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Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies

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Abstract In the rocky subtidal ecosystem of the western North Atlantic outbreaks of the introduced epiphytic bryozoan *Membranipora membranacea* cause defoliation of kelp beds and facilitate the introduction of other non-native benthic species. We quantified size- and temperature-dependent growth rates of *M. membranacea* colonies in the field and the laboratory for durations of 8–23 days. Also, we examined the interaction between food abundance and temperature on growth rates of newly settled colonies in the laboratory. Growth rates were positively related to temperature and increased exponentially with size of colonies over the ranges examined (5.7–16.2°C and 0.5–192 mm, respectively), and were significantly higher in the field than in the laboratory. There was an interactive effect between food and temperature on the size and growth rates of colonies, with the most pronounced effects of food limitation on colonies grown at the warmest temperatures, and no effect of food on colonies grown at the coldest temperatures. Quantifying the growth rates of introduced species is essential to understanding their population dynamics, particularly when outbreaks can have severe impacts on the native community.

Introduction

On the Atlantic coast of North America, heavy encrustations of the non-native epiphytic bryozoan *Membranipora*

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membranacea on kelps cause blades to weaken, fragment and subsequently defoliate during autumn storms (Lambert et al. 1992; Scheibling et al. 1999; Saunders and Metaxas 2008). By removing the competitively dominant kelps, outbreaks of *M. membranacea* have facilitated the introduction and establishment of monospecific stands of the non-native green algae *Codium fragile* ssp. *fragile* (Levin et al. 2002; Scheibling and Gagnon 2006). *C. fragile* is chemically defended against herbivory (Lyons et al. 2007) and its consumption reduces the somatic and gonadic growth of the dominant local grazer, the sea urchin *Strongylocentrotus droebachiensis* (Lyons and Scheibling 2007). *M. membranacea* is native to both the Atlantic coast of Europe and the Pacific coast of North America, and was first observed in 1987 in the Gulf of Maine (Berman et al. 1992), and in 1992 on the southern shore of Nova Scotia (Scheibling et al. 1999). In Nova Scotia, larvae of *M. membranacea* (cyphonautes) begin to settle on kelps in May–July, and reach peak settlement in September–October (Saunders and Metaxas 2007). Upon settlement, larvae metamorphose into a pair of sessile filter-feeding zooids (ancestrula), which then bud asexually to form sheet-like colonies on kelp blades (Fig. 1). After reaching peak coverage on kelps in the autumn (Berman et al. 1992; Saunders and Metaxas 2008), colonies senesce, and <1% of the population overwinters (M. Saunders unpublished data) and provides the larvae for the following year.

Outbreaks of *M. membranacea* resulting in pronounced decreases in kelp canopy cover were observed on the southern shore of Nova Scotia in the autumns of 1993 (Scheibling et al. 1999), 1997, 1999 (Scheibling and Gagnon, unpublished manuscript) and 2006 (Saunders and Metaxas 2008). Outbreaks have been attributed to warmer winters through increases in overwintering survival and earlier and more abundant larval settlement and
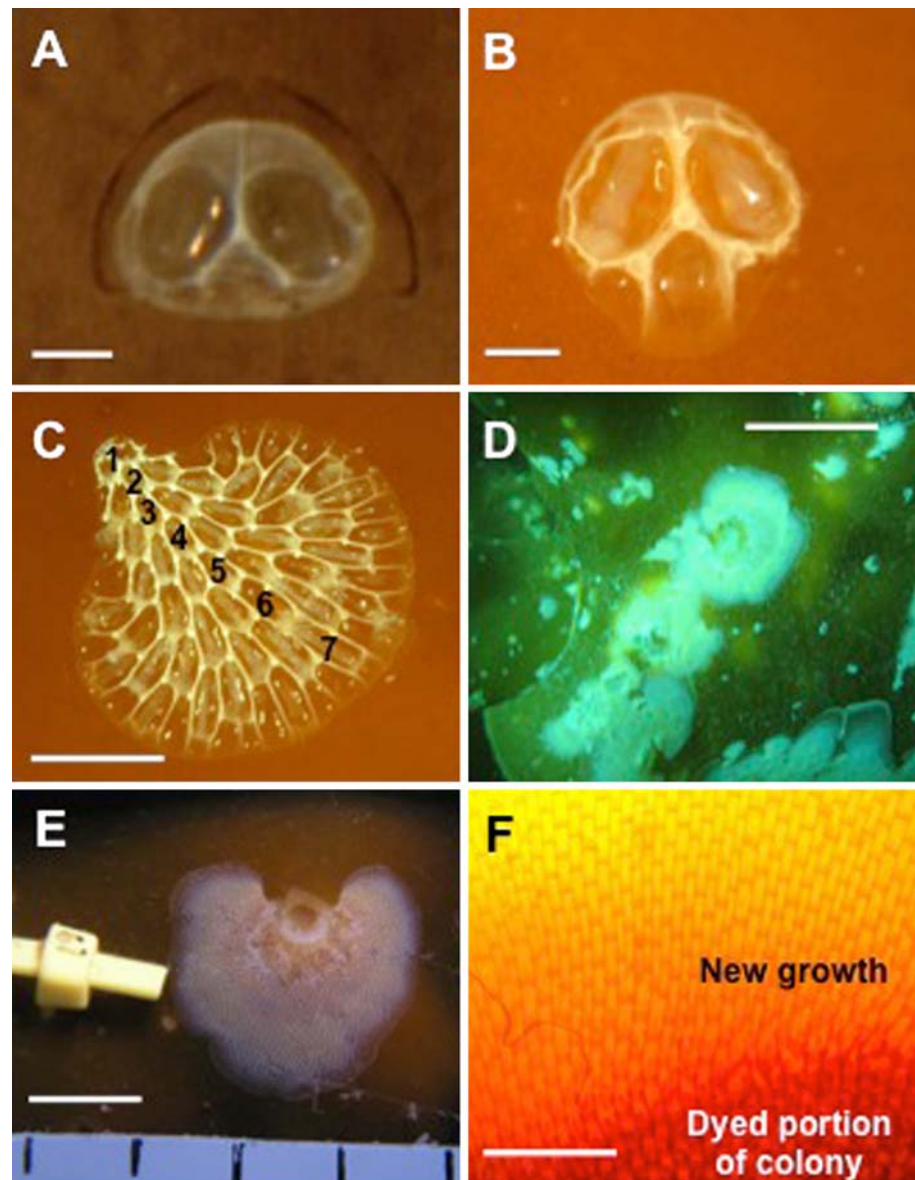
Fig. 1 Colonies of
Membranipora membranacea:
(**a**) newly settled colony
(ancestrula) (with the larval
shell and 2 zooids present,
classified as "0.5 zooid
generation"); (**b**) growing
colony with beginning of a new
zooid (2.5 zooids present,
enumerated as 1.5 zooid
generations); (**c**) colony with
∼45 fully formed zooids,
enumerated as 7.5 zooid
generations [numbers indicate
rows from 1 (ancestrula) to 7 (at
the growing edge)]; (**d**) colonies
on a kelp blade; (**e**) tagged
colony in the field; and (**f**)
zooids within a large colony—
the darker zooids at the bottom
were dyed 2 weeks previously
with Alizarin Red S, and the
lighter portions are new growth.
Scale bars indicate: (**a**, **b**)
200 μm, (**c**) 3 mm, (**d**) 50 mm,
(**e**) 1 cm, (**f**) 5 mm

recruitment (Saunders and Metaxas 2007, 2008). Within a range of thermal tolerance, growth rates of ectothermic organisms are typically positively related to temperature (Trudgill et al. 2005). Consequently, warmer water during the growth period in summer and autumn potentially could result in higher growth rates of colonies and thus contribute to outbreaks. Bryozoan colony growth rates can also be affected by other factors, such as food supply (e.g. O'Dea and Okamura 1999), which could either independently, or in interaction with temperature, contribute to variations in *M. membranacea* abundance.

Measures of growth rates and size at age are critical for modeling population dynamics and, thus, predicting population outbreaks. For many aquatic organisms, it is logistically difficult or even impossible to measure growth rates in the field. Instead, growth rates are measured in the

laboratory and then extrapolated to the wild population. However, laboratory estimates of biological processes may vary considerably compared to field measures. For example, growth and survival rates of *Daphnia ambigua* were higher in the laboratory than in the field, likely as a result of limited food availability in the field (Lei and Armitage 1980), and consumption rates by three crab species were two to four times higher in the laboratory than in the field (Breen and Metaxas 2008). Moreover, we might expect variations in growth rates to be greater in the field than in the laboratory, due to the many abiotic and biotic variables that cannot be controlled in the natural setting.

Growth rates of *M. membranacea* have been measured under a variety of conditions in the laboratory (e.g. Menon 1972; Eckman and Duggins 1993), and on artificial substrates in the field (Harvell and Grosberg 1988; Eckman

and Duggins 1991; Harvell and Helling 1993; Padilla et al.
1996; Grünbaum 1997; Pratt 2008). However, growth rates
of *M. membranacea* have rarely been quantified on natural
substrates in the field (but see Yoshioka 1973; Okamura
and Partridge 1999) and survival rates of *Membranipora*
species are lower on artificial compared to natural sub-
strates (Manriquez and Cancino 1996). Within the native
range, *M. membranacea* growth rates are known to increase
with temperature (Menon 1972) and size (Yoshioka 1973).
However, growth rates within the introduced range in the
western North Atlantic, where food supply, temperature,
predation, and algal substrates differ from other regions,
have not been quantified. Additionally, the combined
effects of temperature and size, the interactive effects of
temperature and food, and the differences between labo-
ratory and field measurements on the growth rate of
M. membranacea are unknown.

We quantified the growth rate [linear extension of col-
ony size (diameter for circular colonies, or maximum
dimension for oblong colonies, mm day^{-1})] of colonies of
M. membranacea using a number of different techniques in
both field and laboratory studies. We examined the effects
of (1) colony size and temperature (field and laboratory),
(2) setting (laboratory versus field), and (3) food avail-
ability (limited and unlimited, laboratory). We predicted
that growth rate would be positively related to size, tem-
perature, food availability for any given temperature, and
higher in the laboratory than in the field.

Materials and methods

Field experiments were conducted in St. Margarets Bay
and Lunenburg Bay, Nova Scotia (NS), Canada, and off
the floating dock at Friday Harbor Laboratories (FHL),
Washington (WA), USA. Laboratory experiments were
conducted at Dalhousie University, NS, and at FHL.
Experiments at FHL were included because: (a) delicate
small (<4 mm) colonies could be tagged without the use of
SCUBA, since bryozoans on kelps are present on the
floating docks at FHL, and (b) the timing and high avail-
ability of competent larvae are predictable at FHL. In all
experiments, growth rate was measured for colonies that
were circular or oblong in shape, and were not crowded by
conspecifics or significantly limited by space. Colonies
<50 mm (approximately) have a relatively circular shape,
whereas those >50 mm become elongated, and maximal
growth typically occurs in the direction of the longest axis,
for those colonies. Temperature was measured at 10-min
intervals using Onset Co. Pendant data loggers (accu-
racy ±0.47°C), and averaged at daily intervals. In the field,
loggers were affixed to the substratum at the same depth
and located at 1–20 m from the marked colonies. In the

laboratory, loggers were placed in the aquaria or incubators
adjacent to the growing colonies.

Laboratory studies

Newly settled colonies, Washington

Settlement-competent *M. membranacea* larvae (cyphona-
utes) were isolated from plankton collected with a 333-
μm net of the floating dock at FHL. Larvae were main-
tained in 80-μm filtered seawater at ambient temperature
(~12°C) in 100-ml glass dishes for up to 12 h prior to
inclusion in experiments. Larvae were introduced into
dishes (1 larva per dish) with a single 1 × 1 cm square
piece of *Laminaria saccharina* resting on the bottom to
induce settlement. The kelp had been maintained in run-
ning seawater for 24 h prior to inclusion to reduce its
production of mucilage. The majority of larvae meta-
morphosed immediately upon introduction to the dishes
with kelp, but the dishes were maintained overnight in the
seawater tables to maximize the total number of settlers.
Colonies on kelps ($N = 36$) were subsequently introduced
into eighteen 100-ml ("food unlimited" treatment) and
eighteen 6-ml ("food limited" treatment) individual
dishes, which were subsequently placed into three incu-
bators (6 colonies per food treatment per incubator)
maintained at each of three temperatures (6, 11, and
14°C; Table 1) with a 14/10-h day/night cycle for
15 days. Seawater was changed on alternate days with:
(a) 100 ml of 3-μm filtered seawater supplemented with
the microalgae *Isochrysis galbana* (4×10^4 cells ml^{-1})
and *Dunaliela tertiolecta* (5×10^3 cells ml^{-1}) ("food
unlimited"); or (b) 6 ml of 80-μl filtered seawater ("food
limited"). The algal concentration in the "food unlim-
ited" treatment (4.5×10^4 ml^{-1}) was within the range of
optimum values reported for various bryozoan species
[1.5×10^3–1.0×10^5 ml (reviewed in Amui-Vedel et al.
2007)] and was at the upper end of the range of phyto-
plankton concentrations observed in coastal Nova Scotia
throughout the year (10^0–10^4 cells ml^{-1}, Metaxas and
Scheibling 1996). Algal cells remained suspended during
the 48-h period between water changes. To maximize
food limitation, the food limited colonies were grown in a
smaller volume of seawater than the food unlimited col-
onies. Since we observed similar growth patterns for
colonies grown in 3-μm filtered seawater in 100 ml con-
tainers (with no added food) as for the "food limited"
treatments (unpublished data), we do not believe that the
container size affected the experimental outcome. Colo-
nies and kelps were cleaned daily by gentle shaking to
remove fecal pellets and sedimentation.

On each day, the maximum number of rows of zooids
("generations") from the ancestrula to the edge of the

Table 1 Details of the field and laboratory experiments to measure growth rate of *M. membranacea* colonies in Nova Scotia (*SMB* St. Margarets Bay, *Lun* Lunenburg Bay, *Dal* Dalhousie) and Washington (*FHL* Friday Harbor Laboratories) in 2005–2007

Location	Time period	Depth (m)	Temperature (°C, mean ± SD)	N	Size of colonies (mm)	Technique
Field						
SMB, NS	15–23 August 2005	4, 6, 8	15.5 ± 0.7, 14.4 ± 0.9, 12.7 ± 1.6	17, 19, 18	4–111	Tag
	12–22 August 2006	4, 8, 12	12.8 ± 1.9, 10.7 ± 1.5, 8.6 ± 0.8	15, 27, 15	5–118	Tag
	22 August–9 September 2006	4, 8, 12	16.2 ± 1.8, 15.1 ± 3.0, 14.1 ± 3.9	12, 25, 12	10–128	Tag
FHL Dock, WA	6–25 May 2006	0.5	9.9 ± 0.2	28	0.5–4	Tag
Lun, NS	25 July–5 August 2007	8	8.9 ± 1.7	5	8–66	Dye
	6–29 August 2007	8	9 ± 1.3	1	55	Dye
Laboratory						
FHL, WA (unlimited food)	10–25 May 2006	NA	5.7 ± 0.9, 10.8 ± 0.7, 14.2 ± 1.0	6, 6, 6	0.5	Settled from larvae
FHL, WA (limited food)	10–25 May 2006	NA	5.7 ± 0.9, 10.8 ± 0.7, 14.2 ± 1.0	6, 6, 6	0.5	Settled from larvae
Dal, NS (Experiment 1)	3–16 August 2007	NA	7.3 ± 0.7, 10.6 ± 0.2, 14.4 ± 0.1	6, 4, 7	11–155	Colonies collected
Dal, NS (Experiment 2)	20 August–1 September 2007	NA	8.5 ± 0.7, 14.1 ± 0.2	15, 12	10–192	Colonies collected

Series of temperature and *N* values correspond to depths (*field*) or temperature (*laboratory*) treatments, respectively. Size of colonies is the range (minimum and maximum) used in each experiment

colony was quantified. For example, settlers which had not yet lost their larval shell (Fig. 1a) were scored as "0.5 generation", ancestrula (one row of zooids with no larval shell) as "1 generation", ancestrula plus one incompletely formed zooid as "1.5 generations" (Fig. 1b), and so on (Fig. 1c). We developed a relationship to convert the number of zooid generations in a colony (Z) to the maximum colony diameter in mm (D), by measuring the maximum dimension of colonies over a range of sizes on day 15 in digital photographs (taken with a Q imaging Micropublisher 3.3 RTV camera through a Nikon Eclipse E600 compound microscope) using Sigma Scan Pro image analysis software ($D = 0.40e^{0.36Z}$, $n = 17$, $R^2 = 0.83$, $P < 0.001$). This technique was employed to reduce handling time each day. Although zooid size in bryozoans can be influenced by temperature (e.g. O'Dea and Okamura 1999; Amui-Vedel et al. 2007), we did not observe such an effect on zooid size in this study (M. Saunders unpublished data), likely because the colonies were small and still within the zone of astogenetic change (Boardman et al. 1970).

Established colonies, Nova Scotia

Large (10–192 mm; Table 1) colonies of *M. membranacea* growing on the distal ends of the kelp *Laminaria digitata* were collected using SCUBA from 5 to 7 m depth at Splitnose Point, Nova Scotia (44°28.6′N, 63°32.7′W) in August 2007. The larger colonies had overwintered from the previous year, and were growing in only one direction, the other side having been fragmented off with the distal end of the kelp fronds as the kelps grew. Colonies on kelps were transported to the laboratory in seawater (4°C) in large plastic tubs, and placed in seawater tables at Dalhousie University's Aquatron facility within 2 h of collection. Colonies were collected for two experiments on 3 August 2007 and 20 August 2007, for Experiments 1 and 2, respectively. Each colony was marked by passing a small numbered cable tie through the kelp at 0.5 mm from the growing edge of the colony. Colonies were assessed daily, and portions of the kelp substrate which were rotten were carefully trimmed off using small scissors (as in Lutaud 1961). Throughout the experiments, colonies that were damaged or were growing on particularly deteriorated kelp were discarded.

Colonies were maintained in three 120-l seawater tables at temperatures of 7, 11, and 14°C for Experiment 1, and 9 and 14°C for Experiment 2, for 13 days (Table 1). In Experiment 1, portions of kelp blades with colonies were maintained in plastic containers [26 × 16 × 18 cm (L × W × H), with 16–18 holes (3 cm diameter) lined with 2-mm mesh to allow water circulation], which were placed in the seawater tables. In

Experiment 2, to maximize flow and minimize algal deterioration, kelps were placed directly in the seawater tables. Colonies were fed *Tetraselmis chuii* ($\sim 1 \times 10^6$ cells ml^{-1} culture) daily through a drip system into the flow-through seawater tables. In total, 6.5 and 4.5 l day^{-1} of culture were added for Experiments 1 and 2, respectively. Flow through each seawater table was approximately 4 l min^{-1}, and thus the volume of seawater in each table was replaced approximately every 30 min. Upon termination of the experiments, colonies were preserved with 7% buffered formalin. Colony growth was measured as the maximum linear distance (mm) from the cable tied at the colony's edge upon collection, to the edge of the recently grown colony, using a clear plastic ruler (accuracy 1 mm).

Field studies

Tagged colonies, Nova Scotia

Colonies (4–128 mm; Table 1) on kelp (*Saccharina longicruris*) were tagged in situ using SCUBA at Lodge (44°33.3′N, 64°01.9′W), on the western shore of St. Margarets Bay, Nova Scotia [see Saunders and Metaxas (2007, 2008) for site descriptions]. Colonies were tagged by carefully threading a small plastic cable tie through the kelp at a distance of 1–3 cm from each colony. Colonies were tagged during 3 measurement periods, at 4, 6 and 8 m depth (15–23 August 2005), and at 4, 8, and 12 m depth (12–22 August 2006, and 22 August–9 September 2006). The size (maximum dimension) of the colony (to the nearest mm) was measured with a clear plastic ruler at the time of tagging, and again at the end of the measurement period. Average daily water temperature during the study periods ranged from 8.6 ± 0.8 to 16.2 ± 1.8°C depending on time and depth (Table 1).

Tagged colonies, Washington

Colonies 0.5–4 mm in diameter (Table 1) were tagged on kelps (*Laminaria saccharina*) at 0.5 m depth on the floating dock at FHL, using small plastic garment tags passed through the kelp ~ 1 cm from the edge of each colony. To do so, rubber tires on which kelps were growing were lifted from the water for ~ 20 min. Colonies were tagged and measured (number of zooid generations) on 6, 7 and 12 May 2006, and final colony size was measured on 25 May 2006 (after 19, 18 and 13 days, respectively). Diameter was calculated based on a known relationship between zooid rows and diameter (mm) (Saunders and Metaxas 2008). Average daily water temperature during the study period was 9.9 ± 0.2°C.

Dyed colonies, Nova Scotia

We employed a novel approach for measuring growth rates of *M. membranacea* in situ. Alizarin Red S is a calcium binding dye that is incorporated into the calcium carbonate structures of actively growing organisms, and has been used extensively to measure growth rates in corals (e.g. Dustan 1975). Colonies (8–66 mm; Table 1) on the kelps *S. longicruris* and *L. digitata* were dyed using SCUBA at Sculpin Shoal, Lunenburg Bay, NS (44°20.3′N, 64°15.0′W). The shoal is located near the centre of the bay, and consists of multiple ridges of granitic bedrock, which are oriented parallel to the mouth the bay. Ridges (2 m deep) are separated by valleys of 5–10 m deep and 10–50 m wide. To dye *M. membranacea* colonies, plastic bags (40 l) were placed over individual kelps, cinched around the stipe, and injected with highly concentrated Alizarin Red S (Sigma Aldrich) in filtered seawater, to achieve an in-bag dye concentration of approximately 100 g ml^{-1}. Bags were closed tightly around the stipe and left for approximately 18 h. The bags were then removed and colonies were allowed to grow. The study was repeated twice (25 July–5 August 2007, and 6–29 August 2007). At the end of the study period, dyed colonies on kelps were collected, returned to the laboratory in coolers (4°C), and preserved in 7% buffered formalin in seawater within 8 h of sampling. Skeletal tissue formed prior to the dye application was stained pink, and any new growth after the removal of dye was white (Fig. 1f), allowing measurement (mm) of both the original colony size and of the new growth.

Data analysis

Growth rate was calculated as the linear extension of colony diameter (for circular colonies) or maximum dimension (for oblong colonies) per day (mm day^{-1}). Using all data except for the growth rates of food limited colonies grown in the laboratory, the effect of setting (field, laboratory) on standardized colony growth rate (day^{-1}) [growth rate (mm day^{-1})/initial colony size (mm)] was examined using one way ANCOVA with average daily temperature (°C) as the covariate. For each setting independently, the effects of initial colony diameter and temperature on growth rate were examined using multiple linear regressions. The effects of temperature (3 levels: 6, 11, 14°C) and food (2 levels: limited, unlimited) on the size of colonies after 15 days were examined using two-way ANOVA. For the colonies grown with limited food, colony size was observed to increase from days 1 to 6, but not later in the study period. To quantify this apparent trend, we calculated growth rates for 50% of the colonies for days 1–6, and for the other 50% of the colonies from days 6 to 15, for each

food treatment level ($n = 3$ per treatment per time period). The effects of time period (2 levels: days 1–6, days 6–15), temperature (3 levels: 6, 11, 14) and food (2 levels: limited, unlimited) on growth rate were examined using three-way ANOVA. Colony diameters were log-transformed for the ANCOVA and for the regressions examining the effects of setting, size, and temperature on growth rate. Colony growth and standardized growth rates were log-transformed for all analyses, except for the analysis of the effect of food treatment, where growth rates were $\log(x + 0.1)$-transformed. The data satisfied the assumption of homogeneity of variances according to Levene's test, but did not satisfy the assumption of normality according to the Shapiro–Wilk test; however, ANOVA is robust to deviate from normality (Zar 1999). The data for the effects of food × temperature × time period on growth rate did not satisfy the assumption of homogeneity of variances according to Levene's test because there was no variability for 2 of the treatments (10°C/No Food/days1–6, and 14°C/Food/days1–6), thus inflating the probability of a type I error (Zar 1999). Consequently, we used a more conservative $\alpha_{crit}(0.01)$. When appropriate, homogeneous subsets were identified using Student–Newman–Keuls test. Statistical analyses were conducted using SPSS 15.0.

Results

Effects of size, temperature and setting on growth of colonies

Colony growth rates of *M. membranacea* ranged from 0.01 to 12 mm day^{-1}. For any particular size, growth rate spanned almost one order of magnitude, depending on setting and temperature. For example, colonies 0.5–0.6 mm in diameter grew 0.01–0.1 mm day^{-1}, and those 100 mm in diameter grew 1–10 mm day^{-1}. Standardized colony growth rate increased with temperature and was significantly higher in the field than in the laboratory (Table 2; Fig. 2). The relationship between growth rate (GR, mm day^{-1}), initial colony size (S, mm) and temperature (T, °C) was described by the equations:

Laboratory :
$$\log(GR) = -1.683 + 0.677 \cdot \log(S) + 0.058(T)$$
$$R^2 = 0.87, \ P < 0.001$$
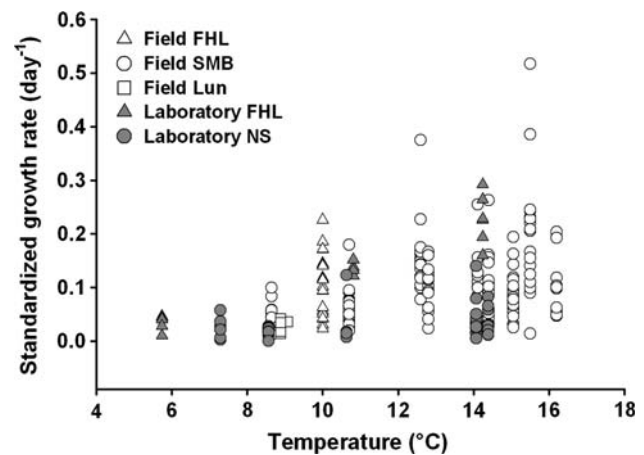
Field: $\log(GR) = -1.665 + 0.719 \cdot \log(S) + 0.072(T)$
$$R^2 = 0.84, \ P < 0.001$$

The standard errors of the regression constant, S parameter and T parameter were 0.137, 0.036, 0.012 (laboratory), and 0.086, 0.032, and 0.008 (field), respectively. In both settings, size explained more of the variability in growth rate

Table 2 Results of ANCOVA examining the effects of setting (laboratory, field) and temperature (covariate) on the log-transformed standardized growth rates (growth rate/initial colony size) of *Membranipora membranacea* colonies grown in Nova Scotia, Canada and Washington, USA

Factor	MS	df	F	P
Setting	0.48	1	4.86	0.028
Temperature	3.174	1	32.1	<0.001
Temperature × setting	0.034	1	0.35	0.556
Error	0.099	249		

Fig. 2 Standardized growth rate (growth rate/initial size) of colonies of *Membranipora membranacea* measured in the field and the laboratory over a range of sizes and temperatures. *NS* Nova Scotia; *SMB* St. Margarets Bay, NS; *Lun* Lunenburg Bay, NS; *FHL* Friday Harbor Laboratories, Washington. Field and laboratory data are indicated in *white* and *gray*, respectively

than temperature (partial correlation: field: S, $r = 0.85$, and T, $r = 0.56$; laboratory: S, $r = 0.93$, and T, $r = 0.55$).
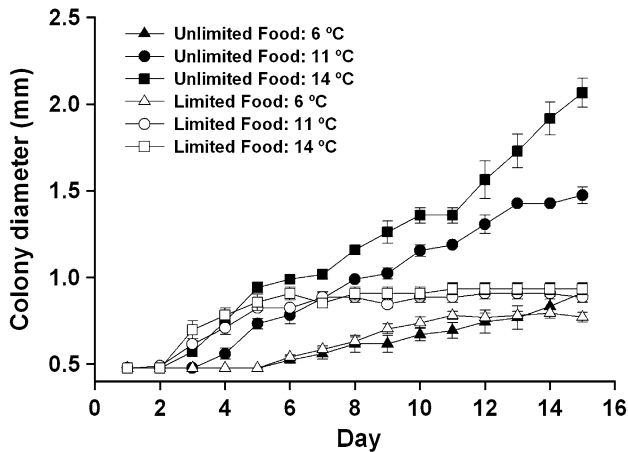
Effects of food and temperature on growth of newly settled colonies

At both food levels, newly settled colonies lost their larval shell after 2 days at 14°C, 2–3 days at 11°C, and 5 days at 6°C. There was a significant 2-way interaction for the effects of food and temperature on the size of colonies at the end of the study period (Table 3). The largest colonies (2 mm) were those grown at 14°C under unlimited food (Fig. 3). Those colonies grown at 11°C under unlimited food were of intermediate size (1.5 mm), and the sizes of all other colonies (unlimited food at 6°C, and limited food at 6, 11, and 14°C) were smallest (0.8–0.9 mm) (SNK tests).

There were significant 3-way and 2-way interactions of the effects of food, temperature, and time period on colony growth rate (Table 4; Fig. 4). Colony growth rate varied,

Table 3 Results of two-way ANOVA examining the effects of temperature and food on the diameter of newly settled colonies of *Membranipora membranacea* after 15 days of growth in the laboratory

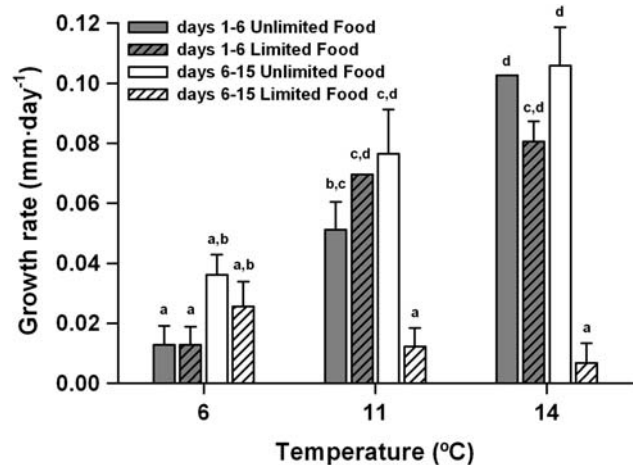| Factor	MS	df	F	P
Food	5.04	1	179	<0.001
Temperature	1.48	2	52.4	<0.001
Temperature × food	0.82	2	29.2	<0.001
Error	0.028	30		

Fig. 3 Diameter (mean ± SE, N = 3) of colonies of *Membranipora membranacea* grown from newly settled larvae for 15 days in the laboratory at 3 temperatures with 2 food treatments

Table 4 Results of three-way ANOVA examining the effects of temperature, food and time period on the growth rates of newly settled colonies of *Membranipora membranacea* in the laboratory

Factor	MS	df	F	p
Food	0.066	1	36	<0.001
Time	0.011	1	5.7	0.023
Temperature	0.062	2	33.7	<0.001
Food × Time	0.066	1	35.9	<0.001
Temperature × Food	0.018	2	9.5	0.001
Temperature × Time	0.025	2	13.3	<0.001
Temperature × Food × Time	0.011	2	5.93	0.009
Error	0.002	24		

only for the food limited colonies, between time periods at 11 and 14°C but not at 6°C. For days 1–6, growth was fastest in the 14°C water and slowest in the 6°C water for both food levels, and there was no effect of food at any temperature. For days 6–15, growth rate was higher for colonies at 11 and 14°C with unlimited food than the growth in all other treatments. At 6°C, there was no effect of food or time period on growth rate; however, at 11 and

Fig. 4 Growth rate (mean + SE, n = 3) of newly settled colonies of *Membranipora membranacea* grown during 2 time periods (days 1–6 and days 6–15) in 3 temperatures, and with 2 food treatments. *Letters* above *bars* indicate homogeneous subsets identified using Student–Newman–Keuls test

14°C, growth during days 6–15 was slower when colonies were grown with limited food than for the other treatments.

Discussion

The combination of colony size and temperature explained 84–87% of the variability in growth rate of relatively uncrowded *M. membranacea* colonies during summer. We observed increasing growth rates with respect to temperature between 5.7 and 16.2°C, which is within the range of temperatures observed in St. Margarets Bay, NS [<0–19°C (Saunders and Metaxas 2007)]. Menon (1972) found a similar result for colonies from the North Sea grown in the laboratory at 6–18°C, and reported the lower lethal temperature as <0°C for colonies acclimated at 6 or 12°C, and 1.5°C for colonies acclimated at 18°C. Because of the strong dependence of growth on temperature, changes in temperature either in winter/spring, when a few remnant colonies are overwintering, or in summer/autumn, when the population is growing, could have a significant impact on the abundance of this non-native species. However, the relative importance of variations in temperature at particular times of year on outbreak dynamics of *M. membranacea* have not been examined. An interaction between temperature variability and the population dynamics of introduced species has been shown for other organisms. In New England, 6 species of non-indigenous and indigenous tunicates had similar growth rates at moderate temperatures, but the non-indigenous species had more pronounced increases in growth rate with temperature than the native species (Stachowicz et al. 2002). In contrast, in Santa Barbara harbor, recruitment and survival of the non-native

kelp *Undaria pinnafida* was linked to periods of cooler water (Thornber et al. 2004). The specific relationships between temperature and growth of introduced species are likely determined by the climate of origin of the species.

There were no significant effects of food on the growth of newly settled colonies at any temperature for the first 6 days, suggesting that there was enough energy from larval reserves and in the 6 ml of 80-μm filtered seawater to sustain growth until a diameter of 0.8 mm was reached. Growth rates of newly settled colonies during the second week of growth with limited food were not affected by temperature, in contrast to colonies grown in unlimited food conditions. In the northwest Atlantic, phytoplankton abundance is typically low in winter, and high in spring and summer (Townsend and Thomas 2001; Yoder et al. 2002), with peaks during the spring and fall blooms. However, event-scale (days–weeks) processes such as upwelling can cause significant changes in phytoplankton species composition and abundance (Côté and Platt 1983), which could in turn, affect the growth rates of filter-feeding species such as *M. membranacea* on short time scales. Each of our field measurements in Nova Scotia integrated growth rate over periods of ∼2 weeks, and were obtained from July to September during 3 years; thus, they incorporate variability in growth due to variations in food during times of relatively abundant food. The relationship between temperature- and size-dependent growth rates may differ during other times of year (e.g., winter) when colonies are senescent or food is particularly scarce. Standardized growth rate was very low at 6°C, and at such temperatures food did not affect growth of newly settled colonies, similarly to established colonies of the bryozoan *Conopeum seurati* in the field (O'Dea and Okamura 1999). This suggests that in Nova Scotia, low food abundance in winter is of much lower importance to colony growth than the extremely cold (−1 to 3°C) temperatures.

The growth rates we report here (up to 12 mm day^{-1}) are higher than those for many other bryozoan species [e.g., *Conopeum seurati*: <1 mm day^{-1} (O'Dea and Okamura 1999), *Pentapora foliacea*: 2 cm year^{-1} (Pätzold et al. 1987)]. Although *r* (intrinsic rate of increase) of this species is of intermediate value relative to other bryozoan species (Hermansen et al. 2001; Amui-Vedel et al. 2007), the growth rates of many other bryozoan species decelerate at a particular size (Cancino and Hughes 1987) or are inversely related to size (Cocito et al. 1998). In contrast, we observed a positive and exponential relationship between size and growth for all colonies within the size range examined (0.5–192 mm). In Nova Scotia, colonies on *Saccharina longicruris* can reach maximum dimensions of almost 400 mm (Saunders and Metaxas 2008), which is 1–2 orders of magnitude larger than typically observed for the other local epiphytic invertebrates (*Electra pilosa*,

Obelia geniculata, Spirorbis spirorbis). In field and laboratory studies, *M. membranacea* had faster growth rates, reached larger sizes, and had higher survival rates than three other bryozoan species (Pratt 2008); as a result, *M. membranacea* is competitively superior to the other epiphytic species on kelps (Berman et al. 1992). In combination with a lack of predators, high growth rates and the ability to reach large sizes contribute to *M. membranacea*'s success as an invasive species in the western North Atlantic.

A number of factors other than those we examined can affect growth rates of bryozoans, and may explain the remaining 15% of the variation in our study not accounted for by temperature and colony size. Growth rate of reproductive colonies can be lower than that of non-reproductive colonies (Harvell and Grosberg 1988), and there are energetic trade offs between growth and defense (tower cells, spines, stolons) (Padilla et al. 1996; Grünbaum 1997). Flow velocity has an unclear effect on *M. membranacea* growth rate, with a negative relationship reported for laboratory (Eckman and Duggins 1993) and field (Pratt 2008) studies in Washington, but with no effect in a field study in Ireland (Okamura and Partridge 1999). When colonies encounter a conspecific or if they reach the edge of a kelp blade, they will cease growth in that direction (Harvell et al. 1990). Colonies may be partially removed when the underlying kelp tissue breaks, and damage in part of a colony causes increased growth in the direction of the surviving edge (Harvell and Helling 1993). Furthermore, colonies can senesce and shrink, a process that tends to occur in the autumn (Harvell et al. 1990). Thus, temperature- and size-dependent growth rates will likely decrease in the fall as colonies become space limited and begin to senesce. Nevertheless, the growth rates reported in this study are based on a wide range of individuals, substrates, times, and geographic locations, and thus incorporate variability generated by many extraneous factors. Consequently, the strong relationships that we observed appear to dominate over other potential sources of variability.

For logistical purposes, we measured the growth rates of very small (0.5–4 mm) colonies in the field and in the laboratory in Washington. It is possible that the growth rates of colonies in this size range are slightly different in Nova Scotia than in Washington. However, it is unlikely that this difference between sites would be significant compared to the 4 orders of magnitude variation in growth we recorded. Furthermore, although temperature data were not available, size-dependent growth rates of *M. membranacea* measured in the field in California (Yoshioka 1973) were within the range as we report. Thus, we believe that a coast-specific temperature- and size-specific growth relationship is unlikely for the size range that we examined.

We determined that over a large size range, the growth rates of *Membranipora membranacea* colonies were significantly higher in the field than in the laboratory. Therefore, for the purposes of generating predictive models of *M. membranacea* outbreak dynamics, field estimates of growth rates are preferable to laboratory estimates. For small colonies, measuring growth rates in the laboratory allowed for more controlled conditions, less physical disruption to the colonies, and more frequent measurement opportunities. It was preferable to measure growth rates of large colonies in the field, however, because transport to the laboratory was more physically disruptive than in situ tagging. Moreover, large colonies were difficult to maintain in the laboratory (Lutaud 1961) for more than 1–2 weeks because of deterioration of their host kelps, which worsened with increasing temperature. An alternative approach could be to run experiments in the laboratory, using colonies on artificial substrates (e.g. Pratt 2008). However, in both the laboratory and the field, survival of *M. isabelleana* was lower on artificial substrates than on algae (Manriquez and Cancino 1996). In the field, the tagging method required only one day for marking, but was not useful when colonies were densely aggregated on a single kelp blade because of the difficulty in identifying the colony which had been tagged, or for exposed locations, because wave action would cause the tags to be lost or to abrade the kelps and colonies. The dye method required 2 days in the field, but was more effective for marking colonies of irregular shapes, because it left a permanent trace of the entire outline of the colony on the day it was dyed. This method is also not recommended for wave exposed locations because when applied in shallower (2 m) water, wave action caused the dye-filled bag to damage the growing edge of the colonies (unpublished data). Otherwise, the dye did not appear to adversely affect the growth of the colonies. When the dyed colonies were preserved in formalin, the dye deteriorated with time; however, the zooids had a noticeably different shape at the location where the dye band had been located, permitting analysis of the samples even after extended (months–years) durations of storage.

In summary, we quantified size- and temperature-dependent growth rates of the bryozoan *Membranipora membranacea*, demonstrated that colonies grew significantly faster in the field than in the laboratory, and found an interactive effect between food availability and temperature. The reported relationships between growth rate, temperature and colony diameter are necessary for developing predictive models of *M. membranacea* outbreaks, and thus understanding the population dynamics of this ecologically damaging species within the introduced range.

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