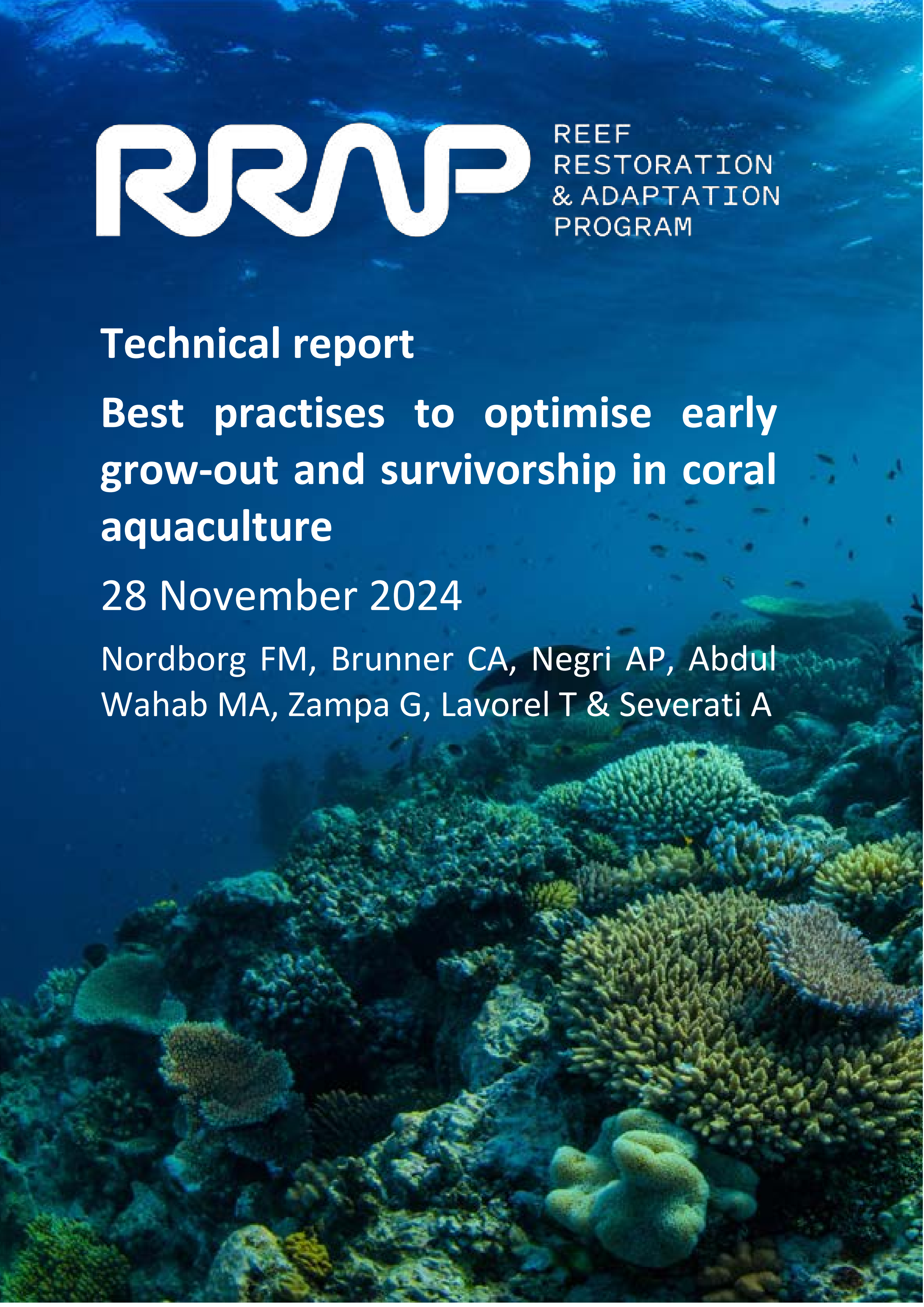

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Technical report

Best practises to optimise early grow-out and survivorship in coral aquaculture

28 November 2024

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Technical report - Best practises to optimise early grow-out and survivorship in coral aquaculture

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[*Cover Page: Coral reef, Credit: Gary Cranitch, Queensland Museum*]

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Location	**Traditional Owner Group**
Palm islands	Manbarra
Davies Reef	Bindal
AIMS HQ Cape Cleveland	Bindal
AIMS vessel facility Townsville	Wulgurukaba

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EXECUTIVE SUMMARY

1 Executive Summary

To achieve meaningful ecological outcomes in coral reef restoration, the scale of restoration efforts is crucial, with expanding spatial coverage identified as a key priority, regardless of the methods employed. (Lamont et al., 2022; Ridlon et al., 2023; Vardi et al., 2021). Despite the apparent benefits of sexual propagation of corals for achieving reef restoration at larger scales, its application has mainly been in research settings (Boström-Einarsson et al., 2020). However, cost-benefit-analyses typically favour sexually produced corals, especially when considering long-term survival and growth (Baria-Rodriguez et al., 2019; Humanes et al., 2021).

Achieving upscaled coral production in *ex situ* coral aquaculture facilities that matches the ambitious, but necessary, reef restoration efforts being called for in Australia and across tropical regions worldwide requires improved cost-efficiency while maintaining, or improving, yields (Banaszak et al., 2023; Gibbs, 2021). Three key target areas for achieving improved cost-efficiency include reducing the spatial footprint of aquaculture systems, improving water efficiency and increasing yield of usable seeding units (Banaszak et al., 2023; Randall et al., 2020; Ridlon et al., 2023). Here we addressed aspects of these target areas by investigating:

(i) the impacts of substrate angle on recruit survival during *ex situ* rearing in the first 3mo post-settlement (Chapter 3)
(ii) potential differences in recruit survival when recruits are reared in semi-recirculating aquaculture systems compared to flow-through (Chapter 4)
(iii) optimal water flow rates for rearing of recently settled recruits, and if an optimal water flow rate for recruit rearing can be determined using acute assays (Chapter 5)

The experimental investigations performed here show that recently settled recruits (0-3 months) of *Acropora* spp. (i) have a higher survival when reared on vertical surfaces compared to lower substrate angles assessed (60° or 0°); (ii) can be reared in semi-recirculating aquaculture systems without any negative impact on recruit survival or the yield of usable seeding units for deployment; (iii) exhibit higher growth rates when reared at higher water flow rates for the velocities assessed (~5-20 cm s^{-1}), but that optimal water flow rates cannot necessarily be determined using acute exposure assays.

These results, combined with information from the literature, indicate that substantial improvements in spatial and water efficiencies can be achieved in coral aquaculture facilities without reductions in yield. In particular, improved efficiency may be achieved by using vertical holding of rearing substrates (10-fold increase in holding capacity compared to horizontal rearing) and semi-recirculating coral aquaculture systems (~2,000L seawater saved per tank and 24 h for the systems used in the present study). However, results also demonstrate the importance of maintaining sufficient water movement across rearing substrates. Therefore, the design and hydrodynamic assessment of large-scale coral recruit rearing systems will be critical to ensure water movement similar to the optimal water flow rate for target species is maintained across all rearing substrates within tanks.

In addition to these abiotic factors, feed of sufficient quantity and quality should be supplied daily, and ideally tank designs should ensure feed is retained within the tank for a period of time to allow prey capture to occur throughout the tank. Reductions of competition with benthic fouling communities through e.g. the choice of rearing substrates, reductions in light intensity, addition of small herbivorous gastropods or echinoderms, and additional filtration to reduce the introduction of spores or larval stages of nuisance species may further enhance the survival and growth of coral recruits during long-term rearing in *ex situ* facilities.

The recommendations provided here may assist in upscaling the sexual propagation capacity of existing aquaculture facilities, or guide the planning of new developments. However, further research is needed to further enhance recruit survival. In particular, continuing to expand the species-specific information required for rearing, optimising feed compositions and regimes, and ways of further reducing competition should be prioritised.

BACKGROUND

2 Background

2.1 Why perform reef restoration?

Coral reefs are one of the most diverse biomes on earth and supply critical ecosystem services to a large proportion of the human population (GCRMN et al., 2021), with close to 1 billion people living in the vicinity of coral reefs (Sing Wong et al., 2022). However, with the impacts of ongoing climate change (IPCC, 2018; IPCC, 2023), local anthropogenic pressures (Caras and Pasternak, 2009; Kroon et al., 2015; MacNeil et al., 2019) and shorter intervals between disturbances and mortality events (Emslie et al., 2024; Hughes et al., 2018), coral reefs are in decline worldwide (GCRMN et al., 2021; Hughes et al., 2017). Coral reef restoration has been identified as one part of the toolkit for maintaining coral reef functioning and services while action is taken to reduce these ongoing stressors, in particular action on global climate change (Banaszak et al., 2023; Boström-Einarsson et al., 2020; Duarte et al., 2020; Hughes et al., 2023; Randall et al., 2020).

Figure 1. Coral reefs support high biodiversity and provide key ecosystem services. Midshelf coral reef community on Bindal sea country, Central Great Barrier Reef. Photo: Dr M Nordborg.

2.2 What is coral reef restoration?

Ecological restoration is defined as "…assisting in the recovery of ecosystems that have been degraded or destroyed, as well as conserving the ecosystems that are still intact." (UN, 2021). Restoration actions are typically classified as passive (e.g. establishment of protected areas) or active (e.g. regreening of deforested areas; UN (2021)). In the context of coral reefs, active restoration efforts often involves the transplantation or 'outplanting' of live corals to increase coral cover at degraded sites (Edwards et al., 2010). The material used in these projects is most commonly produced using asexual propagation of corals, where clonal lines of a limited number of donor colonies are repeatedly fragmented and grown out to a predetermined size and attached to the reef substratum ('coral gardening'; Boström-Einarsson et al. (2020)). Corals produced through sexual propagation, e.g. during mass broadcast spawning events, can also be applied to 'seed' degraded reefs (Banaszak et al., 2023; Randall et al., 2020).

The use of sexual propagation may be more suitable for upscaled production and outplanting over spatial scales that are more likely to result in ecological benefits

2.3 Key reef restoration areas identified as amenable to optimisation

2.3.1 Upscaling

To achieve meaningful ecological outcomes in coral reef restoration, the scale of restoration efforts is crucial, with expanding spatial coverage identified as a key priority regardless of the methods employed. (Lamont et al., 2022; Ridlon et al., 2023; Vardi et al., 2021). Both sexual and asexual approaches offer advantages and disadvantages (Boström-Einarsson et al., 2020; Omori, 2019). Asexual propagation through fragmentation followed by diver facilitated outplanting is labour intensive, and therefore typically limited in terms of spatial scale (Boström-Einarsson et al., 2020; Randall et al., 2020). Sexual propagation may be more suitable for large-scale production and outplanting, which are necessary to achieve meaningful ecological benefits (i.e. >1 hectare; Banaszak et al. (2023); Boström-Einarsson et al. (2020); Randall et al. (2020)). Sexual production of corals is amenable to automated processes including spawn capture and fertilisation (Severati et al., 2024), and other efficiencies such as mass settlement of larvae onto seeding devices (Chamberland et al., 2017; Randall et al., 2023). The use of sexual propagation also maximises the genetic diversity of the outplanted corals, increasing the likelihood of resilience to a wider variety of stressors and conditions (Banaszak et al., 2023; Boström-Einarsson et al., 2020; Ridlon et al., 2023).

Despite the apparent benefits of sexual propagation of corals for reef restoration, its application has mainly been in research settings (Boström-Einarsson et al., 2020). This is largely the result of the higher initial operational costs (Baria-Rodriguez et al., 2019; Omori, 2019) and/or perceived risks associated with conservation aquaculture operations (e.g., spreading diseases or pest species, unintended reductions in genetic diversity; Ridlon et al. (2023)). However, cost-benefit-analyses typically favour sexually produced corals, especially when considering long-term survival and growth. One year after outplanting, costs per surviving coral are lower for sexually propagated individuals (Baria-Rodriguez et al., 2019). Additionally, the use of *in situ* and *ex situ* nurseries to raise early recruits, has been reported to reduce cost per surviving outplanted coral over the long term (Baria-Rodriguez et al., 2019; Humanes et al., 2021).

2.3.2 Cost-effectiveness

The cost of sexually propagated corals can be higher initially, especially if they require *ex situ* facility cultivation before outplanting or transfer to an *in situ* nursery. The majority of the costs are related to facility operation during spawning and recruit rearing (Chamberland et al., 2015; Villanueva et al., 2012), but are also heavily influenced by the cost of labour in the region where the facility is located. The cost of *ex situ* facility operation, and initial infrastructure investment, is also strongly related to the size and technological sophistication of the facility (Lippmann et al., 2023). Improving the cost-effectiveness of coral aquaculture facilities has been identified as another key priority for expanding the use of sexual coral propagation (Banaszak et al., 2023; Randall et al., 2020; Ridlon et al., 2023), and for effectively scaling up reef restoration efforts (Gibbs, 2021; Vardi et al., 2021).

> “...the cost-benefit-analysis of sexual vs. asexual coral propagation for reef restoration favour the use of sexually produced corals if the long-term survival and growth of the outplanted material is considered...”

2.3.3 Yield

Suggestions for improving cost-effectiveness of coral seeding include methodological and technological solutions to enhance the yield at each step of the workflow, such as maximising larval survival in culture, increasing larval settlement success, and boosting recruit survival in nurseries ; Banaszak et al. (2023); Randall et al. (2020); Ridlon et al. (2023)). The application of *in situ* methods, such as slick capture and rearing in larval pools has also been suggested (e.g. Heyward et al. (2002) and Doropoulos et al. (2019)). For *ex situ* facilities, increasing the density of corals per area unit, e.g. through the design of new holding systems or space efficiencies within existing systems, could also reduce the cost per coral.

While efficiencies have already been achieved for some parts of the existing coral propagation workflows (see e.g. Pollock et al. (2017) and Severati et al. (2024)), larval metamorphosis and early recruit survival remain significant bottlenecks due to their complexity. Improvements in coral recruit survival in the first 3 months post-settlement has been identified as having the greatest potential for improving productivity in coral aquaculture (Randall et al., 2020). Both the settlement success of larvae, and the survival and growth of recently settled recruits, are influenced by a large number of environmental and biological factors, including substrate material, microhabitat topography, water temperature, light intensity and quality, feed availability, water flow and quality, microbiome composition, genetics as well as predation from or competition with other species (Leal et al., 2016; Omori, 2019; Randall et al., 2020). Corals are typically reared horizontally in *ex situ* facilities, to enable visual inspection of individual units or colonies, ensure conditions are similar throughout rearing tanks, and optimised based on current knowledge (see e.g. Humanes et al. (2021)). This practice is spatially expensive, but alternative husbandry procedures and culture system configurations carry high risks of productivity loss, and therefore need to be assessed and optimised prior to larger scale investment and implementation.

2.4 Research statement

Here we evaluate the potential to increase cost-effectiveness of *ex situ* coral recruit grow-out by increasing the holding capacity and cost-efficiency per area unit in a land-based facility. Specifically, we assess: (i) the performance of different substrate mounting configurations in coral recruit rearing to optimise the spatial footprint of individual recruit grow-out systems (Chapter 3 Spatial footprint optimisation, page 15); (ii) water saving measures (e.g. the use of semi-recirculating systems during rearing; Chapter 4 Optimising water consumption, page 21); and (iii) investigate optimal water flow rates for coral recruits (Chapter 5 Water movement & flow, page 26).

SYSTEM FOOTPRINT OPTIMISATION

3 System footprint optimisation

3.1 Introduction

Improving the cost-effectiveness of coral aquaculture facilities has been identified as another key priority for expanding the use of sexual coral propagation (Banaszak et al., 2023; Randall et al., 2020; Ridlon et al., 2023), and for effectively scaling up reef restoration efforts (Gibbs, 2021; Vardi et al., 2021). One potential pathway to improve the cost effectiveness of coral conservation aquaculture facilities is to optimise the footprint of the aquaculture systems used, thereby increasing the production capacity per m^2. Corals (adult, propagated fragments and recruits) are typically held horizontally to ensure even light exposure, attractive growth morphologies and ease of monitoring. Although alternative holding systems have previously been proposed for *ex situ* facilities (see e.g. Craggs et al. (2024); Sweet (2023)) the effects of non-horizontal holding has remained largely unstudied. Here we assessed the survival of recently settled *Acropora millepora* recruits for up to 3 months when reared on substrates at three different angles (0°, 60° and 90°) as a potential for increasing the spatial efficiency of aquaria for coral conservation aquaculture.

3.2 Methods

3.2.1 Larval source & settlement

Broodstock colonies were collected by hand on SCUBA from fringing reefs around Falcon Island (18°45’54.8”S 146°31’36.1”E), central Great Barrier Reef (Australia), on the 25th October 2023. Mass larval cultures of *Acropora millepora* were started on the 31st October 2023 using the AutoSpawner method for collection and fertilisation of gametes (Severati et al., 2024). Briefly, gametes from nine broodstock colonies were automatically collected and transferred to a single fertilisation tank as per Severati & Nordborg et al. (2024). In the AutoFertiliser tank, bundles were broken up using gentle aeration and a filtered seawater (FSW) spray-ring and the sperm concentration diluted through addition of FSW (~750 L h^{-1}). Eggs were allowed to fertilise for 10 mins once target sperm concentration was reached (fertilisation success 82.6 % ± 2.9 SEM) and fertilised eggs were transferred to 500 L larval rearing tanks within ~2.5 h of the first bundle release. Larvae were reared for 6-7 days following fertilisation and used for settlement on the conditioned tiles following confirmation of larval settlement competency >80% (assessed using a 10% extract of the crustose coralline algae *Porolithon onkodes* in ethanol; Heyward and Negri (1999)).

Experimental tiles (concrete or PVC) were seawater conditioned for 2 months in a flow-through, 1,000 L system under shaded natural sunlight, cleaned, frozen and stored at -20°C. Prior to settlement, tiles were thawed and rinsed in flow-through FSW (27°C) for ~24 h. Pairs of tiles ($n_{Concrete} = 9$; $n_{PVC} = 17$) of the same material were randomly assigned to 50 L acrylic tanks (two tiles per tank) and placed horizontally on the bottom of tanks using PVC holders, to create a continuous surface. Settlement tanks were maintained at ambient conditions (average of 27.9°C, 35.1 salinity, 8.1 pH and 7.63 mg L^{-1} dissolved oxygen) with a flow rate of 50 L h^{-1} (one turnover per hour) and gentle aeration from multiple points. The outflow of tanks was fitted with a 200 µm mesh filter to prevent loss of larvae during settlement. Light was provided on a 12:12 h dark:light period (3 h ramp:6 h peak:3 h ramp light ramping schedule) using one LED aquarium light (Hydra Aqua Illumination SOL) per tank with a peak intensity of 20 µmol photons m^{-2} s^{-1}.

On the 6th of November 2023, ~1,600 six-day old larvae from a single mass culture tank were added to each 50 L tank. Larvae were added by removing ~2 L of water and then adding 2 L of homogenised larval mixture with a concentration of ~800 larvae L^{-1} to each tank. On the 7th November, outflow filters were removed, settled larvae scraped off of the sides of the acrylic tanks and unsettled larvae flushed out of tanks for ~ 4 h. Cleaned outflow filters were then reinstalled and an additional ~4,000 larvae from the same fertilisation batch were added using the same method previously described (concentration of ~2,700 larvae $tile^{-1}$). Tanks were then left undisturbed for 48 h.

Recruits were inoculated for ~72 h through addition of cultured clade C algal symbionts (*Cladocopium goreaui*) to a final concentration of ~10,000 algal cells mL^{-1} in 50 L acrylic tanks. After 24 h the FSW flow-rate was slowly increased back to 50 L h^{-1} and inoculation continued for an additional 2 days.

3.2.2 Coral recruit rearing

The coral recruits settled in 3.2.1 were then moved, and reared for 3 months in a single 1,000 L fiberglass tank (holding tank turnover = 100% h^{-1}) connected to a semi-recirculating aquarium system. The system consisted of an 800 L sump, 1 micron bag filter and temperature control system (system turnover/top-up rate = 250% per 24 h). Three tiles of each material type were randomly assigned to one of three grow-out angles (0°, 60° or 90°) and racked using a simple fibreglass reinforced racking system. Vertical (90°) tiles were bracketed by an additional four PVC tiles, to ensure the light conditions were similar across all replicate tiles assessed. Recruits were maintained at 27°C and a 12:12 h dark:light regime (4x 300W LED Tunnel Light Panels by Mean Well) with a maximum intensity of 150 µmol photons m^{-2} s^{-1} and a light intensity ramping profile (up:peak:down) of 3:6:3 h. Water movement within the rearing tank was provided by four circulator pumps (Maxspect Gyre XF250) and the water was replaced ~2.5-times every 24 h for the system as a whole. Recruits were fed a mixture of microalgae and rotifers once daily (final concentration of respectively ~500 microalgae cells and ~0.5 rotifers mL^{-1} of tank volume). From ~2 months post-settlement recruits were also fed newly hatched Artemia nauplii (final concentration of ~0.5 nauplii mL^{-1} of tank volume).

3.2.3 Data collection

Following symbiont inoculation and after 4, 8 and 12 weeks of rearing, all tiles (n = 6 per rearing angle) were imaged (n = 28 images per tile) using a Nikon D810 DSLR camera with an AF Micro Nikkor 60mm f2.8 lens and two DS161 Ikelite strobes. A navigational grid was placed on top of each tile during imaging and tiles were submerged in FSW throughout. The number of settled recruits per tile section (~14 x 14 mm) was then assessed using the FIJI Image J software (version 1.54f; Schindelin et al. (2012)). Briefly, the number of recruits in each cell of the 20 x 20 navigational grid was counted using the Cell Counter-plugin. Each recruit was marked and labelled, and markers exported using the inbuilt tools of the Cell Counter-plugin. Recruits where the mouth was obscured by the navigational grid were only counted for the right hand and bottom side of individual sections to avoid double counting.

3.2.4 Statistical analysis

Tile level survival of recruits after 1, 2 and 3 months of rearing was analysed using the software *R* (version 4.2.2; R Core Team (2022)) and *RTools42* (version 42; RTools Team (2022)) through the *RStudio* interface (version 2023.03.0; RStudio Team (2023)). Following data exploration and initial candidate model fitting, model fits were assessed using the inbuilt functions of the *brms* package (Bürkner, 2017) and *rstan* (Stan Development Team, 2023). Initially a linear regression model including substrate angle, rearing time and tile material was fitted. While tile material affected the survival of recruits, the pattern of survival across reading angles and assessment time points was the same for both concrete and PVC tiles. Therefore, a second linear regression model was fitted using only substrate angle and rearing time as fixed factors.

Table 1. A. millepora recruit survival following rearing for up to 3 months on substrates at 0°, 60° or 90° angle, regardless of tile material. Median and 95% credible intervals (in brackets) shown for each treatment combination. Treatments where 95% credible intervals do not overlap are considered statistically different.

Treatment	**Rearing period**		
	1 month	***2 months***	***3 months***
0°	59% (58.3-59.6%)	4.8% (4.6-5.0%)	0.17% (0.16-0.19%)
60°	70.3% (69.6-71.1%)	7.6% (7.3-8.0%)	0.29% (0.27-0.31%)
90°	81.0% (80.5-81.6%)	13.0% (12.6-13.4%)	0.52% (0.49-0.55%)

Statistically significant differences were assessed using the 95% credible intervals (CI) of the median for each combination of predictors, extracted from the posterior probability distributions. Graphical results were produced using *ggplot2* (Wickham, 2016).

3.3 Results

Recruit survival was significantly affected by substrate angle, rearing period and tile material (Figure 2). Recruit survival remained highest on vertical substrates (90°) throughout the rearing period, followed by substrates held at a 60° angle (Figure 2, and Table 1). Recruits reared on horizontal substrates had the lowest survival, regardless of rearing time (Figure 2 and Table 1).

Survival decreased significantly for all tiles between all assessment time points (Figure 2 and Table 1), with the greatest mortality event occurring between the 1 and 2 month assessments (Figure 2). This coincided with a 48 h period where internal circulator pumps were turned off due to the holding requirements for another experiment being conducted in the same system. Recruits reared on concrete tiles had a higher survival than recruits reared on PVC tiles for all timepoints and substrate angles. However, substrate angle affected the recruit survival the same way, regardless of tile material, as exemplified for the 1 month assessment shown in Figure 3.

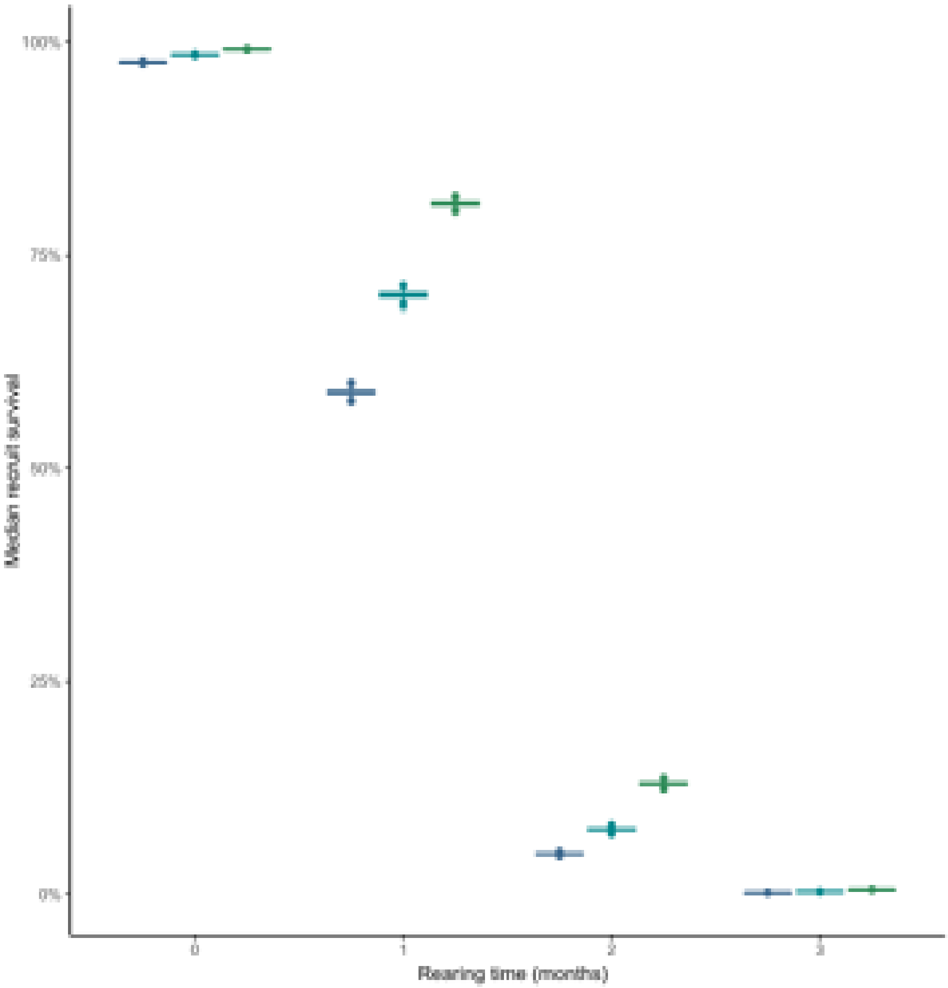

Figure 2. A. millepora recruit survival for recruits reared on substrates at 0° (blue), 60° (teal) or 90° (green) degrees for up to 3 months. Median and 95% credible intervals shown for each combination of substrate angle (indicated by colour) and rearing period (0-3 months).

Coral recruit survival was highest on vertical substrates at all assessment time points

3.4 Discussion

The survival of *A. millepora* recruits varied depending on substrate angle and the material recruits were reared on. Survival was highest for recruits grown on vertical surfaces and lowest on horizontal surfaces, regardless of substrate material. While survival was generally lower on PVC than concrete, the effect of substrate angle during rearing was the same for both materials (e.g. Figure 3).

While performed *ex situ*, the results of the present study agree with previous recruit survival observations from field experiments on the Great Barrier Reef, which reported higher survival of *Acropora kenti* (formerly *Acropora tenuis*) recruits on non-horizontal surfaces (Randall et al., 2021). Early work on *Platygyra sinensis* and *Oxypora lacera* recruits (in situ and *ex situ*) also indicated that recruit survival in the first few months after settlement was higher on non-horizontal surfaces (Babcock and Mundy, 1996). Coral recruits are highly sensitive to sedimentation (Brunner et al., 2021), and sediment exposure has been suggested as a driver of coral recruit mortality on exposed, horizontal surfaces (alongside accidental grazing by herbivorous fish; Babcock and Mundy (1996); Doropoulos et al. (2016); Randall et al. (2021)). However, field experiments assessing the survival of coral recruits (*A. millepora*, *Acropora muricata* and *Montipora aequituberculata*) on vertical and downward facing surfaces on inshore reefs on the southern Great Barrier Reef also indicated that survival was higher on vertical surfaces (Page et al., 2024). Combined, these results highlight that minimizing sediment accrual while maintaining light availability appears to result in the highest recruit survival in the first few months post-settlement.

Vertical rearing has previously been suggested as optimal for *in situ* nursery rearing (Omori and Iwao, 2014). In the context of coral conservation aquaculture facilities these experimental results from the literature indicate that short-term vertical holding of coral recruits would not be expected to negatively impact recruit survival. The results of the present study adds further support to the merits of vertical holding of recently settled coral recruits. In addition to the higher survival observed for recruits reared on vertical surfaces, vertical holding also offers significant space savings (e.g. 10 vertical tiles have a similar horizontal footprint as one tile held horizontally).

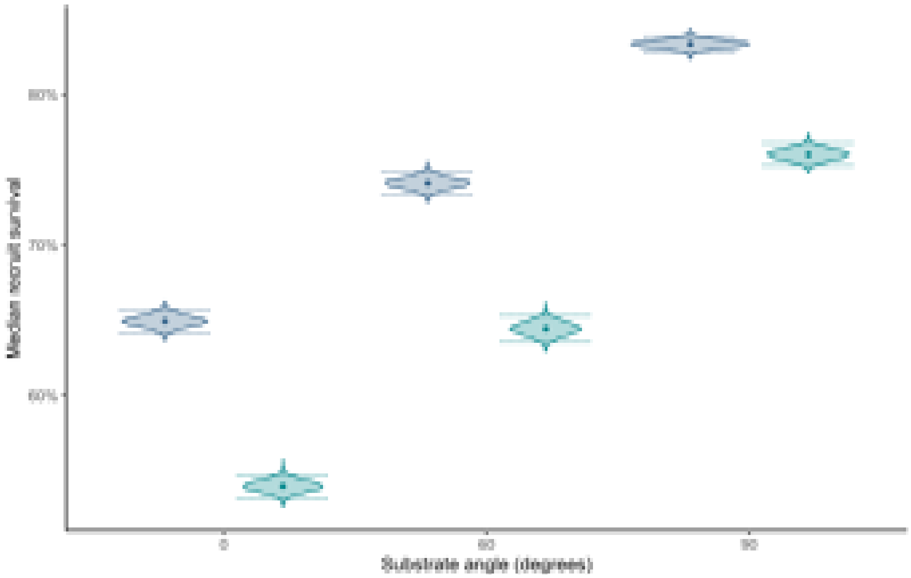

Figure 3. Median survival of Acropora millepora recruits following 1 month of rearing at three different substrate angles (0°, 60° and 90) on concrete (blue) or PVC (teal) tiles. Median (solid circles), 95% credible intervals (dashed lines) and full posterior probability distributions (shaded area) shown for each combination of substrate angle and tile material.

While promising, the implementation of more dense, vertical, *ex situ* rearing systems for coral recruits will likely require further investigation to ensure other factors (e.g. flow past rearing surfaces, light availability) can be sufficiently maintained throughout the system. The effects of other factors, such as water flow rate, on coral recruit survival was exemplified by the sudden mortality of a large proportion of recruits during the second month of rearing, regardless of surface angle (Figure 2). This mortality event occurred shortly after a period of low water velocity within the rearing system where the experiment was taking place (due to the experimental requirements for a different project). Additionally, significant recruit mortality has previously also been observed in large-scale rearing tanks where water velocity past the rearing surfaces was later shown to be low (Nordborg et al, *unpublished*) and the growth of adult corals have been reported to be lower in no-flow conditions (Schutter et al., 2010). Only one study has assessed the effects of water velocity/water flow rate on coral recruit health (Geertsma et al., 2022). However, that study only assessed the recruits in the short term (20 days of rearing) and primarily focused on prey capture ability across the 10 Caribbean species investigated.

While recruit survival across whole tiles was higher for vertical substrates in the present study it is unclear whether there were differences in survival across the surfaces of each tile. For example, vertical tiles experienced a light gradient from top to bottom (data not shown) which may have led to location-dependent variability in survival. Light conditions have previously been confirmed to affect early coral recruit survival, often through increased competition from macroalgae with increased light intensity (e.g. Ramsby et al. (2024)). Further investigations of the interactions between substrate angle, light intensity and benthic community composition during recruit rearing would likely help resolve these uncertainties.

Additional investigations into the optimal water velocities and replacement rates for coral recruit rearing are required to facilitate the design and testing of novel, high-density, vertical rearing systems for coral recruits. Furthermore, the hydrodynamics of any novel system should be assessed, alongside pilot testing with live recruits, prior to full-scale implementation in aquaculture facilities.

OPTIMISING WATER CONSUMPTION

4 Optimising water consumption

4.1 Introduction

A majority of the costs for coral conservation aquaculture are related to facility operation during spawning and recruit rearing (Chamberland et al., 2015; Villanueva et al., 2012). The cost of *ex situ* facility operation, and initial infrastructure investment, is also strongly related to the size and technological sophistication of the facility (Lippmann et al., 2023). Improving the cost-effectiveness of coral aquaculture facilities has been identified as another key priority for expanding the use of sexual coral propagation (Banaszak et al., 2023; Randall et al., 2020; Ridlon et al., 2023), and for effectively scaling up reef restoration efforts (Gibbs, 2021; Vardi et al., 2021). Access to seawater in sufficient quantities and of appropriate quality has long been recognised as a limiting factor for coral aquaculture (Delbeek, 2001). Upscaled coral conservation aquaculture operations will therefore require efficient seawater usage to meet the projected future demand. Recently settled coral recruits are typically held in flow-through aquarium systems to optimise survival and growth. However, flow-through rearing of coral recruits leads to high water usage with a single 1,000 L system with a standard 24 turnovers per day requires over 24,000 L of seawater per day. The use of a semi-recirculating system with 24 tank turnovers per day, but a water replacement rate of 3 turnovers per day (for the system as a whole), could lead to savings of 18,000 L per system and day. To assess the feasibility of increasing the water usage efficiency in coral conservation aquaculture facilities by using semi-recirculating systems we compared the recruit survival of the common species *Acropora spathulata* following 3 months of rearing in flow-through or semi-recirculating aquarium systems.

4.2 Methods

4.2.1 Larval source & settlement

Broodstock colonies of *Acropora spathulata* were collected by hand on SCUBA from Davies Reef (18°49’12.2”S, 147°38’39.4”E), central Great Barrier Reef (Australia) on the 25th November 2023. Mass larval cultures ($n_{\text{Broodstock}}$ colonies = 8 to 12) were started on the 30th November 2023 using manual collection of bundles and an AutoFertiliser tank, as previously described (Severati et al., 2024). Fertilised eggs (>90% fertilisation success) were transferred to 500 L larval rearing tanks within 2.5 h of the first bundle release. Larvae were reared for up to 18 days following fertilisation and used for settlement on the conditioned tiles following confirmation of a settlement competency >80% (assessed using a 10% *Porolithon onkodes* extract and live CCA chips; as per Heyward and Negri (1999); Whitman et al. (2020)).

Concrete experimental tiles, which had been frozen following two months of seawater conditioning in a flow-through, outdoor aquarium system at the National SeaSimulator (Townsville, QLD), were thawed out and rinsed in flow-through FSW (27°C) for ~24 h prior to use in settlement tanks. Individual tiles (n= 42 tiles) were randomly assigned to, and placed horizontally (i.e. 0°) on the bottom of, 50 L acrylic tanks (two tiles per tank) or 90 L fiberglass mass settlement tanks (9 tiles per tank). Tanks were maintained at the same conditions as for larval culture tanks with a flow rate of approximately 1 turnover per hour. The outflow of tanks were fitted with 200 µm mesh filters to prevent loss of larvae during settlement. Light was provided on a 12:12 h dark:light period (3 h ramp:6 h peak:3 h ramp daytime light schedule) using LED aquarium lights (one Hydra Aqua Illumination SOL per 50 L tank, and four Mean Well 300W LED Tunnel Light Panels per fibreglass tank) with a peak intensity of 20 µmol photons m^{-2} s^{-1}.

On the 15th-18th December 2023, 15-18 day old *A. spathulata* larvae from two mass culture tanks were added to each settlement tank at a concentration of ~5,500 larvae $tile^{-1}$. Larvae were concentrated in culture tanks and culture batches mixed prior to addition to experimental tanks. Larvae were added to experimental tanks and then the tanks were left undisturbed for ~48 h. On the 19th December, outflow filters were removed, settled larvae scraped off of the sides of the acrylic experimental tanks (if necessary) and unsettled larvae flushed out. Recruits were then inoculated with cultured clade C algal symbionts (*Cladocopium goreaui*) at a final concentration of ~10,000 algal cells mL^{-1} for ~4 days.

4.2.2 Coral recruit rearing

Recruits were reared in FSW (salinity of 35.0 ± 0.03 and pH 8.1 ± 0.02) at 27.2°C (± 0.1 SEM) under a 12:12h light:dark cycle with a 3:6:3 h light ramp schedule (4x Mean Well 300W LED Tunnel Light Panels, maximum intensity of 20 µmol photons m^{-2} s^{-1}) for 3 months. Recruits were fed daily using a mixture of microalgae and rotifers (final concentration of respectively ~1000 microalgae cells and ~1 rotifers mL^{-1} of tank volume). From ~2 months post-settlement, recruits were also fed newly hatched *Artemia* nauplii (final concentration of ~1 nauplii mL^{-1} of tank volume).

Water quality (temperature, salinity, pH and dissolved oxygen) in rearing tanks was assessed weekly. All parameters remained within acceptable ranges throughout the rearing period for all replicate tanks. Husbandry of rearing tanks (algae removal, sediment siphoning, etc.) was minimal (<1 per week) throughout the rearing period.

4.2.3 Data collection

Following settlement and at the end of the 3-month rearing period, all tiles were imaged (n = 28 images per tile) using a Nikon D810 DSLR camera with an AF Micro Nikkor 60mm f2.8 lens and two DS161 Ikelite strobes. A navigational grid was placed on top of each tile during imaging and tiles were submerged in FSW throughout. The number of settled recruits per tile section (~14 x 14 mm) was then assessed using the FIJI Image J software (version 1.54f; Schindelin et al. (2012)). Briefly, the number of recruits in each cell of the 20 x 20 navigational grid was counted using the Cell Counter-plugin. Each recruit was marked and labelled, and markers exported using the inbuilt tools of the Cell Counter-plugin. Recruits where the mouth was obscured by the navigational grid were only counted for the right hand and bottom side of individual sections to avoid double counting. Total alive recruits per tile and the number of individual units/'tabs' with at least one live recruit at the end of the 3 months were calculated manually.

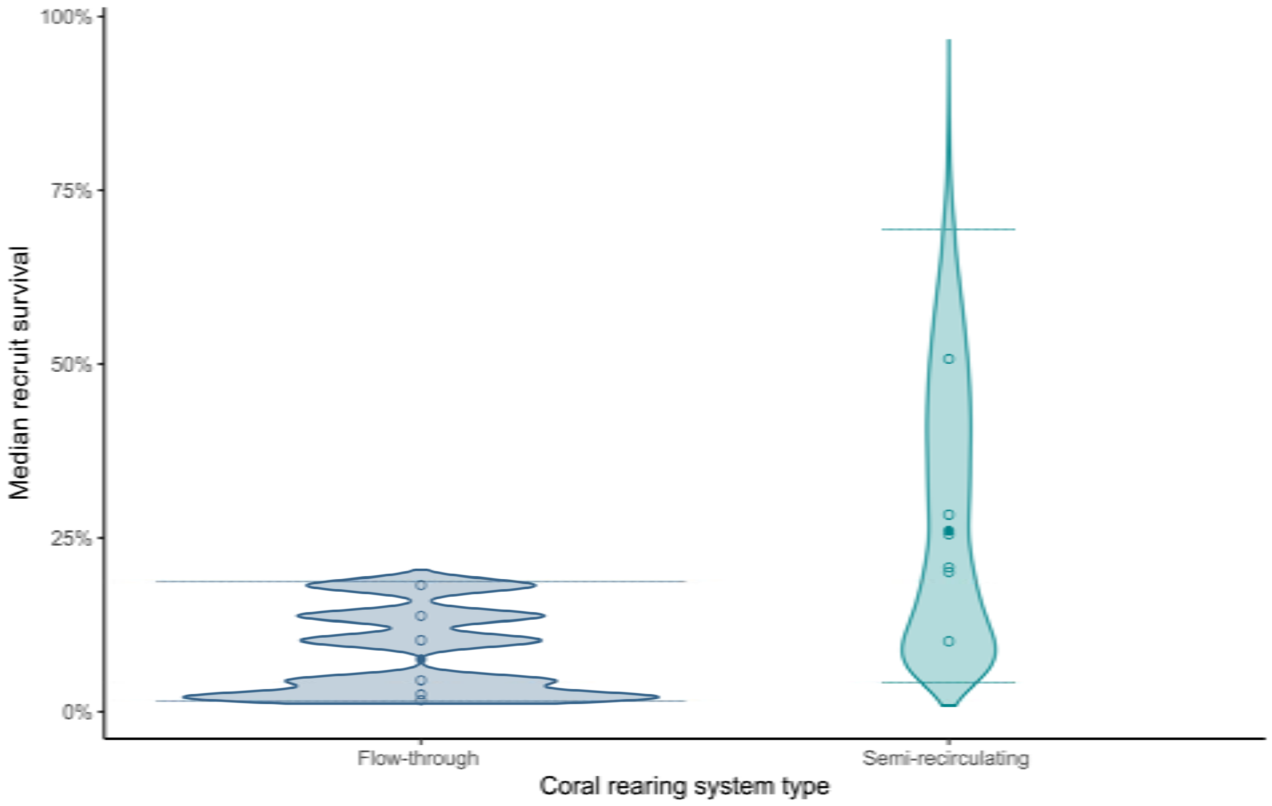

Figure 4. Recruit survival after 3 months of grow-out in flow-through (blue) or semi-recirculating (teal) recruit rearing systems. Model median (solid circle), raw data (open circles; n = 6 tanks), full posterior probability distribution (shaded areas) and 95% credible intervals (dashed lines) shown for the models fitted for each treatment. Treatments considered statistically different if 95% credible intervals do not overlap. Average of 4,600 live coral recruits per tank at start of grow-out period.

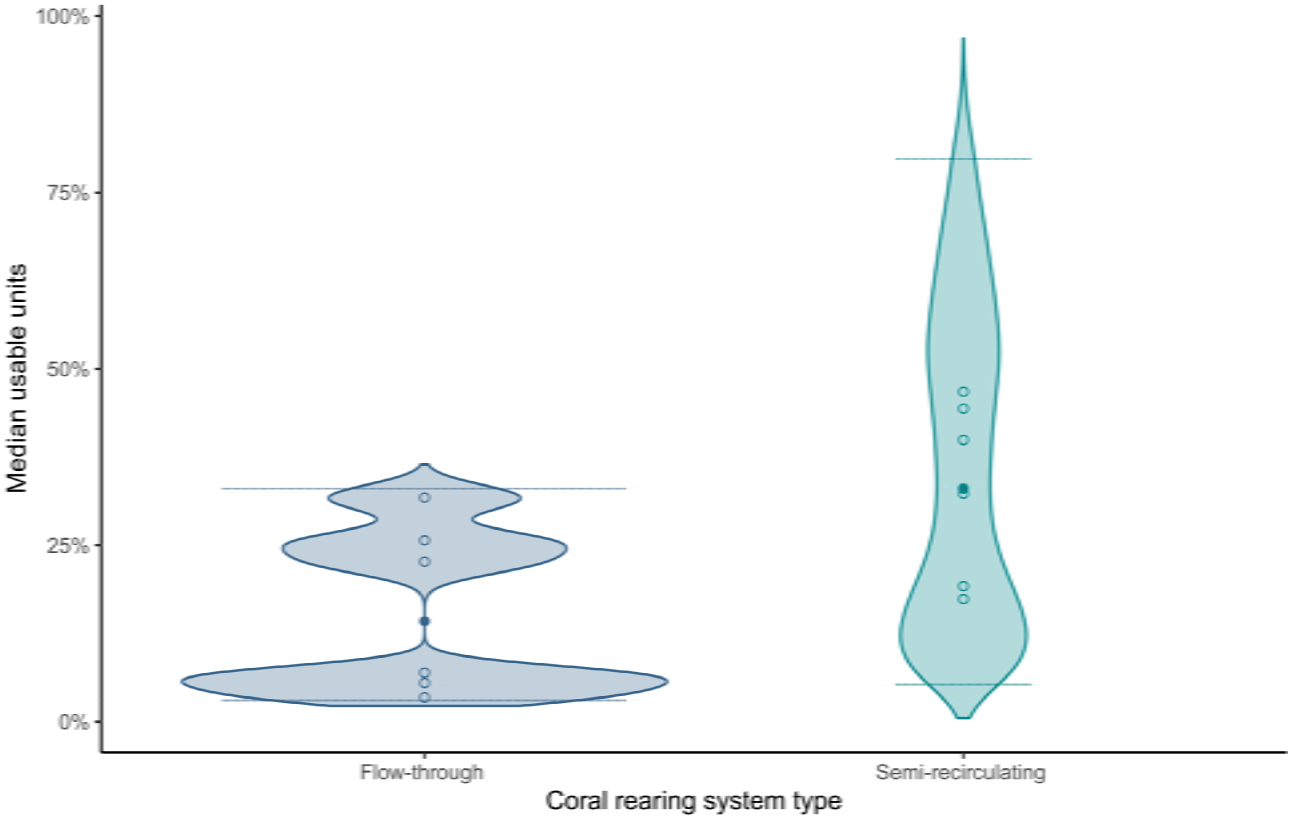

Figure 5. Yield of units (individual concrete 'tabs') suitable for deployment using coral seeding devices (≥1 live recruit present) after 3 months of grow-out in flow-through (blue) or semi-recirculating (teal) recruit rearing systems. Yield calculated as the percent of usable units at 3 mo compared to all units placed into tanks at start of experiment. Model median (solid circle), raw data (open circles; n = 6 tanks), full posterior probability distrubition (shaded area) and 95% credible intervals (dashed lines) shown for the models fitted for each treatment. Treatments considered statistically different if 95% credible intervals do not overlap. Average of 1370 units placed into each tank at start of the grow-out period; however, not all units contained recruits at t0.

4.2.4 Statistical analysis

Tank level coral recruit survival, and yield of deployable units, were analysed using the software *R* (version 4.2.2; R Core Team (2022)) and *RTools42* (version 42; RTools Team (2022)) through the *RStudio* interface (version 2023.03.0; RStudio Team (2023)). Following data exploration and initial candidate model fitting, model fits were assessed using the inbuilt functions of the *brms* package (Bürkner, 2017) and *rstan* (Stan Development Team, 2023). The best model for both data sets used treatment (Flow-through or Semi-recirculating) as a fixed effect and tank_ID as a random effect. Statistically significant differences were assessed using the 95% credible intervals (CI) of the median, extracted from the posterior probability distributions, for each treatment. Graphical results were produced using *ggplot2* (Wickham, 2016).

4.3 Results

No statistically significant difference was observed between the two rearing system types for either the survival of recruits or yield of usable units for deployment at the end of the three month grow-out period (Figure 4 and Figure 5). While not statistically significant, the median survival for *A. spathulata* recruits was higher for the semi-recirculating systems (26%, 4-69% $CI_{95\%}$) than for the flow-through systems (7.5%, 1.5-19% $CI_{95\%}$; Figure 4). Similarly, the median yield of deployable units, relative to the number placed into each tank at the start of the rearing period, was also higher for the semi-recirculating systems (33%, 5-80% $CI_{95\%}$ compared to 14%, 3-33% $CI_{95\%}$; Figure 5). Fouling remained relatively low throughout the rearing period and was dominated by benthic diatoms during the first 2 months of rearing. Qualitative observations indicated that fouling typically occurred in flow-through systems before becoming apparent in semi-recirculating systems. In the final month of rearing, observations of hydroids increased in frequency and hydroids were widespread in most tanks by the end of the rearing period.

Using a semi-recirculating rearing system did not negatively affect recruit survival or seeding unit yield over a 3 month grow-out period

4.4 Discussion

Water usage and access to seawater of sufficient quality has long been recognised as a limiting factor for aquaculture operations, including for coral (Delbeek, 2001). Increasing water usage efficiency without reducing productivity is therefore a critical step in upscaling coral conservation aquaculture operations to meet the projected requirements for future coral reef restoration projects. In the present study there was no statistically significant difference in survival of *A. spathulata* recruits following 3 months of rearing in semi-recirculating compared to fully flow-through aquaculture systems. These results demonstrate that semi-recirculating rearing systems could be used for large-scale grow-out of coral recruits, if sufficient filtration, internal water movement and pest control is applied. Using the present study as an example, water usage could be reduced by approximately 2,000 L per 24 h and rearing tank (where each tank contains 9 tiles, or 3,600 seeding units).

The median coral recruit survival was three times higher in the semi-recirculating systems (26%) than in the flow-through systems (7.5%). The semi-recirculating systems also yielded twice as many units that were suitable for deployment at the end of the 3-month rearing period (33% compared to 14%). The recruit survival observed here is comparable to that reported for other *Acropora* spp. recruits reared in *ex situ* settings (e.g. Fong et al. (2024); Ramsby et al. (2024)). While the recruit survival was lower in the present study, this is likely in part a result of the deliberate decision of minimal husbandry trialled in the present study. Coral recruit rearing for the purposes of research typically involves a large number of husbandry hours per recruit maintained, to ensure that sufficient recruits remain throughout the experimental period and that results are not due to unrelated factors (e.g. mortality as a result of competition with algae). Some investigations into methods to reduce the need for manual husbandry have already been completed, showing that settlement substrate material choices (Fong et al., 2024), light conditions (Ramsby et al., 2024) and co-culture with other organisms (Craggs et al., 2019; Neil et al., 2024; Neil et al., 2021; Toh et al., 2013) can be effective in reducing husbandry requirements. Based on the results from the present study, additional filtration of seawater prior to introduction into tanks may reduce the number of spores or larvae of nuisance species introduced. However, few of these methods have been applied in larger scale coral aquaculture settings, and this should be prioritised moving forward to assess whether the results reported to date are scalable.

WATER MOVEMENT & FLOW

5 Water movement & flow

5.1 Introduction

Coral health is affected by a large number of environmental factors, including light, temperature, salinity, pH and water flow (Gori et al., 2015; Long et al., 2013; Schutter et al., 2010). While less studied, water flow has an important impact on the coral health. The dynamics and velocity of the water flow can significantly affect coral autotrophy (Mass et al., 2010), coral heterotrophy (Geertsma et al., 2022; Gori et al., 2015) and coral gas exchange (Patterson and Sebens, 1989). Previous studies of adult corals have indicated that a lack of water flow can cause significantly reduced growth (Schutter et al., 2010), while the only previous study of recently settled recruits indicated that high flow-rates may cause morphological deformation and that prey capture rates may be affected by flow-rate (Geertsma et al., 2022). Flow rates also affect the diffusive boundary layer, which in turn influences the diffusion of gases across membranes and surfaces (Patterson and Sebens, 1989). Due to these complexities and the limited research performed to date, in particular for early coral life stages post-settlement (Geertsma et al., 2022), the optimal flow conditions for long-term aquaculture of coral recruits remains uncertain. Following observations of high coral recruit mortality during rearing in holding systems with low water velocities (Nordborg et al., *unpublished*), and short-term periods without circulator pumps (Chapter 3 System footprint optimisation) a two part experiment was performed to assess the impact of flow-rate on coral recruit and survival and growth (*Long term rearing*) and to investigate whether morphological deformations observed during acute exposure to a range of water velocities (*Acute exposure*) can be used to predict optimal water flow rates for long term recruit rearing.

5.2 Methods

5.2.1 Larval source & settlement

Gravid *Acropora abrolhosensis* colonies were collected between the 8th and the 16th January from Davies Reef, central Great Barrier Reef (S18° 48.989', E147° 38.644'), at ~9 m water depth and placed into flow-through holding tanks at SeaSim with FSW at ambient temperature (29.0°C).

Spawning occurred on the 29th of February and continued for six nights. The spawn of 8 parent colonies was collected and automatically fertilised in an AutoSpawner system (Severati et al., 2024) and then transferred into an 85 L flow-through culture tank (FSW at 29.0°C). Assays to determine the settlement performance of the fully developed larvae were performed daily. Settlement competency assays were performed by exposing five larvae per petri dish to pieces of preconditioned concrete tiles (*n* = 5). Conditioning was achieved by placing the concrete tiles six weeks prior to the expected coral spawning into an aquarium with a well-developed crustose coralline alga community, including the known coral larvae settlement inducer *Porolithon* cf. *onkodes* (Heyward and Negri 1999). Conditioned concrete tiles were then frozen for at least 24 h prior to use to remove potential competition effects of the benthic algae towards the coral recruits. Mass-settlement on the preconditioned concrete tiles for the experiment was performed in 50 L tanks and was commenced once 80% of larvae in the settlement competency assays' had settled within a 24 h period.

5.2.2 Acute exposure

To investigate the acute morphological responses of recently settled coral recruits to different flow velocities, five one-week-old (post-settlement) *A. abrolhosensis* recruits were simultaneously exposed to a range of flow velocities in a multi-lane flume chamber previously described and hydrodynamically characterised in detail by Illing et al. (2021). The multi-lane chamber can be used to expose multiple replicates simultaneously at water velocities of up to <70 cm s^{-1} while maintaining a highly precise velocity and near laminar flow.

Assessments of the acute morphological responses of recruits were performed as outlined by Geertsma et al. (2022). Briefly, one concrete tab (14x14 mm) with one settled coral recruit was mounted in the center of the water column in each of the five lanes, to avoid effects of fluid friction against the border of the lanes. After 15 min acclimation, the flow velocity was stepwise increased from an initial rate of 1 cm s^{-1} to a final rate of 70 cm s^{-1}. An increment of 1 cm s^{-1} was applied between 1 and 40 cm s^{-1}, followed by increments of 5 cm s^{-1} thereafter. Each increment was executed over a period of 5 minutes, facilitating a smooth transition for the recruits. Additionally, recruits were held at each treatment velocity for 1 minute to allow the capture of photographs of each recruit (8 MP Dino-Lite Edge^{PLUS}). One photo of the recruits was also made one day after the experiment at 1 cm s^{-1} from the top and side view to assess the appearance of the recruits ~24 h post-exposure.

5.2.2.1 Data collection

Recruit morphological responses were assessed qualitatively as per Geertsma et al. (2022) using captured images.

5.2.2.2 Statistical analysis

Statistical analysis was conducted using R (version 4.4.0; R Core Team (2024)) through the RStudio interface (RStudio Team, 2023). Polyp deformation data was analysed using a Kruskal-Wallis test.

5.2.3 Long-term rearing

To investigate the long-term growth and survival of coral recruits under different flow velocities, *A. abrolhosensis* recruits were reared in single-lane flume chambers (n = 3) configured to have a near-linear increase in flow-velocities along the length of each flume, for a 7-week period. One week post-settlement, concrete tiles with a high density of recruits were selected and placed in the measurement area of each single-lane flume chamber (~247 recruits per chamber; Fig 1a).

The flume chamber systems were set up as semi-recirculating systems consisting of one flume chamber, a water bath and a sump. The full details of the system parameters were as described by Illing et al. (2021), but modified internally using a ramp and wall obstruction (Figure 6a), to provide a near linear increase in flow velocity (Figure 6b). Briefly, the flume chamber was operated as a flow-through tank with FSW and approximately linear flow. It was placed in a water bath for stable experimental conditions (27.8 ± 0.07 °C, 35.2 ± 0.54 ppt salinity, 8.04 ± 0.02 pH, 7.76 ± 0.12 mg L^{-1} dissolved O_2). The water bath was supplied by the water exiting the flume chamber, which then exited into a sump containing temperature sensors, heating elements and a water level sensor. The seawater exchange rate for each system as a whole was set to 1 L

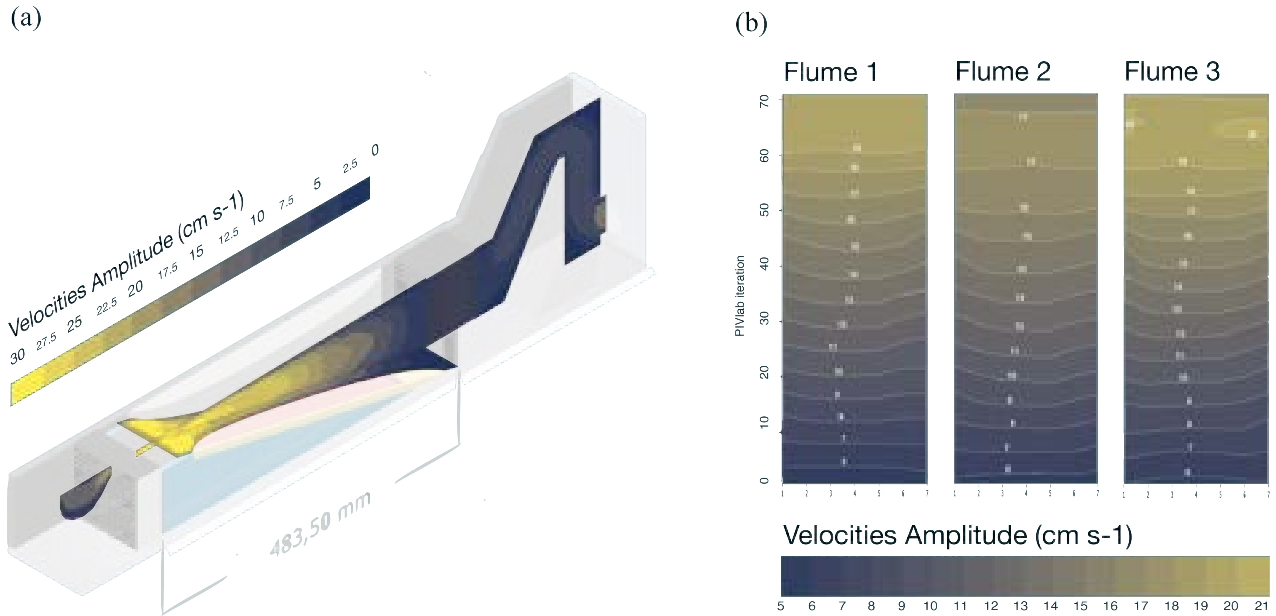

Figure 6: (a) Visualization of the velocity amplitude within flume chambers following modifications of the flume described by Illing et al. (2021). (b) Colormap of the mean (in time) velocities observed along the length of the three flumes resulting from the beads simulation analysis (top view of flumes).

min^{-1} (three turnovers day^{-1}). All of the parameters were automatically controlled and monitored by built-in sensors and a supervisory control and data acquisition system (SCADA; SIMATIC WinCC, Siemens). Additional water quality and nutrient measurements were taken every week using handheld probes alongside water quality samples for dissolved organic carbon (DOC) and dissolved inorganic nitrogen (DIN). Visible light was provided on a 12:12 h light:dark period with a peak intensity of 20 µmol photons m^{-2} s^{-1} (135 W, Hydra 52 HD, Aqua illumination). During system configuration additional light measurements were performed (US-SQS/L serial number: SQSA0133, Walz) to confirm that the light intensities at the bottom, middle and end of the ramp inside of each flume chamber were similar (21.96 ± 2.26 µmol photons m^{-2} s^{-1}). Recruits were fed daily with a 10 mL live feed mix consisting of rotifers (2 mL of enriched L-Rotifer strain *Branchionus plicatilis*, 500-1500 rotifers mL^{-1}) and algae (8 mL of five microalgae species, ~5 million cells/mL). *Artemia salina* was added to the mixture after the first month (2 mL at a concentration of 4000 nauplii mL^{-1}). The feed mixture was injected into the flow upstream from each flume and only passed through the chamber once per feeding occurrence.

5.2.3.1 Data collection

Recruits were photographed fortnightly throughout the rearing period. Eight photos were taken of the measurement area of each flume chamber, following the profile of the ramp, using a DSLR camera (Nikon D810 Camera, Nikkor 60mm f2.8. AF-S Micro Lens). The eight photos were merged using Adobe Photoshop 2023 (Version 24.7.0). To map the location of each recruit within the flume chambers a grid consisting of 8 by 71 cells was created using RStudio (Posit team 2023), corresponding to a velocity grid created for the flow chambers derived in PIVLab. The grid was then deformed in Adobe Illustrator with the tool “Perspective Distortion” to account for the differences in scaling due to the varying distance from the camera due to the slope inside of the chambers for flow manipulation and added to the merged photos of the flumes to fit the tile surface (Adobe Illustrator 2023, Version 27.8.1). Survival data was extracted from the merged and grid-mapped photos, associating the individual coral recruits to the specified scale and measured velocity values. Keeping the velocity value association, growth data were extracted from the eight raw photos to avoid any distortion of the coral recruits during the Photoshop merging algorithm. Growth was measured using the ROI manager tool in the Fiji software (Fiji, Version Fiji for Mac OS X) as described by Nordborg et al. (2022). The scale was calibrated for each photo individually to reduce the bias of the inclined ramp.

5.2.3.2 Statistical analysis

Analysis of the recruit survival and growth was conducted using R (version 4.4.0, R Core Team 2024) through the RStudio interface (Posit team 2023). For the survival, each individual recruit was accounted for, unlike for the growth analysis, which used patches of coral for measuring relative growth. The survival analysis included a total of 720 recruits, with 339 recruits in the first flume, 210 in the second and 171 in the third flume. For the recruit growth analysis, a minimal value of 0.01% growth was assigned to any negative and zero values to enable the use of a gamma distribution during model fitting. The growth analysis included 323 replicates (71% of the total data set) spread among the three flumes. Individual velocity values were assigned to each of the pseudo replicates.

A Bayesian generalized linear multivariate, multilevel model was fitted using the “brms” package (Bürkner 2017) to evaluate the relationship between recruit growth and water flow velocity. The model was fitted using a Gamma distribution with a log link function. Priors were defined as: intercept (normal(-2.3, 1)), coefficients (normal(0.1, 1)), and shape parameter (gamma(0.01, 0.01)). Additionally, the growth data was automatically scaled by the model. Posterior predictions were obtained by descaling the results at the minimum and maximum velocities. Percentage changes in growth and posterior probability distributions were visualized using ggplot2 (Wickham 2016).

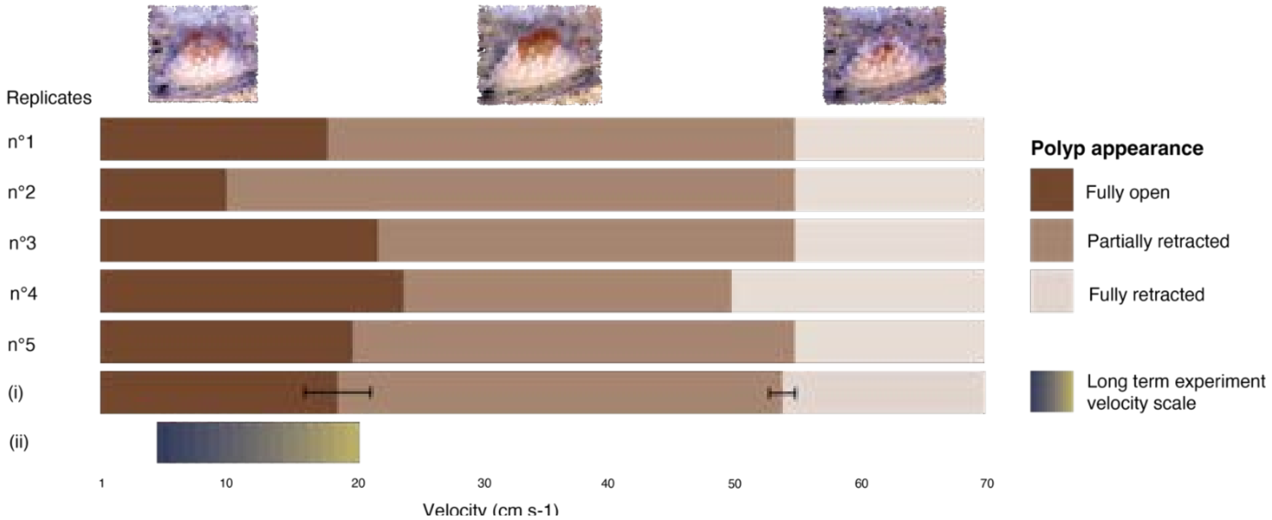

Figure 7. Comparison of morphological reactions of recently settled A. abrolhosensis recruits towards a stepwise increase (1 to 70 cm s-1) in flow velocity; (i) average responses in the polyp appearance when facing different flow velocities; (ii) indicative flow velocity range used in the long-term experiment compared to this short-term experiment. The three informative photos depict the same replicate at 7, 26 and 60 cm s-1, respectively. Error bars show standard deviation.

5.3 Results

5.3.1 Acute exposure

All five one-week old *A. abrolhosensis* recruits tested had fully extended their polyps at the end of the 15 min acclimation period, prior to commencing the acute exposure assay. Three morphological responses were observed to the rising flow velocity. At relatively low velocities (> 18.8 cm s^{-1} ± 5.40) a bending of the tentacles or a partial tissue retraction was observed (Figure 7). At higher flow velocities (> 54 cm s^{-1} ± 2.23) the tentacles were completely retracted into the primary corallite, as well as retraction of the living tissue situated along the outside edges of the coral skeleton, resulting in a jagged or sharp appearance (Figure 7).

5.3.2 Long-term rearing

5.3.2.1 Recruit survival

Acropora abrolhosensis recruits were grown under a gradient of flow velocities in three replicate flumes for the first 7 weeks following settlement. Overall, nine individual recruits died, out of the total 720, at the end of the 7 weeks. Hence, recruit survival was high (97.8, 99.8 and 99.7% for flume 1, 2 and 3 respectively) and no differences in survival could be detected between the flumes (Kruskal-Wallis, p-value = 0.249). Due to the low number of dead recruits, no further statistical analysis was performed for recruit survival.

5.3.2.2 Recruit growth

The growth of *A. abrolhosensis* recruits showed a positive correlation with flow velocity during the 7-week grow out period (Figure 8a). The difference in growth between recruits exposed to the maximum (20.1 cm^{-1}) and the minimum (5.4 cm s^{-1}) flow velocities was not statistically significant; however the median *A. abrolhosensis* recruits had grown more when reared at the maximum flow velocity (109% median relative growth; 104.7-113.8% 95% CIs) compared to when being exposed to minimum flow velocities (106.0% median relative growth; 103.1-109.7%; Figure 8a). Large variability in recruit growth was observed between the three flumes (sd(Intercept) = 0.37) and the overall variability was high (R^2 = 0.04; Figure 8).

Recruit growth over a 7-week rearing period was positively related to the water flow velocity up to the maximum velocity assessed (20 cm s^{-1})

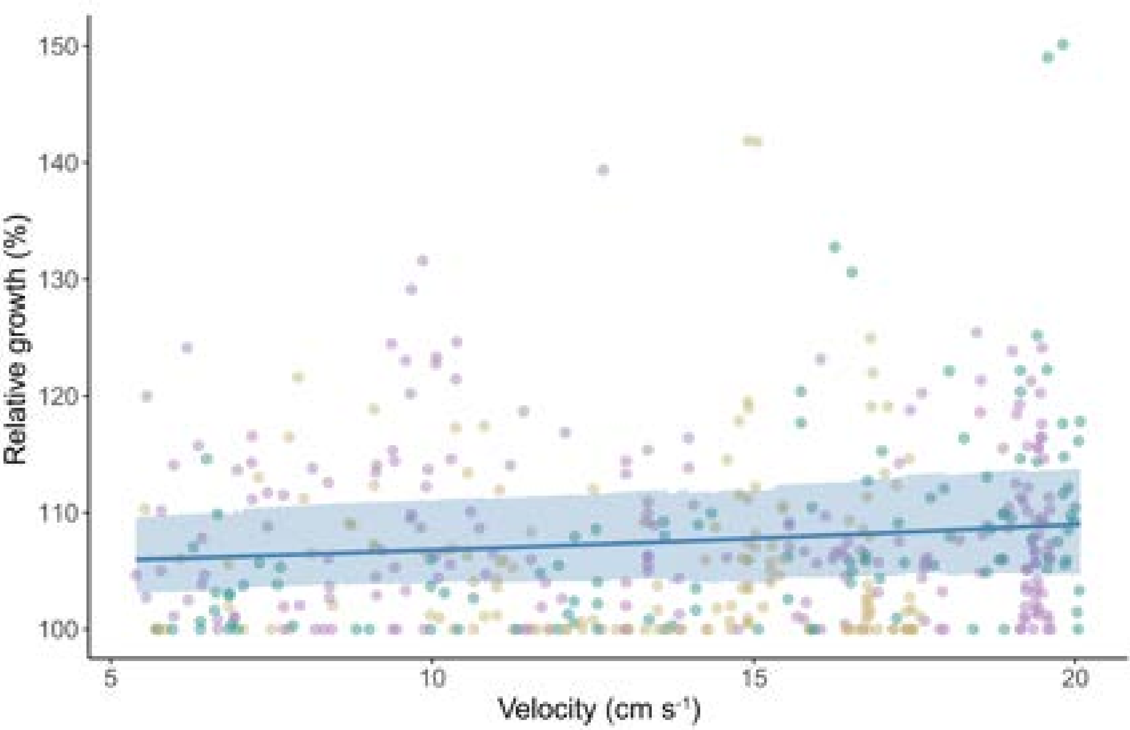

Figure 8. Relative growth of A. abrolhosensis recruits after 7 weeks of exposure to different flow velocities. Data points are shown for three different flumes, indicated by colour (Flume 1: purple, Flume 2: beige, Flume 3: teal). Model predictions (solid blue line) for relative growth (%) as a function of flow velocity (cm s^{-1}) and 95% credible intervals (shaded area) shown for model fitted for all three flumes.

5.4 Discussion

To enable upscaling and improved yield in coral aquaculture facilities all aspects of coral recruit biology need to be considered. Identifying the biological and ecological drivers of growth across gradients is the key to aquaculture success. Among others, understanding the relationship between water flow velocity and coral growth is crucial for designing high-performing aquaculture systems. The results of this study provide insights into the effects of both acute and longer-term exposures to a range of flow velocities on coral recruits. The drastic morphological responses of the *A. abrolhosensis* recruits at a flow velocities >50 cm s^{-1} in the acute exposures, and the positive correlation between water flow rate and recruit growth in the long-term grow-out, suggests that the growth-to-flow velocity response of coral recruits may be nonlinear at higher water flow rates.

Geertsma et al. (2022) suggested that morphological changes during acute exposure of coral recruits to a wide range of water flow rates could be used to determine the optimal water flow rate for long-term recruit rearing. While the polyp deformation results of this study are from a small number of replicates, results show that a partial polyp retraction response occurs at flow velocities of >18 cm s^{-1} for *A. abrolhosensis* recruits, indicating that the optimal water flow rate should be below this value (Geertsma et al., 2022). However, in the long-term grow-out experiment a similar range of flow velocities (>18 cm s^{-1}) resulted in the highest relative growth of *A. abrolhosensis* recruits of the same origin as those used in the acute exposures (Figure 8). Additionally, when comparing the polyp appearance during the two experiments, it is evident that none of the polyps exposed to flow velocities >18 cm s^{-1} in the long-term experiment were retracted or semi-retracted. This suggests that an acclimation period greater than 15 minutes may be required for *A. abrolhosensis* recruits when assessing morphological changes at different flow velocities, and that the short-term polyp deformation assay used in the present study may not be useful in identifying the optimal water flow rate for coral recruits if applied in isolation. Additionally, future recruit rearing studies assessing the optimum flow velocity for aquaculture rearing of coral recruits should include a wider range of flow velocities than those assessed in the long-term experiment of the present study.

KEY FINDINGS & RECOMMENDATIONS

6 Key findings & Recommendations

Achieving upscaled coral production that matches the ambitious, but necessary, reef restoration efforts being called for in Australia and across tropical regions worldwide requires improved cost-efficiency while maintaining or improving yields. Three key target areas for achieving the improvements required include reducing the spatial footprint of aquaculture systems, improving water efficiency and increasing yield of usable seeding units. Here we addressed aspects of these target areas by investigating:

(i) the impacts of substrate angle on recruit survival during *ex situ* rearing in the first 3 months post-settlement (Chapter 3)
(ii) whether there is a difference in recruit survival when recruits are reared in semi-recirculating aquaculture systems compared to flow-through (Chapter 4)
(iii) what the optimal water flow rate is for rearing of recently settled recruits, and if an optimal water flow rate for recruit rearing can be determined using acute assays (Chapter 5)

6.1 Key findings

Recruit survival for *A. millepora* was highest on vertical surfaces (90°) and lowest on horizontal surfaces (0°), regardless of the length of the rearing period of the material of substrates (Chapter 3). However, results highlighted the potential importance of maintaining continuous water flow of sufficient velocity to ensure recruit health, as exemplified by the significant drop in survival between the 1- and 2-month assessment timepoints, regardless of substrate angle (54.2, 62.7 and 68.0% drops for 0°, 60° and 90°, respectively). These results indicate that significant reductions in the spatial footprint of systems could be made (i.e. holding of 10 vertical tiles in the space of a single horizontal tile), without any loss of yield, as long as appropriate biotic and abiotic conditions are maintained within systems.

There was no difference in the *A. spathulata* recruit survival at the end of a 3-month rearing period in semi-recirculating and flow-through aquaculture systems when tank turnover rate and internal circulation are the same (Chapter 4). While not statistically significant, the median survival of recruits in semi-recirculating systems was ~3-fold higher than for the flow-through systems (26% and 7.5%, respectively). Similarly, the median yield of deployable seeding units was higher for semi-recirculating systems (33% and 14%, respectively). Based on the current study, the use of semi-recirculating aquaculture systems could save ~2,000 L of FSW per rearing tank and day without any reduction in yield.

Acute exposure assays of *A. abrolhosensis* recruits (*sensu* Geertsma et al. (2022)) indicated that the optimal water flow rate for rearing recruits of this species should be <18 cm s^{-1} (Chapter 5). However, the medium- to long-term growth of recruits from the same cohort reared under flow rate gradients (~5-26 cm s^{-1}) indicated that the optimal water flow rate for rearing of this species was >18 cm s^{-1} and that coral recruits may require a longer acclimation period than that proposed by Geertsma et al. (2022) prior to assessing morphological deformation of polyps for the purpose of determining optimal water flow rates for long-term rearing.

6.2 Recommendations for *ex situ* coral recruit rearing

The work presented here demonstrate that significant improvements in cost-efficiency and production scale can be achieved in coral aquaculture without a loss of productivity or the need for unfeasible facility expansions. In fact, some of the experimental results produced indicate that in some instances yield may be the same, or higher, for the more cost-efficient option (e.g. Chapter 4).

To optimise the space and cost-efficiency of coral recruit rearing while maintaining a high yield of usable seeding units in *ex situ* coral aquaculture the following recommendations, based on the work completed here and prior works and reviews (e.g. Guest et al. (2010); Omori and Iwao (2014); Randall et al. (2020), may be considered. However, recommendations should be considered in the context of the infrastructure available and objectives of the facility.

Table 2. Overview of recommendations for medium to -long-term coral recruit rearing in ex situ aquaculture facilities. Recommendations based on resulteds reported in the present study and from literature.

Aspect of recruit rearing	**Recommendation**	**Additional comments**	**Source**
Aquaculture system type	Semi-recirculating, system replacement rate of 300% day^{-1}	Given that sufficient filtration is used and a high enough water flow can be maintained within rearing tanks (e.g. using circulator pumps)	Present study
Orientation of recruits/substrate in tank	Vertical	Given that continuous water flow can be maintained across surfaces throughout tank and sufficient feed is used (to compensate for potential differences in light)	Present study; Omori and Iwao (2014)
Water replacement rate for rearing tanks	>100% h^{-1}	Consideration should be given to retention time for feeds within holding tank to ensure recruits have sufficient time to capture enough Replacement rate during feeding, or feeding densities, may be adjusted to compensate Even higher replacement rates are often recommended by/for hobby aquarists, and rates may need to be adjusted based on the biological load	Severati et al. (*personal communication*)
Water movement within tank	Species dependent, generally >5 cm s^{-1}	Should ideally be assessed for key target species, but relatively higher is typically considered better than no-flow	Present study; Geertsma et al. (2022); Rahnke et al. (2022)
Light	Initially low Gradual increases may be beneficial once recruits reach a threshold size	For recently settled recruits, heterotrophy may constitute a larger proportion of energy requirements, keeping light levels low may reduce competition with benthic algae. As algal symbionts populate tissues, symbiont autotrophy becomes a more dominant source of energy and may warrant gradual increases in intensity	Rahnke et al. (2022); Ramsby et al. (2024)
Feed	Mixture of live feeds, or commercially available coral feeds	Starting from when settlement and inoculation with symbiotic microbes have been completed. Live feeds may include rotifers, micro algae and larval crustaceans, e.g. Artemia nauplii	Lippens and Banaszak (2024); Petersen et al. (2008); Schutter et al. (2023)
Co-culture	Small herbivorous snails and young, herbivorous sea urchins, ideally >30 m^2	Size matters, large herbivores may still damage young recruits. Suitable species include: *Mespilia globulus, Calthalotia strigata,Turbo haynesi, Cerithium lutosum* and *Trochus niloticus*	Craggs et al. (2019); Lippens and Banaszak (2024); Neil et al. (2024); Neil et al. (2021); Omori and Iwao (2014)

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