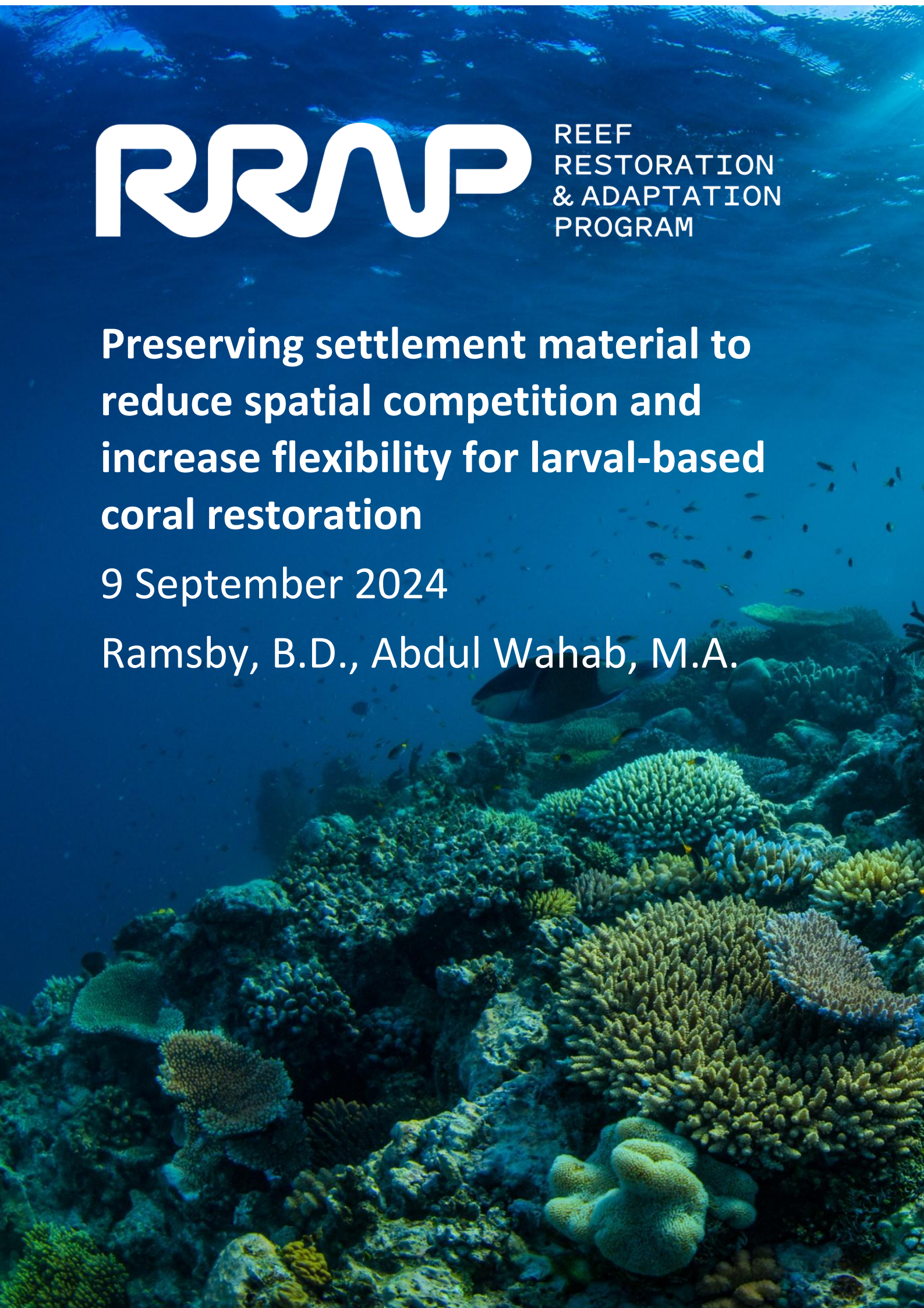

REEF RESTORATION & ADAPTATION PROGRAM

Preserving settlement material to reduce spatial competition and increase flexibility for larval-based coral restoration

9 September 2024

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Preserving settlement material to reduce spatial competition and increase flexibility for larval-based coral restoration

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

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We specifically acknowledge and thank the following Traditional Owners of sea Country that this report relates to:

Location	**Traditional Owner Group**
AIMS Townsville	Wulgurukaba
Davies Reef	Bindal

Table of Contents

1 Abstract

Active management interventions like coral seeding are increasingly being considered to increase coral populations after bleaching events or on degraded reefs. Coral seeding uses the coral reproductive cycle to spawn, fertilize, and settle coral larvae that are then grown into coral. However, since coral spawning typically occurs once per year for a given species (and location), the dependence on coral spawning puts concentrated demand on resources, including the need to efficiently settle up to millions in a short amount of time. Here, we tested four methods to preserve the conditioned settlement material required to settle coral larvae in order to enable the preparation of settlement time at any time or place. To be effective, preserved settlement material must maintain the cue for larval settlement and no promote algal competitors. We compared the efficacy of air dried, frozen, freeze dried, and oven dried settlement material alongside material with live CCA for their ability to settle and grow *Acropora spathulata* spat. In addition, we grew *A. spathulata* larvae on these materials at 5 or 50 µmol to determine whether light intensity affected the effectiveness of tile preservation at promoting spat survival and retarding algal growth. We found that larval settlement was similar between all preservation methods and material with live CCA, indicating that preservation retained settlement cues of the conditioned substratum. There was little difference in spat survival on preserved versus live material over 16 weeks, suggesting that competition was minimal on the live material. In fact, light intensity had a larger effect on survival than tile preservation, as survival was 20% lower at 50 compared to 5 µmol quanta m^{-2} s^{-1}. However, despite reduced survivorship, the spat that survived at 50 µmol were 4 times larger than those at 5 µmol, suggesting that keeping spat under minimal irradiance might have negative effects on long term survival. The largely similar results among preservation methods indicate that the choice of preservation method may be an economic one, as frozen and freeze-dried methods were marginally more effective, but require additional equipment and electricity compared to air drying. The ability to preserve settlement material increases the flexibility for larval restoration for coral reefs where funding, facilities, or time may be constrained.

2 Introduction

Rising sea surface temperatures and associated increases in frequency of mass coral bleachings have led to a poor prognosis for the future of coral reef ecosystems (Hughes et al. 2018). In addition to climbing temperatures, coral reefs continue to be affected by cyclone damage and associated run-off, devoured by crown of thorns starfish, and afflicted with devastating diseases (Muller et al. 2020). Managing bleaching requires reducing ocean temperatures which involves international cooperation to limit greenhouse gas emissions. However, regional outbreaks of crown of thorns starfish can be effectively managed using culling programs (Matthews et al. 2024). Interventions (i.e., human-assisted increases in coral populations) may also be able to mitigate losses of coral, but the feasibility of different actions is still being evaluated (Bay et al. 2023). Reef managers are faced with a predicament: inaction suggests that reefs recover on their own, which they might not be capable of in the future meanwhile the efficacy of management interventions including habitat engineering (Harrison 2024), selective breeding (Drury et al. 2022), and coral seeding (Banaszak et al. 2023), has not been fully vetted, especially over large scales or time periods (Bay et al. 2023).

One promising intervention is to use coral aquaculture to breed corals and return the offspring the reef (Chamberland et al. 2017). This intervention uses the prolific spawning of corals and increases the fertilization, settlement, and survival of larvae that can then be settled on the reef naturally or seeded using artificial substratum (Banaszak et al. 2023). While larval coral restoration has the potential to produce millions of new coral, coral seeding requires research and development for widespread application. The period of operation is constrained and concentrated as reproduction of broadcast spawning corals typically only occurs once per year (Randall et al. 2020). Therefore, understanding the reproductive timing of target species is required, as well as their larval competency periods and cues for settlement (Randall et al. 2024; Abdul Wahab et al. 2023; Baird et al. 2021).

In addition, coral seeding has to contend with large losses of larvae that can occur during culture, settlement, or after settlement (Doropoulos et al. 2016), which limit the production of coral. For example, 50% of settled larvae (or coral spat) can die in the days following settlement (Martinez & Abelson 2013; Cooper et al. 2014) and only ~1% of settled larvae survive to one year (Doropoulos et al. 2016). Therefore, maximizing the odds of spat survival can increase the yield of coral seeding.

On the reef, mortality of coral spat can be due to predation, incidental grazing, or spatial competition (Doropoulos et al. 2016). For example, grazing fish can reduce spat survivorship on artificial substrate by 50% (Whitman et al. 2024). Competition on the settlement substratum can reduce spat survivorship within coral seeding devices and in aquaria (Page et al. 2024; Ramsby et al. 2024). Settlement substrata are typically conditioned by exposure to seawater, which results in the growth of macroalgae and other competitors on the settlement surface, to enhance larval settlement. In particular, the presence of crustose coralline algae (CCA) induces the settlement of many coral species. However after settlement, however , crustose coralline algae can aggressively overgrow spat, especially under greater than 60 µmol quanta m^{-2} s^{-1} irradiance (Ramsby et al. 2024). Light intensity appears to amplify the intensity of competition for coral spat, as coral spat can tolerate higher CCA cover under lower irradiance than under higher irradiance (Ramsby et al. 2024). Therefore, measures to limit algal competition may increase survivorship of coral spat and the yield of coral seeding programs. Alternatively, the settlement material itself can influence competitive dynamics, as crustose coralline algae can colonize and spread on concrete relatively quickly compared to ceramic or calcium carbonate materials (Fong, Jackson, et al. 2024; Fong, Ramsby, et al. 2024). Therefore, the preservation of settlement material, which would kill any competitive algae, may reduce competition for recently settled coral spat and thereby increase their survival. In addition to reducing competition, tile preservation would enable the conditioning and storage of settlement substratum before an annual coral spawning event, which would free up time and aquarium space during a demanding period.

Coral seeding has used concrete for large-scale settlement of larvae (Miller et al. 2022; Chamberland et al. 2017), given its availability and affordability, but it also is rapidly colonized by competitive algae (Fong, Ramsby, et al. 2024; Fong, Jackson, et al. 2024). Recent work indicates that dead CCA (air-dried and

desiccated) can induce coral larval settlement (Lei et al. 2021). In this study, we compared the effectiveness of four preservation methods at inducing larval settlement, limiting fouling by crustose coralline algae, and promoting spat survivorship and growth. If preserved substratum can induce larval settlement, preservation of settlement materials has the potential to improve the survival of coral spat in aquaria and the yield of coral restoration.

3 Methods

3.1 Coral larvae and tile preservation

Twenty four *Acropora spathulata* colonies were collected from holding platforms on Davies Reef, QLD, Australia (2 m depth; 18°49'10.0"S, 147°37'52.5"E) in November 2023. Colonies had been previously collected on SCUBA using hammer and chisel, spawned at the AIMS National Sea Simulator (Townsville, QLD, Australia), and returned to platforms on Davies Reef in 2022. Corals were transported onboard a research vessel in flow-through seawater from the collection site to the AIMS National Sea Simulator where they were held in outdoor aquaria (280 × 100 × 44 cm aquarium in a 2800 L semi-recirculating system with 3 turnovers d^{-1}; 27 ºC, 120 max. µmol quanta m^{-2} s^{-1}). All colonies had emergent bundles and were isolated into individual aquaria 14 nights after the full moon. Bundles of sperm and egg were skimmed from the water surface above each colony using a plastic cup within 1 h of release. Bundles from all coral colonies were added to an 80 L flow-through tank with vigorous aeration to allow them to break and for bulk fertilization of eggs following Severati et al. (2024; AutoFertilizer module). 92% of eggs were fertilized and the zygotes were cultured for 7 days in two 500 L flow-through tanks (~1.2 larvae mL^{-1}) before they were settled on tiles treated using different preservation treatments.

Larvae were settled on conditioned concrete tiles. Three 280×280×10 mm concrete tiles were conditioned for six weeks in an outdoor aquarium with an established fouling community (280 × 100 × 44 cm aquarium in a 2800 L semi-recirculating system with 3 turnovers d^{-1}; 100 max. µmol quanta m^{-2} s^{-1}). The concrete tiles were cut into sixty-six 56×56 mm settlement tiles which were then divided amongst five tile preservation treatments: 1) maintaining them in flow-through aquaria (live CCA control), 2) freezing (48h in -20 ºC), 3) freeze-drying (24 h in -20 ºC freezer followed by 24 h in freeze dryer), 4) drying in air (24 h outdoors followed by 24 h in air conditioning), or 5) drying in an oven for 48 h (37 ºC). Settlement tiles were then rinsed three times in 500 mL 1 µm filtered seawater (FSW) immediately prior to use.

3.2 Larval settlement assays

Settlement tiles (n=32) were assessed for their ability to induce larval settlement. Each settlement tile was placed in individual plastic jars with 500 mL FSW and a layer of glass beads that prevented larval access to the side or bottom of the tile. Jars were kept in a temperature-controlled water bath at 27 ºC. 50 larvae were added to each jar and, after 48 h, the number of settled larvae (either on jar, on top of tile, or on side of tile) were recorded. Multiple settlement controls were assessed, including settlement in empty jars, in jars with only glass beads, and in jars with an unconditioned tile and a chip of CCA (*Porolithon* cf. *onkodes*; 3 x 3 mm). All control jars were filled with 500 mL FSW. Maximum settlement for these larvae was assessed using 10 larvae in 10 mL using control wells (no CCA chip; n=12) and wells with a CCA chip (n=12) in six well plates.

To meet the number of tiles required to assess the survival and growth of spat, larvae were settled on additional tiles (n=38: 8 live, 10 air, 8 oven, 4 frozen, 8 freeze-dried) in 50 L tanks. Live tiles were settled in separate tanks from preserved tiles to promote evenly-distributed settlement.

In the two weeks following the settlement assays, spat were inoculated twice with cultured type C1 Symbiodiniaceae (AIMS ID: SCF049-01). Settled tiles were exposed to 20,000 cells mL^{-1} and 50,000 cells mL^{-1} for 12 h.

Table 1: Sample sizes for settlement assay.

Type	Jar contents	Jars (n)
Settlement tile	Live tile	6
	Air dried tile	5
	Oven dried	8
	Freeze dried tile	6
	Frozen tile	7
Control	Unconditioned tile + CCA chip	6
	Glass beads only	6
	Empty jar	6

3.3 Survival and growth under 5 or 50 µmol

The survival and growth of spat on preserved and live tiles were monitored over 16 weeks. Tiles from the settlement assay and the 50 L settlement were divided amongst eight 30 L tanks (n=7-9 per tank). Each tank contained 1-2 tiles from each preservation treatment, including live CCA. Each tank received flow-through 0.8 L min^{-1} FSW (26.9 ºC ± 0.05 SD), contained an air bubbler for circulation, and received either ~5 or 50 maximum µmol quanta m^{-2} s^{-1} from LED panels. Spat were fed on weekdays with artemia (0.5 nauplii mL^{-1}) and rotifers (1 mL^{-1}).

Tiles were photographed fortnightly for 16 weeks to measure survival and growth. Photographs were captured using either an Olympus TG-6 or Nikon D810 camera at resolutions of ~40 or 100 pixels mm^{-1}. Survival was measured as the proportion of live spat observed at each timepoint out of the maximum number observed on each tile. Clumps of spat were recorded as one spat. The size of individual spat on each tile was measured in Ilastik software by training a pixel classifier to identify spat on the settlement tiles and then using an object classifier to distinguish individual spat from clumps of spat. In addition to identifying spat, a classifier was also trained to identify CCA to calculate percent cover of the tile area. CCA cover was measured as a proportion of pixels classified as CCA out of the total number of pixels of each tile.

3.4 Statistical analysis

CCA cover was analyzed using a generalized linear mixed model with a negative binomial error distribution and log link. Predictors included the source of large tiles used to create settlement tiles, the light treatment, tile preservation treatment, and light×preservation combinations. The model included a random intercept to account for differences in the number of light treatment tanks (n = 8). Pairwise post hoc comparisons were performed to determine significant differences within predictors. P-values were adjusted using the Tukey method when multiple comparisons were performed.

Larval settlement was analyzed using a generalized linear model with a binomial error distribution and log link. Predictors included the source of large tile used to create settlement tiles and the tile preservation treatment.

Spat survival after 16 weeks was analyzed using a generalized linear mixed model with a binomial error distribution and a logit link. Fixed predictors included the tile preservation treatment (live, air

dried, oven dried, freeze dried, frozen), light treatment (5 or 50 µmol m^{-2} s^{-1}), combinations of preservation and light treatments, and the source of the settlement tile. Tank was included as a random intercept.

Recruit size after 16 weeks was analyzed using a linear mixed model with the same predictors as for survival and an additional random intercept for the settlement tile.

For all responses, post hoc comparisons were performed to compare treatments within significant predictors. P-values were adjusted using the Tukey method when multiple comparisons were performed.

Table 2: Number of settlement tiles for survival and spat for growth.

Tile preservation	**5 µmol Tiles / Spat (n)**	**50 µmol Tiles / Spat (n)**
Live tile	6 / 107	7 / 98
Air dried tile	8 / 130	7 / 177
Oven dried	8 / 89	6 / 191
Freeze dried tile	7 / 115	6 / 153
Frozen tile	6 / 80	5 / 63

4 Results

4.1 Tile preservation and CCA cover

At the start of the experiment, settlement-inducing crustose coralline algae (CCA) covered 11% of the live settlement tiles, which was lower than previous studies (e.g., Ramsby et al. 2024). All preserved tiles had no detectable live CCA after preservation.

After 16 weeks under grow-out conditions, the cover of CCA on live conditioned tiles increased to 30-45% regardless of light treatment (Figure 1). Over this time, cover on preserved tiles only increased to 3.6% ± 0.8 SE at 50 µmol and was barely detectable at 5 µmol. These changes suggest that while CCA continued to grow on the live tiles, there is little CCA seeding between live and preserved tiles when both were held in the same experimental grow-out tank, and that higher light intensity can also accelerate CCA seeding. Subtle differences in final CCA cover were detected among preservation treatments (Table 3), including higher CCA cover on frozen versus freeze dried tiles (at 5 µmol) and relatively low CCA cover on oven dried tiles (at 50 µmol; Table 4).

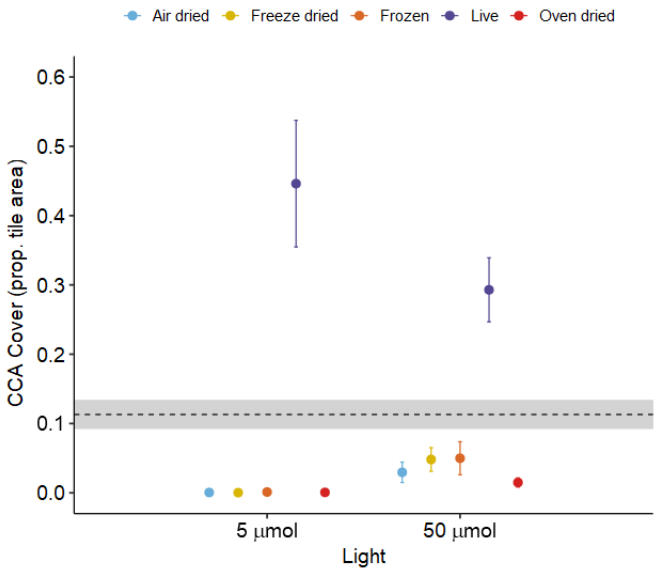

Figure 1. Crustose coralline algae cover on settlement tiles after 16 weeks in aquaria. Tiles were kept at either 5 or 50 µmol. The initial cover on the live-conditioned tiles is indicated by the dashed line and SE by the gray shading. Points represent means and SE among tiles.

Table 3. Statistical results for CCA cover at 16 weeks. CCA cover was analyzed using a generalized linear mixed model with a negative binomial error distribution and log link. Predictors included the large tile used to create settlement tiles, the light treatment, tile preservation treatment, and light×preservation combinations. The table includes the df, chi-square statistic, and p-value from Wald chi-square tests. Significant p values are shown in bold.

Predictor	Df	X^2
Large tile	2	4.6
Light	1	45.4
Tile preservation	4	288.0
Light×Preservation	4	73.5

Table 4. Post hoc comparisons of CCA cover among tile preservations (Pres; FD=freeze dried) within 5 and 50 µmol light treatments. For each comparison, listed are the two preservations being compared, the ratio between estimates for Pres 1/Pres 2, the SE of the ratio, Z ratio, and p value. Significant p values are shown in bold.

		5 µmol					50 µmol			
Pres 1	**Pres 2**	**Ratio**	**SE**	**Z**	**p**		**Ratio**	**SE**	**Z**	**p**
Air	FD	1.38	0.74	0.60	0.98		0.44	0.19	-1.93	0.30
Air	Frozen	0.25	0.13	-2.56	0.08		0.40	0.18	-2.02	0.26
Air	Live	<0.01	0.00	-13.57	**<0.01**		0.06	0.03	-6.56	**<0.01**
Air	Oven	0.60	0.40	-0.77	0.94		1.45	0.57	0.93	0.88
FD	Frozen	0.18	0.09	-3.27	**0.01**		0.91	0.41	-0.21	1.00
FD	Live	<0.01	0.00	-14.24	**<0.01**		0.14	0.06	-4.69	**<0.01**
FD	Oven	0.44	0.30	-1.22	0.74		3.28	1.36	2.87	**0.03**
Frozen	Live	<0.01	0.00	-11.26	**<0.01**		0.16	0.07	-4.09	**<0.01**
Frozen	Oven	2.47	1.67	1.34	0.67		3.61	1.60	2.89	**0.03**
Live	Oven	612.31	384.56	10.22	**<0.01**		23.21	9.31	7.84	**<0.01**

4.1 Settlement

Settlement assays indicated that preserved tiles settled A. spathulata larvae as well as live-conditioned tiles in 500 mL assays and nearly as well as observed in small-volume assays (10 mL). In 10 mL assays, 70±5% of A. spathulata larvae settled when cued by CCA whereas 0% of larvae settled without a CCA cue. In the 500 mL assays, an average of 38-58% of larvae settled depending on the preservation treatment, suggesting that most of the settlement cue was preserved (Figure 2,Table 5). There were some differences in settlement among preservation treatments, as oven dried tiles (36±8%) had significantly lower settlement than live (53±13%), freeze dried (58±6%), or frozen tiles (54±4%). In addition, air dried tiles had significantly lower settlement than freeze dried tiles. These results indicate that the 'cold' preservations (freezing and freeze drying) were somewhat better than 'hot' preservations (oven drying and air drying) and were similar to live conditioned tiles in settlement efficiency (Table 6). In comparison, only 22±4% of larvae settled on unconditioned settlement tiles with a CCA cue.

Settlement was also influenced by variation in the conditioning among the three source tiles used to create settlement tiles, as settlement tiles from one source had 30% lower settlement than tiles from another source regardless of which preservation treatment was used (Table 5, Table 7).

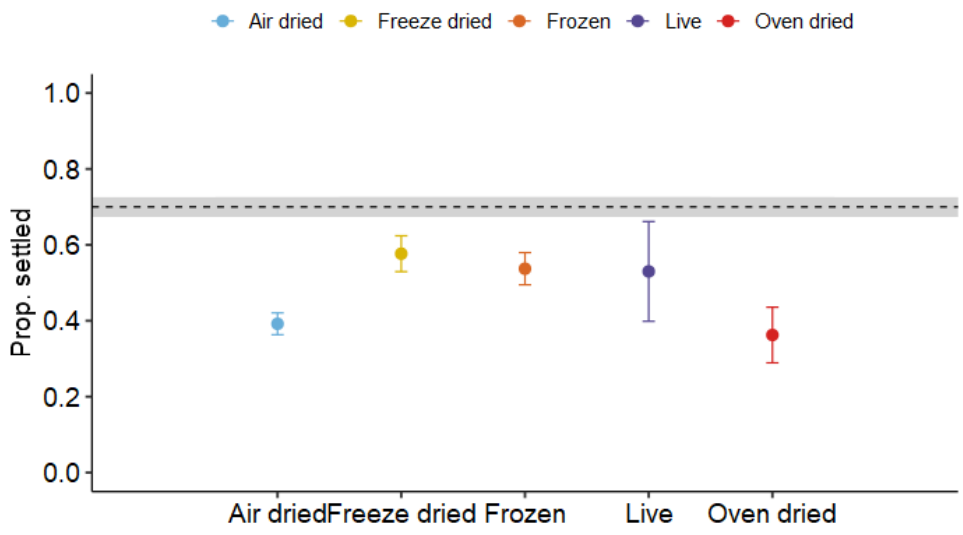

Figure 2. The proportion of larvae settled on tiles preserved using different methods. Points and error bars represent means and SE. The dashed line and gray bay represent the expected maximum mean and SE settlement of the same larvae in 10 mL assays when presented with a live CCA chip.

Table 5. Statistical results for larval settlement. Settlement was analyzed using a generalized linear model with a binomial error distribution and log link. Predictors included the source tile used to create settlement tiles and the tile preservation treatment. The table includes the df, chi-square statistic, and p-value from Wald chi-square tests for each predictor. Significant p values are shown in bold.

	df	X^2	p
Tile preservation	4	21.2	**<0.01**
Tile source	2	9.9	**<0.01**

Table 6. Post hoc comparisons of larval settlement among tile preservation treatments. For each comparison, lists the two preservations being compared, the odds ratio between estimates, the SE of the ratio, Z ratio, and p value. Significant p values are shown in bold. FD – Freeze dried.

Pres. 1	Pres. 2	Odds ratio	SE	Z ratio	*p*
Live	Air	1.40	0.22	2.2	0.19
Live	Oven	1.52	0.22	3.0	**0.02**
Live	FD	0.91	0.12	-0.7	0.95
Live	Frozen	0.99	0.13	-0.1	1.00
Air	Oven	1.10	0.17	0.56	0.98
Air	FD	0.65	0.10	-2.8	**0.04**

Table 7. Post hoc comparisons of larval settlement among sources for settlement tiles. For each comparison, lists the two preservations being compared, the odds ratio between estimates, the SE of the ratio, Z ratio, and p value. Significant p values are shown in bold.

Source 1	Source 2	Ratio	SE	Z ratio	p	Source 1
A	B	1.42	0.16	3.1	**<0.01**	A
A	C	1.08	0.11	0.8	0.73	A
B	C	0.76	0.08	-2.3	**0.05**	B

4.2 Survival

Survival was generally >80% after 16 weeks of grow-out, with tile preservation and light intensity having independent significant effects on spat survival (Figure 3, Table 8). Survival was significantly lower at 50 µmol compared to 5 µmol (by 13%) regardless of the tile preservation treatment. However, there was no significant difference in survival between any two preservation treatments (Table 9). There was little evidence that survival varied among preservation and light treatment combinations or among source tiles (Table 8).

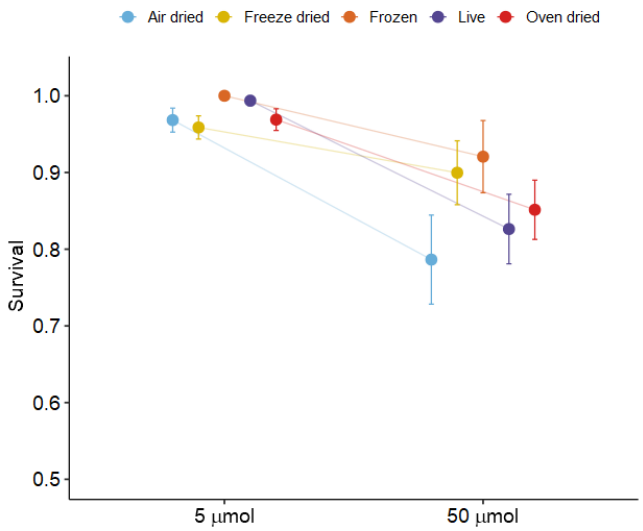

Figure 3. Proportion of spat surviving on live or preserved tiles over 16 weeks at 5 or 50 µmol. Points represent means and error bars represent SE.

Table 8. Statistical results for spat survival after 16 weeks. Survival was analyzed using a generalized linear mixed model with a binomial error distribution and logit link. Predictors included the source tile used to create settlement tiles, the tile preservation treatment, the light treatment, and the combination of preservation and light treatment. In addition, a random intercept was used to account for differences between light treatment tanks. The table includes the df, chi-square statistic, and p value from Wald chi-square tests for each predictor. Significant p values are shown in bold.

	Df	X^2	p
Light	1	16.4	**<0.01**

Tile preservation	4	16.5	**<0.01**
Tile source	2	2.2	0.34
Light×Preservation	4	3.4	0.50

Table 9. Post hoc comparisons of spat survival among tile preservation treatments (FD = freeze dried). For each comparison, lists the two preservations being compared, the odds ratio between estimates, the SE of the ratio, z ratio, and p value.

Pres. 1	**Pres. 2**	**Ratio**	**SE**	**z ratio**	***p***
Air	FD	0.49	0.00	-1.97	0.28
Air	Frozen	0.00	0.00	0.00	1.00
Air	Live	0.48	0.00	-1.32	0.68
Air	Oven	1.00	0.00	0.00	1.00
FD	Frozen	0.00	0.00	0.00	1.00
FD	Live	0.98	1.00	-0.04	1.00
FD	Oven	2.05	1.00	1.81	0.37
Frozen	Live	1.22×10^{4}	5.51×10^{7}	0.00	1.00
Frozen	Oven	2.56×10^{4}	1.16×10^{8}	0.00	1.00
Live	Oven	2.10	1.00	1.28	0.70

4.3 Size

Spat size was strongly influenced by light intensity with some evidence that tile preservation contributed to the difference in spat size between light intensities (Table 10). Spat were nearly four times larger when grown under 50 µmol and recruits at 5 µmol had not grown from their initial size (Figure 4). Tile preservation did not affect recruit size when recruits were grown at 5 µmol, however at 50 µmol recruits on freeze-dried tiles were significantly smaller than recruits on air dried or frozen tiles (Table 11). Notably, there were no significant differences in spat size between live conditioned and preserved tiles (Table 11). The source tile used to make settlement tiles was not associated with significant differences in final spat size (Table 10).

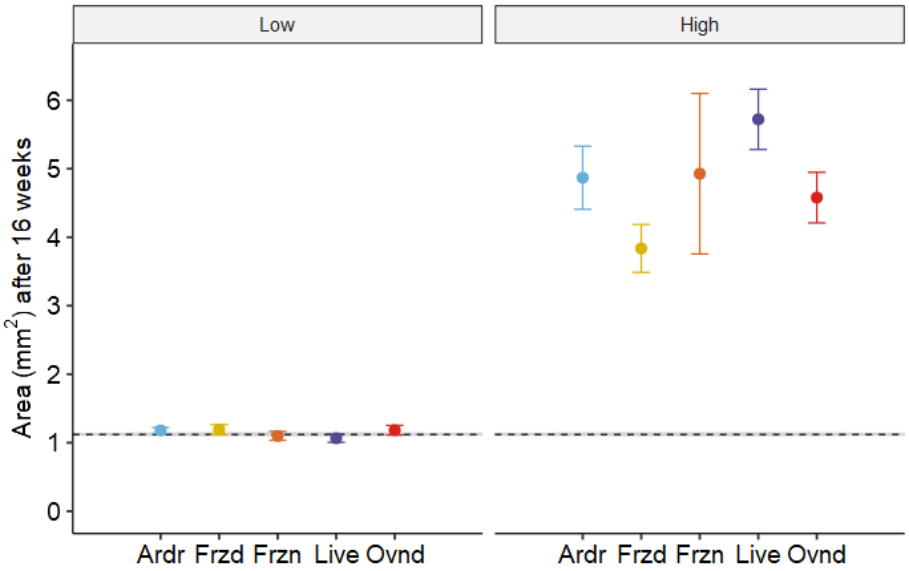

Figure 4. Spat area after 16 weeks at 5 or 50 µmol m^{-2} s^{-1}. Points represent means and error bars represent SE. The dashed line and gray region indicate the mean and SE of spat size 2 weeks after settlement. Tile preservation treatments are abbreviated on the x-axis (Air dried, freeze dried, frozen, live conditioned, and oven dried)

Table 10. Statistical results for spat size after 16 weeks. Size was analyzed using a linear mixed model with a log transformation. Predictors included the source tile used to create settlement tiles, the tile preservation treatment, the light treatment, and the combination of preservation and light treatment. In addition, a random intercept was used to account for differences between light treatment tanks. The table includes the df, chi-square statistic, and p value from Wald chi-square tests for each predictor. Significant p values are shown in bold.

Predictor	Df	X2	p
Light	1	167.5	**<0.01**
Tile pres.	4	4.6	0.33
Tile source	2	0.8	0.68
Light × Pres.	4	11.6	**0.02**

Table 11. Post hoc comparisons of spat size among tile preservation treatments. For each comparison, lists the two preservations being compared, the ratio between estimates (Pres 1/Pres 2), the SE of the ratio, t ratio, and p value.

		5 µmol						50 µmol				
Pres. 1	**Pres. 2**	**Est**	**SE**	**Df**	**t**	**p**		**Est**	**SE**	**Df**	**t**	**p**
Air	FD	<0.01	0.10	43.1	-0.02	1.00		0.30	0.11	37.6	2.75	0.07
Air	Frozen	0.06	0.11	46	0.60	0.97		-0.08	0.12	50.8	-0.69	0.96

Air	Live	0.08	0.11	40.1	0.77	0.94		-0.11	0.12	58.4	-0.93	0.89
Air	Oven	-0.02	0.11	43.5	-0.17	1.00		0.10	0.10	38	1.03	0.84
FD	Frozen	0.07	0.11	47.9	0.58	0.98		-0.38	0.13	50.6	-2.97	**0.03**
FD	Live	0.08	0.11	44.6	0.73	0.95		-0.40	0.12	59.6	-3.25	**0.02**
FD	Oven	-0.02	0.11	45.8	-0.14	1.00		-0.20	0.11	38.1	-1.86	0.36
Frozen	Live	0.02	0.12	45.9	0.15	1.00		-0.02	0.14	68.00	-0.16	1.00
Frozen	Oven	-0.08	0.12	47.1	-0.70	0.95		0.19	0.12	54.9	1.52	0.55
Live	Oven	-0.10	0.12	43.1	-0.87	0.91		0.21	0.11	63.5	1.83	0.37

5 Discussion

The preservation methods tested were effective in retaining the settlement cues that promote metamorphosis of coral larvae directly onto the substrate and minimized the growth of competitive algae. However, the survival and growth of spat was similar between the preserved materials and material with live conditioning, likely due the low initial cover of crustose coralline algae (CCA; ~11%) and the low irradiance (≤50 µmol m^{-2} s^{-1}) that the coral were grown under. There were subtle differences among preservation methods in promoting larval settlement and limiting algal fouling, but not in the survival or growth of coral spat. We anticipate that preserved material would be more effective than live-conditioned material, specifically in controlling competition and overgrowth, if the initial cover of CCA was higher (e.g., 43% in Ramsby et al. 2024). Nonetheless, the preservation of settlement material provides buffering capacity for a conservation aquaculture facility, by enabling year-round conditioning and storage of substrate that are independent to intensive periods of coral spawning and production, and the distribution of these settlement material to other aquaculture facilities.

One of the challenges of larval based coral restoration is that many larval species require CCA to settle (Heyward & Negri 1999; Abdul Wahab et al. 2023; Randall et al. 2024), but the algae can subsequently outcompete coral spat for space and overgrow them post-settlement (Ramsby et al. 2024; Page et al. 2024). Here, the relatively low live CCA cover on the substrate at settlement likely nullified a major advantage of preserved material in limiting competition for settled spat. While preservation was effective, whereby preserved tiles only had 4% live CCA cover after 16 weeks compared to >29% on live tiles, the decision as to which preservation method to use may be based on economics rather than performance. While there were no differences in survival or growth among the preservation treatments, it is notable that oven and air-dried material had lower larval settlement efficiency. The heat from the drying oven may degrade settlement-inducing compounds. In terms of fouling by CCA, oven and freeze dried material had significantly less CCA cover, although cover was <5% among all preservation treatments. Given the similar performance, the low cost and scalability of air drying tiles likely suits many restoration programs, as freezer, freeze dryers, or drying ovens are more expensive and more laborious to preserve large numbers of tiles.

Light had a larger effect on spat survival and growth than the preservation treatments. Keeping spat at 50 µmol translated to ~13% lower spat survival when compared to 5 µmol but were four-fold larger across all preservation treatments. The decline in survivorship in this study was much smaller than when spat were grown at similar irradiance levels on live material (Ramsby et al. 2024). Previously, spat survivorship was XX% at 60 µmol on live material, suggesting that limiting or eliminating competing organisms promoted survival and increased the energy available for growth. Larger spat are more likely to survive (Doropoulos et al. 2012), which suggests that the combination of preserved settlement material and moderate irradiance level would maximize the yield of larval restoration activities.

This study demonstrated that the preservation of settlement material can effectively settle coral larvae and grow coral spat. While several methods were tested here, the choice of preservation method will likely be dictated by resources available to a given project. Tile preservation enables year-round conditioning of settlement tiles and also the transport of settlement material to remote restoration sites. This study also raises important questions of how long preserved material can effectively settle and grow coral spat, as this study tested material that was preserved immediately before use, and what accounts for variation in between effective and ineffective materials that have been preserved in the same way.

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