Journal of Experimental Marine Biology and Ecology 576 (2024) 152015

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

3D printed plant-based biodegradable materials as alternative for PVC panels in monitoring coastal marine communities

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ARTICLE INFO

Keywords:
Artificial settlement
Coral restoration
Marine ecology
Community ecology
Larval recruitment

ABSTRACT

Coastal marine environments are some of the most productive and overexploited ecosystems on earth. Coastal ecosystems, like oyster and coral reefs, need physical structures to establish and habitat destruction has led to the destabilization of reef structures resulting in massive biodiversity losses. For decades, researchers have utilized PolyVinyl Chloride (PVC) panels as artificial larval settlement panels to study recruitment and community dynamics in these incredibly important ecosystems. Unfortunately, PVC itself is relatively toxic, the panels are smooth which makes it difficult for larval settlement, and it can take months to see establishment because larvae need settlement cues on PVC from biofilm development. This study assessed the use of 3D printed panels constructed out of plant-based biodegradable materials as an alternative to PVC panels. Panels constructed of three different corn-based polylactic acid (PLA) materials (white impact modified, gray impact modified, wood pulp) were tested at a well monitored site along the Long Island Sound in Connecticut, US. Overall, the 3D printed panels had equal or higher recruitment compared to the PVC panels. Beyond the normal application of panels for ecological assessment of local settlement, this work highlights that using innovative, biodegradable panels has strong potential in reestablishing these overburdened yet critical ecosystems. Additionally, the 3D printed panels were economically beneficial to use, which increases equity in this conservation crisis field as material expense can be cost prohibitive.

1. Introduction

Coastal marine environments are some of the most productive ecosystems on earth, providing irreplaceable ecosystem services like storm protection and coastline stabilization (Barbier, 2012, 2017; Liquete et al., 2013), yet they are also some of the most exploited regions on earth (Barbier, 2017). In past decades, these systems have faced anthropogenic threats that include pollution, overexploitation, introduction of invasive species, urbanization, and habitat degradation (Beatley, 1991; Crain et al., 2009; Liquete et al., 2013). In this same timeframe, we have seen the destabilization of coral reefs, and massive loss in biodiversity of these incredibly important ecosystems. Indeed, habitat loss and degradation are some of the biggest threats to biodiversity across terrestrial and marine ecosystems (Kingsford et al., 2009; Hanski, 2011; Segan et al., 2016). If the loss of suitable habitats is strong enough, then marine larvae cannot find suitable establishment sites, and recruitment (the total number of organisms that are able to successfully settle in a new area) is severely affected or not even possible.

Foundational marine species, such as oysters and corals, are often impacted by habitat loss and degradation that directly affect their reestablishment (Birkeland, 1977; van der Schatte Olivier et al., 2020; van der Heide et al., 2021), yet they provide essential ecosystem services. They provide increased structural complexity like reef building that establishes accessible habitat for other organisms, e.g. marine grasses, snails, crabs and fish (Angelini et al., 2015; Idjadi and Edmunds, 2006). Not only do they form the physical substrate on which the ecosystem is created, the organisms themselves also often filter the water and help by taking up excess nitrogen and nutrient uptake (Newell and Shumway, 1993; Cranford et al., 2007; Nielsen et al., 2016), and act as ecosystem engineers that help protect the ecosystem from storm damage. This is a substantial benefit to the coastal communities as well, as it prevents further damage to infrastructure and aids in erosion

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https://doi.org/10.1016/j.jembe.2024.152015
Received 10 May 2023; Received in revised form 1 September 2023; Accepted 23 April 2024
Available online 7 May 2024

control (Dame and Patten, 1981; Bible and Sanford, 2016). The loss of these foundational species further decreases habitat complexity, leading to cascading effects on ecosystem biodiversity (Balata et al., 2007; Graham, 2014), necessitating active efforts to restore systems to function.

Establishment of these foundational species is crucial, but in many ecological systems, recruitment is limited by habitat. Humans have challenged natural systems to an extent that we must develop artificial habitats that promote establishment and recruitment, which will then be maintained via succession. This new promising line of work could potentially revolutionize our intentional impacts on habitat restoration. Artificial habitats can facilitate range expansion of some species (Cannizzo and Griffen, 2019) and are a good predictor of ecosystem richness (Janiak and Branson, 2021). Passive sampling by using artificial settlement panels is a widespread technique used to understand community ecology (Ma et al., 2022), but this process is limited by the target species' ability to recruit on the artificial material. Not all materials used for artificial settlement panels are equally as appropriate for recruitment, which could lead to a sampling bias especially against more sensitive species, and perhaps lower settlement of target restoration species from the local larval supply. Additionally, artificial settlement panels have been used to detect invasive species at the early stages of recruitment (Ma et al., 2017; Moore et al., 2014), which is essential to develop effective management or removal protocols. Most of these studies have used polyvinyl chloride (PVC) panels, which has been a powerful tool for assessment, but this material has drawbacks for use in population studies and conservation applications. Most notably, PVC is associated with microplastic pollution, which has been shown to have sub-lethal effects on aquatic invertebrates' growth (see Doyle et al., 2022). Further, PVC breakdown is known to leach toxic materials into marine systems, which is becoming an issue of emergent concern (Lithner et al., 2012).

The use of conventional materials like PVC for marine settlement panels are effective but also contribute their own negative ramifications to the ecosystem we are trying to re-establish and protect. As a response, scientists have turned to a combination of natural and artificial settlement habitats to promote re-establishment (Fitzhardinge and Bailey-Brock, 1989; Spieler et al., 2001; Neo et al., 2009; Colsoul et al., 2020) and reduce unintended negative ramifications. Unfortunately, essential foundational species like oysters and corals, do not always prefer the natural substrates provided for establishment. For example, wood was not an effective material for oyster establishment, but they did settle readily on ceramic tiles (Colsoul et al., 2020), and as mentioned above, corals settled well on PVC panels (Oren and Benayahu, 1997). We must find the balance between foundational species establishment and material ramification reduction.

Newer technologies, like 3D printing, have allowed us to explore new materials and even merge organic and inorganic materials together to create new substrate types. This ability to create artificial settlement panels from mixed materials has major advantages and has been used with some success, but more data are needed to understand their efficacy in recruitment. To this end, we tested the use of three different 3D printed plant-based biodegradable panels as alternatives for PVC panels for monitoring community composition in an intertidal marine system.

2. Materials and methods

2.1. Construction of 3D printed panels

The 3D printed panels were designed with computer aided design software (Wandering Technologist, LLC) and were processed with a 3D printer slicer software. For integrity, each panel was composed of a flat central panel, and to provide appropriate rugosity characteristics, parallel ridges were extruded from that surface on both sides of the panel (ridge depth was: ~1 mm). The panels were each printed using a removable raft for stability while printing. The panels were 10 cm^2 and all panels were made with 1.75 mm corn-based formula PLA (Polylactic Acid) materials (materials maintain dimension accuracy +/− 0.05 mm; all meet ASTM standards for biodegradation (Standard Specification for Labeling of Plastics Designed to Be Aerobically Composted in Municipal or Industrial Facilities, n.d.)). Three different corn-based PLA materials were used, including a white impact modified PLA (white color, designed for increased strength and integrity; 3d Printlife Pro, Southborough, MA), gray impact modified PLA (gray color, designed for increased strength and integrity; 3d Printlife Pro, Southborough, MA), and wood PLA (brown color, 40% walnut pulp combined with corn-based PLA; 3d Printlife Pro, Southborough, MA).

2.2. Sampling protocol

Settlement panels were deployed at the UConn Avery Point Campus in Groton, CT (41.3153° N, 72.0636° W). This site is part of a 20+ year NSF Long-Term Ecological Research in Environmental Biology (LTERB) monitoring program. The three different 3D printed panels (white impact modified, gray impact modified, and wood; n = 36 panels/material) and the PVC panels (control; n = 24 panels) were randomly affixed to 'trees' constructed out of 5 cm PVC pipes (Fig. 1). The structures were deployed off the floating docks at the site and suspended 0.5 m below the water line. Settlement on the panels was tracked weekly by imaging for taxonomic identification. Water parameters were recorded at each sampling event.

2.3. Water sampling

During each sampling event, a YSI probe (Xylem Inc., Ohio, US) was used to measure the water temperature, salinity, pH and dissolved oxygen (DO) adjacent to the panels 0.5 m below the water. Samples were also collected to measure the total suspended solids (TSS) as follows. A Niskin water sampling bottle was used to collect duplicate samples. Samples were placed in dark Nalgene bottles and stored at 4 °C until they were processed in the lab (processing took place within 1 h of sampling). Then, 1 L of seawater was filtered through a pre-weighed glass fiber filter (GF-C, Whatman), rinsed with distilled water to remove salts, and oven-dried at 70 °C until a steady weight was reached. Samples were cooled individually in a desiccator before weighing. Water samples were also collected for suspended chlorophyll *a* measurements

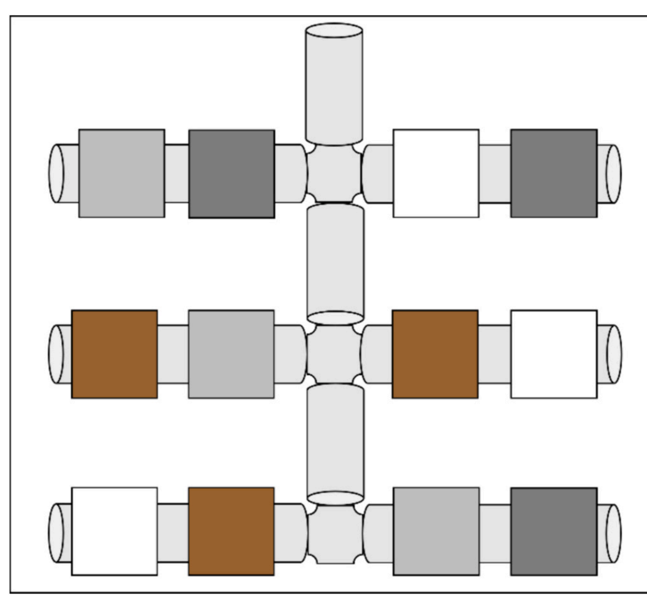

Fig. 1. Schematic of PVC "tree." Each tree was identical had randomly distributed 3D printed and control settlement panels. All panels were distributed along the docks at the Avery Point campus in Groton, CT.

following standard protocols (Holm-Hansen and Riemann, 1978). Briefly, alcohol scrubbed PVC bottles were filled with the water samples and kept on ice and in the dark until processing, which was generally within 1 h of sampling. Then, samples were filtered through glass fiber filters (Whatman GF-F, 25 mm), wrapped in foil and stored at 4 °C until ready for extraction. Once all samples were ready for extraction, they were processed together to minimize variation in sampling. Samples were placed in absolute methanol for 1 h, in the dark at room temperature (21 °C). Filters were then centrifuged at 1000*g* for 5 min and the supernatant transferred to a glass culture tube. The absorbance was measured with a SPECTRONIC™ 200 spectrophotometer (ThermoFisher Scientific) at 440 nm, and compared to a standard curve to determine the chlorophyll *a* concentration.

2.4. Settlement & recruitment monitoring

The artificial trees with the settlement panels (Fig. 1) were monitored weekly by briefly removing them from the water and imaging individual panels for later species identification (Fig. 2). All images included a ruler for scale and a level to ensure the camera was parallel with the plane of the panel. The presence of any motile macroinvertebrates, such as amphipods and snails, was recorded at time of imaging. The panels were placed back in the water in the same location and were not out of the water for >10 min weekly. Each image was analyzed by three researchers who all independently identified and quantified settlement, and an average abundance was determined from these data. All species were identified to species when possible, and to genus level at a minimum. ImageJ (NIH) was used to quantify the abundance of each species and calculate percent cover using established methods (Schneider et al., 2012).

2.5. Data processing and statistical analysis

Statistical analyses were run using RStudio (RStudio Team, 2020). Model fitting using AICc values was used to determine the best fit model for the data (package: stats, function: extractAIC). A zero-inflated negative binomial generalized linear model (package: glmmTMB, function: glmmTMB; family: nbinom2; nesting within specific panel ID) was used to determine if panel material (white impact modified PLA, gray impact modified PLA, wood PLA, and PVC) and time significantly impacted organism abundance for barnacles, *Botryllus schlosseri*, *Molgula manhattensis*, and filamentous algae. A negative binomial generalized linear model (package: glmmTMB, function: glmmTMB; family: nbinom2; nesting within specific panel ID) was used to determine if panel material and time significantly impacted organism abundance for *Diplosoma listerianum*, *Botrylloides violaceus, Mytilus edulis*, Bryozoans, Amphipoda, and *Ilyanassa obsoleta*. Analyses for each organism were run separately. For each analysis, an estimated marginal means test was run to determine pairwise comparisons among treatments (package: emmeans, functions: emmeans and pairs).

2.6. Presence of predation

Model fitting using AICc values was used to determine the best fit model for the data (package: stats, function: extractAIC). To determine if there was an effect of predator *I. obsoleta* on the abundance of barnacles, *Botryllus schlosseri*, *Mo. manhattensis*, and filamentous algae a zero-inflated negative binomial generalized linear model (package: glmmTMB, function: glmmTMB; family: nbinom2; nesting within specific panel ID) was used, and to determine if there was an effect of predator *I. obsoleta* on the abundance of *D. listerianum*, *Botrylloides violaceus, My. edulis*, Bryozoans, Amphipoda a negative binomial generalized linear model (package: glmmTMB, function: glmmTMB; family: nbinom2; nesting within specific panel ID) was used. Analyses for each organism were run separately and time and substrate were used as covariates in these analyses.

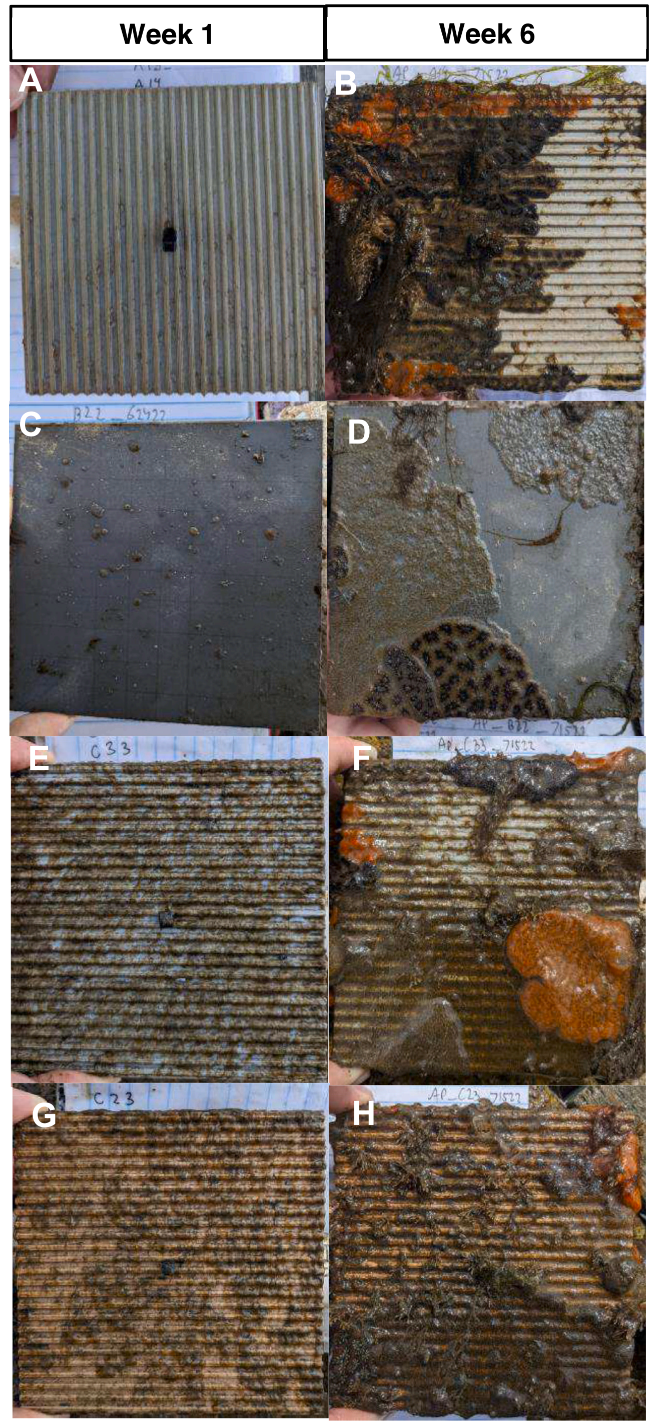

Fig. 2. Photo images of 3D printed and control settlement panels during the experimental deployment. Panels on the left (A, C, E, G) were imaged 1-week post-deployment, and panels on the right (B, D, F, H) were imaged six weeks post-deployment. Panel materials are A-B: Gray impact modified PLA; C-D: PVC; E-F: White impact modified PLA; and G-H: Wood PLA.

2.7. Growth over time

To determine if there were differential growth rates across time for all organisms, a generalized linear model (package: stats, function: glm) was used to determine if there was an effect of panel material on the overall growth of organisms over time. The analyses were again rerun with all 3D printing materials aggregated together and compared to PVC. For ease of communication, and because only one species per genus was found, each species will be referred to by genus in the following sections.

3. Results

3.1. Water sampling

The average water temperature was 20.5 °C, DO was 6.67 mg l^{-1}, salinity was 30.1 PPT, pH was 7.95, TSS was 6.2 mg l^{-1}, and chl*a* 0.06 mg l^{-1} (Table 1).

3.2. Recruitment on panels

There was no effect of panel material and there was an effect of time on the abundance of barnacles (Fig. 3: $\chi^2_1 = 4.97$, $p = 0.174$ and $\chi^2_1 = 22.51$, $p < 0.001$, respectively; Table S1), the golden star tunicate *Botryllus schlosseri* (Fig. 4: $\chi^2_1 = 1.57$, $p = 0.665$ and $\chi^2_1 = 59.48$, $p < 0.001$, respectively; Table S1), the colonial ascidians *D. listerianum* (Fig. 5: $\chi^2_1 = 1.66$, $p = 0.647$ and $\chi^2_1 = 67.03$, $p < 0.001$, respectively; Table S1), *Botrylloides violaceus* (Fig. 6: $\chi^2_1 = 0.26$, $p = 0.968$ and $\chi^2_1 = 55.73$, $p < 0.001$, respectively; Table S1). There was an effect of panel material but not time on abundance of amphipoda (Fig. 7: $\chi^2_1 = 8.23$, $p = 0.04$ and $\chi^2_1 = 0.24$, $p = 0.623$, respectively; Table S1). There was an effect of both panel material and time on the abundance of the solitary tunicate *Mo. manhattensis* (Fig. 8: $\chi^2_1 = 29.83$, $p < 0.001$ and $\chi^2_1 = 44.47$, $p < 0.001$, respectively; Table S1), bryozoans (Fig. 9: $\chi^2_1 = 14.24$, $p = 0.003$ and $\chi^2_1 = 29.50$, $p < 0.0001$, respectively; Table S1), the blue mussel *My. edulis* ($\chi^2_1 = 11.51$, $p = 0.009$ and $\chi^2_1 = 10.69$, $p = 0.001$, respectively; Table S1) and percent cover of filamentous algae (Fig. 10: $\chi^2_1 = 11.06$, $p = 0.011$ and $\chi^2_1 = 8.52$, $p = 0.003$, respectively; Table S1). There was no effect of panel material or time on the Eastern mud snail *I. obsosleta* ($\chi^2_1 = 7.11$, $p = 0.069$ and $\chi^2_1 = 1.79$, $p = 0.180$, respectively; Table S1).

3.3. Growth over time

Panel material did affect growth (abundance over time) of Bryozoans and *My. edulis* ($\chi^2_1 = 10.45$, $p = 0.015$ and $\chi^2_1 = 13.92$, $p = 0.003$, respectively), but did not differentially affect the growth of barnacles ($\chi^2_1 = 7.54$, $p = 0.057$), *Botryllus schlosseri* ($\chi^2_1 = 4.62$, $p = 0.202$), *D. listerianum* ($\chi^2_1 = 5.70$, $p = 0.127$), *Mo. manhattensis* ($\chi^2_1 = 5.25$, $p = 0.154$), *Botrylloides violaceus* ($\chi^2_1 = 0.955$, $p = 0.812$), filamentous algae ($\chi^2_1 = 5.54$, $p = 0.136$), or amphipoda ($\chi^2_1 = 2.32$, $p = 0.51$). When the 3D printed materials were aggregated together and compared as a group to the PVC material, the 3D printed materials had a positive effect on barnacle and bryozoan growth ($\chi^2_1 = 4.73$, $p = 0.029$ and $\chi^2_1 = 7.33$, $p = 0.007$, respectively). There was no effect of material when comparing aggregated 3D printed materials to the PVC materials for any of the other settled organisms (*Botryllus schlosseri:* $\chi^2_1 = 1.39$, $p = 0.238$, *D. listerianum*: $\chi^2_1 = 1.18$, $p = 0.277$, *Mo. manhattensis*: $\chi^2_1 = 3.18$, $p = 0.074$, *Botrylloides violaceus:* $\chi^2_1 = 0.039$, $p = 0.842$, *My. edulis*: $\chi^2_1 = 1.99$, $p = 0.158$, Filamentous algae: $\chi^2_1 = 2.99$, $p = 0.084$, amphipoda: $\chi^2_1 = 0.419$, $p = 0.51$, and *I. obsosleta:* $\chi^2_1 = 1.97$, $p = 0.161$).

3.4. Presence of predation

There was an effect of the abundance of the snail *I. obsosleta* on the abundance of *Botryllus schlosseri* ($\chi^2_1 = 6.92$, $p = 0.009$), but there no correlation with any other species examined (barnacles: $\chi^2_1 = 3.48$, $p = 0.01$; *Botrylloides violaceus*: $\chi^2_1 = 0.126$, $p = 0.722$, *D. listerianum:* $\chi^2_1 = 0.152$, $p = 0.696$; filamentous algae: $\chi^2_1 = 1.12$, $p = 0.290$; *Mo. manhattensis:* $\chi^2_1 = 0.297$, $p = 5.86$; *My. edulis*: $\chi^2_1 = 0.017$, $p = 0.896$; Bryozoans: $\chi^2_1 = 0.509$, $p = 0.476$; and amphipoda: $\chi^2_1 = 0.814$, $p = 0.367$).

4. Discussion

In this study, we screened whether 3D printed biodegradable plant-based panels were suitable for recruitment and establishment of marine species in an LTERB site. We found that they were effective, which could have a positive impact on the field of habitat restoration, because these panels are easy to produce, the shape and size can be manipulated, and they are light-weight making for easy travel and deployment. Additionally, given that the material traditionally used, PVC, has been shown to be toxic to marine organisms, these 3D printed biodegradable panels may be a better choice ecologically, as well.

Recruitment studies like this one are important because many marine species, particularly sessile forms, have a planktonic larval stage (Pineda et al., 2007), which must settle and establish in a suitable habitat for recruitment success. Generally, for these invertebrate species recruitment and survival (i.e. settlement) is related to the rate of biomass accumulation (Birkeland, 1977) and so these field studies can yield a strong view of recruitment success in these habitats.

Generally, we found equal or higher abundance of many of our foundational species on the biodegradable 3D printed substrates compared to the PVC panels. We found some species had a more generalist response, including barnacles, *Botryllus schlosseri*, *D. listerianum*, and *Botrylloides violaceus*, and panel material did not affect their settlement success. This meant that the 3D printed materials were equally as effective as the traditional PVC materials. The most common organisms to settle on all of the panels were barnacles. The colonial ascidian *Botrylloides violaceus* appears to be more of a generalist here, as it was found on all four panel types with no significant differences in abundance among the panels. This is interesting because other studies have found that this ascidian will preferentially settle on specific substrates, e.g. concrete. Perhaps this settlement preference is associated with differences in substrate roughness (Chase et al., 2016). One major advantage of the 3D printed panels is that future work could mimic the substrate roughness of other materials to see if settlement rates can be increased.

Some of the species studied here were more specialists and had differential success across settlement material, for example *My. edulis, I. obsoleta, Mo. manhattensis,* Bryozoans, filamentous algae and amphipods. For example, there was a positive effect of the 3D printed panels on solitary ascidian *Mo. manhattensis* abundance when compared to the PVC panels. Interestingly, while *Mo. manhattensis* were first observed on the PVC panels, their density decreased on the PVC panels throughout the experiment, while it increased in the 3D printed panels. The 3D printed panels may be more effective settlement sites for these

Table 1
Measured water quality parameters during the sampling period at Avery Point, Groton, CT US.

	Temperature (°C)	Dissolved Oxygen (mg/L)	Salinity (PPT)	pH	TSS (mg/L)	Chlorophyll *a*
Week 0 (start)	20.0	7.1	28.4	7.9	NA	NA
Week 1	18.1	6.8	29.4	8.0	8.2	0.022
Week 2	19.9	6.6	30.3	7.9	7.5	0.041
Week 3	21.7	6.9	30.5	7.9	4.0	0.049
Week 4	21.5	6.5	31.0	8.0	7.4	0.155
Week 5	21.9	6.1	31.2	8.0	3.9	0.012

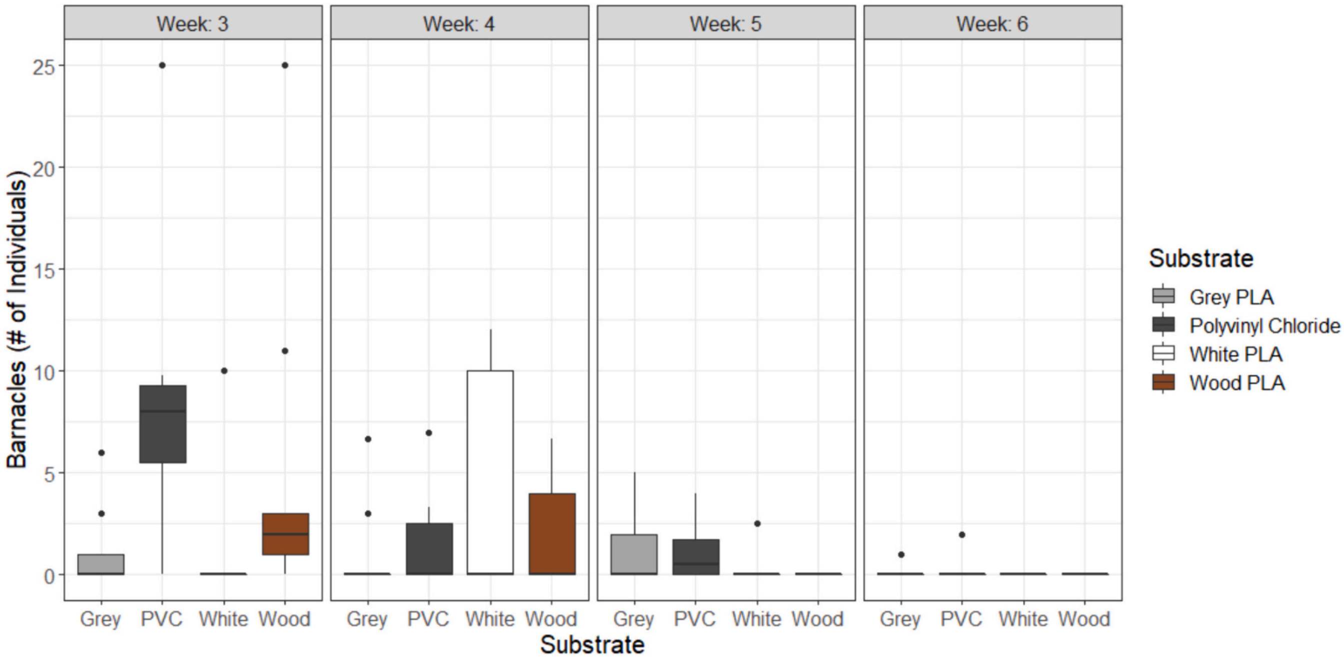

Fig. 3. Settlement of barnacles on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the number of barnacles, $n = 5$. There was an effect of time on the abundance of barnacles ($\chi_1^2 = 22.51$, $p < 0.001$), but no effect of panel material ($\chi_1^2 = 4.97$, $p = 0.174$).

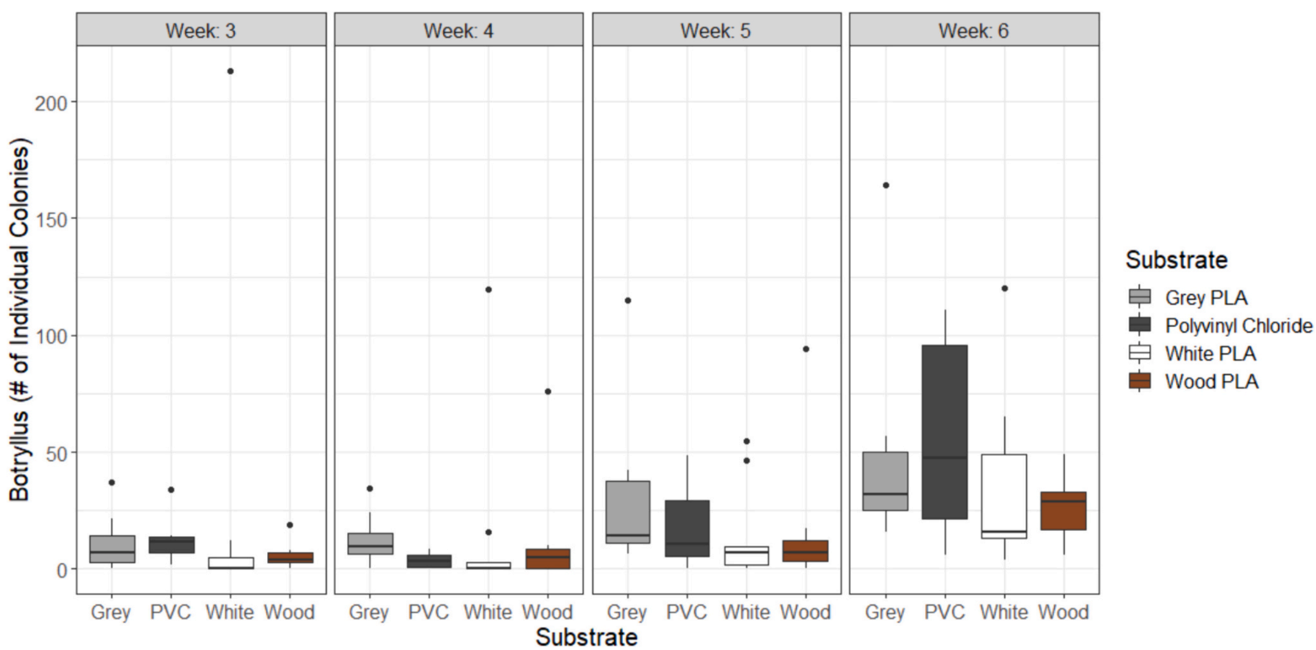

Fig. 4. Settlement of the golden star tunicate, *Botryllus schlosseri* on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the number of individual colonies; n = 5. There was an effect of time on the abundance of *Botryllus schlosseri* ($\chi_1^2 = 59.48$, $p < 0.001$), but no effect of panel material ($\chi_1^2 = 1.57$, $p = 0.665$).

organisms especially considering that *Mo. manhattensis* can become reproductive within three weeks of settling (Grave, 1933). Interestingly, this ascidian is typically seen in low abundance on traditional panels because they lack chemical defenses and tend to be predated at higher rates than other solitary and colonial ascidians in the area. Because of this, their presence and growth on the 3D printed panels is particularly noteworthy as it more closely reflects their abundance.

Beyond differential settlement and establishment of some species, we also recorded impacts of panel material on organism growth (measured as abundance over time) in several species, including Bryozoans and *My. edulis*. The wood panel material significantly increased Bryozoan growth during this study period compared to the PVC panels, though it was not different from the other 3D printed materials. On the other hand, after establishment of the *My. edulis* population on the panels, there was a significant decrease in this population on the white impact modified panels compared to the PVC. While decreased abundance may seem negative, *My. edulis* is a strong competitor for space, and a reduction in this species may actually allow for higher biodiversity in the long run.

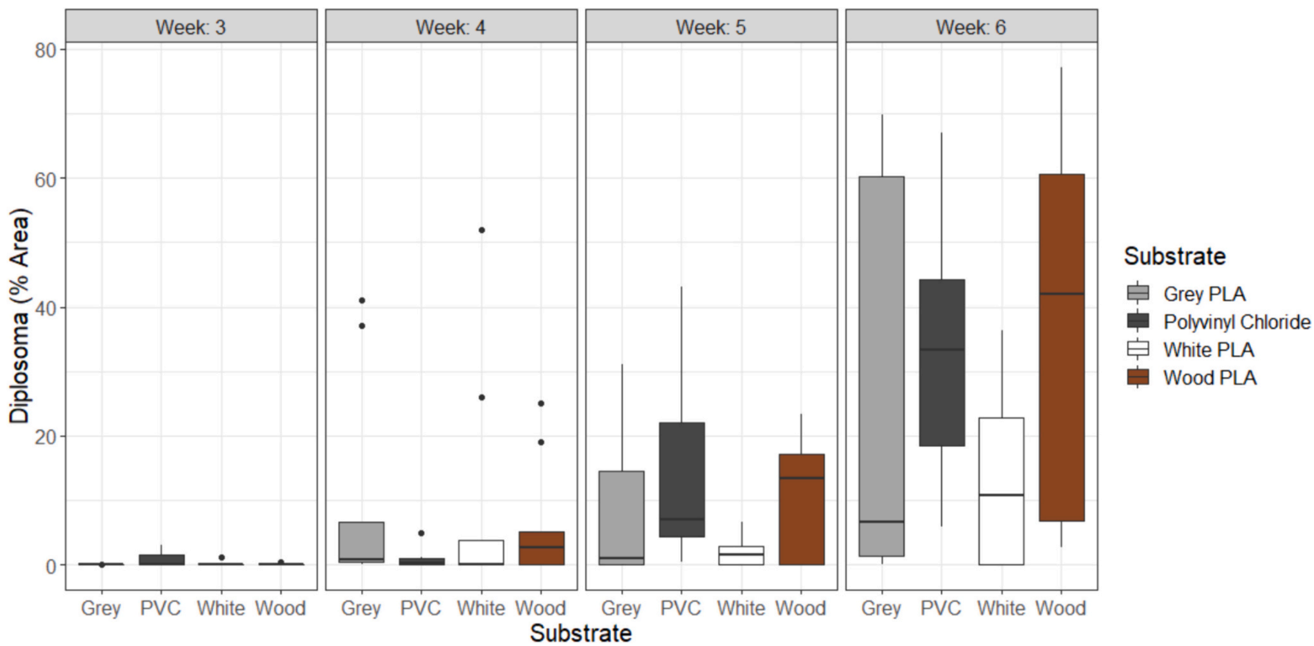

Fig. 5. Settlement of the colonial ascidian *Diplosoma listerianum* on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as percent area; n = 5. There was an effect of time on the abundance of *D. listerianum* ($\chi^2_1 = 67.03$, $p < 0.001$), but no effect of panel material ($\chi^2_1 = 1.66$, $p = 0.647$).

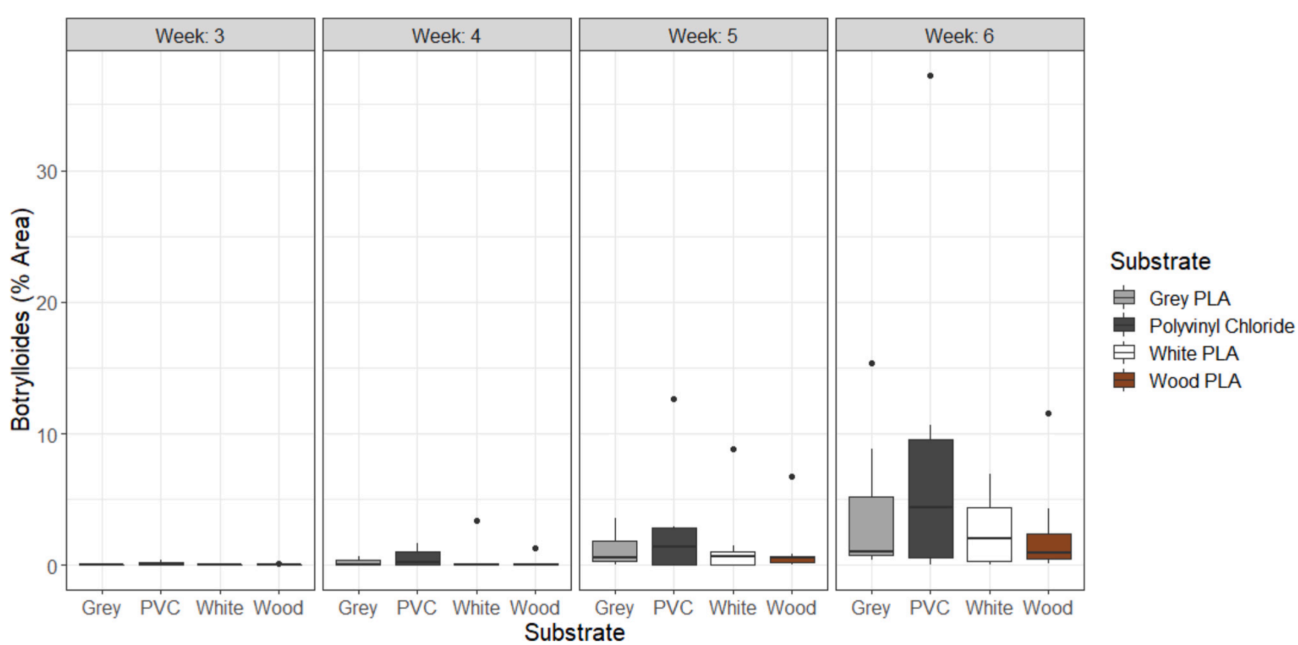

Fig. 6. Settlement of the colonial ascidian *Botrylloides violaceus* on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as percent area; n = 5. ($\chi^2_1 = 2.23$, $p = 0.527$ and $\chi^2_1 = 44.66$, $p < 0.001$). There was an effect of time on the abundance of *Botrylloides violaceus* ($\chi^2_1 = 55.73$, $p < 0.001$), but no effect of panel material ($\chi^2_1 = 0.26$, $p = 0.968$).

It is critical to look across multitrophic levels when considering ecosystem health because the presence of a predator can have direct and indirect impacts on species biodiversity, as well. Here, we found the abundance of the Eastern mud snail *I. obsoleta* significantly impacted the abundance of barnacles, *Botryllus schlosseri,* and *Botrylloides violaceus* but not all in the same way. Indeed, increased presence of *Ilyanasa* was correlated with a decrease in barnacle abundance, but it was positively associated with *Botryllus schlosseri* and *Botrylloides violaceus* abundance. Previous work in the area, which transplanted ascidian recruits settled on PVC panels between a site protected from predators and one with multiple predator guilds, found that predation on newly settled and juvenile life-stages resulted in differences in community development and species composition (Osman and Whitlatch, 2004). Similarly, differing predator densities have been found to have a significant effect on controlling ascidian density settled on PVC panels at Avery Point, the site of this study (Whitlatch, 2009). Predation can increase biodiversity in many ways (Ives et al., 2005), for example, by reducing the abundance of a competitively dominant species, e.g. barnacles and mussels, or by altering consumer pressure on primary producers, e.g. filamentous algae. While it is unlikely that competition played a significant role in

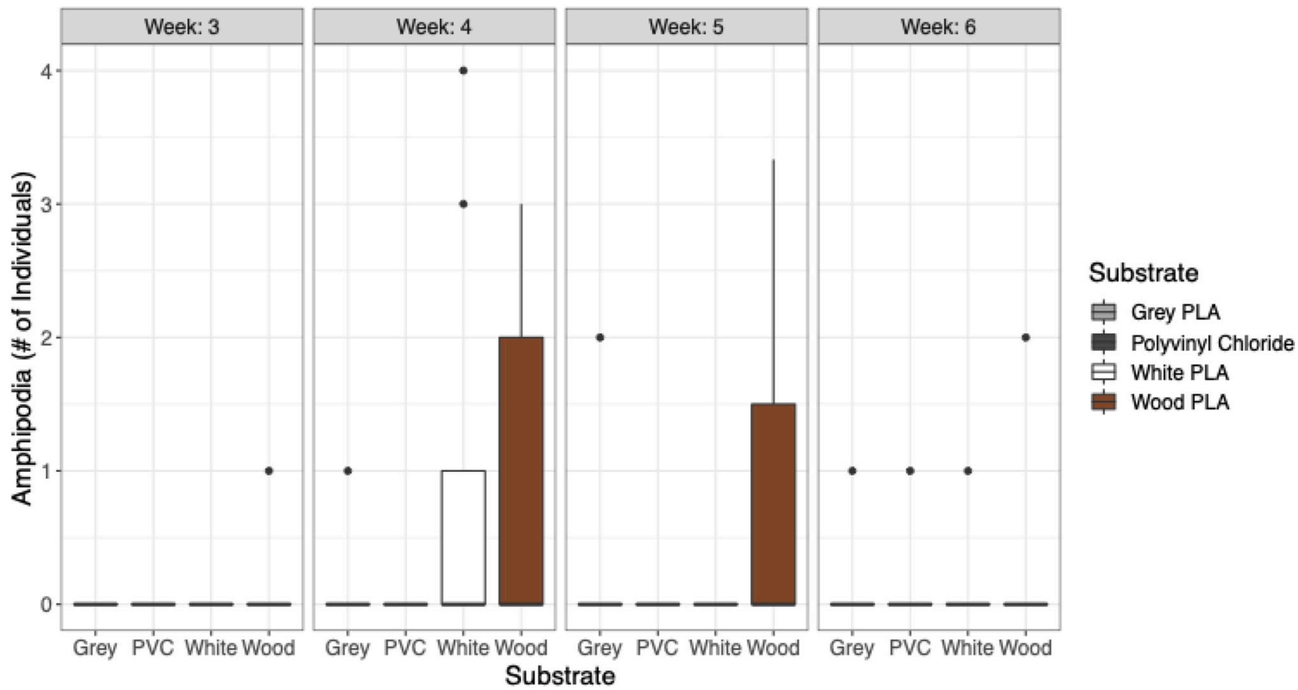

Fig. 7. Appearance of individual animals on the genus *Amphipoda* on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the number of individuals; $n = 4$. There was an effect of both panel material and time on the abundance of ($\chi_1^2 = 8.23$, $p = 0.04$ and $\chi_1^2 = 0.24$, $p = 0.623$).

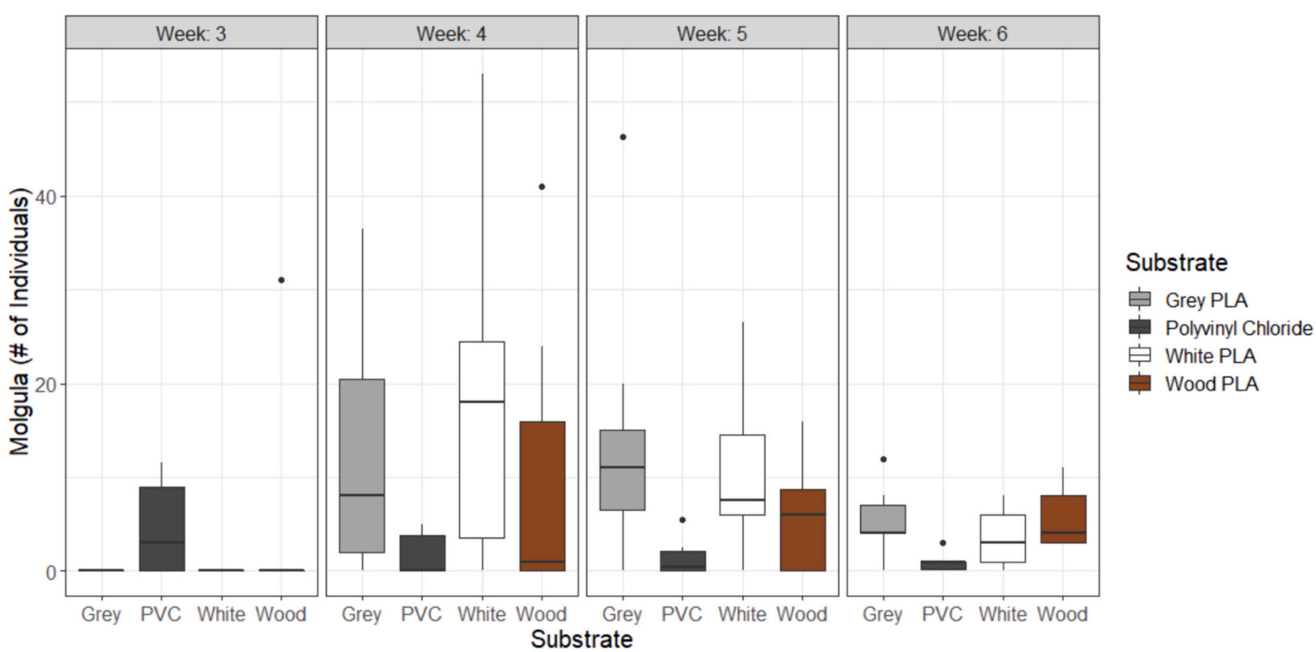

Fig. 8. Settlement of the solitary tunicate *Molgula manhattensis* on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the number of individuals; $n = 5$. There was an effect of both panel material and time on the abundance of ($\chi_1^2 = 29.83$, $p < 0.001$ and $\chi_1^2 = 44.47$, $p < 0.001$).

the observed settlement patterns in the ascidians, as previous work in the area found that the presence of established ascidians did not affect larval settlement of new recruits on PVC panels (Bullard et al., 2004), it is possible that the removal of barnacles allowed for more space for a higher diversity of organisms.

The use of 3D printed materials is a promising line of research that may change future conservation efforts. Workers have had success bio-printing materials with coral-algal symbionts to mimic coral environments (Wangpraseurt et al., 2022). The scaffolding described in this work makes it possible to further study the interactions of coral-host symbionts, and creates a good mimic of this natural relationship. Work with composite substrates found that the presence of soluble inorganic minerals such as silica (SiO_2) and lime mortar ($CaCO_3$) increased settlement of larvae of the elkhorn coral *Acropora palmata* and the grooved brain coral *Diploria labyrinthiformis* (Levenstein et al., 2022). Though this work is promising, more research is needed to understand the

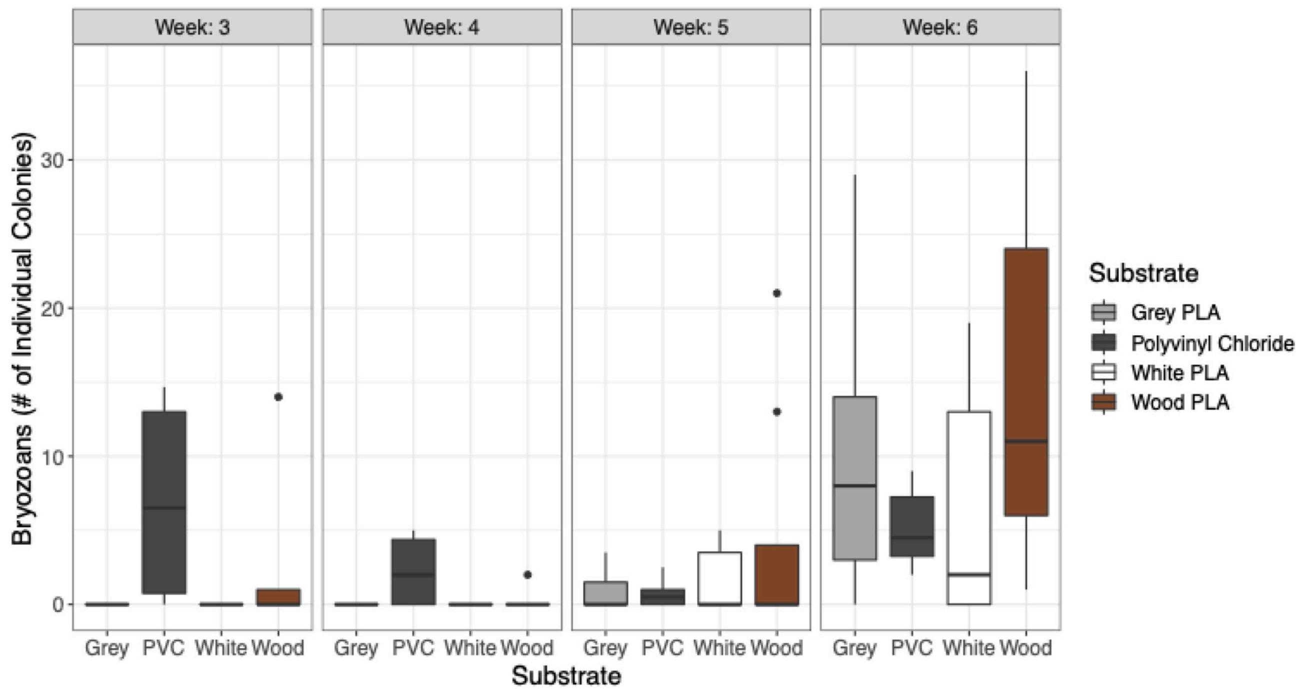

Fig. 9. Settlement of encrusting bryozoans on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the percent area of individual colonies on the panels. There was an effect of both panel material and time on the abundance of ($\chi^2_1 = 14.24$, $p = 0.003$ and $\chi^2_1 = 29.50$, $p < 0.0001$).

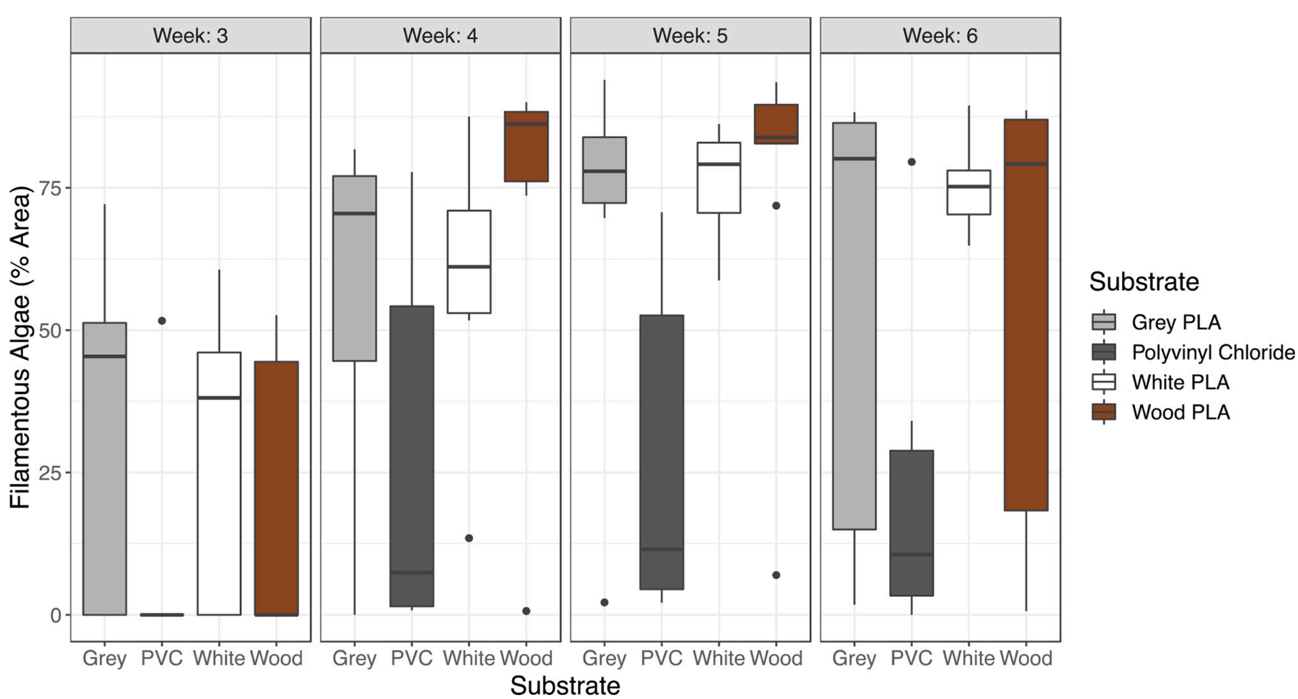

Fig. 10. Settlement of filamentous algae on the different panels (white impact modified PLA, gray impact modified PLA, wood pulp PLA and PVC) during the six-week experimental period. Data are shown as the percent area of algae on the panels. There was an effect of both panel material and time on the abundance of ($\chi^2_1 = 11.06$, $p = 0.011$ and $\chi^2_1 = 8.52$, $p = 0.003$).

chemical cues produced by printed materials, and if they can mimic the natural chemical cues that have been found to enhance settlement in larval invertebrates (Fitt and Coon, 1992; Hadfield and Koehl, 2004). Cues that enhance larval settlement have a rich literature (Hadfield and Koehl, 2004, Neo et al., 2009, Chase et al., 2016, Gómez-Lemos et al., 2018, de la Cruz and Harrison, 2020, Jorissen et al., 2021, Levenstein et al., 2022), and now that we have evidence of the 3D printed panels enhancing recruitment, possible cues mediating this process could be an important avenue for further study.

This work illustrates the potential of using 3D printed panels as recruitment materials in temperate ecosystems, because they were equally or more effective than the traditional PVC materials used and they are easier to manipulate and deploy. Future research will explore the efficacy of the 3D panels for studies of community ecology in tropical marine systems, as well as their potential use as a basis for restoration and conservation.

Author statement

All authors have agreed to be listed and approved the submitted version of the manuscript. In terms of authorship, the concept, experimental framework, funding, and major writing was done by MR, TM and GG contributed to the conceptual framework and development & printing of the 3D panels, data collection and analysis was done by TE, GR, ML, and NS. Statistical analysis was performed by TM, first draft was written by MR, all authors contributed to writing and editing of the manuscript. This is an original manuscript not submitted elsewhere, or previously submitted to a different journal. None of the authors have a conflict of interest or ethical concerns with the work.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge all the organisms that were utilized during this work. Funding was provided by Research Matters (TAM, MR), Connecticut College STEM Summer Research Institute, the Benneck-Polan Foundation (GR, MS, and NS), the National Science Foundation (TE: NSF REU #1950415 to UConn-Mystic Aquarium; TAM: IOS-2113544) and the National Institutes of Health (TAM: 1R01GM135935-01). The authors would like to thank Dr. David Hudson for reviewing an earlier version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2024.152015.

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