Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 48, No. 3, September 2019
pages (262-269)

ISSN 1730-413X
eISSN 1897-3191

Recruitment patterns of the solitary ascidian *Phallusia nigra Savigny*, 1816 on artificial substrates submerged in the central Red Sea, Saudi Arabia

by

Abdul Mohsin A. Al-Sofyani,
Sathianeson Satheesh*

DOI: **10.2478/ohs-2019-0023**
Category: **Original research paper**
Received: **November 05, 2018**
Accepted: **January 21, 2019**

King Abdulaziz University,
Faculty of Marine Sciences,
Department of Marine Biology,
80207 Jeddah, Saudi Arabia

Abstract

The solitary ascidian *Phallusia nigra* is commonly found on hard substrates along the Jeddah coastal waters of the central Red Sea. In this study, the recruitment pattern of *P. nigra* on artificial substrates was assessed in relation to their type, surface color and orientation. The results showed a higher recruitment rate of the ascidian species on concrete and dark panels. The abundance of the ascidian on test panels varied between the four seasons. The orientation (vertical or horizontal) of the panels did not show any major difference in the recruitment. Significant effects of light intensityon the recruitment of *P. nigra* were observed on test panels, with higher abundance on panels submerged in the shade. In conclusion, this study clearly indicates the importance of the type and color of substrates in the recruitment of ascidians on artificial materials.

Key words: biofouling, bioinvasion, ascidians, Ascidiacea, artificial reefs, Red Sea

* Corresponding author: *satheesh_s2005@yahoo.co.in; ssathianeson@kau.edu.sa*

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at www.oandhs.pl

Introduction

The substrate selection by larval forms of marine organisms is an important factor that may to some extent determine the patterns of spatial distribution in adult populations (Pineda et al. 2010). In general, the substrate selection is influenced by various physical, biological and chemical factors such as the type of substrate, its surface properties, composition, color, and microbial biofilms (Rodriguez et al. 1993; Azevedo et al. 2006; Swain et al. 2006; Walker et al. 2007; Satheesh & Wesley 2010; Dobretsov et al. 2013; Whalan et al. 2015; Siddik et al. 2018). Research on the settlement of larvae on different substrates is important for those organisms that are suspected of being invasive, as this will help to understand the potential effects on native communities and establish environmental control policies (Mizrahi et al. 2014).

Ascidians (Phylum: Chordata, Class: Ascidiacea) are commonly found on hard substrates in seawater and are the major part of fouling assemblages on artificial and natural substrates (Holmstrom & Kjelleberg 1994; Swami & Chhapgar 2002; Tracy et al. 2017). Due to their fast growth rate (Lambert 2002) and superior colonization capacity on substrates (Dean 1981; Satheesh & Wesley 2011), ascidians play an important role in determining the structure of fouling communities developed on artificial materials submerged in seawater (Bullard et al. 2007; Satheesh & Wesley 2011). Being widespread in biofouling assemblages, ascidians are easily transferred to new locations through vessel fouling, movement of fouled aquaculture nets as well as oil and gas structures (Tracy et al. 2017). Further, bioinvasion of ascidians to new habitats may lead to significant economic and ecological damage (for a review, see Zhan et al. 2015).

The solitary ascidian *Phallusia nigra* Savigny, 1816 occurs mainly in shallow waters in tropical and sub-tropical regions throughout the world (Vandepas et al. 2015; Kim et al. 2016). The species (*P. nigra*) was reported as an introduced species in the Mediterranean Sea, the Pacific and Indian Oceans (Shenkar 2012; Vandepas et al. 2015; Kim et al. 2016). *P. nigra* is considered native to the Red Sea and the Indian Ocean or the tropical western Atlantic Ocean (De Felice et al. 2001; Kondilatos et al. 2010). Despite the importance of *P. nigra* in biofouling communities, research on recruitment patterns on hard substrates has received little attention compared to other fouling organisms such as barnacles. In their recent study, Salama et al. (2018a) identified ascidians as one of the important fouling organisms on aquaculture cage nets in the central Red Sea. In addition, ascidian recruitment patterns are mainly species-specific, which may differ between different substrates, their orientation, and seasons (Shenkar et al.2008). Therefore, this study assesses the recruitment patterns of *P. nigra* on artificial substrates in the central Red Sea waters. The questions addressed were as follows: 1) Does the recruitment of *P. nigra* depend on the type of substrate? 2) Does the substrate orientation affect the recruitment of *P. nigra*? 3) Does the color of the substrate affect the recruitment of ascidian species and 4) Does the abundance of ascidian species on substrates vary depending on the season? The results obtained in this study will improve our knowledge about the settlement behavior of *P. nigra*, a cosmopolitan ascidian species considered invasive in many regions.

Materials and methods

The experiment was conducted at Obhur Creek (21°42′33.52″N, 39°5′45.71″E) in the central Red Sea, Jeddah, Saudi Arabia. Obhur Creek was selected as a study site due to the presence of jetties and pontoons that offer a potential site for the settlement of sessile organisms. The experimental design comprised three different aspects, i.e. the effects of the type, orientation and color of substrates. To test the effects of the substrate type and orientation, artificial test panels (15 × 15 cm) were prepared using acrylic (colorless, transparent sheet, ISO 9001 grade), concrete blocks, ceramic tiles (white polished surface on one side) and stainless steel plates. The panels were mounted on a PVC frame (raft) to be submerged in the sea. Each raft contained four panels (one from each material) and a total of eight rafts were prepared. Of these, four rafts were submerged in the Creek waters in the vertical position, while the remaining four rafts were kept in a horizontal position. The panels were submerged at a depth of 2 m in Obhur Creek in winter (December 2016), autumn (February 2017), spring (April 2017) and summer (July 2017). The test panels (n = 4 for each material and position) were retrieved from the seawater after four weeks of submersion in each season. They were immediately transferred to the laboratory and kept in a refrigerator (for a maximum duration of 24 h) below 4°C for further analysis (Glasby & Connell 2001).

Artificial test panels (15 × 15 cm; total surface area of 225 cm^2) were prepared using acrylic sheets with four different colors (red, blue, green and yellow) to study the effects of surface color on ascidian settlement. Control test panels were also prepared using a colorless (transparent) acrylic sheet. Each raft consisted of five panels (one panel for each color and one colorless panel). A total of eight rafts were prepared for submersion. Four rafts (n = 4 for each color) were submerged in a vertical position at a depth of 2 m (exposed to light) at Obhur Creek. The remaining four rafts were submerged under a jetty (shade) in a vertical position at a depth of 2 m. The experiment was carried out in spring (April 2017) and summer (July 2017). The light intensity at the submersion zone of the panels were 26 μmol m^{-2} s^{-1} (under the jetty, shade) and 450 μmol m^{-2} s^{-1} (open waters, the site where panels were exposed to light). The light intensity was measured using a portable underwater light meter (Underwater Light Sensor, Skye Instruments Ltd). The panels were retrieved after four weeks of submersion each season and immediately transported to the laboratory, where they were refrigerated before analysis as described above.

The recruited *P. nigra* individuals were counted manually on all test panels (only one side of the panels was analyzed; the upper side in the case of panels placed in a horizontal position). The panels were also observed under a stereomicroscope (Leica) to check for newly settled individuals of *P. nigra*. The taxonomic characters described by Vandepas et al. (2015) for the identification of *P. nigra* were used to distinguish this species from other ascidians (both solitary and colonial) recruited on the panels. The mean (± SE) abundance for each treatment was calculated and presented as the number of individuals per 225 cm^2 of panel surface area.

Statistical analysis

Three-way factorial ANOVA (analysis of variance) was used to analyze the differences in the recruitment of the ascidian species on the test panels prepared from different materials. For this purpose, the type of substrate and its orientation as well as seasons were used as factors. In addition, three-way ANOVA was employed using the seasons, substrate color and light as factors to understand the effects of surface color on ascidian recruitment. ANOVA was conducted using the STATISTICA software (version 13). The interaction plots for the abundance of *P. nigra* on different treatment panels were prepared using Fast Statistics (version 2.0).

Results

P. nigra recruitment on different materials

Among different materials submerged in horizontal and vertical positions, the recruitment of *P. nigra* showed a maximum of 3.75 individuals per 225 cm^2 on concrete panels deployed in a horizontal position (Fig. 1). The results also indicate that *P. nigra* recruitment was high on concrete panels, followed by stainless steel (Fig. 2). The recruitment of ascidians

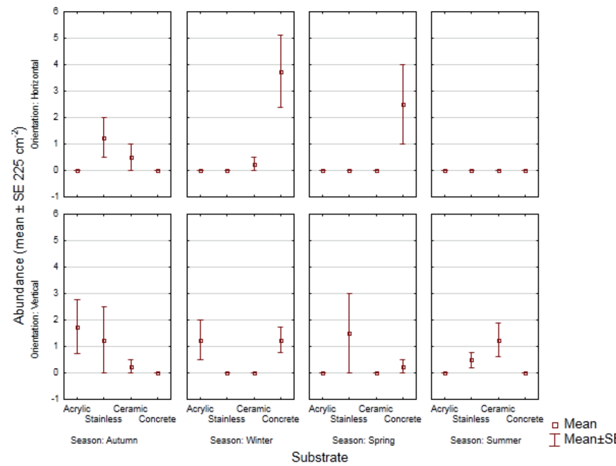

Figure 1

P. nigra recruitment pattern (number of individuals, mean ± SE, n = 4) on artificial substrates submerged in vertical and horizontal positions

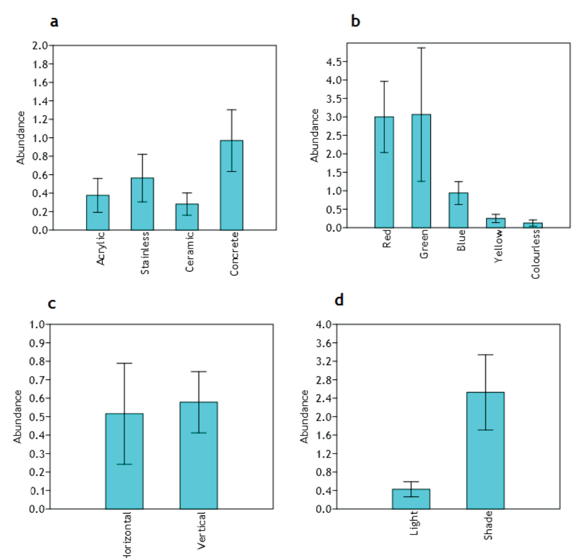

Figure 2

Abundance of *P. nigra* on different treatment panels used in this study. Abundance (mean ± SE 225 cm^{-2}) data were pooled for each factor and all treatments. a) Recruitment pattern on different substrate types. b) Abundance of the ascidian on substrates indifferent colors. c) Abundance of the ascidian on panels submerged in vertical and horizontal orientation. d) Recruitment pattern on panels submerged in the shade and exposed to light was high in winter and low in summer (Fig. 3). The interaction plot revealed a higher abundance of ascidians on vertical panels except for concrete ones (Fig. 4). Further, the recruitment of the *P. nigra* was not observed on panels deployed in a horizontal position during the summer season. Three-way ANOVA results showed no significant variation in *P. nigra* recruitment on substrates in relation to such factors as the season, the type of substrate and its orientation (Table 1). However, the abundance of *P. nigra* revealed a significant interaction effect between the type of substrate and the season and between the type of substrate and its orientation (Table 1).

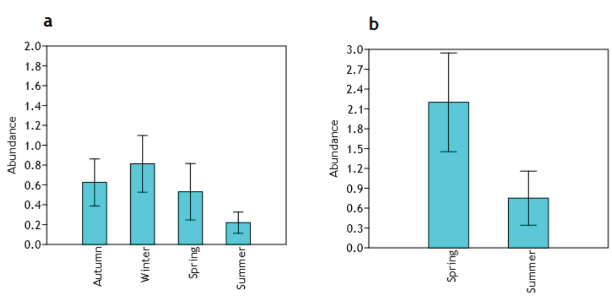

Figure 3

Seasonal recruitment pattern of *P. nigra* on artificial materials submerged in the central Red Sea. a) Pooled data for the seasonal abundance of the ascidian on different types of substrate b) Pooled data for the seasonal abundance of the ascidian on substrates in different colors. Abundance (mean ± SE) for the panel surface area of 225 cm^2

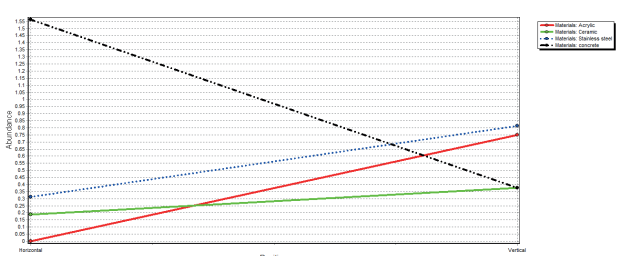

Figure 4

Interactive plot of *P. nigra* recruitment on different substrates submerged in vertical and horizontal orientations

Recruitment of *P. nigra* on substrates indifferent colors

The recruitment of ascidians showed a significant difference between the panels submerged in the shade and exposed to light (Table 2). The interaction plots revealed that the abundance of ascidians was high on the test panels deployed in the shade (Fig. 5). Among the test panels indifferent colors, the green and red panels submerged in the shade showed higher abundance – 11.5 and 6.5 individuals per 225 cm^2, respectively (Fig. 6). A maximum of 2.25 individuals per 225 cm^2 was observed on the blue test panels submerged in the shade. On the other hand, among the panels exposed to light, the red panels showed a

Table 1

Three-way ANOVA results of *P. nigra* recruitment on different types of substrate ($p < 0.05$ = significant effect). Season, substrate type and orientation were used as factors for ANOVA

	SS	Df	MS	F	p
Season	5.9063	3	1.9687	1.0989	0.353
Substrate	8.9063	3	2.9687	1.6570	0.181
Orientation	0.1250	1	0.1250	0.0697	0.792
Season*Substrate	43.1562	9	4.7951	2.6765	0.008
Season*Orientation	3.9375	3	1.3125	0.7326	0.535
Substrate*Orientation	17.9375	3	5.9791	3.3374	0.022
Season*Substrate*Orientation	18.2500	9	2.0277	1.1318	0.348
Error	173.7813	97	1.7915		

Table 2

Three-way ANOVA results for the ascidian *P. nigra* recruitment on substrates in different colors submerged in the shade and exposed to light. Season, substrate color and light were used as factors ($p < 0.05$ = significant effect)

	SS	Df	MS	F	p
Season	42.0500	1	42.0500	3.5207	0.065
Color	135.3250	4	33.8312	2.8326	0.031
Light	88.2000	1	88.2000	7.3848	0.008
Season*Color	105.0750	4	26.2687	2.1994	0.079
Season*Light	9.8000	1	9.80000	0.8205	0.368
Color*Light	97.9250	4	24.4812	2.0497	0.098
Season*Color*Light	137.0750	4	34.2687	2.8692	0.030
Error	728.5500	61	11.9434		

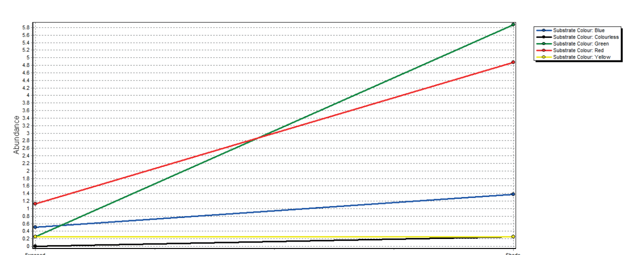

Figure 5

Interactive plot of *P. nigra* recruitment on substrates in different colors submerged in the shade and exposed to light maximum of 2.25 individuals per 225 cm². In general, red and green panels supported higher abundance of ascidians compared to panels in other colors (Fig. 2). Three-way ANOVA (Table 2) indicated a significant variation in the recruitment of the *P. nigra* on the test panels depending on the color of the substrate and light intensity (light or shade). The submersion season of the test panels showed no significant effect on the recruitment of *P. nigra*, but the interaction of season, color and light had a significant effect.

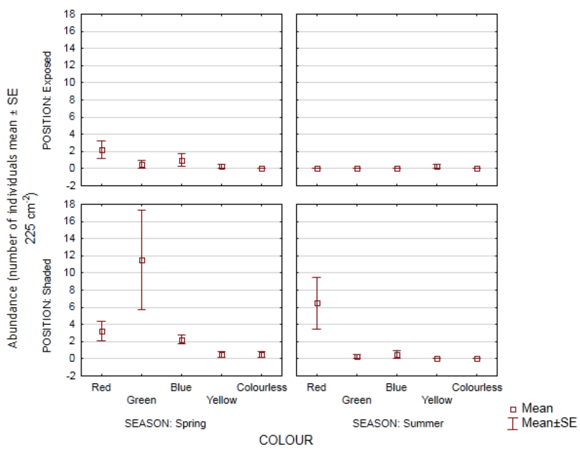

Figure 6

Abundance (number of individuals, mean ± SE, n = 4) of *P. nigra* on panels in different colors submerged in the shade and exposed to light

Discussion

Understanding the recruitment pattern of ascidians on different materials submerged in seawater is essential due to their superior invasive potential in new habitats (Marins et al. 2010; Novak et al. 2017; Tracy et al. 2017). The results of this study revealed considerable variations in the abundance of *P. nigra* on different treatment panels submerged in the Creek, i.e. factors such as substrate color and light have a significant effect on the recruitment of *P. nigra* on artificial materials. The significant pair-wise interactive effect was also observed between the type of substrate and its orientation. A number of studies have previously reported temporal and spatial dynamics in the recruitment of ascidians on test materials in different geographical locations (Oren & Benayahu 1998; Swami & Chhapgar 2002; Satheesh & Wesley 2008; 2011; Bullard et al. 2013). However, most of these studies were conducted using only one type of material as a test substrate. Benthic assemblage recruitment assessment, using different materials and multiple treatment factors, may provide valuable information compared to studies conducted with either one material or one factor (Shenkar et al. 2008; Siddik et al. 2018). Of all the materials used in this study, *P. nigra* recruitment was high on concrete andstainless steel panels. These two materials (concrete and stainless steel) are commonly used to createman-made structures in coastal habitats around the world (Bulleri & Chapman 2010; Valdez et al. 2016). The preference of concrete plates by the ascidian *Botrylloides violaceus* was previously reported based on a laboratory study by Chase et al. (2016). While many factors have been reported to affect the settlement of benthic organisms on substrates (for a review, see Rodriguez et al. 1993), the ascidian abundance pattern on different substrates in this study showed that the surface roughness (though not analyzed in this study) seems to have played a major role in the increased settlement rate on concrete (see Chase et al. 2016) and stainless steel panels. Moreover, the surface roughness of the concrete (which supported the maximum number of individuals) may be high (Azevedo et al. 2006) compared to other panels used in this study. The possible role of surface roughness in the recruitment pattern of *P. nigra* on different substrates observed in this study is further supported by data from the two other panels (ceramic and acrylic), which have a polished surface and supported lower recruitment.

The orientation of substrates has been reported in previous studies as one of the factors affecting the recruitment of ascidians (Glasby 2000; Knot et al. 2004). Although no significant difference was observed in the abundance between vertically and horizontally deployed panels in this study, more individuals were found on the concrete panels submerged in a horizontal position. This observation was in contrast with other panels that supported the maximum abundance on vertical panels. Many previous studies have also reported higher abundance of benthic organisms on vertical substrates (Van Dolah et al. 1988; Bayness 1999; Walker et al. 2007). The variations in the benthic community composition between vertically and horizontally orientated substrates are mainly attributed to the difference in light intensity (horizontal surfaces receive direct sunlight), which may favor increased algal growth on horizontal surfaces (Miller & Etter 2008). In general, ascidians favored vertical substrates due to the shade, reduced sedimentation and low competition from algal species (Young & Chia 1984). Further, the bottom side of the horizontally submerged panels may attract a larger number of organisms due to limited stress from physical and biological factors (Sokolowski et al. 2017). The bottom side of the panels placed in the horizontal position was not analyzed in this study due to the differences in surface topography between both sides of ceramic tiles.

The results obtained in this study revealed that the light intensity and surface color have significant effects on the recruitment of *P. nigra* on the test substrates. Biofouling organisms generally prefer dark surfaces rather than light-colored surfaces (Satheesh & Wesley 2010; Dobretsov et al. 2013). Only a few studies have previously addressed the effects of substrate color on the recruitment of ascidians (e.g. Ells et al. 2016). While some earlier studies have reported that larval forms of fouling organisms show preferences for substrates with a specific color (e.g. Satheesh & Wesley 2010; Dobretsov et al. 2013), few studies have shown no significant correlation between substrate color and settlement (e.g. Ells et al. 2016). Although the results of this study have confirmed the preference of *P. nigra* for dark substrates (red, green and blue) during the settlement process, its abundance differed between the panels submerged in the shade and those exposed to light. As the abundance of *P. nigra* on the colored test panels varied depending on the shade and light, it is important to differentiate between the response of larvae to spectral composition (color) and light intensity (Crisp 1976; Ells et al. 2016). The results of this study showed higher abundance of the ascidian on panels submerged under the jetty (shade) compared to those exposed to light. This difference may be due to the photonegative behavior of the larvae (Darbyson et al.2009),which enables them to settle on shaded surfaces. Interestingly, the large difference in the abundance was observed on red and green panels submerged under the jetty (shade) and exposed to light. Further, the abundance on the two other panels, yellow and colorless, did not show a large difference between the panels submerged in the shade and those exposed to light. Ascidian larvae may avoid environments with too high or too low light intensity (Van Duyl et al. 1981) and the results obtained from the panels submerged in the shade and those exposed to light confirmed that *P. nigra* preferred panels submerged in the shade over those exposed to light.

Although ANOVA did not reveal a significant difference in the abundance of *P. nigra* between panel submersion seasons, considerable variations in the recruitment pattern were observed between the seasons. The abundance of *P. nigra* on different treatment panels showed that the recruitment was low in summer (Fig. 3), which indicates that seasonal factors may also play an important role in the ascidian recruitment on artificial materials. Previous studies from the central Red Sea also confirmed the significant seasonal variations in the abundance of most biofouling organisms on the artificial substrate (Salama et al. 2018b). For instance, Salama et al. (2018a) described solitary ascidians as dominant fouling organisms on aquaculture nets submerged in the central Red Sea and found their higher abundance in summer and autumn. Another study also reported seasonal variations in the abundance of invertebrates on artificial materials submerged in the central Red Sea (Siddik et al. 2018). In this study, the recruitment of *P. nigra* was very low in the summer season. In general, seasonal changes in environmental factors play a key role in the settlement of benthic communities on artificial substrates (Satheesh & Wesley 2008; 2011). The lower abundance of the *P. nigra* in the summer season on the materials submerged in this study raises further questions regarding the effect of multiple factors (including food availability; Lopez-Legentil et al. 2005), because higher temperature (during summer months in the central Red Sea, Salama et al. 2018b) usually favors an increase in the recruitment of benthic organisms on hard substrates (Satheesh et al. 2008).

In conclusion, the present study shows that the recruitment of the ascidian *P. nigra* varied between different substrates, with higher abundance observed on concrete panels. Furthermore, *P. nigra* preferred dark substrates over yellow and colorless panels. While the orientation of substrates did not show significant differences in the recruitment, light intensity plays an important role, which was evidenced by higher recruitment on panels submerged under the jetty (shade). As *P. nigra* is native to the Red Sea and considered potentially invasive in other regions, the results obtained in this study may not be extrapolated to new habitats due to local environmental conditions and other biotic factors in a given area. However, they confirmed that some substrates (e.g. concrete and stainless steel) are more susceptible to *P. nigra* colonization than others. In addition, sites with limited light availability may be suitable for the settlement of *P. nigra*. Further long-term research on the interaction of *P. nigra* with other fouling organisms and the role of environmental factors in the recruitment may improve our knowledge about the invasion success in new habitats.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G-222-150-38. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

References

Azevedo, F.B.B., Carloni, G.G. & Carvalheira, L.V. (2006). Colonization of benthic organisms on different artificial substratum in Ilha Grande bay, Rio de Janeiro, Brazil. *Braz. Arch. Biol. Technol.* 49: 263–275. DOI: 10.1590/S1516-89132006000300012.

Baynes, T.W. (1999). Factors structuring a subtidal encrusting community in the southern Gulf of California. *Bull. Mar. Sci.* 64: 419–450.

Bullard, S.G., Lambert, G., Carman, M.R., Byrnes, J., Whitlatch, R.B. et al. (2007). The colonial ascidian *Didemnum* sp. A: current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. *J. Exp. Mar. Biol. Ecol.* 342: 99–108. DOI: 10.1016/j.jembe.2006.10.020.

Bullard, S.G., Davis, C.V. & Shumway, S.E. (2013). Seasonal patterns of ascidian settlement at an aquaculture facility in the Damariscotta River, Maine. *J. Shellfish. Res.* 32(2): 255–264. DOI: 10.2983/035.032.0202.

Bulleri, F. & Chapman, M.G. (2010). The introduction of coastal infrastructure as a driver of change in marine environments. *J. Appl. Ecol.* 47(1): 26–35. DOI: 10.1111/j.1365-2664.2009.01751.x.

Chase, A.L., Dijkstra, J.A. & Harris, L.G. (2016). The influence of substrate material on ascidian larval settlement. *Mar. Pollut. Bull.* 106(1–2): 35–42. DOI: 10.1016/j.marpolbul.2016.03.049.

Darbyson, E., Locke, A., Hanson, J.M. & Willison, J.M. (2009). Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. *Aquat. Invasions* 4(1): 87–94. DOI: 10.3391/ai. 2009.4.1.9.

De Felice, R.C., Eldredge, L.G. & Carlton, J.T. (2001). Nonindigenous marine invertebrates. In L.G. Eldredge & C. Smith (Eds.), *A Guidebook of Introduced Marine Species in Hawaii* (pp. bi–bvi). Honolulu: Bishop Museum Technical Report.

Dean, T.A. (1981). Structure aspects of sessile invertebrates as organizing forces in an estuarine fouling community. *J. Exp. Mar. Biol. Ecol.* 53: 163–180.

Dobretsov, S., Abed, R.M. & Voolstra, C.R. (2013). The effect of surface colour on the formation of marine micro and macrofouling communities. *Biofouling* 29(6): 617–627. DOI: 10.1080/08927014.2013.784279.

Ells, V., Filip, N., Bishop, C.D., DeMont, M.E., Smith-Palmer, T. et al. (2016). A true test of colour effects on marine invertebrate larval settlement. *J. Exp. Mar. Biol. Ecol.* 483: 156–161. DOI: 10.1016/j.jembe.2016.07.011.

Glasby, T.M. (2000). Surface composition and orientation interact to affect subtidal epibiota. *J. Exp. Mar. Biol. Ecol.* 248: 177–190.

Glasby, T.M. & Connell, S.D. (2001). Orientation and position of substrata have large effects on epibiotic assemblages. *Mar. Ecol. Prog. Ser.* 214: 127–135.

Holmstrom, C. & Kjelleberg, S. (1994). The effect of external biological factors on settlement of marine invertebrate and new antifouling technology. *Biofouling* 8(2): 147–160.

Kim, I.H., Cruz-Rivera, E., Sherif, M.E.D. & El-Sahhar, S. (2016). Cyclopoid copepods (Ascidicolidae, Notodelphyidae) associated with *Phallusia nigra* Savigny, 1816 (Ascidiacea) in the Red Sea: a new ascidicolid and first descriptions of the males from two notodelphyids. *J. Crustac. Biol.* 36(4): 553–566. DOI: 10.1163/1937240X-00002439.

Knott, N.A., Underwood, A.J., Chapman, M.G. & Glasby, T.M. (2004). Epibiota on vertical and horizontal surfaces on natural reefs and on artificial structures. *J. Mar. Biol. Ass. UK* 84: 1117–1130.

Kondilatos, G., Corsini-Foka, M. & Pancucci-Papadopoulou, M.A. (2010). Occurrence of the first non-indigenous ascidian *Phallusia nigra* Savigny, 1816 (Tunicata: Ascidiacea) in Greek waters. *Aquat. Invasions* 5(2): 181–184. DOI: 10.3391/ai.2010.5.2.08.

Lambert, G. (2002). Nonindigenous ascidians in tropical waters. *Pacific. Sci.* 56: 291–298.

Lopez-Legentil, S., Ruchty, M., Domenech, A. & Turon, X. (2005). Life cycles and growth rates of two morphotypes of Cystodytes (Ascidiacea) in the western Mediterranean. *Mar. Ecol. Prog. Ser.* 296: 219–228.

Marins, F.O., Novaes, R.L., Rocha, R.M. & Junqueira, A.O. (2010). Non indigenous ascidians in port and natural environments in a tropical Brazilian bay. *Zoologia* 27(2): 213–221. DOI: 10.1590/S1984-46702010000200009.

Miller, R.J. & Etter, R.J. (2008). Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. *Ecology* 89: 452–462.

Mizrahi, D., Navarrete, S.A. & Flores, A.A. (2014). Uneven abundance of the invasive sun coral over habitat patches of different orientation: An outcome of larval or later benthic processes? *J. Exp. Mar. Biol. Ecol.* 452: 22–30. DOI: 10.1016/j.jembe.2013.11.013.

Novak, L., Lopez-Legentil, S., Sieradzki, E. & Shenkar, N. (2017). Rapid establishment of the non-indigenous ascidian *Styela plicat*a and its associated bacteria in marinas and fishing harbors along the Mediterranean coast of Israel. *Mediterr. Mar. Sci.* 18(2): 324–331. DOI: 10.12681/mms.2135.

Oren, U. & Benayahu, Y. (1998). Didemnid ascidians: rapid colonizers of artificial reefs in Eilat (Red Sea). *Bull. Mar. Sci.* 63(1): 199–206.

Pineda, J., Porri, F., Starczak, V. & Blythe, J. (2010). Causes of decoupling between larval supply and settlement and consequences for understanding recruitment and population connectivity. *J. Exp. Mar. Biol. Ecol*. 392: 9–21. DOI: 10.1016/j.jembe.2010.04.008.

Rodriguez, S.R., Ojeda, F.P. & Inestrosa, N.C. (1993). Settlement of benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* 97(2): 193–207.

Salama, A.J., Satheesh, S. & Balqadi, A.A. (2018a). Biofouling community development on nylon net panels submerged in the central Red Sea coast, Saudi Arabia. *Cah. Biol. Mar.* 59: 517–525. DOI: 10.21411/CBM.A.EA4208FE.

Salama, A.J., Satheesh, S. & Balqadi, A.A. (2018b). Development of Biofouling Communities on Nylon Net Panels Submerged in the Central Red Sea: Effects of Season and Depth. *Thalassas* 34(1): 199–208. DOI: 10.1007/s41208-017-0052-z.

Satheesh, S. & Wesley, S.G. (2008). Seasonal variability in the recruitment of macrofouling community in Kudankulam waters, east coast of India. *Estuar. Coast. Shelf. S.* 79(3): 518–524. DOI: 10.1016/j.ecss.2008.05.008.

Satheesh, S. & Wesley, S.G. (2010). Influence of substratum colour on the recruitment of macrofouling communities. *J. Mar. Biol. Ass. UK* 90(5): 941–946. DOI: 10.1017/S0025315410000032.

Satheesh, S. & Wesley, S.G. (2011). Influence of submersion season on the development of test panel biofouling communities in a tropical coast. *Estuar. Coast. Shelf. S.* 94(2): 155–163. DOI: 10.1016/j.ecss.2011.06.011.

Shenkar, N., Zeldman, Y. & Loya, Y. (2008). Ascidian recruitment patterns on an artificial reef in Eilat (Red Sea). *Biofouling* 24(2): 119–128. DOI: 10.1080/08927010801902083.

Shenkar, N. (2012). Ascidian (Chordata, Ascidiacea) diversity in the Red Sea. *Marine Biodiversity* 42: 459–469. DOI: 10.1007/s12526-012-0124-5.

Siddik, A.A., Al-Sofyani, A.A., Ba-Akdah, M.A. & Satheesh, S. (2018). Invertebrate recruitment on artificial substrates in the Red Sea: role of substrate type and orientation. *J. Mar. Biol. Ass. UK*. DOI: 10.1017/S0025315418000887.

Sokołowski, A., Ziółkowska, M., Balazy, P., Kukliński, P. & Plichta, I. (2017). Seasonal and multi-annual patterns of colonisation and growth of sessile benthic fauna on artificial substrates in the brackish low-diversity system of the Baltic Sea. *Hydrobiologia* 790: 183–200. DOI: 10.1007/s10750-016-3043-9.

Swain, G., Herpe, S., Ralston, E. & Tribou, M. (2006). Short-term testing of antifouling surfaces: the importance of colour. *Biofouling* 22: 425–429. DOI: 10.1080/08927010601037163.

Swami, B.S. & Chhapgar, B.F. (2002). Settlement pattern of ascidians in harbour waters of Mumbai, west coast of India. *Indian J. Mar. Sci.* 31(3): 207–212.

Tracy, B.M., Larson, K.J., Ashton, G.V., Lambert, G., Chang, A.L. et al. (2017). Northward range expansion of three non-native ascidians on the west coast of North America. *BioInvasions Rec.* 6(3): 203–209. DOI: 10.3391/bir.2017.6.3.04.

Valdez, B., Ramirez, J., Eliezer, A., Schorr, M., Ramos, R. et al. (2016). Corrosion assessment of infrastructure assets in coastal seas. *J. Mar. Eng. Technol.* 15(3): 124–134. DOI: 10.1080/20464177.2016.1247635.

Van Duyl, F.C., Bak, R.P.M. & Sybesma, J. (1981). The ecology of the tropical compound ascidian *Trididemnum solidum*. I. Reproductive strategy and larval behaviour. *Mar. Ecol. Prog. Ser.* 35–42.

Van Dolah, R.F., Wendt, P.H., Knott, D.M. & Wenner, E.L. (1988). Recruitment and community development of sessile fouling assemblages on the continental shelf off South Carolina, USA. *Estuar. Coast. Shelf. S.* 26(6): 679–699.

Vandepas, L.E., Oliveira, L.M., Lee, S.S.C., Hirose, E., Rocha, R.M. et al. (2015). Biogeography of *Phallusia nigra*: is it really black and white? *Biol. Bull.* 228: 52–64.

Walker, S.J., Schlacher, T.A. & Schlacher–Hoenlinger, M.A. (2007). Spatial heterogeneity of epibenthos on artificial reefs: fouling communities in the early stages of colonization on an East Australian shipwreck. *Marine Ecology* 28: 435–445.

Whalan, S., Wahab, M.A.A., Sprungala, S., Poole, A.J. & De Nys, R. (2015). Larval settlement: the role of surface topography for sessile coral reef invertebrates. *PloS one* 10: e0117675.

Young, C.M. & Chia, F.S. (1984). Microhabitat-associated variability in survival and growth of subtidal solitary ascidians during the first 21 days after settlement. *Mar. Biol.* 81(1): 61–68.

Zhan, A., Briski, E., Bock, D.G., Ghabooli, S. & MacIsaac, H.J. (2015). Ascidians as models for studying invasion success. *Mar. Biol.* 162: 2449–2470. DOI: 10.1007/s00227-015-2734-5.