


<https://doi.org/10.15517/rev.biol.trop..v71iS1.54864>

Settlement and post-settlement survival of *Orbicella annularis* and *Orbicella faveolata* (Scleractinia: Merulinidae) on substrates with coatings

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Received 30-IX-2022.

Corrected 07-III-2023.

Accepted 13-III-2023.

ABSTRACT

Introduction: One of the main bottlenecks in restoration projects based on sexual reproduction is post-settlement survival, mainly due to competition for substrate with fleshy algae and predation. Therefore, substrates of different shapes and materials have been created and tested, seeking to optimize these processes with attractive surfaces for the larvae and structures where the recruits are protected from predation, and competition is reduced.

Objective: To improve settlement and post-settlement survival of two important Caribbean reef-building corals, using different coatings on substrates.

Methods: To determine whether substrate coatings properties are favourable to larval settlement in *Orbicella annularis*, and *O. faveolata*, collected in Puerto Morelos, Mexican Caribbean, we evaluated their settlement for three weeks on six coatings with a combination of properties. Each coating was designed to provide a combination of two out of three properties: 1) water repellence (hydrophobicity), 2) phosphorescence-based colour, and 3) mineral-enriched surface chemistry. In a separate experiment larvae settlement was tested using coatings with a single property. Finally, we determined the post-settlement survival of *O. annularis* and *O. faveolata* on the different coatings for seven weeks.

Results: The combination of high hydrophobicity and light blue phosphorescent microparticles and high hydrophobicity and red-orange phosphorescent microparticles resulted in a higher settlement of *O. annularis* and *O. faveolata* when compared with other coatings (30.8 - 66.7 % higher). No significant differences were found in the number of larvae settled when the water-repellence and the phosphorescence-based were evaluated independently. Post-settlement survival time on substrates was low, with a maximum of 34 days after settlement for *O. annularis* and 42 days for *O. faveolata*.

Conclusions: In terms of the larval settlement, the combination of the coatings properties appears to play an essential role in the choice of microhabitat for both *O. annularis* and *O. faveolata*. But individually these properties did not generate an advantage in the larval settlement. Moreover, some chemical components associated with the coatings may be counterproductive to the survival of the polyps over time.

Key words: reef restoration; sexual reproduction; scleractinian corals; reef conservation; coral larvae.



RESUMEN

Asentamiento y supervivencia post-asentamiento de *Orbicella annularis* y *Orbicella faveolata* (Scleractinia: Merulinidae) sobre sustratos con recubrimientos.

Introducción: Uno de los principales cuellos de botella en proyectos de restauración basada en reproducción sexual es la supervivencia de las larvas posterior al asentamiento, principalmente por la competencia por el sustrato con algas filamentosas y la depredación. Por ello, se han creado y analizado sustratos de diferentes formas y materiales, buscando optimizar estos procesos con superficies atrayentes para las larvas, y estructuras donde los reclutas se encuentran protegidos de la depredación y se disminuya la competencia.

Objetivo: Mejorar el asentamiento y la supervivencia de dos importantes corales formadores de arrecifes del Caribe, utilizando diferentes recubrimientos en sustratos.

Métodos: Para determinar si las propiedades de la superficie del sustrato son favorables para el asentamiento de larvas de *Orbicella annularis* y *O. faveolata*, recolectadas en Puerto Morelos, Caribe mexicano, evaluamos su asentamiento durante tres semanas en seis recubrimientos con una combinación de propiedades. Cada recubrimiento fue diseñado para proporcionar una combinación de dos de tres propiedades: 1) repelencia al agua (hidrofobicidad), 2) fosforescencia y 3) química superficial enriquecida con minerales. En un experimento separado se evaluó el asentamiento de larvas en sustratos con recubrimientos de una sola propiedad. Finalmente, se determinó la supervivencia posterior al asentamiento de *O. annularis* y *O. faveolata* sobre los diferentes recubrimientos durante siete semanas.

Resultados: La combinación de alta hidrofobicidad y micropartículas fosforescentes azules y alta hidrofobicidad y micropartículas fosforescentes rojo-naranja dio como resultado un mayor asentamiento de *O. annularis* y *O. faveolata* en comparación con otros recubrimientos (30.8 - 66.7 % mayor). No se encontraron diferencias significativas en el número de larvas asentadas cuando se evaluaron de forma independiente la repelencia al agua y la fosforescencia. El tiempo de supervivencia posterior al asentamiento en los sustratos fue bajo, con un máximo de 34 días después del asentamiento para *O. annularis* y 42 días para *O. faveolata*.

Conclusiones: En el asentamiento de larvas, la combinación de las propiedades del recubrimiento parece desempeñar un papel importante en la elección del microhábitat tanto para *O. annularis* como para *O. faveolata*. Pero de forma individual estas propiedades no generaron una ventaja en el asentamiento larvario. Además, algunos componentes químicos asociados con los recubrimientos pueden ser contraproducentes para la supervivencia de los pólipos a lo largo del tiempo.

Palabras clave: restauración de arrecifes; reproducción sexual; corales escleractinios; conservación de arrecifes; larvas de coral.

INTRODUCTION

Coral reefs are characterized by high biodiversity, hosting 32 % of all known marine species and provide essential ecosystem services that contribute to the well-being of millions of people (Fisher et al., 2015; Knowlton et al., 2010; Woodhead et al., 2019). They are considered endangered ecosystems (Carpenter et al., 2008) and, most importantly, one of the most sensitive ecosystems to climate change (Riegl et al., 2009). Since 1970, coral cover in the Caribbean has decreased by 80 %, mainly due to the high mortality of reef-building corals, which has led to a phase-shift from coral to algae, and a loss of ecological functionality, as the result of both natural and anthropogenic impacts (Álvarez-Filip et al.,

2022; Arias-González et al., 2017; Aronson & Precht, 2001; Jackson et al., 2014; Rioja-Nieto & Álvarez-Filip, 2019). This has brought about the loss of recruitment for the main reef-building corals (Edmunds & Elahi, 2007; Edmunds et al., 2011; Hernández-Delgado et al., 2014; Quinn & Kojis, 2005; Williams et al., 2008), which include the species *Orbicella annularis* (Ellis & Solander, 1786) and *Orbicella faveolata* (Ellis & Solander, 1786).

Larval settlement as well as survival and growth of coral species, are integral to the ongoing maintenance of coral populations and necessary for the recovery of coral reefs (Ritson-Williams et al., 2009). Many factors can impact these processes, such as the abundance of fleshy algae, competition with other benthic invertebrates, predation, and sedimentation

(Pizarro et al., 2007). To assist in the recruitment of corals, restoration strategies have become more critical over the past decades and have involved culturing coral settlers from gametes to improve the genetic diversity of coral population (Baums et al., 2019). However, one of the significant bottlenecks in this process is post-settlement survival and coral settler growth (Ritson-Williams et al., 2009), mainly due to competition with fleshy algae and predation (Edwards & Gomez, 2007), but also include important abiotic factors such as water clarity, sedimentation, nutrient enrichment, salinity, and temperature (Richmond, 1997).

In controlled conditions (*Ex-situ*) settlement may be enhanced by using substrates with surfaces containing specific larvae-attracting attributes, including varying colour phosphorescence properties. Phosphorescence is a specific type of photoluminescence whereby, once the light has been absorbed, it is emitted and continues to be emitted even after excitation has ceased (Valeur & Berberan-Santos, 2011). It is a property that can generate micro-environments of coloured light in otherwise dark conditions, sometimes preferred by certain species as a condition for settlement (Mason et al., 2011; Strader et al., 2015).

Post-settlement survival may also be enhanced by using substrates with surface properties that may promote a reduction in competition, nutrient supply, and coral skeleton growth. This can be achieved by engineering substrate coatings with different chemical properties and hydrophobicity (Levenstein et al., 2022). Hydrophobicity can play an important role in reducing competition with algae from the genus *Ulva*, as it can affect algal spore adhesion and the strength of the adhesion (Chaudhury et al., 2006; Finlay, 2002; Fletcher & Callow, 1992). On the other hand, the presence of hydroxyapatite on the substrate's surface can be a source of phosphate released as the hydroxyapatite potentially dissolves in low phosphate ion concentration scenarios in seawater (Atlas & Pytkowicz, 1977; Stumm & Morgan, 1996). This nutrient may be taken up by symbiotic dinoflagellates and converted into

essential organic molecules (Ezzat et al., 2016). Similarly, the presence of calcium carbonate on a substrate's surface provides a source of carbonate and calcium ions, the building blocks of aragonite-based coral skeletons, which can be rapidly released upon $\text{CaCO}_{3(s)}$ dissolution in response to local variations in seawater chemistry, affecting $\text{CaCO}_{3(s)}$ saturation state, such as acidity build up in the vicinity of the coral tissue-seawater interface (Venn et al., 2013).

The research presented here aims to investigate the synergistic effects of specific physical-chemical properties (hydrophobicity, phosphorescence and mineral-enriched surface chemistry) on settlement as well as post-settlement survival for *O. annularis*, and *O. faveolata* from Puerto Morelos, Mexico.

MATERIALS AND METHODS

Substrate fabrication: In 2019, coatings were applied to commercially available floor tiles to test a combination of properties and their potential effect on settlement and post-settlement survival of coral larvae. Each coating was designed to provide a combination of two out of three properties: 1) water repellence (as determined by its degree of hydrophobicity), 2) phosphorescence-based colour, and 3) mineral-enriched surface chemistry. The ceramic floor tiles (Interceramic[®], Mexico 5 cm × 5 cm × 5 mm) were used as the substrate to which six sets of coatings (C1 to C6) were applied (Table 1). The tile surfaces were roughened using water-based sandpaper to improve adhesion before applying the coating. A hydrophobic polyester resin coating served as a base for the application of micro or nanoparticles and was applied to all tiles except C6. Tiles C1-C3 contained microparticles of strontium aluminate salts with varying relative concentrations of rare earth elements europium and trivalent dysprosium ($\text{SrxEuYDyZAl}_2\text{O}_4$), each yielding specific colour phosphorescent properties (Distribuidora Química Textil S.A. de C.V., Mexico): light blue (C1), red-orange (C2) and turquoise (C3). Phosphorescent microparticles in tiles C2 also contained a



TABLE 1
Properties and characteristics of substrate coatings.

Substrate ID	White paint	Hydrophobic coatings		Characteristics of micro or nanoparticles	
		Intermediate hydrophobicity polyester resin	Superhydrophobic polysiloxane coating	Size particles	Properties
C1	No	Yes	Yes	Micro	Light blue phosphorescent
C2	No	Yes	Yes	Micro	Red-orange phosphorescent
C3	No	Yes	Yes	Micro	Turquoise phosphorescent
C4	No	Yes	No	Nano	Hydroxyapatite
C5	No	Yes	No	Nano	Amorphous calcium carbonate
C6	No	No	Yes	Nano	Amorphous calcium carbonate
T1	Yes	Yes	Yes	No	No
T2	Yes	Yes	No	Micro	Light blue phosphorescent
T3	Yes	Yes	No	Micro	Red-orange phosphorescent
T4	Yes	Yes	No	No	No
T5	Yes	No	No	No	No
T6	No	No	No	No	No

coumarin-type red-emitting fluorescent pigment. On tiles C4, 60 nm hydroxyapatite nanoparticles [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, 96 % purity] (MK Impex Corp., Canada, MKN-HXAP-060) were applied, whereas 80 nm nanoparticles of amorphous calcium carbonate [CaCO_3 , > 98 % purity] (MK Impex Corp., Canada, MKN-CaCO3-080) were applied to tiles C5 and C6. A commercially available superhydrophobic polysiloxane-based coating (Eronde, USA, 9HMR-FIX) was applied on the dry surface of the polyester resin of tiles C1, C2, and C3. In the case of C6, the superhydrophobic coating was applied directly onto the tile surface and served as a base for applying the CaCO_3 nanoparticles.

In 2021, we evaluated the individual properties of coatings on settlement preference of *Orbicella* spp. larvae to determine if it is determined by a combination of properties or by an individual property. We focused on substrates C1 and C2 because they elicited greater settlement compared to the other tiles that were tested. The same brand of ceramic floor tiles from the previous experiment was used. We prepared six treatments (T1 to T6, Table 1). All the treatments had a hydrophobic polyester

resin coating. For treatment T1 a superhydrophobic polysiloxane-based coating (same as the previous experiment) was applied. Treatments T2 and T3 contained microparticles of strontium aluminate salts with light blue and red-orange colours, respectively. Treatment T4 had a hydrophobic polyester resin coating applied. White paint was added to T5 and T6 consisted of the bare ceramic floor tile without coatings.

Substrate conditioning: Ten replicates of each treatment (T1 to T6) were placed in flow-through seawater aquaria (480 L) for seven weeks to pre-condition them and allow biofilm formation (Sneed et al., 2014). Sea water was filtered to 1 μm and set to the following conditions: temperature 27–28.5 °C, salinity 35–36 psu, pH 7.9–8.3. Concentrations of ammonia, nitrite, and nitrate were undetectable using standard methods (Grasshoff et al., 1999). Therefore, the remaining ten substrates of each treatment were maintained without conditioning. Substrate conditioning was evaluated for *Orbicella* spp. in 2021 (Table 2). It was not tested in 2019, because we did not have enough replicates.

TABLE 2
Experimental design of coatings with combinations of properties (2019) and coatings with a single property (2021).

Species, year	Containers	Number of substrates	Larvae per ml	Variables			
				Coatings		Conditioning	
				Treatments	Replicates per treatment	Treatments	Replicates per treatment
<i>O. annularis</i> - <i>O. faveolata</i> 2019	5 (6 L)	12 substrates per container, 2 per coating	0.2	C1 to C6	10		
<i>O. annularis</i> - <i>O. faveolata</i> , 2021	10 (2 L)	6 substrates per container, 1 per coating	0.1	T1 to T6	10	C, UNC	30

Abbreviations: Conditioned (C), and Unconditioned (UNC).

Collection of gametes, assisted fertilization, and care of embryos: One week after the full moon in September 2019, gamete bundles were collected from four colonies of *Orbicella annularis* from Jardines Reef (20°49'53.0" N, 86°52'28.2" W), as well as three colonies of *O. faveolata* from La Bocana Reef (20°52'25.7" N, 86°51'04.2" W). In September 2021 gamete bundles were collected from 10 colonies of *O. annularis* and two colonies of *O. faveolata* from Jardines Reef. The gametes were collected using nets that concentrated the gamete bundles into small plastic containers at the top of the nets. These reefs are located in the Puerto Morelos Reef National Park, Mexican Caribbean. Afterwards, all the gametes were transported to the laboratory, where gamete predators (crustaceans, polychaetes, and fish larvae) were removed using transfer pipettes, and gametes from the different colonies of the same species were mixed in a single 6 L container to assist fertilization. Subsequently, excess sperm was removed, and the developing embryos were transferred to a culture system, where they developed into planula larvae.

Choice experiment: Three days after spawning, 6 000 *O. annularis* larvae were distributed evenly into five replicate sets of 6 L plastic containers (1 200 larvae per container). Each plastic container had two substrates of each type of treatment (C1 to C6). The same experimental design was repeated for *O. faveolata* (Table 2). The settlement was recorded on days eight, 13, and 18 after spawning. One day

after the last count of the substrates, those with *O. annularis* and *O. faveolata* primary polyps were transferred to flow-through aquaria with access to natural light, food, and symbionts. Substrates were scored for survival 13, 20, 27, 34, and 41 days after settlement.

Three days after spawning, 2 000 *O. annularis* larvae were distributed evenly into ten replicate sets of 2 L plastic containers (200 larvae per container). Each container had six substrate coatings (T1 to T6). Five containers had conditioned substrates, and five containers had unconditioned substrates (Table 2). On days eight, 13, and 18 after spawning, *O. annularis* settlement was recorded. One day after the last count, substrates with *O. annularis* primary polyps were transferred to flow-through aquaria. The substrates were scored for survival seven, 14, 21, 28, 35, and 42 days after settlement. The same experimental setup and methodology were used for *O. faveolata*.

Statistical analysis: A generalized linear model (GLM) with a negative binomial distribution and a logit link was used to analyse settlement in the *O. annularis* and *O. faveolata* choice experiment between substrate coatings with combinations of properties (C1 to C6, 6 levels) and in the *O. annularis* and *O. faveolata* choice experiment to determine the effect of substrate coatings with a single property (T1 to T6, six levels) and substrate conditioning (Conditioned and unconditioned, two levels). The models are selected by the Bayesian Information Criterion (BIC). Model



validations revealed that there was no over-dispersion. Likelihood ratio chi-square (LR Chisq) was performed for statistical inference. Tukey tests for post hoc analysis were then used. Kaplan-Meier survival analysis was performed to determine differences in survivorship between substrate coatings (C1 to C6 and T1 to T6, six levels) in the *O. annularis* and *O. faveolata* choice experiments. Survival between the substrate coatings (C1 to C6 and T1 to T6, six levels) was compared using log-rank tests. All statistical tests were carried out using R (R Core Team, 2019), and the results were plotted using the ggplot2 package (Wickham, 2009). Data are presented as mean \pm standard error (SE).

RESULTS

Coatings with combinations of properties

For *Orbicella annularis* a maximum of 258 settlements was recorded on the coatings, representing 4.30 % of the 6 000 larvae placed in the containers. A maximum of 171 *O. faveolata* settlers was recorded on the substrate coatings, representing 2.85 % of the 6 000 larvae used in the experiment.

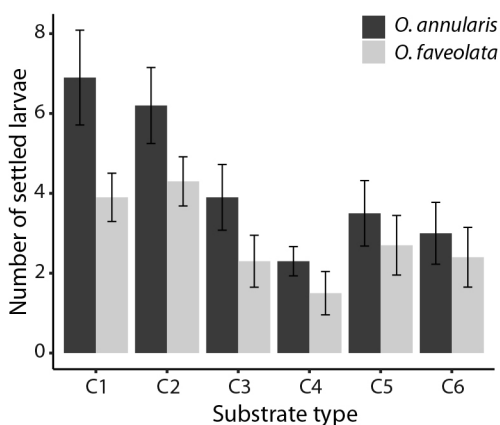


Fig. 1. The maximum number of *O. annularis*, and *O. faveolata* larvae settled on substrates with coatings with combinations of properties. Values are means (N = 10), and error bars represent \pm standard error.

Choice experiment: Despite the low settlement, distinguishable differences were found in the number of *O. annularis* settled larvae (Fig. 1) (LR Chisq = 25.74, $P = 0.0001$), with a greater number of settlers found on substrate C1 when compared to substrate C4 (Tukey, $P = 0.0015$), and C6 (Tukey, $P = 0.02$). The number of settlers was significantly greater on substrate C2 when compared to substrate C4 (Tukey, $P = 0.007$). A total of 196 (3.3 ± 0.43) settlers were observed on the coatings of the substrates on day eight after spawning, 133 (2.0 ± 0.25) on day 13, and 58 (0.9 ± 0.18) on day 18. This shows that the highest number of settlements for this experiment occurred between day three and day 12 after spawning under these conditions. Similarly, for *O. faveolata* significant differences were found in the number of settled larvae (Fig. 1) (LR Chisq = 12.27, $P = 0.03$), with lower settlement on substrate C4 when compared to substrate C2 (Tukey, $P = 0.04$). A total of 27 (0.4 ± 0.10) settlers were observed on the upper surface of substrates on day eight after spawning, 139 (2.2 ± 0.27) on day 13, and 109 (1.8 ± 0.27) on day 18. This shows that the highest number of settlements for this experiment occurred between days nine and 13 after spawning under these conditions.

Post-settlement survival: The highest number of polyps settled was on substrates C1 and C2 compared to other substrates. The Kaplan-Meier survival curves demonstrated a mean survival time of 14.00 ± 0.68 days after settlement on substrate C1, 13.37 ± 0.37 days on substrate C2, 13.00 ± 0.00 days on substrate C3, 16.89 ± 1.23 days on substrate C5 and 18.6 ± 4.08 days on substrate C6 (Fig. 2A). The survival time was significantly lower in substrate C2 than substrate C5 (Log-rank test, $P = 0.02$).

A total of 109 *O. faveolata* primary polyps were transferred to aquaria after the last count, distributed between the substrate types, mostly on C1 and C2 substrates. The Kaplan-Meier survival curves revealed a mean survival time of 13.00 ± 0.00 days after settlement on substrate C1, C4, and C5, 14.17 ± 0.68 days on

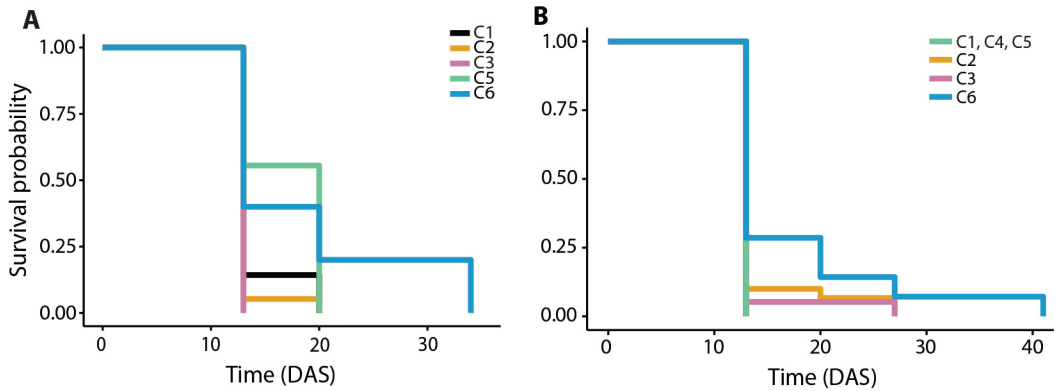


Fig. 2. Survival probability of primary polyps on substrates with coatings with combinations of properties (C1 to C6) for 41 days after settlement (DAS). **A.** *O. annularis*. **B.** *O. faveolata*.

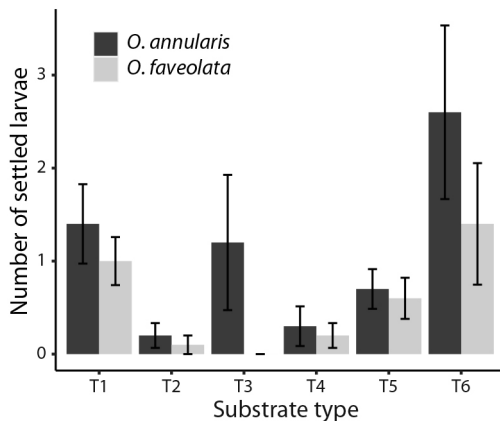


Fig. 3. The maximum number of *O. annularis* and *O. faveolata* larvae settled on substrates with coatings with a single property. Values are means (N = 10), and error bars represent standard error.

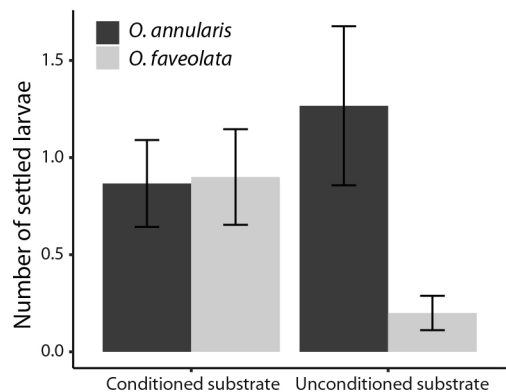


Fig. 4. The maximum number of *O. annularis*, and *O. faveolata* larvae settled on conditioned and unconditioned substrates. Coatings with a single property (T1 to T6). Values are means (N = 30), and error bars represent standard error.

substrate C2, 13.74 ± 0.74 days on substrate C3 and 17.00 ± 2.17 days on substrate C6 (Fig. 2B). No significant differences were found in the survival time between substrate coatings (Log-rank test, $P = 0.08$).

Coatings with a single property

For *O. annularis*, a maximum of 64 settlers was recorded on the substrate coatings, representing 3.20 % of the 2000 larvae originally placed in all containers. A maximum of 33 *O. faveolata* settlers was recorded on the substrate

coatings, representing 1.65 % of the 2000 larvae placed in the containers.

Choice experiment: Although the settlement was low, differences were found in the number of *O. annularis* settled larvae between the coatings (Fig. 3) (LR Chisq = 18.96, $P = 0.002$), with a significantly greater number of settlers found on substrate T6 when compared to substrate T2 (Tukey, $P = 0.03$), and T4 (Tukey, $P = 0.046$). No significant difference was found between substrate conditioning and lack of conditioning (Fig. 4) (LR Chisq = 0.63,

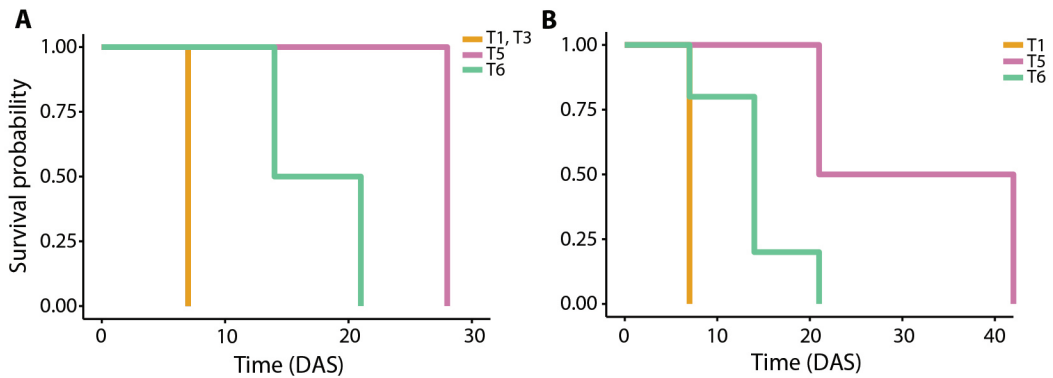


Fig. 5. Survival probability of primary polyps on substrates with coatings with a single property (T1 to T6) throughout 42 days after settlement (DAS). **A.** *O. annularis*. **B.** *O. faveolata*.

$P = 0.43$). A total of 16 (0.27 ± 0.09) settlers were observed on the coatings of the substrates on day eight after spawning, 38 (0.63 ± 0.15) on day 13, and 11 (0.18 ± 0.07) on day 18. This shows that the highest number of settlers for this experiment occurred between day nine and day 13 after spawning under these conditions.

For *O. faveolata* no significant difference was found in the number of settled larvae between the substrate coatings (Fig. 3) (LR Chisq = 1.90, $P > 0.05$). However, differences were found between substrate conditioning (Fig. 4) (LR Chisq = 13.92, $P = 0.0002$), with a significantly greater number of settlers found on conditioned substrates compared to unconditioned substrates. A total of 17 (0.28 ± 0.11) settlers were observed on the coatings of the substrates on day eight after spawning, 21 (0.35 ± 0.11) on day 13, and 9 (0.15 ± 0.07) on day 18. This shows that the highest number of settlers for this experiment occurred between days nine and 13 after spawning under these conditions.

Post-settlement survival: A total of 11 *O. annularis* primary polyps were transferred to aquaria after the last count, distributed between T1, T3, T5, and T6 substrate types, with the highest number of polyps settled on substrates T1. The Kaplan-Meier survival curves demonstrated a mean survival time of 7.00 ± 0.00 days after settlement on substrate T1 and T3,

28.00 ± 0.00 days on substrate T5 and 17.50 ± 3.50 days on substrate T6 (Fig. 5A). No significant differences were found in the survival time between substrate coatings (Log-rank test, $P > 0.05$).

Nine *O. faveolata* primary polyps were transferred to aquaria after the last count, distributed between T1, T5, and T6 substrate types, mostly on T6 substrates. The Kaplan-Meier survival curves revealed a mean survival time of 7.00 ± 0.00 days after settlement on substrate T1, 31.50 ± 10.5 days on substrate T5, and 14.00 ± 2.21 days on substrate T6 (Fig. 5B). No significant differences were found in the survival time between substrate coatings (Log-rank test, $P > 0.05$).

DISCUSSION

Overall, our results show that the presentation of substrates with distinct chemical coatings and combinations to two species of coral larvae results in low but distinct settlement patterns and post-settlement survival.

For *O. annularis*, the substrate with the combination of properties with a high level of hydrophobicity combined with light blue phosphorescent micro-particles (C1) and the substrate with a high level of hydrophobicity and red-orange phosphorescent micro-particles (C2) were the preferred settlement substrates when compared to the other combinations.

Nevertheless, the experiment with a single property revealed that the ceramic control (T6) was preferred over intermediate hydrophobicity (T4) and light blue phosphorescent microparticles (T2). For *O. faveolata*, we found that a high level of hydrophobicity, combined with the red-orange (C2) phosphorescent microparticles, was the preferred combination as a settlement substrate when compared to the substrate with intermediate hydrophobicity and hydroxyapatite coating (C4). This means that combinations of properties generate successful settlement on these substrates. Individual properties did not generate this effect. The fact that the greatest number of settlers was observed on substrates with phosphorescent microparticles, and a high level of hydrophobicity confirms that these species use photosensitivity and respond to colour, in this case, light blue and red-orange, to fine-tune the selection of appropriate substrates or microhabitats (Mason et al., 2011). This photosensitivity towards certain wavelengths of light may assist larvae in selecting a microhabitat (Strader et al., 2015), serving as a form of depth gauge (Mason & Cohen, 2012) or guide, given that the distribution of light on coral reefs is highly heterogeneous at the microhabitat level due to depth, water clarity, bottom slope, substrate type and exposure (Brakel, 1979).

The substrate types determined the survival rates of *O. annularis* and *O. faveolata* recruits. On transferring the recruits to aerated aquaria, the number of polyps that survived on the substrates dropped abruptly, especially for those coated with polyester resin. This was evidenced by the fact that in the experiment with coatings with combinations of properties, the longest survival time (41 days after settlement) was associated with the only substrate without a polyester resin coating (C6). In the experiment with coatings with a single property, we observed the same pattern; the longest survival time (42 days after settlement) was on a substrate without this polyester resin coating (T5). The polyester resin used is somewhat soluble in water; hence the resin may have slowly dissolved, resulting in an unstable substrate,

possibly preventing viable adhesion and compromising long-term post-settlement survival. Additionally, hydrophobic surfaces generate a contact angle with the water greater than 90°. When this contact angle is greater, the adhesion force to this surface will be less (Lejars et al., 2012). Therefore, hydrophobicity generates a weaker adhesion force between the recruit and the substrate and possibly, when generating movement in the aquarium system or due to aeration, the settlers detached from the substrate possibly due to a weak attachment. Therefore, using these hydrophobic surfaces to prevent the colonization of other organisms, such as filamentous algae, can also be detrimental to coral survival since they function as antifouling coatings that allow for easy release when water motion is applied (Lejars et al., 2012). This would mean that, even though the combination of high hydrophobicity and phosphorescent micro-particles presents the most favourable settlement option compared to other combinations, the component used to create intermediate hydrophobicity (polyester resin) and high hydrophobicity may have been detrimental to settler attachment.

However, even though substrate conditioning is known to encourage settlement (Erwin et al., 2008), in the experiment with coatings with combinations of properties, it should also be noted that none of the substrates used in the *O. annularis* and *O. faveolata* experiments were conditioned; hence no biofilm could have formed at their surfaces, yet larvae settled successfully, which means that conditioning is unnecessary for these substrates. This represents an advantage over other substrates (Harrison & Wallace, 1990; Patterson et al., 2016; Petersen et al., 2005) that require conditioning prior to larval settlement. However, for the experiment with coatings with a single property, we found that the settlement of *O. faveolata* was greater on conditioned substrates; this may indicate that these substrates with a single property are not attractive enough on their own to larvae and require conditioning to generate an adequate settlement cue for coral larvae.



Orbicella annularis and *O. faveolata* larvae showed different behaviours, most notably in their settlement patterns. For *O. faveolata*, most larvae settle within the first two weeks if an appropriate site is found, with 80 % of larvae remaining in the plankton for more than eight days before attempting to settle (Szmant & Meadows, 2006). Our results show that the peak settlement period for *O. faveolata* larvae was between nine and 13 days after spawning. For *O. annularis*, the settlement peak was between three and eight days after spawning. This behaviour may also affect the affinity that *O. annularis* larvae have for the substrates with the combination of properties. More *O. annularis* larvae settled and they settled more rapidly when compared to *O. faveolata* larvae. Furthermore, we did not find this pattern in *O. annularis* in the single-property experiment, possibly because the larvae were not attracted to the substrates. Thus, they took more time to settle and were therefore settled in a time frame similar to *O. faveolata* larvae.

In conclusion, this is the first study to report the ability of *O. annularis* and *O. faveolata* to detect phosphorescence and hydrophobicity during the substrate selection process, which could be key to micro-habitat selection. Furthermore, this is the first time that the effects of substrate surface properties have been tested on these species, with varying effects. On the one hand, microparticles with blue and red-orange phosphorescence and high hydrophobicity favour settlement in *O. annularis* and *O. faveolata*, but some of the components used to coat the substrates may be detrimental to the survival of the polyps over time. These issues need to be addressed as long-term survival is critical in restoration projects involving the outplanting of coral settlers. Finally, it is essential to keep testing different combinations of substrate surface properties to determine the ones that are most conducive to larval settlement and polyp survival as well as being the least favourable to fleshy algal settlement. This will help us to identify the most suitable substrates for each species and is vital for optimizing future restoration efforts.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

ACKNOWLEDGEMENTS

We wish to thank members of the Corallium laboratory for sharing their knowledge and support in the field and the lab, particularly Gandhi Ramírez, Raul Tecalco, and Sandra Mendoza. Also, to Pablo Andrés Guzmán for his help with statistical analyses and expertise in R Software.

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