Size matters: Microherbivores make a big impact in coral aquaculture

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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 we 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*. Our results demonstrate microherbivores have potential for application in aquaculture to promote production, while also reducing labour costs.

1. 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 and 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 <5-10 mm 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

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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 and McCook, 2003; McCook et al., 2001; Nugues and 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 and 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 and 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 and 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 and 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., 2013; Villanueva et al., 2013), juvenile sea urchin (Barrows et al., 2023; Craggs et al., 2019; Serafy et al., 2013; Toh et al., 2013) 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., 2013; 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). In addition, due to the current high cost of coral aquaculture, it would be beneficial if herbivorous co-culture organisms were easy to obtain and could be sold as a secondary product (Craggs et al., 2019; Toh et al., 2013). Many species of small herbivorous gastropods are commonly found in marine aquaculture systems and are capable of reproducing with minimal husbandry (Watson et al., 2018). Hermit crabs can remove significant amounts of algal biomass and are relatively common in the marine ornamental trade (Altman-Kurosaki et al., 2018; Francis et al., 2019). Juveniles of edible sea urchins have also been suggested as potential grazers that could provide a secondary source of income (Craggs et al., 2019). In this study, we 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. Material and methods

2.1. 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 Supplementary Materials. 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.

2.2. 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 under AIMS general collection permit (G21/38062.1). 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 Supplementary Table A 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. 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 Supplementary Table 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 to 1600 with 1-h 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 (Supplementary Fig. 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 3:00 pm. 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.

2.4. 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 and 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 Supplementary Fig. 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.

2.5. 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 and Legendre, 2009)), and pairwise comparisons made with permutational multivariate analysis of variance (PERMANOVA) using distance matrices (adonis) with a Bonferroni correction.

3. Results

3.1. 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 ($p > 0.05$), 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 ($p < 0.05$), though some of the replicates for *C. strigata* did have similar densities to these hermit tanks ($p = 0.0562$). 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 ($p < 0.001$), averaging 28.0 ± 1.5 mins for total cleaning required each week (Fig. 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 ($p < 0.05$). 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 ($p > 0.05$). 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.

3.2. Coral recruit survival and growth

Acropora millepora averaged $37.2 \pm 2.34\%$, $36.5 \pm 5.78\%$ and $30.0 \pm 9.62\%$ (mean $\pm$ SEM) survival at 112 days under *Calthalotia*, Aquarist and *Clibanarius* treatments respectively, which was significantly higher ($p < 0.05$) when compared to all other grazing treatments (Fig. 2A). *Echinometra* produced significantly lower survival ($p < 0.05$) for *A. millepora* than all other treatments, while there was no evidence for differences in survival ($p > 0.05$) among Control, *Turbo* and *Tripneustes*

treatments. *A. millepora* also experienced its highest growth in the Aquarist tanks compared to all other treatments (Fig. 3A), with a final average size of 1.5 ± 0.1 mm^2 (mean $\pm$ SEM, See Table 1) ($p < 0.01$). *Clibanarius* depressed growth of *A. millepora* ($p < 0.01$) compared to *Calthalotia*, *Tripneustes* and *Turbo* treatments, but there was no evidence for differences in growth between the Control and any other grazing treatment ($p > 0.05$). 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 ($p > 0.05$).

After 112 days, *Acropora kenti* experienced highest survival in the Aquarist treatment ($36.2 \pm 3.48\%$), which was significantly higher than in all other treatments ($p < 0.05$) except *Calthalotia* ($23.0 \pm 1.78\%$) and Control ($26.0 \pm 4.64\%$) ($p > 0.05$). *Calthalotia* and Control, however, only represented a moderate increase in survival compared to the *Echinometra* and *Clibanarius* treatments ($p < 0.05$) (Fig. 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* ($p = 0.6289$) or *Tripneustes* ($p = 0.3491$) 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 (Fig. 3B). Growth in these treatments was higher than in the Control, *Echinometra* and *Turbo* treatments ($p < 0.05$), though growth in the *Tripneustes* treatment was not significantly different from the *Turbo* tanks ($p = 0.0506$). 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) ($p = 0.5659$).

The highest survival of *Goniastrea retiformis* recruits was observed in the *Tripneustes* treatment ($48.3 \pm 15.3\%$ at 112 days), which was greater ($p < 0.001$) than survival in all other treatments except the Aquarist ($43.0 \pm 12.9\%$; Fig. 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), significantly higher ($p < 0.0001$) than all other treatments, with the
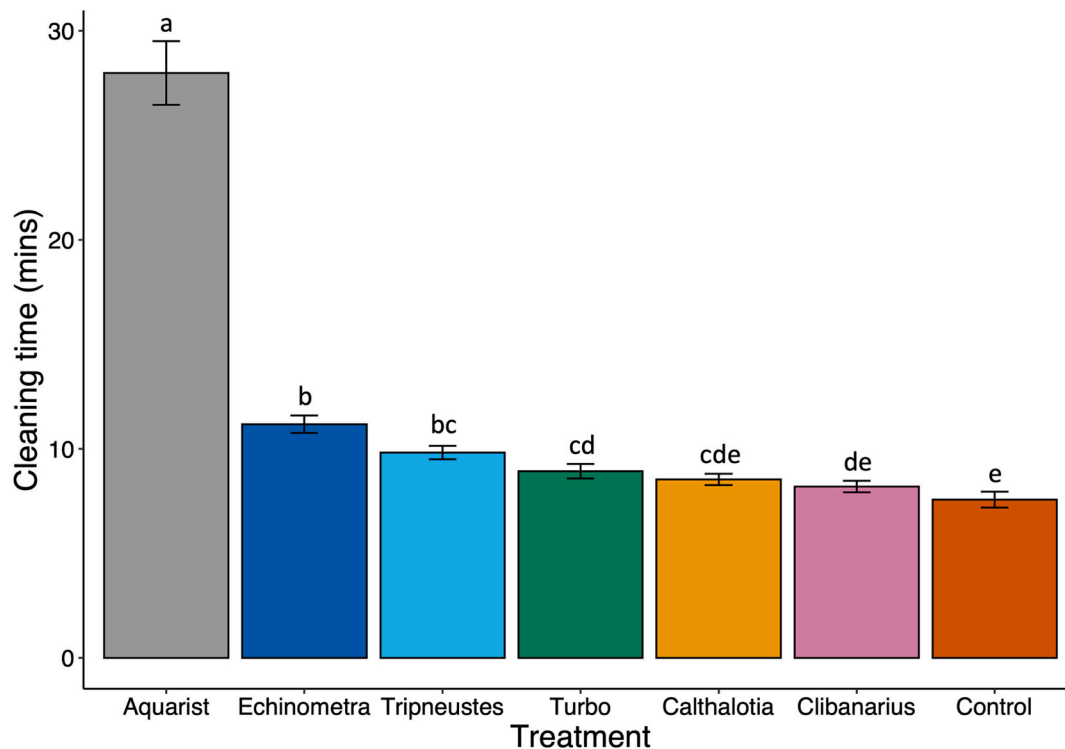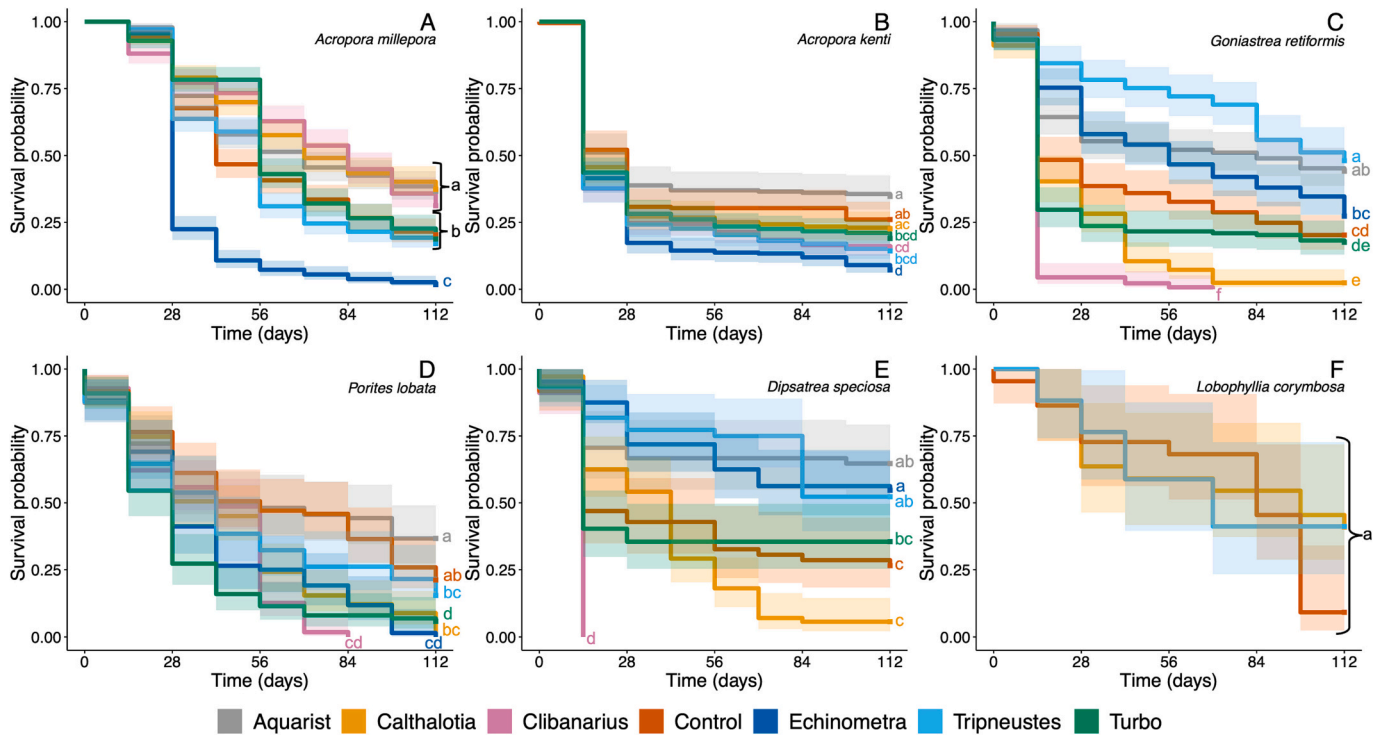

Fig. 1. Time (minutes) required to manually clean replicate tanks of fouling organisms in different grazing treatments. Letters represent significance levels based on results from pairwise comparison of estimated marginal means of linear mixed effects model. Data are means $\pm$ SEM, $n = 4$.

Fig. 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.
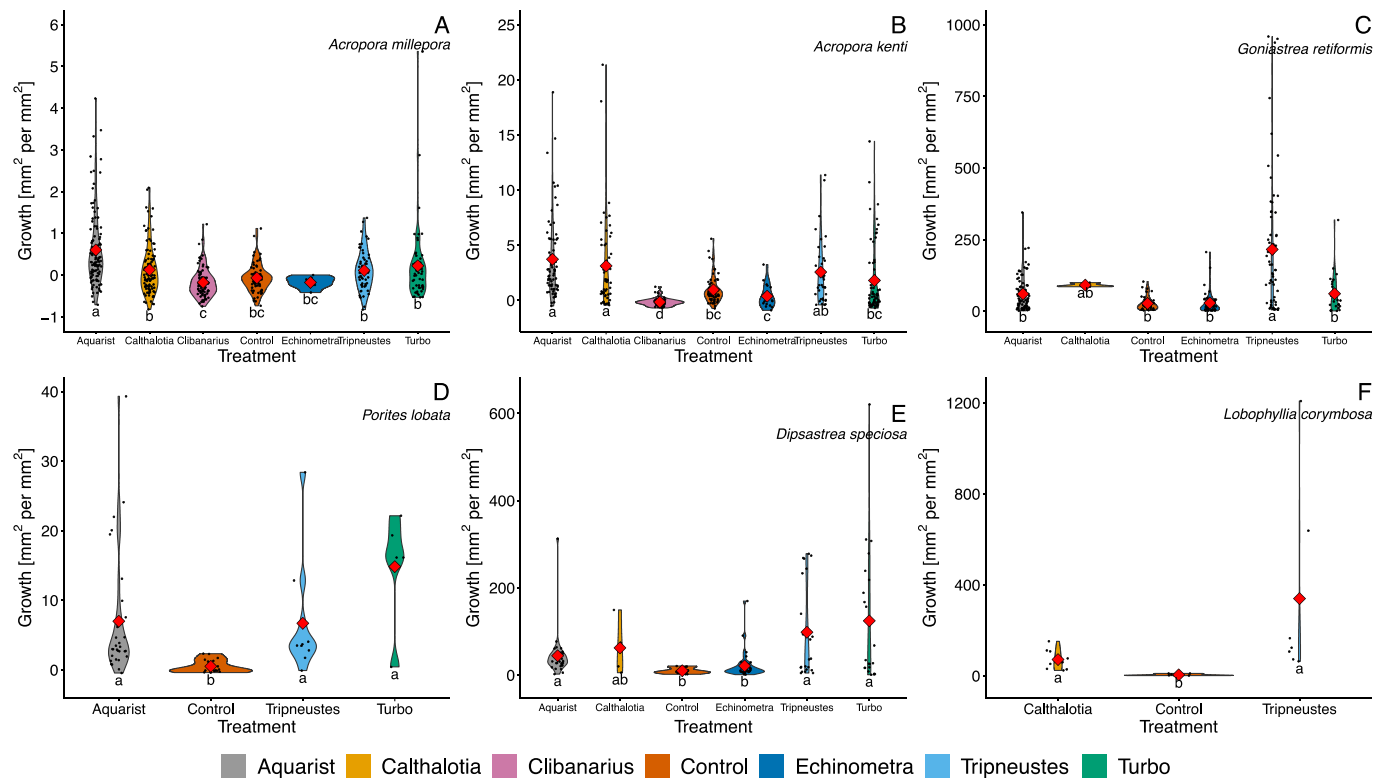
Fig. 3. Violin plots of relative growth of coral recruits under different grazing treatments from day 0 to 112. A) *Acropora millepora*, B) *Acropora kenti*, C) *Goniastrea retiformis*, D) *Porites lobata*, E) *Dipsastraea speciosa* and F) *Lobophyllia corymbosa*. Growth is measured as relative change 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Basal surface area of coral recruits (mean ± sem) 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 (mm²) (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 (mm²)						
Aquarist[a]	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[b]	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[c]	0.781 ± 0.030 *n* = 85	0.802 ± 0.057 *n* = 49	na	na	na	na
Control[d]	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[e]	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[f]	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[g]	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

na = no data available due to high mortality in these treatments.

[a] Aquarist = manual cleaning by an aquarist.
[b] Calthalotia = Calthalotia strigata gastropods.
[c] Clibanarius = Clibanarius cf. taeniatus hermit crabs.
[d] Control = no cleaning of coral plugs.
[e] Echinometra = Echinometra mathaei sea urchins
[f] Tripneustes = Tripneustes gratilla sea urchins.
[g] Turbo = Turbo haynesi gastropods.

exception of recruits subjected to *Calthalotia* grazing (24.3 ± 2.4 mm², *p* = 0.9994). However, growth in the *Calthalotia* treatment was not significantly different to growth in the other treatments (*p* > 0.05) (Fig. 3C). Productivity was also highest in *Tripneustes* (1868.1 ± 1036.4 mm² per 100 recruits), though there was no evidence this was significantly higher than in the other treatments with surviving recruits (*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 (*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 (*p* = 0.2669) (Fig. 2D). *Calthalotia* and *Tripneustes* treatments had similar survival curves to the Control (p > 0.05), while *Clibanarius*, *Echinometra* and *Turbo* all had significantly lower survival than the Control treatment (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 (p < 0.05), though there were no differences among the grazed treatments themselves (p > 0.05) (Fig. 3D), with final respective basal surface areas of 1.1 ± 0.2 mm², 1.1 ± 0.3 mm² and 2.8 ± 0.67 mm². Productivity was also higher in Aquarist (46.4 ± 19.2 mm² per 100 recruits), *Turbo* (45.8 ± 0 mm² per 100 recruits) and *Tripnesutes* (34.3 ± 13.2 mm² per 100 recruits) than Control (4.4 ± 2.4 mm² per 100 recruits), though there was no evidence for differences between the treatments (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%, *p* = 0.9943), *Tripneustes* (54.5 ± 18.6%, *p* = 0.9932) or *Turbo* (41.9 ± 21.2%, *p* = 0.0570) (Fig. 2E). All these treatments did, however, display significantly higher survival compared to the Control (36.9 ± 22.1%) (p < 0.05). No *D. speciosa* recruits survived to day 112 under the *Clibanarius* treatment. *D. speciosa* growth was significantly higher (p < 0.05) in the Aquarist (final mean size 11.4 ± 1.0 mm²), *Tripneustes* (33.1 ± 7.5 mm²) and *Turbo* (36.3 ± 9.2 mm²) treatments compared to *Echinometra* and Control. However, growth was not statistically different among Aquarist, *Calthalotia*, *Tripneustes* or *Turbo* treatments (p > 0.05)

(Fig. 3E). Similarly, though there was no evidence for statistical differences (p > 0.05), productivity was highest in *Tripneustes* (3011.8 ± 1270.8 mm² per 100 recruits) and *Turbo* (2353.4 ± 1938.9 mm² 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 (p > 0.05)(Fig. 2F). *L. corymbosa* growth in *Tripneustes* and *Calthalotia* treatments was significantly greater compared to the Control treatment (p < 0.05), though not statistically significant between the individual grazer treatments (p > 0.05), with corals in *Tripneustes* and *Calthalotia* treatments growing to 83.2 ± 11.9 mm² and 50.6 ± 9.0 mm² respectively (Fig. 3F). *Tripneustes* productivity (4518.8 ± 815.5 mm² per 100 recruits) was also significantly higher than the Control (*p* = 0.0486), though not than *Calthalotia* (2891.4 ± 943.2 mm² per 100 recruits).

3.3. 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. 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*, *Audouinella*), 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
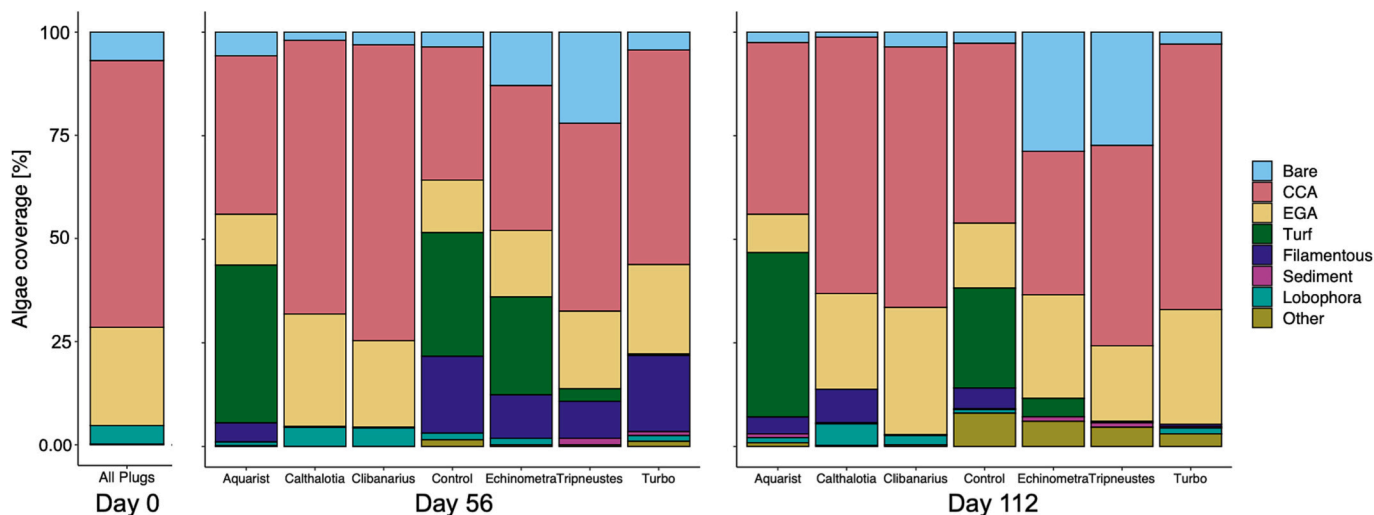
Fig. 4. Stacked barplots summarising coverage of algae assemblages on blank tiles housed in the seven grazing treatments at day 0, day 56 and day 112. Day 0 summarises all plugs, as there were no differences detected between the different treatments at this timepoint. For day 112, points from *Calthalotia* 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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. 3). 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 and 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).

4. Discussion

4.1. 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 the 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.

4.2. 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 and 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).

4.3. 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 ~40m^2) compared to natural grazing conditions on the GBR (e.g. 5.2m^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 and 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.

4.4. 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 min of cleaning per week, compared to 8.5 min *C. strigata*'s for tanks. Based on minimum hourly wage of $14.59 USD,[1] aquarist tanks require an additional weekly investment of $4.74 USD per 50 L tank to achieve similar results to *C. strigata* grazing. This equates to an additional $247 USD per tank per year – a significant cost if coral aquaculture is to expand to large-scale production. 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 and García, 2004; Neil et al., 2021) or have little additional benefits to single species (Atalah et al., 2016), sequential application of different types of grazers may be a better solution. In this experiment, we 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 our results indicate grazer applications must be tailored to different species. Results from the present study suggest co-culture benefits of 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

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[1] 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/

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 we 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.

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, our results indicate co-culture is a scalable, cost-effective method to improve survival rates of aquacultured corals in their early life stages.

CRediT authorship contribution statement

Rachel C. Neil: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Jonathan A. Barton:** Investigation, Writing – review & editing. **Winona Dougan:** Investigation, Writing – review & editing. **Symon Dworjanyn:** Resources, Writing – review & editing. **Andrew Heyward:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing. **Benjamin Mos:** Resources, Writing – review & editing. **David G. Bourne:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Craig Humphrey:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available via the AIMS Data Repository at https://doi.org/10.25845/RPRZ-BT30

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740402.

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