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Colonization of Artificial Substrates at Dauphin Island, Alabama: A Comparison of *Balanus* (Cirripedia), *Membranipora tenuis* (Bryozoa), and *Conopeum tenuissimum* (Bryozoa) Settlement in 1999-2000 and 2010-12

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Colonization of Artificial Substrates at Dauphin Island, Alabama: A Comparison of *Balanus* (Cirripedia), *Membranipora tenuis* (Bryozoa), and *Conopeum tenuissimum* (Bryozoa) Settlement in 1999–2000 and 2010–12

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Glass slides were used as artificial substrates to collect settling bryozoan and barnacle larvae during two collection periods, in 1999–2000 and 2010–12. This study follows up a previous report of *Balanus* settlement in Mobile Bay and now includes two bryozoan species. Slides were immersed at the Dauphin Island Sea Lab (Alabama) for 1 wk each month for 17–18 mo in each study, and then collected for staining and counting. The bryozoans *Conopeum tenuissimum* and *Membranipora tenuis* were both present in 1999–2000, though in 2010–12 *C. tenuissimum* was rarer and only six organisms were observed. In general the bryozoan colonization period extended throughout the spring, summer, and fall, with peak settlement in May–Aug. Barnacle cyprids and metamorphed stages colonized the substrates in July–Sept. and Feb.–March in 1999–2000, but in 2010–12 they were present in the summer and fall months and did not have a Feb.–March settlement. Colonization by both bryozoans and barnacles correlated statistically with temperature, and *M. tenuis* correlated negatively with salinity as its colonization density increased following the decreased salinity in the spring. In 1999–2000 only *M. tenuis* correlated with temperature. This study reports settlement periods for these invertebrates in Alabama and provides new data for colonization studies in Mobile Bay. Additionally, we document the successful colonization of substrates by these invertebrates immediately following the Deepwater Horizon oil spill.

Few studies have investigated the annual reproductive cycles of bryozoans or barnacles, despite the vast literature on these organisms that reports settling behavior, substrate selection, larval development and behavior, tidal influences on settlement, the influence of biofilms, food availability, salinity, temperature, larval age, and many other aspects of colonization and growth (Menon, 1972; Crisp, 1974; Winston, 1976, 1977; Hines, 1978; Vail and Wass, 1981; Yoshioka, 1982; Costlow and Tipper, 1984; Anil et al., 1995; Satuito et al., 1997; Hills et al., 1998; Olivier et al., 2000; Desai et al., 2006; Saunders and Metaxas, 2007; and many others). Those reports that do focus on seasonal or temporal settlement success have shown that these biofouling organisms have varied reproductive seasonal patterns that differ between species and locality. For example, the barnacle *Balanus glandula* is known to produce broods during the winter months in Southern California, but produces broods from Feb. to Dec. in Washington (Hines, 1978; Branscomb and Vedder, 1982). Bryozoan reproduction varies as well, either by species, location, or temperature. For example, warmer temperatures favor *Conopeum tenuissimum* in Florida, whereas the winter months favor *Conopeum seurati* at the same location (Winston, 1995). The literature for these important organisms is extensive, and demonstrates that each bay or locality has its own seasonal, biotic, and abiotic factors that influence the biofouling community. An understanding of the reproductive seasons and health of sessile filtering animals such as bryozoans and barnacles is important because of their role in the ecosystem in reducing bacterial and phytoplankton levels and providing food for grazers, and because of their potential to be used as bioindicators of environmental stress.

Landers and Phipps (2003) examined the settlement of *Balanus* from Dauphin Island and Weeks Bay, AL, and reported two major reproductive seasons: Feb.–March and late summer. During the 2010 Deepwater Horizon oil spill disaster, Alabama's shoreline was heavily oiled. The Operational Science Advisory Team reports 1 and 2 (OSAT, 2010, 2011) provided details of the extent of this oiling: polycyclic aromatic hydrocarbons were detected in numerous Alabama near-shore (within 3 nautical miles) water and sediment samples, including samples taken from Mobile Bay, some of which were at levels that exceeded the Environmental Protection Agency's aquatic life benchmark values (OSAT, 2010). Additionally, significant oiling was reported from representative case study beaches at Grand Isle, LA; Petit Bois Island, MS; Bon

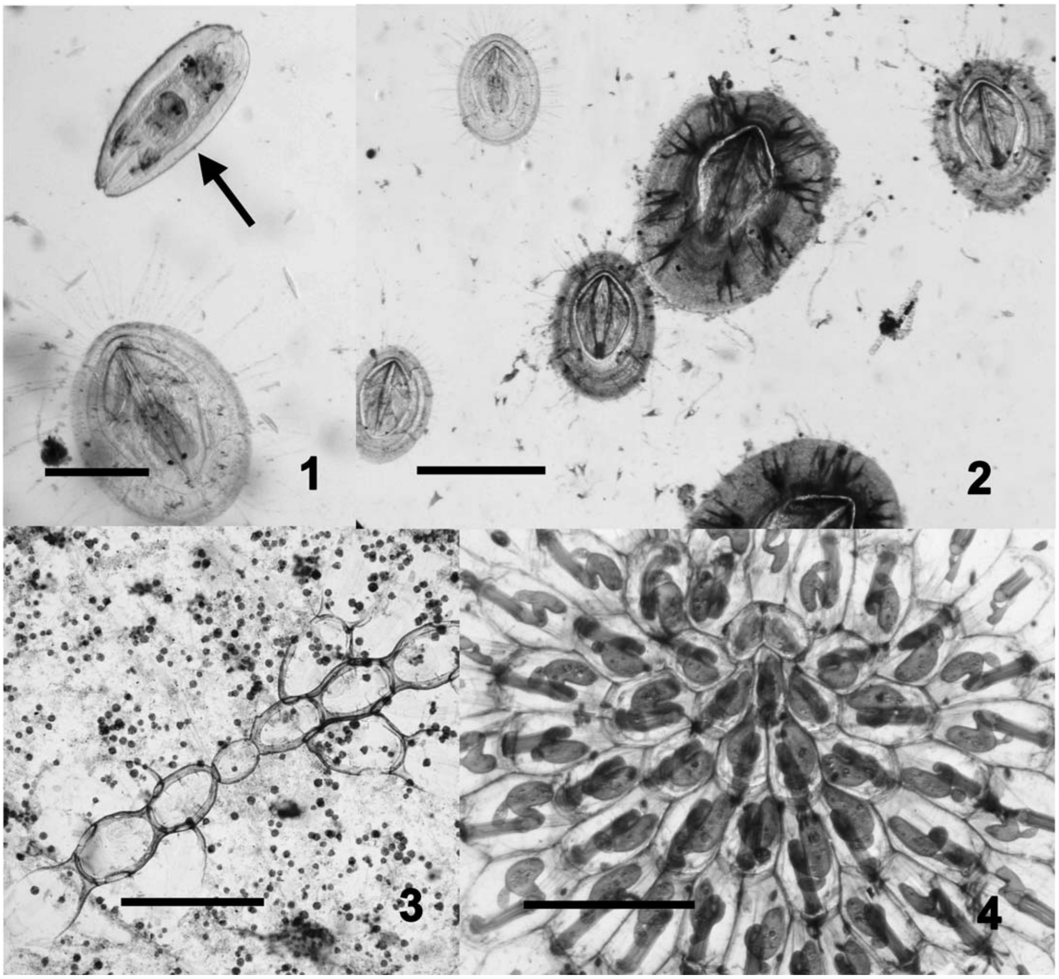
TABLE 1. 1999–2000. Average number of individuals or colonies (per cm^2) and environmental data.

Date	Total bryozoans	*Conopeum tenuissimum*	*Membranipora tenuis*	*Balanus*	Temperature (C)	Salinity (PSU)
June 1999	0	0	0	0.07	27.5	14.2
July 1999	0.72	0.34	0.38	1.33	29.2	13.3
Aug. 1999	0.57	0.53	0.04	10.3	30	23.3
Sept. 1999	0.22	0	0.22	21.333	29	23.5
Oct. 1999	0	0	0	0.833	17	27.5
Nov. 1999	0	0	0	0.037	19.5	26
Dec. 1999	0.04	0.04	0	0	18	30.5
Jan. 2000	0	0	0	0	14	27.5
Feb. 2000	0	0	0	28.38	16	28
March 2000	0.185	0.09	0.09	38.01	21	20
April 2000	0	0	0	0.047	14	10.5
May 2000	1.61	0.78	0.83	0	27	25.5
June 2000	0	0	0	0.12	28	28.5
July 2000	0.03	0.03	0	0.347	29	27
Aug. 2000	0.1	0.1	0	0.937	30	25
Sept. 2000	0	0	0	0.758	27	26
Oct. 2000	0.05	0.05	0	0.28	17	30
Average	0.207	0.115	0.092	6.046	23.129	23.900

Secour, AL; and Fort Pickens, FL (OSAT, 2011), located to the east and west of Dauphin Island. A further detailed report of the heavily oiled Alabama beaches near Dauphin Island (Hayworth et al., 2011) as well as bacterial analyses of the tar balls washed ashore on Dauphin Island from the Deepwater Horizon oil spill (Tao et al., 2011) followed. Earlier studies have reported impacted recruitment of bryozoans (*Membranipora savartii*) after oil exposure (Banks and Brown, 2002). Barnacles have been reported to have a variable response to oil depending on the species and situation. Direct oiling of *Balanus improvisus* resulted in greatly reduced coverage by the animals in Panama (Jackson et al., 1989), but variable results—including increased recruitment—were reported for barnacles (*Semibalanus balanoides*, *B. glandula*, *Chthamalus dalli*) following the *Exxon Valdez* spill (Highsmith et al., 1996) and *Balanus eburneus* showed increased recruitment following oil exposure in Louisiana (Banks and Brown, 2002).

TABLE 2. 2010–12. Average number of individuals or colonies (per cm^2) and environmental data.

Date	*Membranipora tenuis* A	*Membranipora tenuis* B	*Balanus* A	*Balanus* B	Temperature (C)	Salinity (PSU)
Aug. 2010	0	0	3.379	1.064	31	22.7
Sept. 2010	0.370	0.324	3.240	9.490	30.25	22.05
Oct. 2010	0.046	0	0.046	0	24.15	25.05
Nov. 2010	0	0.046	0.046	0.416	20.55	25.05
Dec. 2010	0	0.046	0	0	9.75	23.1
Jan. 2011	0	0	0	0	11.7	26.25
Feb. 2011	0	0	0.046	0	16.1	20.45
March 2011	0	0	0	0	19.5	9.2
April 2011	0.324	0	0.046	0	23.7	7.05
May 2011	0.833	0.231	0	0	28.15	19.9
June 2011	0.925	2.083	0.601	0.740	31.25	19.55
July 2011	0.509	0.601	1.203	1.203	30	20.34
Aug. 2011	0.046	0.138	0.185	0.138	31.5	23.15
Sept. 2011	0.046	0.046	0.648	0.694	26.15	18.9
Oct. 2011	0	0.138	0.046	0	18.75	24.5
Nov. 2011	0	0	0	0	19.6	27.8
Dec. 2011	0	0	0	0	14.35	23.7
Jan. 2012	0	0	0	0	14.21	20.74
Average	0.172	0.203	0.527	0.763	22.25	21.08

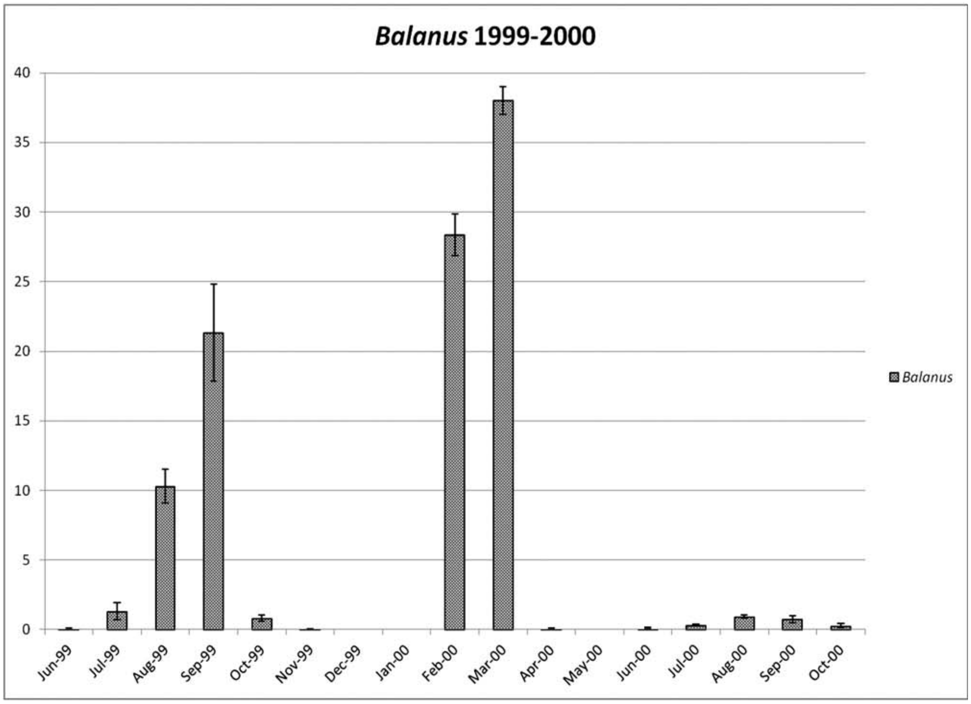


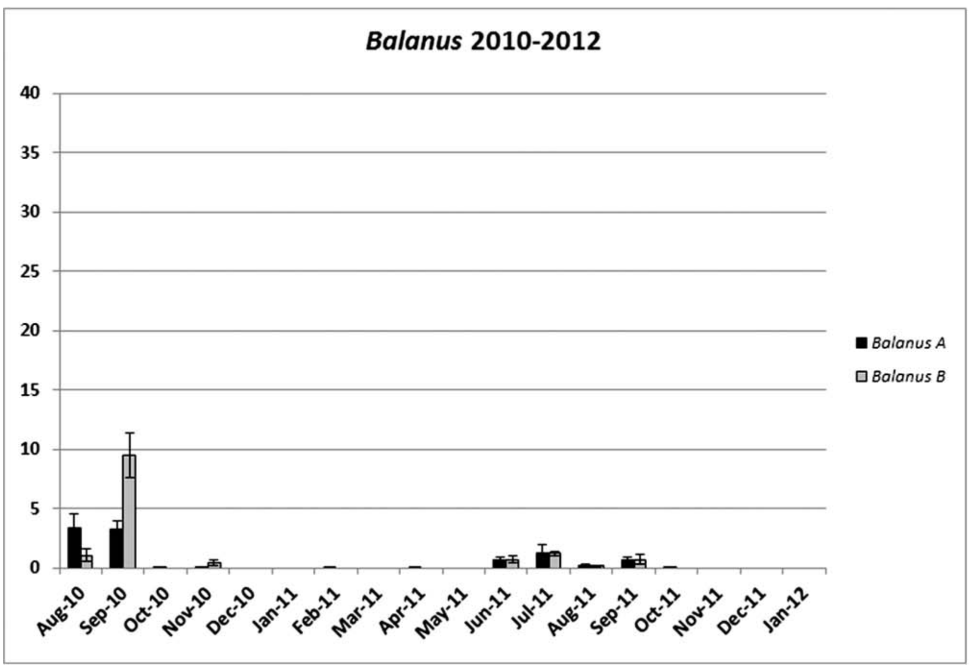
Figs. 1–4. Settled barnacles and bryozoans on glass slides from Dauphin Island Alabama. 1. Early settlement stages of *Balanus.* A cyrpid larva is indicated (arrow). Bar = 300 µm. 2. More advanced stages of metamorphosed *Balanus* settlers. Bar = 600 µm. 3. *Conopeum tenuissimum* colony, revealing the thick cystid wall of individual zooids (zooid polypides not stained, only cystid wall). Bar = 600 µm. 4. A large fan-shaped colony of *Membranipora tenuis.* Bar = 600 µm.

Due to the potential effect of the oil on invertebrate reproductive success and larval survival, the authors began a new study of biofouling organisms at Dauphin Island in August 2010. The aim of the study was to compare postspill *Balanus* settlement to the historical record for the same location. During the study, data on bryozoans were collected and compared to bryozoan settlement from archived slides from the earlier 1999–2000 report.

Materials and Methods

Glass slides were loaded into slide traps as previously described (Landers and Phipps, 2003). The slides were submerged at the Dauphin Island Sea Lab boat dock and left for 7 d each month for 17–18 mo in each study. No two collection weeks were continuous and the week chosen each month was random. Slide traps were loaded with an excess of slides in case some were broken. After collecting the slides each month they were fixed in formalin and stained with hematoxylin and fast green for permanent preparations (Galigher and Kozloff, 1971). Three slides were randomly chosen from each collection so that the animals could be sampled in triplicate. All settled barnacles, attached cyprids, barnacle scars, and bryozoans (colonies or individual zooids) that were located under the cover slip of each processed slide were counted (colonies were counted as one settler), and the data were converted to settlers/cm^2. Barnacles settled on glass slides were consistent with characteristics of the genus *Balanus*, but too





Figs. 5–8. Bar graphs showing barnacle and bryozoan settlement (individuals or colonies/cm^2). SE is indicated on each bar. 5. *Balanus* settlement in 1999–2000 (data from Landers and Phipps, 2003). 6. *Balanus* settlement at two adjacent sites (A and B) in 2010–12. 7. *Membranipora tenuis* and *Conopeum tenuissimum* settlement in 1999–2000. 8. *Membranipora tenuis* settlement at two adjacent sites (A and B) in 2010–12.

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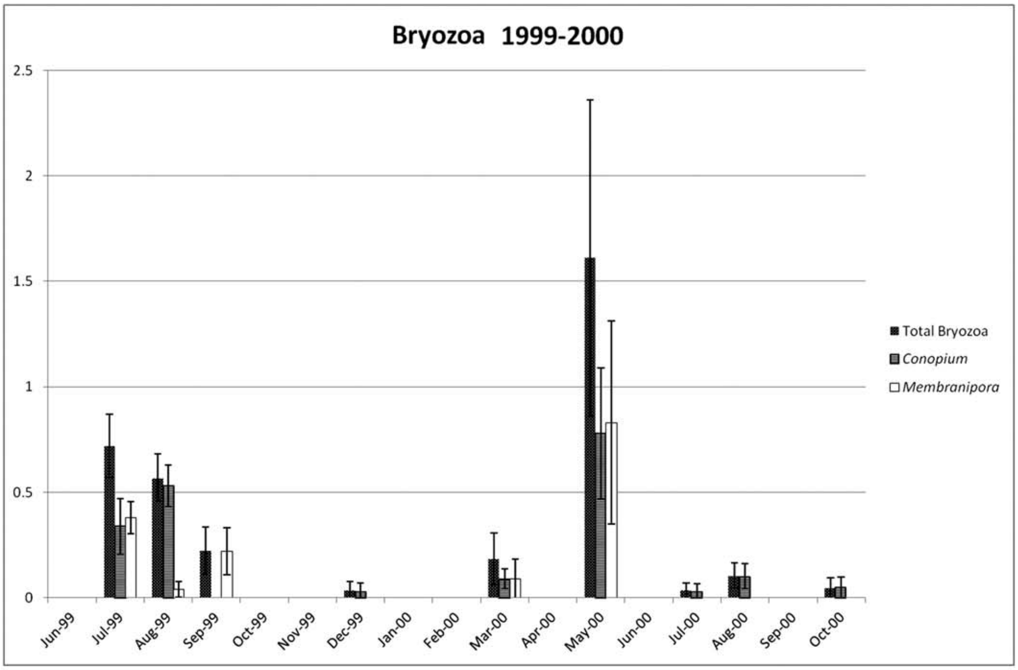

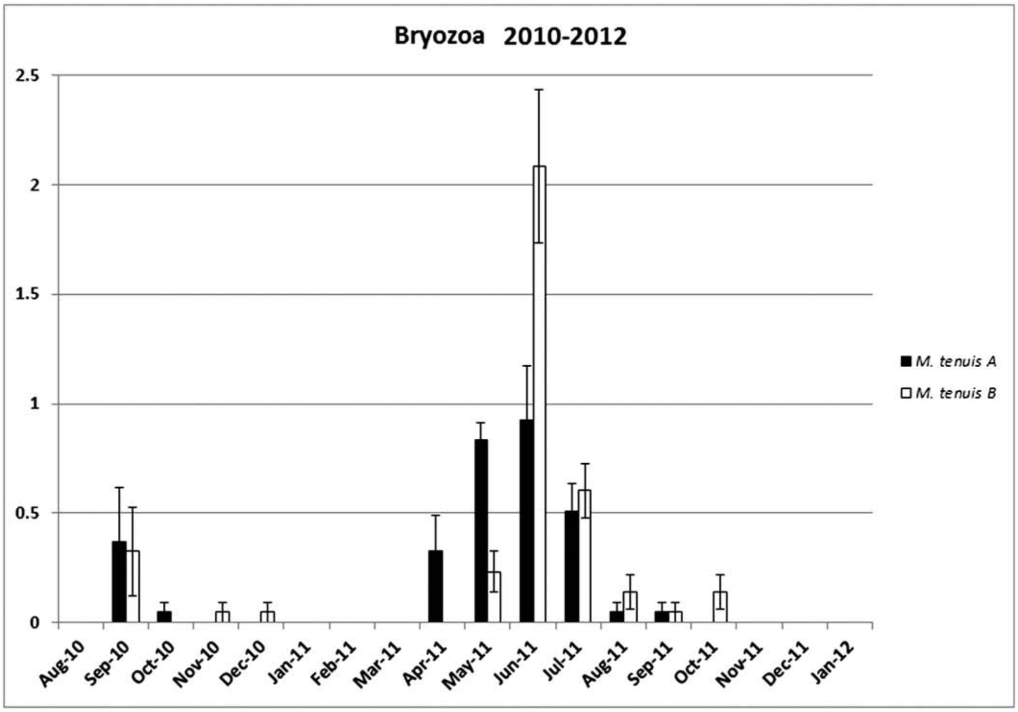

Figs. 5–8. Continued.

small to confirm identification using the tergum and scutum. However, adult barnacles recovered from the sea lab boat dock were reported to different species of *Balanus* earlier (Landers and Phipps, 2003). Those identifications suggested that multiple species may be settling on the artificial substrates. Bryozoans were identified by a collaborating expert (see Acknowledgments). Photographs

TABLE 3. Significant statistical correlations between settling invertebrate larvae and environmental factors (Spearman's rho).

Variable 1	Variable 2	Corr. coefficient	Sig. (2-tailed)
1999–2000			
Total Bryozoans	Temperature	r = 0.591	p = .013
Membranipora	Temperature	r = 0.487	p = .047
Membranipora	*Conopeum*	r = 0.538	p = .026
2010–2012			
Membranipora A	Temperature	r = 0.725	p = .001
Membranipora A	Salinity	r = −0.503	p = .033
Membranipora A	*Membranipora* B	r = 0.669	p = .002
Membranipora A	*Balanus* A	r = 0.470	p = .049
Membranipora B	Temperature	r = .0540	p = .021
Membranipora B	*Balanus* B	r = 0.592	p = .010
Balanus A	Temperature	r = 0.766	p = <.001
Balanus A	*Balanus* B	r = 0.869	p = <.001
Balanus B	Temperature	r = 0.741	p = <.001

were taken on either a Nikon SMZ1000 or E600 microscope with a digital camera, and were adjusted for brightness, contrast, and gamma with Adobe Photoshop® Elements 6.0.

Water temperature and salinity were measured using a refractometer and a salinity/oxygen probe (1999–2000) or the data were obtained from the Mobile Bay National Estuary Program Weather Station at Dauphin Island (mymobilebay.com, 2010–12). Readings were taken at the beginning and/or end of the 7-d collection period each month. If two readings (start and finish) were available for a collection week the data were averaged. Data used for statistical analysis included total bryozoan density (colonies/cm^2), *Balanus* density, *Conopeum* density, *Membranipora* density, temperature (C), and salinity (practical salinity units = PSU). The data were analyzed using the Spearman correlation on SPSS® 11.0.

Site description.—For this study a comparison was made between the ''deep'' site from 1999 to 2000 (Landers and Phipps, 2003) and a similar site at the same boat dock in 2010. The boat dock at Dauphin Island Sea Lab is approximately 0.5 km from the mouth of Mobile Bay (connection to the Gulf of Mexico), where the salinity is influenced by the fresh water draining from the bay to the gulf. The average salinity during the two study periods was 23.9 and 21.1 PSU and the average water temperature was 23.1°C and 22.3°C (Tables 1 and 2). The area is surrounded by sandy substrate, though slabs of cement and large rocks line the shoreline at the dock.

1999–2000: Slide traps at a single site were suspended by wire approximately 0.6 m below the water surface at mean low tide, approximately 23 m from shore.

2010–12: Site A traps were suspended by wire 1.0 m below the water surface. Site depth was 2.3 m. Site B traps were suspended 1.7 m below the water surface. Site B depth was 3.0 m. The slides were not exposed to air during the collection period and remained submerged even at low tide.

RESULTS

Balanus settlement (Figs. 1, 2, 5, 6; Tables 1 and 2).—In 1999–2000 *Balanus* larvae colonized the slides during two primary settlement periods: July–Oct. and Feb.–March. Settlement density reached a peak in March 2000 with 38 settlers/cm^2. The summer settlement event was present but very low in 2000 compared to summer 1999. In 2010–11 *Balanus* primarily settled during the summer, from June to Sept., reaching a settlement peak density of 9.5 settlers/cm^2 at site B in Sept. 2010. No spring reproductive or settlement event was detected in Feb.–March.

Conopeum tenuissimum and Membranipora tenuis settlement (Figs. 3, 4, 7, 8; Tables 1 and 2).—Settlement occurred during the summer months in 1999–2000, reaching a peak of approximately 0.8 settlers/cm^2 for each species in May 2000. *Conopeum tenuissimum* was present during more months in 1999–2000 than *M. tenuis.* In 2010–12 *M. tenuis* was the dominant bryozoan observed (146 organisms), with only six *C. tenuissimum* observed in the same time period. Bryozoan colonization was mostly successful during the summer and fall, with colonization densities reaching 2.1 settlers/cm^2 at site B in June 2011.

Statistical analysis (Table 3).—Spearman analysis of settler densities and abiotic factors revealed significant correlations during each study. In 1999–2000 (n = 17 mo) the total bryozoan density and *M. tenuis* density each correlated positively with temperature. Additionally, *M. tenuis* and *C. tenuissimum* densities correlated positively with each other. *Balanus* settlement density did not correlate with the bryozoan densities or with salinity or temperature, due to the two reproductive periods.

In 2010–12 (n = 18 mo) *M. tenuis* was dominant and the statistical analysis involved only that species of bryozoan. Colonization density correlated positively with temperature at both sites A and B, though at site B the correlation was driven by a single outlying data point. *Membranipora tenuis* also correlated positively with the settlement of *Balanus* at both sites, and was negatively correlated with salinity at site A. The barnacle *Balanus* correlated positively with temperature at both sites. Sites A and B correlated positively with each other for each invertebrate (*Balanus* at site A correlated with *Balanus* at site B, and *M. tenuis* at site A correlated with *M. tenuis* at site B), demonstrating consistency between the sites.

Discussion

Two significant changes were observed in this study when comparing barnacle and bryozoan settlement between the two study periods. The first was the absence of a *Balanus* Feb.–March settling event in 2010–12 that occurred earlier. The second significant change was the low number (six) of *C. tenuissimum* in the current study. In 1999–2000 *C. tenuissimum* and *M. tenuis* were both common. In 2010–12 *M. tenuis* was dominant and was the species used in the 2010–12 statistical analysis. In both studies *Membranipora* settlement correlated positively with temperature.

Similar results regarding bryozoan settlement trends can be found in the literature. *Membranipora membranacea* is a well-studied species implicated as a cause of defoliation and fragmentation of kelp in the northern Atlantic (Saunders and Metaxas, 2008, 2009). This bryozoan preferentially attaches to *Saccharina longicruris* blades and weakens the host tissue, leading to increased blade breakage. The bryozoan reproduces via a cyphonautes larva whose planktonic existence before settlement is approximately 4 wk (Yoshioka, 1973) and whose settlement correlates with higher water temperatures on a seasonal basis in the north Atlantic, particularly when settlement is viewed in terms of thermal history (Saunders and Metaxas, 2007, 2008, 2009). Conversely, colonization of the same species on *Macrocystis pyrifera* in southern California increases during the winter months (Yoshioka, 1982). *Membranipora tenuis* is a common estuarine species known from U.S. Atlantic and Gulf coast estuaries (Winston, 1977), and was the most common bryozoan at Dauphin Island during 2010–12. *Conopeum tenuissimum* was also common, but in 1999–2000 at Dauphin Island. This species is also known from estuaries along the U.S. Atlantic and Gulf coasts (Winston 1977). *Conopeum tenuissimum* is most common in the spring and summer in the Indian River (Florida), whereas *C. seurati* is most common in winter months at the same Florida location (Winston, 1995). In agreement with *C. seurati* in Florida, zooid size for this species is negatively related to temperature in England (O'Dea and Okamura, 1999). Thus a relationship between bryozoan reproduction/colonization and temperature (and salinity [Winston, 1977]) is known for marine bryozoa, though the nature of the relationship (positive or negative) varies from species to species. In this paper we report that the settlement of *C. tenuissimum* did not correlate to temperature (1999–2000 data only) though *M. tenuis* settlement did in both study periods. Interestingly, the correlation may relate to the thermal history of the larva and the water temperature (i.e., warm temperatures in one month may relate to settling success the next, particularly given the potentially long larval existence).

The settlement of *Balanus* is complicated because the species identification of the animal cannot be confirmed with microscopic cyprids and postmetamorphosis stages. Earlier, Landers and Phipps (2003) analyzed the adult resident barnacles on the Dauphin Island Sea Lab boat dock and reported *B. eburneus*, *B. improvisus*, and possibly *Balanus venustus* at that location. All of these species are common in the Gulf of Mexico (Gittings et al., 1986). A mix of species may therefore be responsible for the settlement density and seasonal occurrence of *Balanus*, which may have changed in 2010. This possible change may explain the lack of a Feb.–March settlement event in the 2010–12 data, leading to a strong correlation between *Balanus* settlement and temperature. Earlier work on *B. eburneus* (Scheltema and Williams, 1982) and *Balanus amphitrite* (Qiu and Qian, 1999; Desai et al., 2006) has demonstrated increased reproductive success or larval survival in warmer temperatures, although a complex relationship exists between salinity and temperature that affects survival (Hines, 1978; Anil et al., 1995). Overall, *Balanus* reproduction is clearly regional and interpretations from other studies need to be used with caution. As with bryozoan settlement, the planktonic existence of the larva can vary, as *Balanus* goes through six naupliar stages before the development of the nonfeeding cyprid. The development of the cyprid stage can be as short as 3 d, possibly providing a more direct relationship between environmental conditions and settlement.

Though many factors influence bryozoan and barnacle settlement (see Introduction), our study has maintained consistency in the use of substrates and sampling locations for direct comparison purposes. We discovered that the estuarine bryozoan *M. tenuis* may be influenced to reproduce when exposed to lower salinities, as settlement increased in 2011 following the spring rains that lead to lower salinity in the bay. This study also found that an important environmental determinant for *M. tenuis* and *Balanus* at Dauphin Island was temperature. No inferences can be made regarding the differences between 1999–2000 and 2010–12 with regard to the Deepwater Horizon oil spill, though in light of the absent Feb.–March *Balanus* reproductive event and rarity of *Conopeum* in 2010–12 there may be a new mix of biofouling species in Mobile Bay that would be worth investigating. Oil exposure has variable effects on barnacles (Highsmith et al., 1996) and has been shown to reduce recruitment in bryozoans (Banks and Brown, 2002). The variability reported in the current study assuredly results from a number of variables including temperature, food availability, salinity, and possibly pollution effects. These factors affect not only larval settlement and substrate selection, but also egg and larval production. Controlled laboratory experiments may provide the best way to investigate select factors. Currently though, we are able to report the successful breeding and settlement of *Membranipora tenuis* and *Balanus* following the spill, which is a reflection of their general health. This study in Mobile Bay will be useful for future research as it establishes breeding periods for invertebrate genera in the area.

Acknowledgments

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