Marine Pollution Bulletin 106 (2016) 35–42

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

The influence of substrate material on ascidian larval settlement

Anna L. Chase [a,*], Jennifer A. Dijkstra [b], Larry G. Harris [a]

[a] Department of Biological Sciences, University of New Hampshire, 46 College Road, Durham, NH 03824, United States
[b] Center for Coastal and Ocean Mapping, University of New Hampshire, 24 Colovos Road, Durham, NH 03824, United States

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 14 March 2016
Accepted 20 March 2016
Available online 1 April 2016

Keywords:
Larval substrate preference
Ascidians
Artificial substrate
Surface roughness
Botrylloides violaceus
Ciona intestinalis

ABSTRACT

Submerged man-made structures present novel habitat for marine organisms and often host communities that differ from those on natural substrates. Although many factors are known to contribute to these differences, few studies have directly examined the influence of substrate material on organism settlement. We quantified larval substrate preferences of two species of ascidians, *Ciona intestinalis* (cryptogenic, formerly *C. intestinalis* type B) and *Botrylloides violaceus* (non-native), on commonly occurring natural (granite) and man-made (concrete, high-density polyethylene, PVC) marine materials in laboratory trials. Larvae exhibited species-specific settlement preferences, but generally settled more often than expected by chance on concrete and HDPE. Variation in settlement between materials may reflect preferences for rougher substrates, or may result from the influence of leached chemicals on ascidian settlement. These findings indicate that an experimental plate material can influence larval behavior and may help us understand how substrate features may contribute to differences in settlement in the field.

1. Introduction

Most benthic marine organisms have a biphasic life cycle, including a pelagic larval phase and a demersal, sessile, or sedentary adult phase. Life history strategies vary widely between taxa, but pelagic larval stages often facilitate the dispersal of juvenile organisms. Larvae can spend minutes to months in the water column before metamorphosing and settling onto the seafloor and other surfaces. The site of this eventual settlement is of particular importance for sessile organisms, which are unable to change their location following substrate attachment and metamorphosis. Accordingly, the initial settlement patterns play a large role in the structuring of benthic communities, though these assemblages are also influenced by post-settlement mortality, competition, and growth (Connell, 1985; Hunt and Scheibling, 1997; Fraschetti et al., 2002). Given the influence of settlement site selection on organism survival and fitness, it is unsurprising that many sessile animals exhibit active settlement preferences (e.g., tube-building polychaetes *Hydroides dianthus* and hydroid *Ectopleura crocea*, Mullineaux and Garland, 1993; colonial ascidian *Diplosoma similis*, Stoner, 1994; hydroid *E. crocea* and barnacle *Balanus* sp., Lemire and Bourget, 1996; barnacle *Balanus crenatus*, Miron et al., 1996). Numerous factors, including light intensity (Thorson, 1964; Durante, 1991; Rius et al., 2010), proximity of prey or host organisms (Pawlik, 1992) or conspecifics (reviewed in Burke, 1986 and Pawlik, 1992), surface microtopography (Wethey, 1986; Kerr et al., 1999; Lemire and Bourget, 1996) and substrate chemical composition (Kerr et al., 1999; Bavestrello et al., 2000) are known to influence sessile organism settlement.

Many benthic taxa exhibit dramatic differences in abundance on natural and man-made structures (e.g., ascidians, Lambert, 2002; Simkanin et al., 2012), often causing resultant variation in fouling community composition on these surfaces (Holloway and Connell, 2002; Bulleri, 2005; Wilhelmsson and Malm, 2008). These community differences are frequently driven by non-native species, which are often more prevalent on man-made marine structures than on natural surfaces (Lambert and Lambert, 2003; Simkanin et al., 2012). Anthropogenic structures are at high risk of colonization by non-indigenous species, as these constructions are often located in high-traffic areas and exposed to direct influxes of non-native propagules via aquaculture, shipping, and recreational boating activities (Carlton, 1989; Carlton and Geller, 1993; Floerl and Inglis, 2005). Disparities in community structure and species composition may result from several factors that tend to vary systematically between natural and man-made substrates, including surface orientation and light exposure (Vandermeulen and Dewreede, 1982; Glasby and Connell, 2001; Thomason et al., 2002; Miller and Etter, 2008) proximity to the sea floor or water surface (Glasby and Connell, 2001; Holloway and Connell, 2002), and predation exposure (Otsuka and Dauer, 1982; Dumont et al., 2011), all of which are known to influence community development. Substrate type and composition are other factors that may contribute to observed differences in fouling on natural and man-made surfaces (Bavestrello et al., 2000). Though multiple studies have compared fouling community assemblages on different materials in the field (McGuinness, 1989; Anderson and Underwood, 1994; Tyrrell and Byers, 2007; Andersson et al., 2009; Vaz-Pinto et al., 2014), relatively few studies have directly

---

* Corresponding author.
E-mail address: ali269@wildcats.unh.edu (A.L. Chase).

http://dx.doi.org/10.1016/j.marpolbul.2016.03.049

examined larval settlement preferences for different materials (scyphozoa, Holst and Jarms, 2006; Hoover and Purcell, 2008).

Laboratory experiments that examine larval behavior are a valuable complement to field-based community studies and can help identify whether variation in initial settlement density and/or post-settlement mortality and growth rates are responsible for observed differences in community composition in the field. Laboratory studies have discovered that many invertebrates, including most ascidians, exhibit preferential settlement on non-illuminated surfaces (Durante, 1991; Rius et al., 2010). This adaptation may serve to encourage settlement in areas where competition with, and overgrowth by, macroalgae is less likely. Substrate microtopography, which can influence flow conditions, shear stress, and the availability of dissolved gasses and food particles (Vogel, 1996), can enhance or reduce larval settlement (Howell and Behrends, 2006; Orlov, 1996). Rougher surfaces, which have more turbulent boundary layer flows, are known to facilitate the settlement of certain organisms, including hydroids, barnacles (Mullineaux and Butman, 1991; Wright and Boxshall, 1999), bivalves (Bologna and Heck, 2000), and polychaetes (Hurlbut, 1991; Walters et al., 1999). However, many other species preferentially settle on smooth substrates (ascidians, bryozoans, polychaetes, Osman and Whitlatch, 1995a,b; barnacles, Lemire and Bourget, 1996). Material characteristics, including substrate color and chemical composition, are also known to impact settlement, though few studies have directly examined these topics (Satheesh and Wesley, 2010; Bavestrello et al., 2000).

To determine if larval settlement preferences may contribute to observed differences in ascidian abundance on natural- and man-made surfaces in the Gulf of Maine, we examined larval settlement in two commonly occurring species. These organisms, the solitary *Ciona intestinalis* (Linnaeus, 1767) and the colonial *Botrylloides violaceus* (Oka, 1927), produce larvae that are easily obtained in quantities suitable for manipulative experimentation. Larvae were exposed to settlement plates composed of concrete, granite tile, high-density polyethylene (HDPE), and polyvinyl chloride (PVC) during controlled laboratory trials. As changes in biofilm composition and thickness over time can influence larval settlement (Wieczorek and Todd, 1997), trials were conducted using plates subjected to two different durations of pre-trial soaking. In the Gulf of Maine, *B. violaceus* and *C. intestinalis* are non-native and cryptogenic, respectively. These and other ascidian species frequently dominate sessile communities on man-made marine structures in the Gulf of Maine (e.g., Dijkstra and Harris, 2009). Therefore, we hypothesized that larvae of both species would settle more frequently on artificial substrates than on a pseudo-natural substrate (granite tile).

2. Methods

2.1. Study species

Both *C. intestinalis* and *B. violaceus* commonly occur on man-made structures, also colonizing natural substrates, in the southwestern Gulf of Maine. In 2015 it was recognized that *C. intestinalis* included two distinct species, *C. intestinalis* (formerly *C. intestinalis* type B) and *Ciona robusta* (formerly *C. intestinalis* type A; Brunetti et al., 2015). Species-defining molecular or morphological characters were not assessed, but all animals used in this study were collected in New Hampshire, Maine, and Massachusetts in the fall of 2014. Only *C. intestinalis* is known to occur within this range (Zhan et al., 2010), therefore we assume that the animals studied were *C. intestinalis*. *C. intestinalis* was present in the Gulf of Maine, where it is considered cryptogenic, before the 1940s (Miner, 1950) and has become a common and often dominant fouling community constituent on floating docks in sheltered coastal areas. Fertilization of *C. intestinalis* eggs occurs in the water column following gamete release during summer and fall months. The duration of embryonic development is highly dependent upon temperature and can range from 18 h at 18–20 °C to 48 h at 12 °C (Dybern, 1965; Bullard and Whitlatch, 2004). *C. intestinalis* larvae are very small (0.88–1.28 mm in length) and nearly transparent with a larval phase ranging from 24 h at high temperatures (18–20 °C) to 5 days at low temperatures (10–12 °C; Dybern, 1965; Bullard and Whitlatch, 2004).

Our second study species, *B. violaceus*, is a colonial ascidian that is likely native to Japan and has been established in the Gulf of Maine since 1981 (Berman et al., 1992). *B. violaceus* is a common fouling organism and is found on both man-made and natural substrates including floating docks, pilings, subtidal rocks and algae, and *Mytilus edulis* shells (Carver et al., 2006). This species produces very large (length <3 mm) and often brightly colored orange, red, pink, or purple tadpole larvae that are brooded within the colony and released throughout the summer and fall (Bullard and Whitlatch, 2004). The *B. violaceus* larval phase is very short, lasting from several minutes to hours after release from the parent colony (Lambert, 1990).

2.2. Specimen collection

C. intestinalis gametes were obtained from individuals collected from HDPE floating docks at Hampton River Marina, Hampton Beach, NH and Salem Marina, Salem, MA and from HDPE floating docks and concrete and steel underwater structures at the University of New Hampshire Coastal Marine Lab Pier in Newcastle, NH. *B. violaceus* larvae were obtained from mature colonies collected from floating docks composed of HDPE at Wentworth Marina in New Castle, NH, from shallow subtidal rock surfaces and algae at Odiorne Point State Park, Rye, NH, and from subtidal rocks and algae via scuba diving at Cape Neddick, York, ME.

2.3. Experimental substrates

Four materials were selected to represent natural and man-made substrates typical of Gulf of Maine coastal systems. Concrete (commercial grade Quickrete® quick-setting cement) and black high-density polyethylene (King StarBoard® marine building material, HDPE) were chosen as experimental substrates because these materials are extremely common in developed marine areas. Gray chemical-resistant type 1 PVC was included in this study because it is a commonly used settlement plate material in scientific studies examining marine fouling communities (Osman and Whitlatch, 1995a; Stachowicz et al., 2002; Blum et al., 2007; Osman and Whitlatch, 2007; Janiak et al., 2013; Simkanin et al., 2013). Granite substrates were included because this mineral is the primary component of ledge and bedrock in the southwestern Gulf of Maine. Standardized natural rock samples could not be obtained, so the unpolished sides of dark gray granite tiles were used as pseudo-natural substrates.

2.4. Larval acquisition

2.4.1. C. intestinalis

Eggs and sperm were obtained via dissection of mature individuals and fertilized in vitro (procedure adapted from Cirino et al., 2002). A longitudinal incision was made through the tunic of each individual *C. intestinalis* and eggs were removed from the oviduct using a pipette. Eggs from 6 to 10 individuals were hydrated in dishes containing approximately 100 ml of seawater for 15–30 min before sperm addition. One drop of sperm was removed from 4 to 7 individuals and mixed with 100 ml of fresh seawater approximately 5 min before gamete mixing to promote maximum motility. A sample of the sperm mixture (~5 ml) was added to each dish of eggs and embryogenesis was monitored until larvae were fully developed. Excess sperm was removed 1 h after fertilization to minimize embryo mortality by removing several ml of water from each dish and replenishing containers with fresh seawater. The required number of larvae was removed prior to the start of trials.