

# Lack of substrate specificity contributes to invasion success and persistence of *Membranipora membranacea* in the northwest Atlantic

Danielle Denley\*, Anna Metaxas

Department of Oceanography, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada

**ABSTRACT:** Selective settlement by planktonic larvae plays a significant role in determining the distribution and abundance of many species of marine invertebrates. For non-indigenous species, larval settlement behavior can determine invasive potential by influencing initial invasion success, secondary spread, and persistence of species outside their native environments. *Membranipora membranacea* is an ecologically significant invasive bryozoan in the northwest Atlantic, where settlers are most abundant on some but not all species of kelp. Whether the increased abundance of *M. membranacea* on select kelp species is the result of larval settlement preference remains unknown. In this study, we examine selective settlement by larvae of *M. membranacea* by (1) quantifying settlers in mixed kelp beds and determining whether larvae settle preferentially with respect to kelp species, (2) conducting laboratory settlement preference experiments using the most abundant kelp substrates in Nova Scotia, and (3) examining whether the presence of kelp beds provides a settlement cue for larvae by quantifying settlement of *M. membranacea* on plates deployed within and outside of kelp beds. Contrary to settlement behavior described for native populations, our results suggest that larvae of *M. membranacea* in invaded habitats do not exhibit preference for settling on particular kelp species or within kelp beds. Instead, larvae settle on substrates extending furthest above the primary substratum. Lack of substrate specificity suggests that *M. membranacea* will continue to persist in the northwest Atlantic despite significant declines in regional kelp abundance. Our results emphasize the importance of early life-history strategies in contributing to invasion success.

**KEY WORDS:** Invasive · Selective settlement · Invertebrate larvae · Life-history strategies · Bryozoans

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## INTRODUCTION

The successful recruitment of marine invertebrates with planktonic larvae is strongly dependent on the location of larval settlement at scales ranging from millimeters (e.g. Lathlean et al. 2013) to kilometers (e.g. Hernández et al. 2010). Because of the strong association between the location of settlement and post-settlement survival, substrate selection by larvae of some marine invertebrates is thought to have evolved in response to specific cues affiliated with preferred juvenile or adult habitats

(reviewed by Burke 1983). These cues may be directly related to characteristics of the substrate (e.g. macro-algae, Ryland 1959; crustose coralline algae, Harrington et al. 2004) or the local environment (e.g. cues released by adult conspecifics, Knight-Jones 1953; or preferred prey species, Morse & Morse 1984, Lambert & Todd 1994), but can also involve more general stimuli, such as light (reviewed by Thorson 1964) or gravity (Naylor 2006). Such stimuli or cues can elicit larval behavioral responses that increase the probability of a competent larva encountering preferred habitat.

Selective settlement of marine invertebrate larvae plays a critical role in predicting shifts in species distributions and abundances of adult populations in response to habitat alteration at local to regional scales. Quantifying the influence of specific cues on larval settlement is particularly important with respect to the establishment and spread of non-indigenous species, since invasion success is strongly related to conditions of the recipient region, including the availability of suitable substrates for settlement (Carlton 1996, Folino-Rorem et al. 2006). Differences in settlement behavior among ecologically similar invasive species can determine which species persists in the invaded habitat (e.g. Zabin 2009). However, larvae of many globally invasive species have been shown to successfully recruit onto a variety of substrates or under a wide range of environmental conditions (e.g. Creed & De Paula 2007, Rius et al. 2010). Consequently, lack of strong settlement preference may also enhance invasion potential.

*Membranipora membranacea* is a cosmopolitan invasive bryozoan that exhibits many of the life-history characteristics common to successful invaders, including strong competitive ability (Pratt 2008, Yorke & Metaxas 2011), rapid growth (Pratt 2008, Saunders & Metaxas 2009a, Yorke & Metaxas 2011), high recruitment peaks (Saunders & Metaxas 2007), and widespread dispersal of long-lived planktonic larvae (Yoshioka 1973). *M. membranacea* is native to the Pacific coast of North America and the Atlantic coast of Europe, and is an ecologically significant invasive species in the northwest Atlantic where periodic population outbreaks have been linked to massive defoliation of kelp beds (Lambert et al. 1992, Levin et al. 2002, Saunders & Metaxas 2008, Scheibling & Gagnon 2009). There is evidence to suggest that cyphonautes larvae from native populations of *M. membranacea* may be induced to settle by the presence of specific algal substrates (Ryland 1962, Stricker 1989, Matson et al. 2010). However, patterns in the distribution of settlers of *M. membranacea* within its native habitats have also been attributed to both passive filtration by the kelp canopy, and active behavior by the free-swimming larvae in response to ocean temperature (Bernstein & Jung 1979, Yoshioka 1986).

Off the coast of Nova Scotia, settlers of *M. membranacea* are generally most abundant on some kelps during periods of both early and peak settlement (Saunders & Metaxas 2009b, Yorke & Metaxas 2012). This may have significant implications for the persistence and spread of *M. membranacea* in the northwest Atlantic, given its propensity to negatively impact kelps, its purported primary host substrate

(Saier & Chapman 2004, Krumhansl & Scheibling 2011, Krumhansl et al. 2011). In particular, the 3 most abundant kelp species in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) differ in their susceptibility to defoliation following a *M. membranacea* outbreak. *S. latissima* appears to suffer the most from encrustation by *M. membranacea* (Saunders & Metaxas 2008, 2009b), whereas loss of *A. clathratum* following *M. membranacea* outbreaks is typically less severe, and *L. digitata* is fairly resilient to high levels of encrustation (Saunders & Metaxas 2009b). Differential vulnerability to recurrent *M. membranacea* outbreaks may result in a shift in the distribution and abundance of kelp species within kelp beds in Nova Scotia, as described for the Gulf of Maine in the 1990s (Harris & Tyrell 2001).

Should kelp beds in the northwest Atlantic continue to decline, in part due to recurring outbreaks of *M. membranacea* (Filbee-Dexter et al. 2016), the persistence of *M. membranacea* will depend on its capacity to colonize alternative substrates, both as stepping stones to facilitate spread between patchy kelp beds, as well as refuges during periodic or persistent loss of kelp habitat. However, studies of settlement by larvae of *M. membranacea* in its native habitat are inconclusive (Ryland 1962, Bernstein & Jung 1979, Yoshioka 1986, Stricker 1989, Matson et al. 2010), and whether the increased abundance of *M. membranacea* on select kelp species in its invaded habitat is the result of selective larval settlement remains unknown.

In this study, we examine selective settlement by larvae of *M. membranacea* to determine whether larvae are capable of detecting and responding to settlement cues associated with specific kelp substrates, or more general cues affiliated with the presence of mixed kelp beds. To achieve this, we address the following questions: (1) Do larvae of *M. membranacea* settle preferentially with respect to kelp species? (2) Can observed distributions of newly settled colonies in the field be explained by selective larval settlement? And (3) Does the presence of kelp beds provide a settlement cue for larvae of *M. membranacea*? We address these questions by (1) quantifying the distribution of newly settled colonies within mixed kelp beds in the field and comparing the observed distribution of newly settled colonies among the 3 dominant kelp species with that expected under a random distribution, (2) conducting laboratory settlement preference experiments, and (3) quantifying settlement of *M. membranacea* relative to that of other bryozoan epiphytes onto settlement plates deployed in locations within and outside of kelp beds.

Table 1. Field sampling and experiments used to measure (a) selective settlement by larvae of *Membranipora membranacea* within kelp beds, and (b) the effect of understory kelp on bryozoan settlement. TL: The Lodge; PH: Paddy's Head, SC: Sandy Cove

Measurement	Location	Depth (m)	Collection method	Duration and frequency	Analysis	Comparison	Results
(a)							
No. of settlers m <sup>-2</sup> kelp for each kelp species	TL PH	4, 8, 12 4, 8, 12	10–15 individuals of each kelp species randomly selected along a 30 m transect	18 Jun 2012 to 2 Aug 2013, ~every 6 wk	Goodness of fit (G-tests)	Observed no. of settlers vs. random distribution	Table 2
Total surface area (m <sup>2</sup> ) of each kelp species	SC	4, 8	All individual kelps collected from within 8–11 haphazardly placed 0.5 m <sup>2</sup> quadrats		Binomial sign tests	No. of times kelp species is preferred or avoided vs. expected by chance	Table 3 Fig. 2
Total no. of settlers on all kelp species							
(b)							
No. of <i>M. membranacea</i> colonies	TL	8, 12	Settlement collectors deployed within and outside of kelp beds	Deployed Sep 2012 Replaced 13 Nov 2012, 1 Jun, 2 Sep, 22 Nov 2013	Mixed effects models Zero-inflated negative binomial models	Effect of presence of kelp bed, distance above substratum, and depth on the no. of colonies	Table 5 Supplement 3 Figs 4 & 5
No. of <i>M. membranacea</i> , <i>Electra pilosa</i> , and <i>Cryptosula pallasiana</i> colonies	SC TL SC	4, 8 8, 12 4, 8		Replaced 25 Nov 2012, 14 Jun, 5 Sep, 13 Nov 2013 2 Sep, 22 Nov 2013 5 Sep, 13 Nov 2013			

Our study provides evidence that the behavior of generalist settlement manifested by *M. membranacea* can contribute towards the persistence and spread of invasive marine invertebrates with planktonic larvae, emphasizing the importance of early life-history strategies in determining invasion success. Our results can be used to predict the response of invasive populations of *M. membranacea* to changing availability of substrates with implications for the recovery or continued decline of kelp beds throughout the northwest Atlantic.

## MATERIALS AND METHODS

### Selective settlement by *Membranipora membranacea* larvae within kelp beds

We collected individuals of the 3 most abundant kelp species in Nova Scotia (*Saccharina latissima*, *Laminaria digitata*, and *Agarum clathratum*) at 3 sites on the southwestern shore of Nova Scotia, Canada: The Lodge (44° 33' 3" N, 64° 1' 9" W) on the western shore of St. Margarets Bay, Paddy's Head (44° 31' 6" N, 63° 57' 2" W) on the eastern shore near the mouth of St. Margarets Bay, and Sandy Cove (44° 27' 6" N, 63° 42' 4" W) in Terence Bay, 20 km to the northeast of St. Margarets Bay, approximately every 6 wk from June 2012 to August 2013 (for a map of the study sites, see Saunders & Metaxas 2009b). At The Lodge and Paddy's Head, 10–15 kelps of each species were randomly collected along each of 4, 8, and 12 m depth contours, for a total of 90–135 kelps at each sampling time (Table 1). At Sandy Cove, we collected all kelps from 8–11 haphazardly placed 0.5 m<sup>2</sup> quadrats at each of 4 and 8 m (the seabed becomes sandy beyond this depth), for a total of 84–184 kelps at each sampling time (our Table 1; for detailed methods, see Denley & Metaxas 2016). Collected kelps were transported to the Aquatron facility at Dal-

housie University in plastic tubs without seawater, where they were maintained in aquaria with running ambient seawater.

For *Membranipora membranacea*, we defined newly settled colonies as colonies <1 cm in diameter (<2 wk old). Post-settlement mortality is very low for *M. membranacea* (Yoshioka 1982, 1986), and size-specific mortality rates were negligible for small (<1 cm diameter) colonies in Nova Scotia (Denley & Metaxas 2016). Consequently, our definition of newly settled colonies (from here on referred to as settlers) accurately represents settlement of *M. membranacea* in the field.

We counted the number of settlers of *M. membranacea* on both sides of the blades of all collected kelps and then photographed each kelp blade to determine its surface area. We measured the surface area of individual kelp blades from photographs by tracing the outline of each blade in ImageJ software and multiplying the resulting area by 2 to yield the total surface area including both sides of the blade. Blades of *S. latissima* are highly crenulated; therefore, we corrected surface area measurements for this species using location- and depth-specific correction factors (Saunders & Metaxas 2007). For *A. clathratum*, we also measured the biomass of all collected thalli using a triple beam balance (accuracy: 0.005 g). The perforated nature of *A. clathratum* makes accurate measurement of its surface area using image analysis difficult. Thus, we were only able to obtain accurate measurements of surface area using ImageJ for a subset of the collected individuals ( $n = 172$  kelps, spanning all sites, depths, and sampling times). To estimate the surface area of the remainder, we used the measurements of surface area and biomass from the subset of 172 kelps to generate a relationship between kelp biomass and surface area (Fig. S1 in Supplement 1 at [www.int-res.com/articles/suppl/m580p117\\_supp.pdf](http://www.int-res.com/articles/suppl/m580p117_supp.pdf)). We then applied this relationship to convert our measurements of biomass to surface area for all remaining blades of *A. clathratum* for which surface area could not be measured accurately using image analysis.

From kelp collected at each depth, site, and sampling time, we calculated (1) the number of settlers per  $m^2$  kelp for each individual kelp, (2) the total surface area of each kelp species by summing individual surface areas, and (3) the total number of settlers on all kelp species combined (Table 1). To determine whether larvae of *M. membranacea* settle preferentially with respect to kelp species, we compared observed numbers of settlers (per  $m^2$  kelp) with the number of settlers (per  $m^2$  kelp) expected under a

random distribution using goodness-of-fit tests ( $G$ -tests) (Table 1). If larvae settle randomly, we would expect the proportion of settlers on each kelp species to be equal to the proportion of the total surface area accounted for by that kelp species. We calculated the expected number of settlers for each depth, site, and sampling time by multiplying the proportional surface area of each kelp species (e.g. proportional surface area of *S. latissima* in  $m^2 = \text{surface area of all } S. \text{ latissima collected in } m^2 \div \text{total surface area of all kelp collected in } m^2$ ) by the total number of settlers. Within each site, only depths and sampling times at which all 3 kelp species occurred simultaneously were considered for analyses.

To reduce the probability of type I error, only combinations of depth, site, and sampling time for which the total number of settlers exceeded 25 ( $n > 25$ ) were included in goodness-of-fit tests; in addition, the Williams' correction was applied for all cases where  $n < 200$  settlers (Sokal & Rohlf 1981). Within each site, there was significant heterogeneity among replicate combinations of depth and sampling times (The Lodge:  $G_H = 826$ ,  $df = 22$ ,  $p < 0.0001$ ; Paddy's Head:  $G_H = 109$ ,  $df = 10$ ,  $p < 0.0001$ ; Sandy Cove:  $G_H = 102$ ,  $df = 6$ ,  $p < 0.0001$ ). Therefore, we conducted separate  $G$ -tests for each depth and sampling time.

For each site, we also used binomial sign tests of the difference between observed and expected numbers of settlers on each kelp substrate at each depth and sampling time to determine whether any of the 3 kelp species were preferred (positive difference, scored as '+') or avoided (negative difference, scored as '-') more often than expected by chance (Table 1).

### Selective settlement by *Membranipora membranacea* larvae in the laboratory

We isolated competent larvae of *M. membranacea* from plankton samples collected from St. Margarets Bay in October–November 2012 and October 2013. Kelp (*S. latissima*, *L. digitata*, *A. clathratum*) was collected over the same periods from 8 m depth at The Lodge and/or Sandy Cove. We conducted settlement preference experiments in the laboratory using paired sample combinations of kelp substrates in 3 'choice' and 3 'no-choice' treatments (Fig. 1). In each paired sample combination, we randomly assigned two  $2 \times 2$  cm kelp segments to 250 ml beakers filled with  $1 \mu\text{m}$  filtered seawater. Kelp segments were excised from the central portion of each blade to ensure tissues were of similar intermediate age. Segments were cut 24 h prior to experiments and maintained in

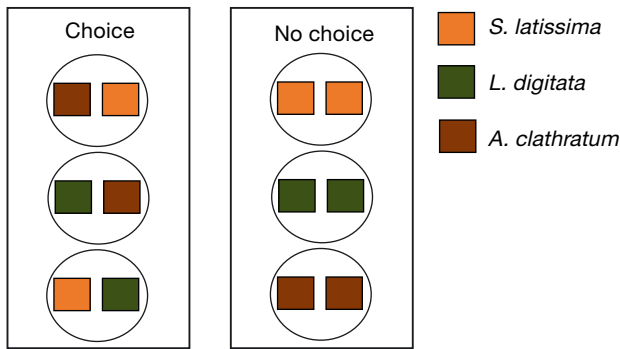


Fig. 1. Experimental design for laboratory settlement preference experiments. Choice treatments include all paired combinations of kelp substrates (*Agarum clathratum*, *Laminaria digitata*, *Saccharina latissima*). No-choice treatments consist of paired samples of the same kelp species. For no-choice treatments, a single kelp segment was randomly chosen from each beaker for analyses (linear mixed effects models, *t*-tests, ANOVA) to maintain independence of replicates. See 'Materials and methods' for full description of experimental procedure and statistical analyses

flow-through seawater tables to allow for removal of mucus exudates following cutting. We identified competent larvae of *M. membranacea* using a Nikon SMZ1500 stereomicroscope (20 $\times$  magnification) based on the size and shape of the shell and the appearance of ornamentation along the basal edge of the shell, all indicators that larvae are ready to metamorphose (Atkins 1955). We introduced 30 competent larvae into each beaker and allowed larvae to settle for 72 h at ambient seawater temperature. Filtered seawater was manually replenished in each beaker daily to avoid anoxia. After 72 h, we examined the kelp under the microscope and counted the number of larvae that had settled on each segment. High temporal variability in larval developmental stage and abundance in the field prevented us from completing an entire replicate of all choice and no-choice combinations in a single trial (3 choice treatments + 3 no-choice treatments  $\times$  30 larvae per treatment = 180 competent larvae per replicate). Therefore, replicates were conducted over the course of 8 separate trials from October–November 2012 and in October 2013.

We used linear mixed effects models (LMMs) to compare the number of larvae that settled on each substrate when given a choice of substrates to the number of larvae that settled on that same substrate in the absence of choice. Treatment (choice, no-choice) was included as a fixed factor in the model, with separate intercepts for the random effect of trial. The effect of trial was not significant for any kelp substrate ( $p = 0.396$ – $0.794$ ). Therefore, we compared the number of settlers between choice and no-choice

treatments for each paired combination of substrates pooled across all trials using 2-tailed independent samples *t*-tests. Lastly, we determined the effect of kelp substrate on the number of settlers over all trials with 1-way ANOVA. Beakers in which no settlement occurred were excluded from all analyses.

### Effect of understory kelp on bryozoan settlement

We deployed 20 settlement collectors at each of 8 and 12 m depths at The Lodge, and 4 and 8 m at Sandy Cove in September 2012 (Table 1). At each depth, 10 collectors were placed within a kelp bed and 10 collectors in areas naturally clear of kelp (density of kelp outside of kelp beds: 0 kelps  $m^{-2}$ ; for details on the density of kelp within kelp bed treatments, see Supplement 2). Each collector held a pair of vertically suspended 10  $\times$  15 cm plates cut from Sintra plastic PVC sheets, one at  $\sim$ 1 m above the substratum, thereby extending out of the kelp canopy, and the other within 10 cm of the substratum, well below the canopy. Plates were switched 4 times between September 2012 and November 2013 to capture seasonal variation in settlement (Table 1). Recovered plates were placed individually in labeled plastic bags and transported to the Aquatron facility at Dalhousie University, where they were maintained in aquaria with running ambient seawater until processing was completed ( $\sim$ 1–2 d). The number of colonies of *M. membranacea* on each plate was recorded for all collection dates. Colonies of the bryozoans *Electra pilosa* and *Cryptosula pallasiana*, which were also observed on the settlement plates, were recorded in September and November 2013 to allow us to distinguish settlement characteristics that may be unique to *M. membranacea* from those shared with the other 2 species (Table 1). We measured bryozoan settlement as the number of colonies of all sizes, rather than colonies  $<$ 1 cm in diameter, because we believe this accurately represents total settlement of bryozoan larvae between collection dates. Settlement plates were switched frequently enough that (1) colonies on plates remained whole and unfragmented, as indicated by intact growing margins; (2) whole-colony mortality, which occurs primarily due to loss of kelp substrate (Denley & Metaxas 2016), was negligible on the artificial substrate; and (3) available space for settlement was not limited on any individual plate.

For each bryozoan species, we examined the main effects of understory kelp (treatment: within kelp bed, outside kelp bed), distance above the substratum (position: top plate, bottom plate), and depth

(shallow, deep) on the number of colonies using mixed models (Table 1). Site and date of collection were considered as crossed random factors, with the random effect of individual settlement collector nested within site. For all 3 species of bryozoans, the number of colonies was  $\log(x + 0.01)$ -transformed to better approximate a normal distribution. Residual plots indicated heterogeneity of variance for *M. membranacea* and *E. pilosa* that could not be alleviated through transformation. Heterogeneity of variance for these species was due to a high number of plates containing no colonies (zero-inflated data). Accordingly, we also analyzed the main effects on the number of settlers using zero-inflated negative binomial models (after Zuur et al. 2009) (Table 1). Results

were consistent with mixed models for *M. membranacea*, and similar for *E. pilosa* (Supplement 3). Therefore, we chose to present results from mixed models and adopted a more conservative p-value ( $\alpha = 0.01$ ) for analyses involving these 2 species.

## RESULTS

### Selective settlement by *Membranipora membranacea* larvae within kelp beds

Settlers of *Membranipora membranacea* were not randomly distributed among kelp substrates, except at The Lodge and Sandy Cove at 8 m depth in July–

Table 2. G-tests for goodness of fit comparing observed distribution of settlers of *Membranipora membranacea* among 3 kelp species with a random distribution. Expected number of settlers under a random distribution calculated for each kelp species based on proportional surface area (see 'Materials and methods'). For each site, only date and depth combinations where all 3 kelp species were present and the number of settlers of *M. membranacea* exceeded 25 ( $n > 25$ ) are included. Significant p-values in **bold** ( $\alpha = 0.05$ ); for  $p < 0.05$ , 'higher' indicates a greater number of settlers observed than expected under a random distribution, 'lower' indicates fewer settlers observed than expected under a random distribution. na: not applicable

Date	Depth (m)	df	G	p	<i>Saccharina latissima</i>	<i>Laminaria digitata</i>	<i>Agarum clathratum</i>
<b>The Lodge</b>							
Jun–Jul 2012	8	2	32.3	<b>&lt;0.0001</b>	Higher	Lower	Lower
	12	2	122	<b>&lt;0.0001</b>	Higher	Lower	Lower
Jul–Aug 2012	8	2	55.3	<b>&lt;0.0001</b>	Higher	Lower	Lower
	12	2	278	<b>&lt;0.0001</b>	Higher	Higher	Lower
Sep 2012	8	2	514	<b>&lt;0.0001</b>	Higher	Lower	Lower
	12	2	69.7 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Lower	Higher
Nov–Dec 2012	12	2	55.5 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Lower	Lower
Mar–Apr 2013	12	2	40.6 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Higher	Lower
Jun 2013	4	2	44.7	<b>&lt;0.0001</b>	Higher	Lower	Lower
	8	2	9.56	<b>0.008</b>	Lower	Higher	Lower
Jul–Aug 2013	8	2	1.39 <sup>a</sup>	0.499	na	na	na
Pooled		2	1042	<b>&lt;0.0001</b>	Higher	Lower	Lower
<b>Paddy's Head</b>							
Jun–Jul 2012	12	2	23.2 <sup>a</sup>	<b>&lt;0.0001</b>	Lower	Higher	Lower
Sep 2012	8	2	49.8 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Higher	Lower
	12	2	33.8 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Lower	Lower
Nov–Dec 2012	12	2	190	<b>&lt;0.0001</b>	Higher	Higher	Lower
Jun 2013	12	2	93.1 <sup>a</sup>	<b>&lt;0.0001</b>	Lower	Higher	Lower
Jul–Aug 2013	12	2	38.0 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Lower	Lower
Pooled		2	327	<b>&lt;0.0001</b>	Higher	Lower	Lower
<b>Sandy Cove</b>							
Jun–Jul 2012	8	2	9.32 <sup>a</sup>	<b>0.010</b>	Lower	Higher	Lower
Sep 2012	4	2	25.4 <sup>a</sup>	<b>&lt;0.0001</b>	Higher	Lower	Lower
Jun 2013	8	2	120 <sup>a</sup>	<b>&lt;0.0001</b>	Lower	Higher	Lower
Jul–Aug 2013	8	2	1.73 <sup>a</sup>	0.422	na	na	na
Pooled		2	43.6	<b>&lt;0.0001</b>	Lower	Higher	Lower

<sup>a</sup>William's correction for  $n < 200$

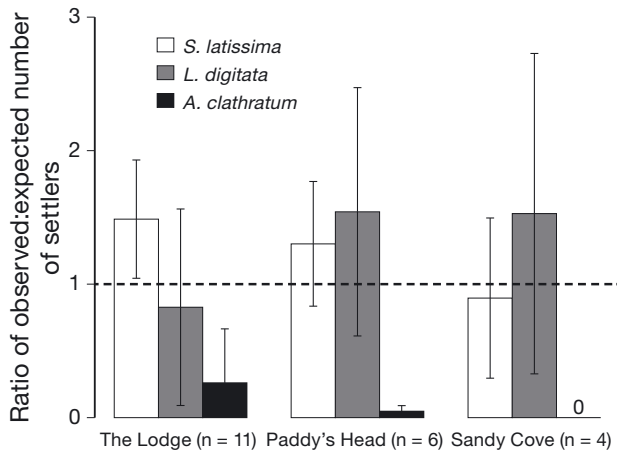


Fig. 2. Ratio (mean  $\pm$  SD) of observed versus expected number of settlers of *Membranipora membranacea* on 3 kelp species (*Agarum clathratum*, *Laminaria digitata*, *Saccharina latissima*) collected at 3 sites and across 2–3 depths per site (4 and 8 m at Sandy Cove; 4, 8, and 12 m at The Lodge and Paddy's Head) approximately every 6 wk from Jun 2012 to Aug 2013. A ratio of 1 (dashed line) indicates observed number of settlers is equal to expected number of settlers based on available surface area of the kelp species; ratios  $<1$  indicate fewer settlers were observed than expected, and ratios  $>1$  indicate more settlers were observed than expected. Zero indicates no settlement of *M. membranacea* on *A. clathratum* at Sandy Cove. For each site, only date and depth combinations included in goodness-of-fit and binomial analyses are shown (see 'Materials and methods')

August 2013 (Table 2). The observed number of settlers differed from the expected number of settlers for both *Saccharina latissima* and *Laminaria digitata* at all depths and sites for most dates when sampling occurred, although not in a consistent direction, i.e. sometimes showing preference while not at others (Table 2). However, when pooled across depths and sampling times, the ratio of the observed versus the expected number of settlers for each species did not differ significantly from 1, except for *S. latissima*, which was preferred at The Lodge (Fig. 2, Table 3). However, neither of these kelp species was preferred significantly more or less often than expected by chance based on available surface area (Table 3). Conversely, *Agarum clathratum* was preferred significantly less often than expected by chance at The Lodge and Paddy's Head (Table 3), and the ratio of observed versus expected number of settlers was significantly  $<1$  at both sites for all depths and sampling times combined (Fig. 2, Table 3). Although *A. clathratum* was never preferred at Sandy Cove (Fig. 2, Tables 2 & 3), significant differences in preference may not have been

detectable using sign tests due to the low number of total settlers at this site.

### Selective settlement by *Membranipora membranacea* larvae in the laboratory

Contrary to patterns of settlement preference observed in the field, where *A. clathratum* was consistently less preferred, the laboratory experiments suggest that *S. latissima* may be less preferred than *L. digitata* by larvae of *M. membranacea* (Fig. 3, Table 4). There was no effect of kelp substrate on the rate of settlement (1-way ANOVA:  $F_{2,15} = 1.08$ ,  $p = 0.36$ ; number of settlers  $\text{cm}^{-2}$ , mean  $\pm$  SE: *S. latissima*:  $0.319 \pm 0.056$ ; *L. digitata*:  $0.225 \pm 0.115$ ; *A. clathratum*:  $0.156 \pm 0.079$ ); however, settler abundances were an order of magnitude greater than those observed in the field (e.g.  $0.063 \text{ cm}^{-2}$  on *S. latissima* during peak settlement at The Lodge in September 2016). To ensure that the lack of preference was not affected by the strength of the settlement cue (the concentration of which would be affected by the amount of kelp tissue present), we ran additional settlement preference experiments examining the effect of size of kelp segment ( $1 \times 1$  versus  $2 \times 2$  cm)

Table 3. (a) Binomial sign tests of difference between observed and expected number of settlers of *Membranipora membranacea*, and (b) 1-sample *t*-test comparing ratio of observed versus expected number of settlers to a value of 1 for each kelp substrate. For each site, only date and depth combinations where all 3 kelp species were present and the number of settlers of *M. membranacea* exceeded 25 ( $n > 25$ ) are included ( $N =$  sample size). na: no settlement of *M. membranacea* on *Agarum clathratum*. Significant *p*-values in **bold** ( $\alpha = 0.05$ )

(a)	No. of times preferred	N	<i>p</i>	(b)	$t_{(df)}$	<i>p</i>
<b>The Lodge</b>						
<i>S. latissima</i>	9	11	0.065		3.65 <sub>(10)</sub>	<b>0.004</b>
<i>L. digitata</i>	4	11	0.549		-0.780 <sub>(10)</sub>	0.454
<i>A. clathratum</i> <sup>a</sup>	1	11	<b>0.012</b>		-6.36 <sub>(10)</sub>	<b>&lt;0.0001</b>
<b>Paddy's Head</b>						
<i>S. latissima</i>	4	6	0.688		1.58 <sub>(5)</sub>	0.175
<i>L. digitata</i>	4	6	0.688		1.43 <sub>(5)</sub>	0.213
<i>A. clathratum</i>	0	6	<b>0.031</b>		-56.1 <sub>(5)</sub>	<b>&lt;0.0001</b>
<b>Sandy Cove</b>						
<i>S. latissima</i>	2	4	1.00		-0.348 <sub>(3)</sub>	0.751
<i>L. digitata</i>	2	4	1.00		0.882 <sub>(3)</sub>	0.443
<i>A. clathratum</i>	0	4	0.125		na	na

<sup>a</sup>For *A. clathratum* at The Lodge only, the ratio of observed versus expected number of settlers was  $\log(x + 0.01)$ -transformed to fit the normal distribution (Shapiro-Wilk test,  $p = 0.108$ )

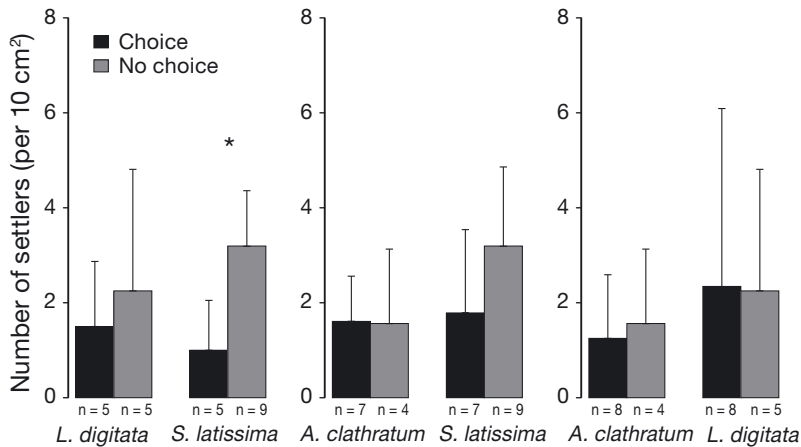


Fig. 3. Laboratory settlement preference experiments. Settlement of larvae of *Membranipora membranacea* (mean  $\pm$  SD) in choice compared to no-choice treatments for all paired combinations of 3 kelp species (*Agarum clathratum*, *Laminaria digitata*, *Saccharina latissima*). For each kelp species in each paired combination: if number of settlers in choice treatment > number of settlers in no-choice treatment, species is preferred; if number of settlers in choice treatment < number of settlers in no-choice treatment, species is less preferred compared to the alternative; and if number of settlers in choice treatment = number of settlers in no-choice treatment, species is equally preferable to the alternative. Data pooled over 8 trials. \*Significant difference ( $\alpha = 0.05$ ) detected by 2-tailed independent samples *t*-tests (Table 4) (see 'Materials and methods')

Table 4. Laboratory settlement preference experiments: 2-tailed independent samples *t*-tests comparing number of settlers of *Membranipora membranacea* between choice (C) and no-choice (NC) treatments for paired combinations of kelp substrates. Beakers in which no settlement occurred not included in the analysis. Mean differences between choice and no-choice treatments (C – NC; settles per 10 cm<sup>2</sup>) and significance statistics are given. SL: *Saccharina latissima*; LD: *Laminaria digitata*; AC: *Agarum clathratum*. Significant *p*-values in **bold** ( $\alpha = 0.05$ )

Treatment	Alga	Mean difference	<i>t</i> <sub>(df)</sub>	<i>p</i>
SL/LD	SL	-2.19	-2.64 <sub>(12)</sub>	<b>0.022</b> NC > C
	LD	-0.75	-0.577 <sub>(8)</sub>	0.580
SL/AC	SL	-1.40	-1.64 <sub>(14)</sub>	0.123
	AC	0.11	0.060 <sub>(9)</sub>	0.954
LD/AC	LD	0.09	0.049 <sub>(11)</sub>	0.962
	AC	-0.31	-0.362 <sub>(10)</sub>	0.725

on settler abundance (Supplement 4). We did not detect a significant effect, supporting the outcomes of the preference experiments.

#### Effect of understory kelp on bryozoan settlement

Settlement of *M. membranacea* did not vary between kelp and no-kelp habitats (Fig. 4, Table 5).

Instead, settlement of *M. membranacea* increased significantly at deeper depths, and was significantly greater on top than bottom settlement plates at both depths and sites across all sampling dates (Fig. 4, Table 5). In contrast, settlement of *Electra pilosa* did not vary with position of the settlement plate or among kelp treatments (Fig. 5, Table 5). The number of settlers of *E. pilosa* tended to be greater at the shallow than deeper depths; however, this difference was not significant at our more conservative  $\alpha = 0.01$  (Fig. 5, Table 5). The presence of kelp significantly affected settlement of *Cryptosula palasiensis*, but this effect depended on the position of the settlement plate. On bottom plates, the number of settlers was greater in the absence of kelp than within the kelp bed, but there was no difference in the number of settlers on top plates between within and outside kelp beds (Fig. 5, Table 5). (For results using zero-inflated negative binomial models, see Table S2 in Supplement 3).

## DISCUSSION

### Settlement by *Membranipora membranacea* larvae in invaded habitat

In the field, larvae of *Membranipora membranacea* settled preferentially on the kelps *Saccharina latissima* and *Laminaria digitata* over *Agarum clathratum*. This preference was consistent across 3 sites that differed substantially with respect to the bathymetric distribution and relative abundance of the 3 kelp species (see Appendix S2 in Denley & Metaxas 2016). *Agarum* spp. have high phlorotannin concentrations (Steinberg 1985), which may inhibit fouling by marine epiphytes (reviewed by Amsler & Fairhead 2006). The absence of *M. membranacea* on *A. fimbriatum* in its native range off the coast of British Columbia has been attributed to toxicity of polyphenolic compounds (Durante & Chia 1991). However, blades of *A. clathratum* and *S. latissima* in the northeast Pacific contain similar phlorotannin content (Dubois & Iken 2012).

The distribution of settlers of *M. membranacea* in the field, where there were significantly fewer settlers on *A. clathratum* than expected, could not be



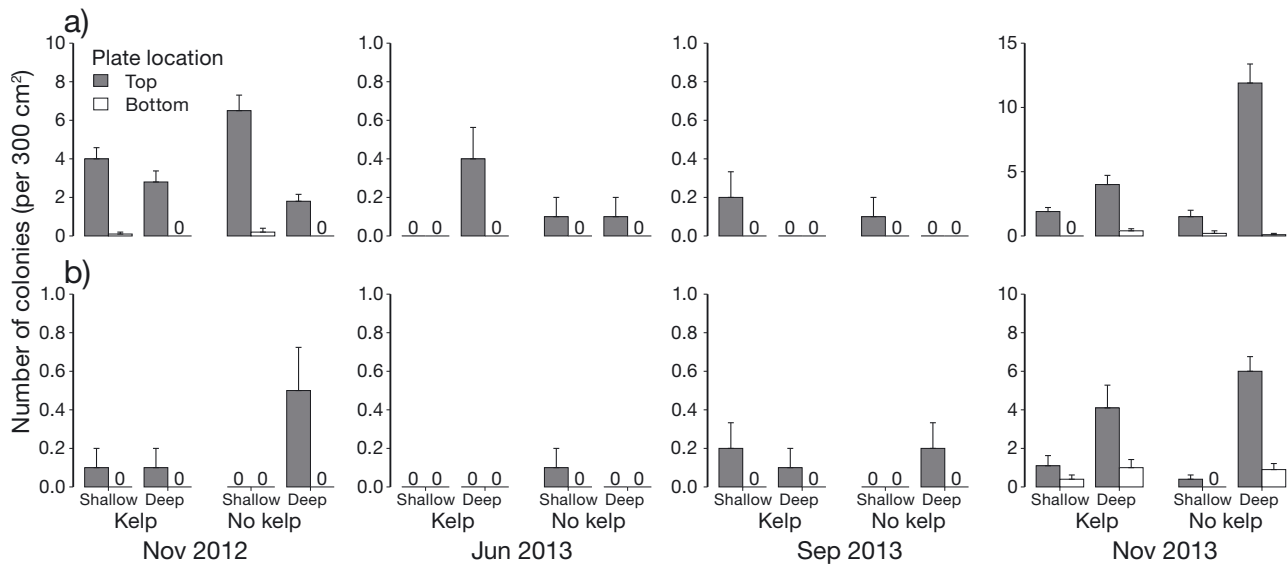


Fig. 4. Settlement of *Membranipora membranacea* in presence and absence of understory kelp canopies at (a) The Lodge at 8 m (shallow) and 12 m (deep), and (b) Sandy Cove at 4 m (shallow) and 8 m (deep) from Nov 2012 to Nov 2013. Data are mean number of colonies ( $\pm$ SE) per 300 cm<sup>2</sup> settlement plate ( $n = 10$  per treatment combination, see 'Materials and methods'). Zeros indicate no settlement of *M. membranacea* over the time interval between deployment and collection of settlement plates. Note different y-axis scales

explained by the results of laboratory settlement preference experiments in which neither *S. latissima* nor *L. digitata* were preferred over *A. clathratum*. Discordance between species distributions in the field and selective settlement in the laboratory can result from aspects of the natural environment that may act to obscure preference, such as the relative availability, and consequently the probability of encountering, preferred substrate. For example, larvae of *Bugula neritina* prefer to settle on surfaces coated with primary biofilms, but will settle in similar abundance on clean surfaces when their preferred substrate is not available (Miller et al. 1948). Although at The Lodge and Paddy's Head, kelp substrates were not necessarily sampled in proportion to their relative abundance in the field in our study, similar patterns in the distribution of settlers among algal substrates were observed at multiple sites which varied in both relative and absolute abundances of kelps. Alternatively, laboratory experiments can exaggerate preference for a particular substrate beyond what is observed in the field, since the chemical cues released by algal substrates and the probability of a larva encountering a preferred substrate are often artificially amplified within the confines of the laboratory (Moore 1975). However, there was no effect of the area of algae used in settlement experiments, incorporating both strength of the cue and the probability of encounter, on the rate of settlement of *M. membranacea* larvae.

Interestingly, there was no effect of the presence of understory kelp beds on settlement of *M. membranacea* in the field. Nor was there any indication that passive filtration by the kelp canopy prevents larvae of *M. membranacea* from settling on under-canopy substrates, since the number of settlers was consistently greater on the top plates both within and outside of kelp beds. For comparison, the number of settlers of *Cryptosula pallasiana* was greater on top plates than on bottom plates within kelp beds, but did not differ between top and bottom plates outside of kelp beds, suggesting passive filtration of *C. pallasiana* larvae. Different patterns of settlement observed for the 2 other species of bryozoans examined, *Electra pilosa* and *C. pallasiana*, substantiate that these results are indicative of settlement characteristics unique to *M. membranacea* rather than being an artifact of the experimental manipulation.

Considering our results in their totality, we suggest that at scales larger than individual algal blades, settlement of *M. membranacea* larvae is not selective with respect to algal substrate in its invaded habitat. Instead, increased abundance of settlers on some kelp species may be the result of larval behavioral responses that are not directly related to algal substrate. Kelp beds create areas of weak circulation beneath the canopy and reduce mass transport at the substratum (Eckman et al. 1989). In previous field experiments in Washington, USA, patterns of settlement of *M. membranacea* were consistent with passive trans-

Table 5. Mixed effects models examining the fixed effects of treatment (within kelp bed, outside kelp bed), position (top plate, bottom plate), and depth (shallow, deep), and random effects of site, date, and collector (nested within site) on settlement of invasive (*Membranipora membranacea*) and native (*Electra pilosa*, *Cryptosula pallasiana*) bryozoan larvae. Number of colonies was  $\log(x + 0.01)$ -transformed to better approximate a normal distribution. Significant p-values in **bold** (*M. membranacea* and *E. pilosa*:  $\alpha = 0.01$ , *C. pallasiana*:  $\alpha = 0.05$ ). See 'Materials and methods' for specific sampling dates

	$\chi^2_{(df)}$	p	Tukey's HSD
<b><i>M. membranacea</i></b>			
Fixed effects			
Treatment	0.048 <sub>(1)</sub>	0.827	
Position	136 <sub>(1)</sub>	<b>&lt;0.0001</b>	Top > Bottom
Depth	14.8 <sub>(1)</sub>	<b>0.0001</b>	Deep > Shallow
Treatment × Position	0.361 <sub>(1)</sub>	0.548	
Treatment × Depth	1.31 <sub>(1)</sub>	0.253	
Position × Depth	3.04 <sub>(1)</sub>	0.081	
Treatment × Position × Depth	1.48 <sub>(1)</sub>	0.223	
Random effects			
Site	22.8 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Date	182 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Collector	0.00 <sub>(1)</sub>	1.00	
<b><i>E. pilosa</i></b>			
Fixed effects			
Treatment	0.005 <sub>(1)</sub>	0.946	
Position	0.153 <sub>(1)</sub>	0.696	
Depth	5.56 <sub>(1)</sub>	0.018	
Treatment × Position	2.04 <sub>(1)</sub>	0.154	
Treatment × Depth	1.09 <sub>(1)</sub>	0.292	
Position × Depth	0.645 <sub>(1)</sub>	0.422	
Treatment × Position × Depth	2.33 <sub>(1)</sub>	0.129	
Random effects			
Site	0.00 <sub>(1)</sub>	1.00	
Date	36.2 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Collector	1.98 <sub>(1)</sub>	0.160	
<b><i>C. pallasiana</i></b>			
Fixed effects			
Treatment	19.4 <sub>(1)</sub>	<b>&lt;0.0001</b>	Top: Kelp = No kelp Bottom: No kelp > Kelp
Position	23.4 <sub>(1)</sub>	<b>&lt;0.0001</b>	Kelp: Top > Bottom No kelp: Top = Bottom
Depth	14.0 <sub>(1)</sub>	<b>0.0002</b>	Shallow > Deep
Treatment × Position	27.2 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Treatment × Depth	0.164 <sub>(1)</sub>	0.685	
Position × Depth	0.832 <sub>(1)</sub>	0.362	
Treatment × Position × Depth	0.808 <sub>(1)</sub>	0.369	
Random effects			
Site	33.3 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Date	16.7 <sub>(1)</sub>	<b>&lt;0.0001</b>	
Collector	0.038 <sub>(1)</sub>	0.846	

port of larvae, with settlement being greater on plates in higher flow environments outside of kelp beds than within kelp beds (Duggins et al. 1990). However, settlement plates deployed by Duggins et al. (1990) were all located within 15 cm of the substratum, limiting measurements of settlement within kelp beds to

below the kelp canopy without accounting for settlement of *M. membranacea* larvae onto overlying kelp fronds. In our study, consistently higher rates of settlement at ~1 m above the substratum, regardless of the presence of kelp beds, suggest that competent larvae may settle on the first substrate they encounter as they descend from the overlying water column. This is consistent with the distribution of settlers of *M. membranacea* among kelp substrates in the field, where blades of *A. clathratum* lie just above the substratum (within ~10 cm), while *S. latissima* and *L. digitata* extend their blades above the substratum, often projecting up to 1 m into the water column.

Settlement by larvae of *M. membranacea* onto substrates extending into the water column may be in response to increasing flow and thus food particle flux (Cancino & Hughes 1987, but see Okamura 1985, 1988, 1992). *M. membranacea* can effectively capture particles at higher flow velocities compared to other bryozoans (Pratt 2008), possibly conferring a competitive advantage on *M. membranacea*. In addition to increased particle flux, elevation above the primary substratum may also reduce rates of sedimentation, which negatively affect settlement (Ryland 1960, Duggins et al. 1990), growth (Eckman & Duggins 1991, Genovese & Witman 1999), and survival (Bak & Engel 1979, Keough 1986) of benthic marine invertebrates.

### Invasive potential and implications for persistence and spread

The apparent lack of preference by larvae of *M. membranacea* for specific algal substrates likely contributed to its successful introduction and rapid spread in the northwest Atlantic. Similarly, the recent introduction and spread of 2 widely invasive bryozoans in the Netherlands, Belgium, and France differed based on their level of substrate specificity (De Blauwe & Faasse

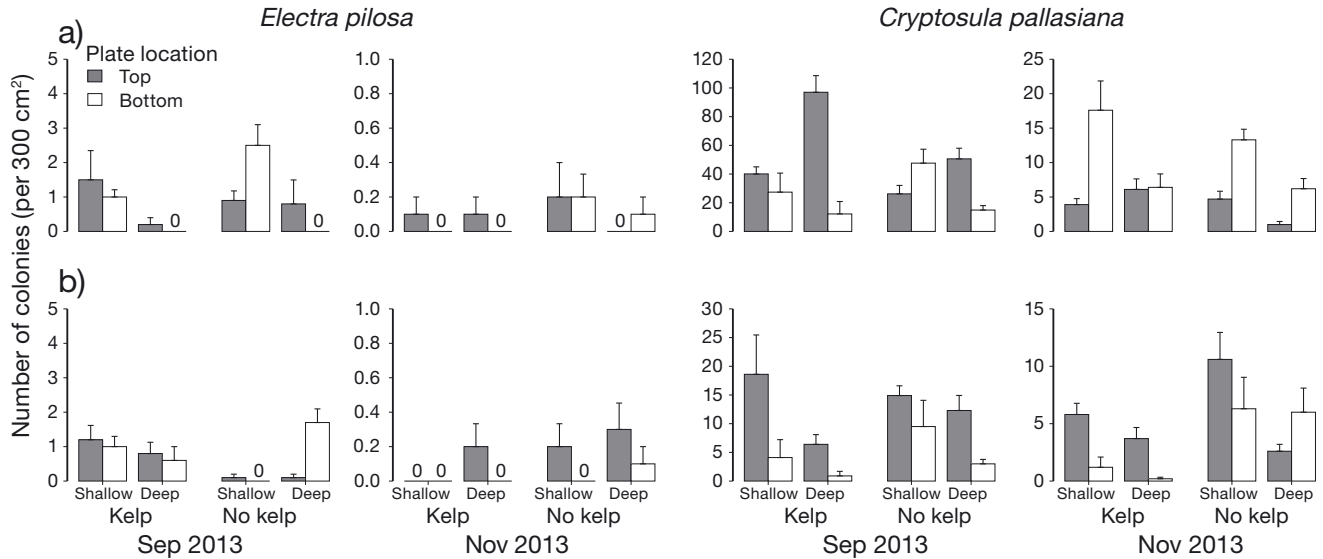


Fig. 5. Settlement of *Electra pilosa* and *Cryptosula pallasiana* in presence and absence of understory kelp canopies at (a) The Lodge at 8 m (shallow) and 12 m (deep), and (b) Sandy Cove at 4 m (shallow) and 8 m (deep) in Sep and Nov 2013. Data are mean number of colonies ( $\pm$ SE) per 300 cm<sup>2</sup> settlement plate ( $n = 10$  per treatment combination, see 'Materials and methods'). Zeros indicate no settlement of *E. pilosa* over the time interval between deployment and collection of settlement plate. Note different y-axis scales

2001). The generalist invader *Tricellaria inopinata* is now widespread and abundant throughout the south-western Netherlands, also occurring in Belgium and France, and is expected to continue to spread northward. In contrast, the more selective *B. simplex* was found in abundance at only one location in the Netherlands. Consequently, *B. simplex* is not considered an ecologically significant invasive species in the region, and its spread throughout the northeast Atlantic is predicted to be limited due to its preference for specific substrate.

Generalist settlement behavior by larvae of *M. membranacea* is also consistent with observations that *M. membranacea* populations do not appear to be in decline in Nova Scotia, despite concurrent declines in kelp (D. Denley pers. obs.). Lack of suitable substrate can act as a dispersal barrier limiting secondary spread of even the most well-established invasives (e.g. Bohn et al. 2015). However, this is not likely to be the case for *M. membranacea* in the northwest Atlantic Ocean, given its capacity to settle and grow on alternative natural (e.g. *Fucus* spp., Yorke & Metaxas 2012) and artificial (e.g. settlement plates, the present study) substrates. While *M. membranacea* seems to prefer habitat within the water column, the occurrence of reproductive colonies on *A. clathratum* and *F. evanescens* (Denley & Metaxas 2017) suggests other substrates that provide even minimal extension above the primary substratum are sufficient for persistence of the population. It is possi-

ble that extensive barrens may slow the spread of *M. membranacea* in the northwest Atlantic, particularly within the Gulf of St. Lawrence (Himmelman et al. 1983, Dumont et al. 2004, Gagnon et al. 2004), since colonies have yet to be observed on bedrock or crustose coralline algae, at least in our region (D. Denley pers. obs.). However, artificial structures that provide submerged surface area above the substratum for colonization, such as pontoons, buoys, navigational markers, and floating or subtidal aquaculture structures, may facilitate the introduction and spread of *M. membranacea* into regions where natural substrates are sparse (e.g. Simons et al. 2016).

The ability to successfully colonize artificial substrate is widespread among invasive epiphytes (Dafforn et al. 2009). There is evidence that *Membranipora* spp. may benefit from their association with kelp substrates by absorbing kelp exudates as an additional source of nutrients (De Burgh & Frankboner 1978, Manríquez & Cancino 1996), although the magnitude of this benefit likely varies depending on particulate-food concentrations. Further investigation into the growth and fecundity of *M. membranacea* on natural versus artificial substrates would be helpful in determining the significance of algal substrate as a potential food source.

Lack of a strong preference for settling on specific substrates appears to be another life-history characteristic exhibited by *M. membranacea* that is common of marine invasive species (e.g. De Blauwe &

Faasse 2001, Creed & De Paula 2007, Rius et al. 2010, Lezzi et al. 2016). Our results suggest more generalist settlement behavior of *M. membranacea* larvae in Nova Scotia compared to within *M. membranacea*'s native range in the northeast Pacific (e.g. Bernstein & Jung 1979, Yoshioka 1986, Stricker 1989, Matson et al. 2010) and northeast Atlantic (e.g. Ryland 1962). Based on our findings, *M. membranacea* provides a key example of how variation in life-history strategies can alter population dynamics of non-indigenous species outside of their native habitats, leading to potentially significant and unforeseen effects on the invasive potential of introduced species.

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