at room temperature for 5 minutes, then absorbance at 562nm measured using a microplate reader. Using bovine serum albumin standards of known protein concentration, a standard curve was constructed, and the protein concentration within each of the samples calculated along it.

Supplementary Table 1: Bayesian hierarchical model parameters

Response	Coral	Model form	Distribution	Link	Priors	Iterations (warmup)	Thinning
Proportional Growth	***Pocillopora verrucosa***	prop_monthly ~ treatment*month + (treatment|replicate) shape ~ treatment*month*genotype	Gamma	log	prior(normal(0.024, 0.1), class = 'Intercept') + prior(normal(0, 0.1), class = 'b') + prior(student_t(3, 0, 0.1), class = 'sd') + prior(student_t(3, 0, 0.1), class = 'b', dpar = 'shape')	10,000 (2,500)	10

	Acropora kenti	prop_monthly ~ treatment*month + (treatment|replicate:genotype) shape ~ treatment*month	Gamma	log	prior(normal(0.020, 0.1), class = 'Intercept') + prior(normal(0, 0.1), class = 'b') + prior(student_t(3, 0, 0.1), class = 'sd') + prior(student_t(3, 0, 0.1), class = 'b', dpar = 'shape')	10,000 (2,500)	10
	Porites lutea	prop_monthly ~ treatment*month + (treatment|replicate:genotype) shape ~ treatment*month	Gamma	log	prior(normal(0.045, 0.1), class = 'Intercept') + prior(normal(0, 0.1), class = 'b') + prior(student_t(3, 0, 0.1), class = 'sd') + prior(student_t(3, 0, 0.1), class = 'b', dpar = 'shape')	10,000 (2,500)	10
	Platygyra daedalea	prop_monthly ~ treatment*month + (treatment|replicate) shape ~ treatment*month	Gamma	log	prior(normal(0.012, 0.1), class = 'Intercept') + prior(normal(0, 0.1), class = 'b') + prior(student_t(3, 0, 0.1), class = 'sd') + prior(student_t(3, 0, 0.1), class = 'b', dpar = 'shape')	10,000 (2,500)	10
Photosynthetic efficiency (Fv/Fm)	***Pocillopora verrucosa***	Y_na ~ treatment*month +	Gaussian	identity	prior(normal(0.7, 0.03), class = 'Intercept') + prior(normal(0, 0.2), class = 'b') +	5,000 (2,500)	5

		(1|tank_rep_rand:genotype) sigma ~ month			prior(student_t(3, 0, 0.03), class = 'sigma') + prior(student_t(3, 0, 0.03), class = 'sd')		
	Acropora kenti	Y_na ~ treatment*month + (1|tank_rep_rand:genotype) phi ~ treatment*month	Beta	logit	prior(normal(0.77, 0.2), class = 'Intercept') + prior(normal(0, 0.8), class = 'b') + prior(student_t(3, 0, 0.2), class = 'sd') + prior(student_t(3, 0, 0.2), class = 'b', dpar = 'phi')	7,000 (2,500)	5
	Porites lutea	Y_na ~ treatment*month + (1|tank_rep_rand) phi ~ month*treatment*genotype	Beta	logit	prior(normal(0.44, 0.2), class = 'Intercept') + prior(normal(0, 0.8), class = 'b') + prior(student_t(3, 0, 0.2), class = 'sd') + prior(student_t(3, 0, 0.2), class = 'b', dpar = 'phi')	7,000 (2,500)	10
	Platygyra daedalea	Y_na ~ treatment*month + (1|tank_rep_rand) sigma ~ treatment*month*genotype	Gaussian	identity	prior(normal(0.70, 0.03), class = 'Intercept') + prior(normal(0, 0.3), class = 'b') + prior(student_t(3, 0, 0.03), class = 'sd') + prior(student_t(3, 0, 0.03), class = 'b', dpar = 'sigma')	10,000 (4,000)	10

Protein ($\mu g\ cm^{-2}$)	***Pocillopora verrucosa***	protein_ugSA ~ treatment + (1|tank_rep_rand)	Gaussian	identity	prior(normal(315, 12), class = 'Intercept') + prior(normal(0, 300), class = 'b') + prior(student_t(3, 0, 12), class = 'sd') + prior(student_t(3, 0, 12), class = 'sigma')	5,000 (2,500)	5
	Acropora kenti	protein_ugSA ~ treatment + (1|tank_rep_rand)	Gaussian	identity	prior(normal(292, 42), class = 'Intercept') + prior(normal(0, 180), class = 'b') + prior(student_t(3, 0, 42), 'sigma') + prior(student_t(3, 0, 42), class = 'sd')	7,000 (2,500)	5
	Porites lutea	protein_ugSA ~ treatment + (1|tank_rep_rand)	Gamma	log	prior(normal(7, 0.8), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 0.6), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	8,000 (2,500)	10
	Platygyra daedalea	protein_ugSA ~ treatment + (1 | tank_rep_rand)	Gamma	log	prior(normal(484, 160), class = 'Intercept') + prior(normal(0, 700), class = 'b') + prior(student_t(3, 0, 160), 'sigma') + prior(student_t(3, 0, 160), class = 'sd')	10,000 (2,500)	10
Symbionts (cm^{-2})	***Pocillopora verrucosa***	zoox_SA ~ treatment + (1 | tank_rep_rand)	Gaussian	identity	prior(normal(1100000, 125000), class = 'Intercept') + prior(normal(0, 1000000), class = 'b') +	6,000 (3,000)	5

					prior(student_t(3, 0, 125000), 'sigma') + prior(student_t(3, 0, 125000), class = 'sd'		
	Acropora kenti	zoox_SA ~ treatment + (1|tank_rep_rand)	Gaussian	identity	prior(normal(323000, 150000), class = 'Intercept') + prior(normal(0, 700000), class = 'b') + prior(student_t(3, 0, 150000), 'sigma') + prior(student_t(3, 0, 150000), class = 'sd')	5,000 (2,500)	5
	Porites lutea	zoox_SA ~ treatment + (1|tank_rep_rand)	Gaussian	identity	prior(normal(1650000, 1400000), class = 'Intercept') + prior(normal(0, 1100000), class = 'b') + prior(student_t(3, 0, 1100000), 'sigma') + prior(student_t(3, 0, 1100000), class = 'sd')	5,000 (2,500)	5
	Platygyra daedalea	zoox_SA ~ treatment + (1|tank_rep_rand)	Gaussian	identity	prior(normal(1100000, 260000), class = 'Intercept') + prior(normal(0, 700000), class = 'b') + prior(student_t(3, 0, 260000), 'sigma')+ prior(student_t(3, 0, 260000), class = 'sd')	5,000 (2,500)	5

NH_4 (μmol L^{-1})	**na**	NH4 ~ treatment + (1|tank_rep_rand:sample)	Gamma	log	prior(normal(-2.1, 0.5), class = 'Intercept') + prior(normal(0, 1.5), class = 'b') + prior(student_t(3, 0, 0.5), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape'	10,000 (2,500)	10
NO_2 (μmol L^{-1})	**na**	NO2 ~ treatment + (1 | tank_rep_rand:sample)	Gamma	log	prior(normal(-3.22, 0.9), class = 'Intercept') + prior(normal(0, 0.8), class = 'b') + prior(student_t(3, 0, 0.5), class = 'sd') + prior(gamma(2, 1), class = 'shape')	5,000 (2,500)	5
NO_3 (μmol L^{-1})	**na**	NO3 ~ treatment + (1|tank_rep_rand:sample)	Gamma	log	prior(normal(-0.332, 0.6), class = 'Intercept') + prior(student_t(3, 0, 0.9), class = 'b') + prior(student_t(3, 0, 0.4), class = 'sd') + prior(gamma(2, 1), class = 'shape')	10,000 (2,500)	5
PO_4 (μmol L^{-1})	**na**	PO4 ~ treatment + (1|tank_rep_rand:sample)	Gaussian	identity	prior(normal(0.17, 0.05), class = 'Intercept') + prior(normal(0, 0.07), class = 'b') + prior(student_t(3, 0, 0.05), class = 'sd') + prior(student_t(3, 0, 0.05), class = 'sigma')	10,000 (2,500)	10

DOC **(mg L^{-1})**	**na**	DOC ~ treatment + (1|tank_rep_rand:sample) sigma ~ treatment	Shifted Log-normal	identity	prior(normal(-1.8, 0.6), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 0.1), class = 'sd') + prior(student_t(3, 0, 0.1), class = 'b', dpar = 'sigma') + prior(uniform(0, 0.91), class = 'ndt')	10,000 (2,500)	10
PC **(μg L^{-1})**	**na**	PC ~ treatment + (1|tank_rep_rand:sample) shape ~ treatment	Gamma	log	prior(normal(2.56, 0.8), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 0.8), class = 'sd') + prior(normal(0, 1), class = 'b', dpar = 'shape')	10,000 (2,500)	10
PN **(μg L^{-1})**	**na**	PN ~ treatment + (1|tank_rep_rand:sample)	Gamma	log	prior(normal(1.06, 0.6), class = 'Intercept') + prior(normal(0, 0.6), class = 'b') + prior(student_t(3, 0, 0.5), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	10,000 (2,500)	10
N:P	**na**	NP ~ treatment + (1|tank_rep_rand:sample)	Gamma	log	prior(normal(1.87, 0.5), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 0.5), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	10,000 (2,500)	10

Appendix E Supplementary Information for Chapter 5

Pilot coral Y_2O_3 detection results

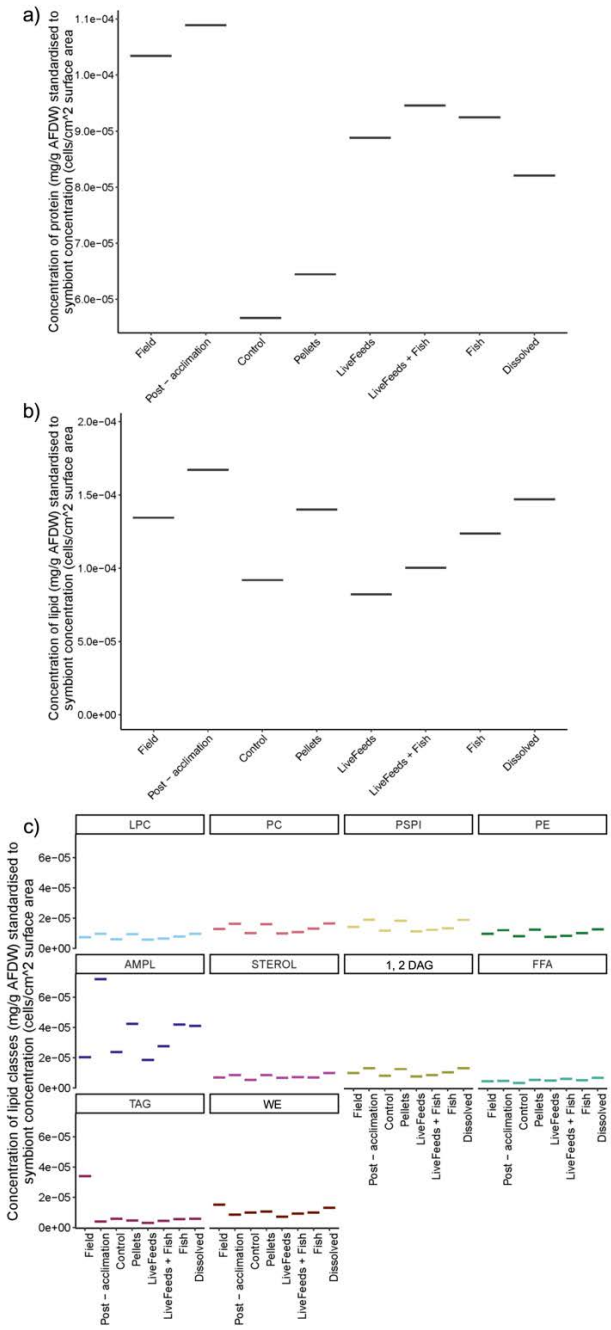
To test whether indigestible marker Yttrium Oxide (Y_2O_3) could be detected in coral fragments after heterotrophic feeding, two fragments of *Stylophora pistillata* (Family Pocilloporidae) were sourced from general coral stock held in the National SeaSimulator Facility, and were held in a 50 L flow-through acrylic tank supplied with filtered, 27.5 °C sea water at 0.8 L min^{-1}. Both fragments were each fed daily at 3pm with ~0.2g of Aquaforest® AF LPS Food top coated with indigestible marker Yttrium Oxide (Y_2O_3) at 0.1% w/w, by gently sprinkling pellets directly over the top of each fragment then siphoning away excess feed after ~1 hour. Water flow within the tank was minimal, as it was only supplied by the incoming water, thus the *S. pistillata* could not access the other's pellets supply. The fragments were held in the pilot for three days in total; both fragments were fed on Day 1, then only one fed for second time on Day 2, then on Day 3 finally both colonies were sampled and processed for Y_2O_3 content via the methods presented in the main manuscript. The fragment that was fed on both Day 1 and 2 recorded a Y_2O_3 concentration of 0.420 ± 0.000 ppm, whilst the colony that was starved for a day prior to sampling recorded a Y_2O_3 concentration of 0.230 ± 0.010 ppm (limit of detection 0.1 ppm), indicating that Y_2O_3 could be detected in colonies after heterotrophic feeding and still be detected after a period of starvation.

Supplementary Table 1: Bayesian model parameters						
Response variable	**Model form**	**Distribution**	**Link**	**Priors**	**Iterations (warmup)**	**Thinning**
Ash	ash_DW_mgg ~ treatment + (1|tank_rep_rand), sigma ~ genotype	Gaussian	identity	prior(normal(936, 18), class = 'Intercept') + prior(normal(0, 20), class = 'b') + prior(student_t(3, 0, 16), class = 'b', dpar = 'sigma') + prior(student_t(3, 0, 16), class = 'sd')	7000 (2500)	10
Protein	protein_AFDW_mgg ~ treatment + (1|tank_rep_rand:genotype)	Gaussian	log	prior(normal(4, 0.5), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 0.5), 'sigma')+ prior(student_t(3, 0, 0.5), class = 'sd')	7000 (2500)	7
Total Lipid	lipid_AFDW_mgg ~ treatment + (1|tank_rep_rand:genotype)	Gamma	log	prior(normal(4.5, 0.3), class = 'Intercept') + prior(normal(0, 1.5), class = 'b') + prior(student_t(3, 0, 0.3), class = 'sd') + prior(gamma(2, 1), class = 'shape')	7500 (2500)	10
SE	SE ~ treatment + (1|tank_rep_rand:genotype)	Gaussian	identity	prior(normal(10.9, 3), class = 'Intercept') + prior(normal(0, 6), class = 'b') + prior(student_t(3, 0, 3), class = 'sd') + prior(student_t(3, 0, 3), class = 'sigma')	5000 (2500)	10
TAG	TAG ~ treatment + (1|tank_rep_rand:genotype)	Gamma	log	prior(normal(1.8, 0.4), class = 'Intercept') + prior(normal(0, 1.5), class = 'b') + prior(student_t(3, 0, 0.4), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	5000 (2500)	10

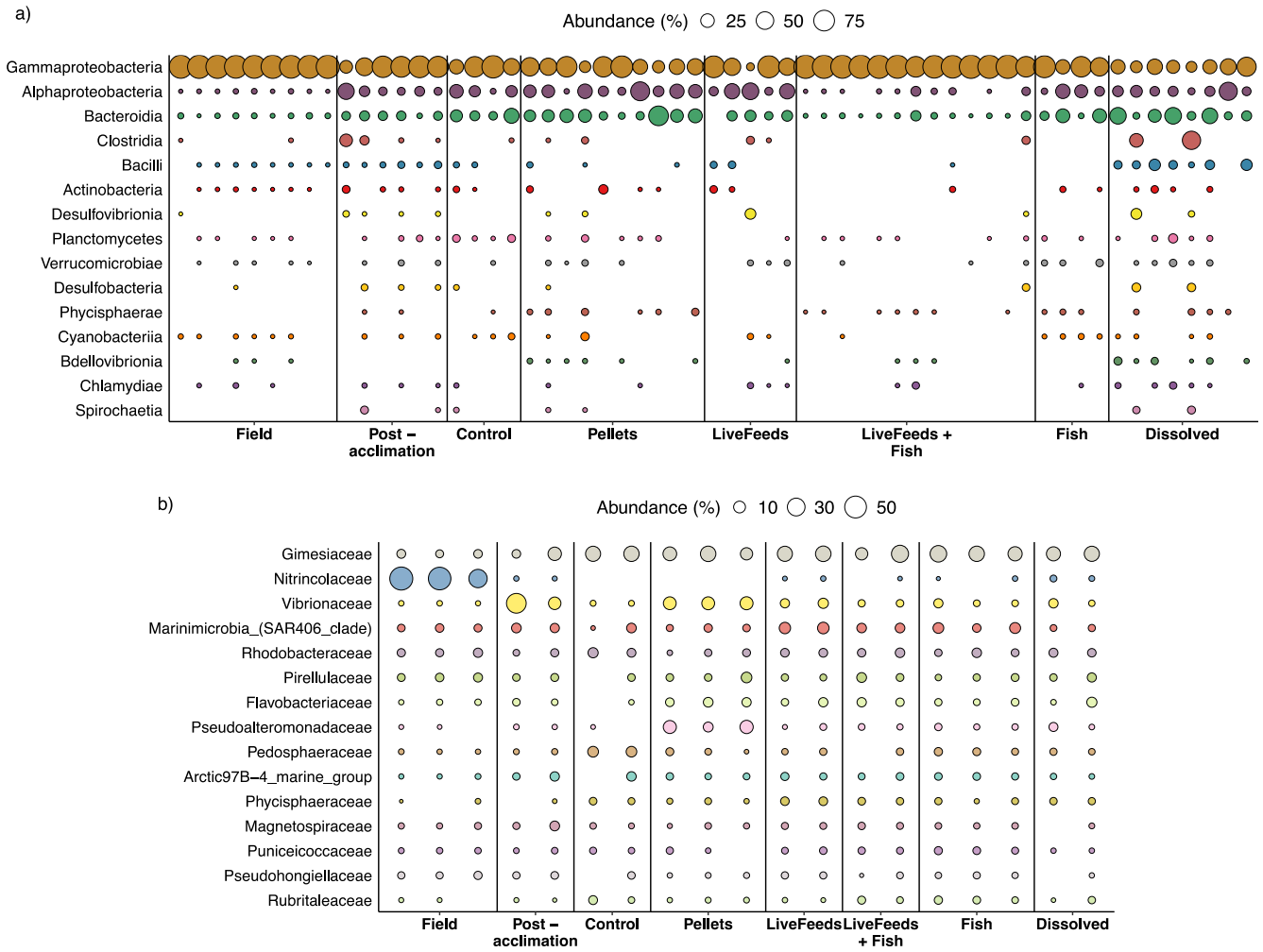
1,2-DAG	DAG ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(8.9, 3), class = 'Intercept') + prior(normal(0, 5), class = 'b') + prior(student_t(3, 0, 1.1), class = 'sd') + prior(student_t(3, 0, 1.1), class = 'b', dpar = 'sigma')	10000 (2500)	15
FFA	FFA ~ treatment + (1|tank_rep_rand:genotype)	Gaussian	identity	prior(normal(3.5, 1.5), class = 'Intercept') + prior(normal(0, 4), class = 'b') + prior(student_t(3, 0, 1.2), class = 'sd') + prior(student_t(3, 0, 1.2), class = 'sigma')	7000 (2500)	5
STEROL	STEROL ~ treatment + (1|tank_rep_rand:genotype)	Gaussian	identity	prior(normal(6.0, 2), class = 'Intercept') + prior(normal(0, 3.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(student_t(3, 0, 1), class = 'sigma')	10000 (2500)	10
AMPL	AMPL ~ treatment + (1|tank_rep_rand), shape ~ treatment*genotype	Gamma	log	prior(normal(3.1, 0.5), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 0.3), class = 'sd') + prior(normal(0, 1), class = 'b', dpar = 'shape')	5000 (2500)	5
PE	PE ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(8.8, 3), class = 'Intercept') + prior(normal(0, 4), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(normal(0, 1), class = 'b', dpar = 'sigma')	7000 (2500)	5
PSPI	PSPI ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(13.0, 4), class = 'Intercept') + prior(normal(0, 9), class = 'b') + prior(student_t(3, 0, 1.5), class = 'sd') + prior(normal(0, 1.5), class = 'b', dpar = 'sigma')	8000 (2500)	10

PC	PC ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(11.3, 4), class = 'Intercept') + prior(normal(0, 6), class = 'b') + prior(student_t(3, 0, 1.5), class = 'sd') + prior(normal(0, 1.5), class = 'b', dpar = 'sigma')	8000 (2500)	10
LPC	LPC ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(6.7, 3), class = 'Intercept') + prior(normal(0, 4), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(normal(0, 1), class = 'b', dpar = 'sigma')	8000 (2500)	10
Storage	Storage ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(30, 6), class = 'Intercept') + prior(normal(0, 20), class = 'b') + prior(student_t(3, 0, 6), class = 'sd') + prior(normal(0, 6), class = 'b', dpar = 'sigma')	9000 (2500)	10
Structural	Structural ~ treatment + (1|tank_rep_rand:genotype), sigma ~ treatment	Gaussian	identity	prior(normal(70, 6), class = 'Intercept') + prior(normal(0, 20), class = 'b') + prior(student_t(3, 0, 6), class = 'sd') + prior(normal(0, 6), class = 'b', dpar = 'sigma')	9000 (2500)	10
TAG as proportion of total lipid	TL_TAG ~ treatment + (1|tank_rep_rand:genotype	Gamma	log	prior(normal(1.6, 0.9), class = 'Intercept') + prior(normal(0, 1.3), class = 'b') + prior(student_t(3, 0, 0.5), class = 'sd') + prior(gamma(0.01, 0.01), class = 'shape')	7500 (2500)	10
PUFA	PUFA ~ treatment + (1|tank_rand:genotype)	Gaussian	identity	prior(normal(32.7, 5), class = 'Intercept') + prior(normal(0, 20), class = 'b') + prior(student_t(3, 0, 4.5), class = 'sd') + prior(student_t(3, 0, 4.5), class = 'sigma')	7500 (2500)	10

MUFA	MUFA ~ treatment + (1|tank_rand:genotype), shape ~ treatment	Gamma	log	prior(normal(1.1, 0.2), class = 'Intercept') + prior(normal(0, 1), class = 'b') + prior(student_t(3, 0, 0.2), class = 'sd') + prior(normal(0, 1), class = 'b', dpar = 'shape')	7500 (2500)	10
SFA	SFA ~ treatment + (1|tank_rand:genotype	Gaussian	identity	prior(normal(55.3, 6), class = 'Intercept') + prior(normal(0, 20), class = 'b') + prior(student_t(3, 0, 6), class = 'sd') + prior(student_t(3, 0, 6), class = 'sigma')	7500 (2500)	10
PUFA n-3:n-6	PUFA_n3_n6 ~ treatment + (1|tank_rand:genotype), shape ~ treatment	Gamma	log	prior(normal(0.6, 1), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(normal(0, 0.6), class = 'b', dpar = 'shape')	7500 (2500)	10
LC PUFA n-3:n-6	LC_n3n6 ~ treatment + (1|tank_rand:genotype), shape ~ treatment	Gamma	log	prior(normal(0.7, 1), class = 'Intercept') + prior(normal(0, 0.5), class = 'b') + prior(student_t(3, 0, 1), class = 'sd') + prior(normal(0, 0.5), class = 'b', dpar = 'shape')	7500 (2500)	10



Supplementary Figure 1: a) Plot of the concentration of mean protein content (mg g^{-1} AFDW) for each treatment standardised to the mean symbiont counts (cells per cm^2 surface area) for each treatment from chapter 4. b) Plot of the concentration of the mean total lipid content (mg g^{-1} AFDW) for each treatment standardised to the mean symbiont counts (cells per cm^2 surface area) for each treatment from chapter 4. c) Plot of the concentration of the different lipid classes (calculated as the mean total lipid concentration (mg g^{-1} AFDW) for each treatment x the relative concentration of each lipid class) standardised to the mean symbiont counts (cells per cm^2 surface area) for each treatment from chapter 4.


a)
Abundance (%) 25 50 75
Gammaproteobacteria
Alphaproteobacteria
Bacteroidia
Clostridia
Bacilli
Actinobacteria
Desulfovibrionia
Planctomycetes
Verrucomicrobiae
Desulfobacteria
Phycisphaerae
Cyanobacteriia
Bdellovibrionia
Chlamydiae
Spirochaetia
Field
Post – acclimation
Control
Pellets
LiveFeeds
LiveFeeds + Fish
Fish
Dissolved
b)
Abundance (%) 10 30 50
Gimesiaceae
Nitrincolaceae
Vibrionaceae
Marinimicrobia_(SAR406_clade)
Rhodobacteraceae
Pirellulaceae
Flavobacteriaceae
Pseudoalteromonadaceae
Pedosphaeraceae
Arctic97B–4_marine_group
Phycisphaeraceae
Magnetospiraceae
Puniceicoccaceae
Pseudohongiellaceae
Rubritaleaceae
Field
Post – acclimation
Control
Pellets
LiveFeeds
LiveFeeds + Fish
Fish
Dissolved

Supplementary Figure 2: a) Bubble plot of relative abundance of top fifteen bacterial classes identified from ASVs in coral samples from the various treatments. b) Bubble plot of relative abundance of top fifteen bacterial families identified from ASVs in water samples from the various treatments. 'Field' sample were taken from temporary holding tanks corals were held in after initial microbial and biochemical sampling.

Supplementary Table 2: Lipid class of *Pocillopora verrucosa* fragments (mg g^{-1} lipid: modelled median and 95% credibility interval)

Treatment	SE	TAG	1,2-DAG	FFA	STEROL	AMPL	PE	PSPI	PC	LPC	Storage	Structural	Storage: Structural
Field n= 10	111.8 96.4 – 128.6	252.0 184.4 – 320.2	73.5 62.4 – 82.4	31.8 22.3 – 41.3	51.9 40.0 – 62.8	145.4 92.2 – 226.1	72.5 63.6 – 82.0	106.4 91.3 – 121.7	95.0 81.8 – 107.2	54.9 47.8 – 62.3	394.3 330.3 – 446.0	606.2 546.5 – 662.9	0.679 0.544 – 0.865
Post-acclimation n= 10	52.6 37.1 – 67.8	23.8 17.3 – 30.4	78.0 61.4 – 94.5	27.6 18.2 – 37.2	50.7 39.6 – 62.1	416.1 233.6 – 643.5	73.1 52.4 – 93.2	113.3 86.7 – 138.5	96.8 75.7 – 117.4	57.7 45.0 – 70.0	102.1 77.9 – 129.6	896.8 870.9 – 923.0	0.117 0.090 – 0.146
Control n= 20	105.9 94.6 – 116.0	63.3 52.5 – 75.7	86.6 79.5 – 94.9	36.3 30.4 – 43.3	57.6 49.6 – 65.2	251.6 195.5 – 320.7	86.7 79.3 – 94.4	126.4 115.2 – 138.0	109.9 101.4 – 119.1	65.0 59.3 – 70.3	206.9 184.8 – 230.0	792.9 770.4 – 818.6	0.265 0.225 – 0.307
Pellets n= 20	76.8 66.9 – 88.1	32.9 27.6 – 39.6	89.5 79.6 – 100.4	38.5 31.8 – 45.3	60.9 52.4 – 68.1	279.2 196.0 – 370.5	87.9 76.9 – 98.3	130.0 113.9 – 146.0	114.7 101.8 – 129.5	67.0 59.1 – 75.2	147.6 129.9 – 167.0	852.3 832.8 – 870.1	0.175 0.151 – 0.205
LiveFeeds n= 20	87.2 75.9 – 97.4	36.6 30.3 – 44.5	93.2 87.1 – 99.3	58.6 51.9 – 65.2	81.9 74.6 – 89.8	222.9 176.4 – 280.0	91.8 85.5 – 97.6	136.7 127.9 – 145.3	119.1 111.8 – 126.4	69.3 64.4 – 73.6	182.7 161.1 – 202.9	817.2 797.2 – 837.5	0.227 0.200 – 0.264
LiveFeeds + Fish n= 20	91.0 80.6 – 102.3	44.1 36.5 – 53.5	84.6 78.1 – 90.6	58.7 52.2 – 65.5	70.4 62.4 – 78.8	270.9 194.5 – 354.3	82.8 76.5 – 89.3	123.0 113.4 – 132.5	107.6 99.3 – 116.4	63.9 59.1 – 68.6	194.7 168.2 – 221.4	805.2 777.5 – 831.7	0.248 0.208 – 0.288
Fish n= 20	80.1 69.5 – 91.6	43.3 36.0 – 51.9	82.8 70.7 – 95.0	40.1 33.6 – 47.1	56.1 47.8 – 63.6	298.1 216.2 – 397.3	81.9 70.0 – 94.2	106.5 85.3 – 131.9	105.4 90.6 – 120.7	62.4 53.9 – 71.4	163.8 140.3 – 187.2	835.2 810.4 – 859.4	0.202 0.171 – 0.238
Dissolved n= 20	89.4 78.9 – 99.8	38.2 31.2 – 45.6	88.7 77.9 – 100.6	45.2 38.5 – 51.9	66.1 57.8 – 73.7	253.8 181.7 – 339.1	86.5 76.1 – 97.3	128.5 112.4 – 144.1	112.3 100.0 – 127.1	65.7 58.2 – 73.8	173.9 146.6 – 200.4	826.3 800.1 – 853.3	0.217 0.176 – 0.259

Supplementary Table 3: Proximate composition of *Pocillopora verrucosa* fragments from different sample points and treatments (modelled median and 95% credibility interval)			
Treatment	**Ash (mg g^{-1} dry weight)**	**Protein (mg g^{-1} ash-free dry weight)**	**Total Lipids (mg g^{-1} ash-free dry weight)**
Field n= 10	940.3 928.3 – 951.3	75.2 53.6 – 97.6	100.7 73.3 – 128.8
Post-acclimation n= 10	949.1 938.8 – 959.5	89.7 67.7 – 114.9	140.0 101.7 – 184.8
Control n= 20	938.9 932.4 – 945.4	59.2 47.5 – 73.2	95.4 78.2 – 113.7
Pellets n= 20	949.6 943.1 – 956.2	71.2 55.9 – 86.5	162.1 132.7 – 194.4
Live Feeds n= 20	943.2 936.2 – 949.9	128.9 110.5 – 148.2	120.9 100.2 – 147.5
LiveFeeds + Fish n= 20	944.0 937.3 – 950.3	108.5 92.6 – 125.7	114.8 96.5 – 139.2
Fish n= 20	946.8 940.0 – 954.0	108.2 91.1 – 125.7	146.8 122.2 – 176.8
Dissolved n= 20	947.8 941.5 – 945.4	99.5 83.8 – 116.3	177.3 144.3 – 213.1

Supplementary Table 4: Water quality C:N:P ratios calculated from data in Appendix D Table 2.							
Treatment	**DOC**		**N (µmol/L)**	**PO4 (µmol/L)**	**Ratio**		
	mg/L	µmol (mg/L * 1000 / 12.011)	(NH4 + NO2 + NO3)		C	N	P
Control	1.130	94.080	1.079	0.169	556.7	6.4	1.0
Pellets	1.110	92.415	1.383	0.171	540.4	8.1	1.0
LiveFeeds	1.130	94.080	1.212	0.178	528.5	6.8	1.0
LiveFeeds + Fish	1.070	89.085	1.928	0.184	484.2	10.5	1.0
Fish	1.130	94.080	1.626	0.179	525.6	9.1	1.0
Dissolved	1.160	96.578	1.128	0.141	685.0	8.0	1.0