

Jan M. Conitz^{a,*}, Robert Fagen^b and Michael S. Stekoll

Effects of density and substrate type on recruitment and growth of *Pyropia torta* (Rhodophyta) gametophytes

Abstract: Does density affect recruitment and growth in the annual, blade phase of *Pyropia* sp., does self-thinning occur, and does substrate affect recruitment? These questions were investigated in laboratory-cultured *Pyropia torta*, a naturally occurring species in Alaska with mariculture potential. Three density levels were produced from conchospores. Measurements were made, initially at settlement and germination and, subsequently, at approximately 3-week intervals, in 12 randomly selected cultures from each density level. Settled spores, germlings, or growing blades were counted microscopically and standardized to unit area. Blade surface area was measured microscopically using image analysis software. Three density levels were still distinct at germination, but the high and medium levels were not significantly different. The germination rate of conchospores was highest at the medium density level, suggesting facilitation at moderate densities but inhibition at higher densities. Significant self-thinning occurred at each density level but differed among levels, while overall blade growth was about 10-fold greater at low density than at the other two levels. In a separate experiment, counts of attached spores per unit area on artificial substrate materials were greatest on materials with interstitial spaces large enough to trap spores until they become firmly attached.

Keywords: density dependent; mariculture; *Porphyra*; *Pyropia*; substrate.

^a**Current address:** Alaska Department of Fish and Game, Commercial Fisheries Division, 333 Raspberry Road, Anchorage, AK 99518, USA.

^b**Current address:** 9084 Sheiye Way, Juneau, AK 99801, USA.

***Corresponding author: Jan M. Conitz**, Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Road, Juneau, AK 99801, USA, e-mail: jan.conitz@alaska.gov

Robert Fagen: Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Road, Juneau, AK 99801, USA

Michael S. Stekoll: Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Road, Juneau, AK 99801, USA; and Department of Natural Sciences, University of Alaska Southeast, 11120 Glacier Hwy., Juneau, AK 99801, USA

Introduction

Mariculture of *Pyropia* (including species formerly classified in *Porphyra*) and *Porphyra* has been practiced for over 300 years in Japan, China, and Korea and is supported today with a highly refined body of scientific and practical knowledge (Mumford and Miura 1988, Blouin et al. 2011). By contrast, the commercial mariculture potential of these species on the Pacific coast of North America is undeveloped (Lobban and Harrison 1994). Experimental determination of optimal conditions for growth and reproduction of selected eastern Pacific *Pyropia* species is a prerequisite for development of cultivars and is ongoing (Waaland et al. 1986, Hannach 1989, Hannach and Waaland 1989, Conitz 1999, Lin 1999, Stekoll et al. 1999, Lindstrom et al. 2008). In Japan, density-dependent growth has been studied in domesticated *Pyropia* (*Py.*) and *Porphyra* (*P.*) species (Yoshida 1972), and practical knowledge guides the production of seeded nets at the correct density for nori cultivation (Noda and Iwata 1978). For *Py. torta* and other *Pyropia* species native to Alaska, no field studies, experimental results, or practical experience were available to indicate appropriate density levels or substrate types for controlled experiments. The experiments described here were undertaken to gain understanding of the effects of these initial conditions upon growth in culture.

Intraspecific competition for resources, especially light and nutrients, in actively growing stands of plants can result in reduced growth and increased mortality. In early studies of terrestrial plants, the relationship between density and mean mass (or biomass) was characterized as a linear regression with a slope of -1.5 (or equivalently -0.5 using total biomass) on a bilogarithmic scale. Because many species approximately conformed to this model, the slope value was postulated to be universal, and the relationship came to be known as the $-3/2$ power self-thinning rule (Yoda et al. 1963, Harper and White 1974, Harper 1977). However, more recent experimental work and extensive review of a wide variety of species supports the concept of “dynamic thinning lines” whose parameters can vary among species or within a single species due to natural

variation in genetic composition or environmental conditions (Weller 1987, 1991).

Density-dependent growth and self-thinning relationships can likewise vary in natural populations of seaweeds, depending on species, habitat, growth conditions, and plant morphology (Schiel and Choate 1980, Ang and DeWreede 1992, Flores-Moya et al. 1996). Complex density-dependence relationships have been demonstrated in new recruits and early growth stages of seaweeds outplanted in the field on settling plates, recruited onto cleared surfaces, or grown in laboratory tanks (Kendziorrek and Stekoll 1984, Reed 1990, Ang and DeWreede 1992, Kendrick 1994, Creed et al. 1996, 1998, Andrew and Viejo 1998, Scrosati and DeWreede 1998). Variable effects of abiotic factors, interactions among different life-history stages within the same stand, and positive density-dependence through facilitation or protection can all modulate density-dependent responses. Substrate is among those factors that affect density and survival of natural populations (Cousens and Hutchings 1983, Reed 1990, Brawley and Johnson 1991). Studies have focused on optimal surface relief dimensions for recruitment on artificial substrates, with naturally recruiting algae in an intertidal environment (Harlin and Lindbergh 1977), and with *Fucus gardneri* propagated in the laboratory (van Tamelen et al. 1997).

The primary goal of this study was to gain understanding of the relationships among density, growth, and survival in the recruitment and early blade growth of *Pyropia torta*. More specifically, we considered the following research questions. What density levels are associated with the highest germination rates, i.e., is germination facilitated or inhibited at certain density levels? Will self-thinning occur in juvenile *Py. torta* blades and, if so, at what density levels? What density level(s) are associated with optimum early blade growth? Analysis of density-size relationships in the cultures focused on exploring functional relationships, rather than on prediction or formal hypothesis testing. We also investigated the relative success of spore settlement, attachment, and germination on various types of artificial substrate.

Materials and methods

Culture material

A culture of the conchocelis phase of *Pyropia torta* (Krishnamurthy) S. C. Lindstrom (as *Porphyra torta*) (culture PtCH13a) was cloned from a single zygospore

released from a blade collected at Chaichei Islands, Alaska (20 April, 1995; 57°4'N, 135°28'W), and grown in culture until the start of the experiment. On 25 February 1997, conchocelis fragments from this culture were induced to release conchospores (Lindstrom et al. 2008), from which experimental cultures were grown.

Density effects experiment

Initial experiences in culturing *Pyropia torta* in our laboratory guided decisions about the amounts of conchocelis to use to provide a suitable range of blade densities for the experiment. Likewise, we used culture methods and conditions for *Py. torta* that were developed prior to this investigation in our laboratory (Conitz 1999, Lin 1999, Stekoll et al. 1999, Lindstrom et al. 2008). Low, medium, and high levels of initial spore density were produced in batch cultures using conchocelis fragments weighing 0.002, 0.02, and 0.2 g (fresh weight) to seed the cultures. Substrate material consisted of 24 plain glass microscope half-slides (25 mm×37.5 mm), attached with silicon grease to the bottom of each of three clear, 2.4-l flat-bottom container (15 cm×15 cm). Provasoli's enriched seawater (Provasoli 1968) at 1/8 concentration (PES/8) was provided as the culture medium. Conchospore release was induced under a photoperiod of 8 h light:16 h dark (8:16 L:D), irradiance of 40–45 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and temperature of 11°C. Cultures were placed on 3-D rotary platforms set at 30 rpm to provide water motion. Spores were released after 1 week, and the majority of spores had germinated after 3 weeks. At 3 weeks, the microscope half-slides were transferred to individual 60×15 mm Petri dishes with 10 ml enriched seawater (PES/8). The 24 individual cultures comprising each density group were incubated under 16:8 L:D at 45 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 11°C, without water motion.

Initial counts were made immediately following transfer to individual culture dishes at 3 weeks. From a grid overlaid on each slide, 12 counting areas were selected randomly, and within each selected area, all attached spores and germlings were counted in one field-of-view (2.54 mm²). The counts from all 12 areas were summed and standardized to a count cm⁻², hereafter referred to as density. Settlement density included all attached spores and germlings; germling density included germlings only. Germination rate was the proportion of germlings in the total settled spores and germlings in each culture. Differences in total settlement density, germling density, and germination rates among low, medium, and high density treatment groups were analyzed using one-way analysis of variance. The count data were square root transformed

to meet the assumptions for one-way analysis of variance. The germination rates, expressed as a ratio of quantities in identical units, were arc-sine transformed. Multiple comparison tests were conducted using Tukey's "Honest Significant Differences" (Tukey HSD) method (D. Bates, R Documentation, <http://127.0.0.1:10925/library/stats/html/TukeyHSD.html>).

At 2, 4¹/₂, 7¹/₂, and 12 weeks following transfer to individual culture dishes, 12 cultures were selected at random from each treatment group for counts and blade area measurements. Blade surface area, rather than weight, was used as a measure of size to permit nondestructive sampling. Microscopic images of all blades found along each long edge and three lengthwise transects on each slide were recorded for later measurement. Blade counts were normalized as number of blades cm². Blade surface areas were measured digitally from the recordings using Optimas 4.0 image analysis software (Media Cybernetics, Rockville, MD, USA), calibrated with a micrometer scale. To confirm that blade surface area was a valid surrogate for weight, *Py. torta* blades cultured separately, but in the same manner, were measured as described above, then harvested, dried for 24 h at 30°C, and weighed in groups of six blades. Summed dry weights were compared against summed surface area measurements for the same groups using linear regression, and blade weight was found to be significantly correlated with surface area ($r=0.897$, $p<0.001$).

The relationship of blade size to density was analyzed for each treatment group using ranged major axis regression of blade area against density on the combined data from all four sampling dates, using a log₁₀ transformation in both variables. Ranged major axis regression is a form of Model II regression appropriate when both regression variables are random or subject to measurement error and not measured in the same units (Sokal and Rohlf 1995, Legendre 2008). Prior to conducting the regression analyses, the log₁₀ transformed data were examined using exploratory data analysis methods in R to see if they met the necessary condition of being approximately bivariate normal and to check for outliers (Legendre 2008).

The self-thinning regression is valid only for plants that are actively growing under crowded conditions, and data from uncrowded, prethinning conditions (or from senescent or postthinning conditions) should be excluded (Weller 1987, Karez 2003 Steen and Scrosati 2004). In order to determine which data, if any, to exclude from our analysis, trajectories of density and mean blade size over time and trajectories of log₁₀ transformed mean blade size against density were plotted for each density group. Self-thinning was indicated by a shift to steeper declines in density over time and with increasing blade size. A shift to

slower blade growth prior to self-thinning was an early indication of crowding. A relative competition-density (RCD) index was also estimated as a more quantitative indicator of density-dependent growth (Steen and Scrosati 2004). For this purpose, we assumed mortality and growth in the low-density culture group to be minimally affected by crowding. The survival rate and mean blade area in this group over a given period were considered to be the potential survival (S_p) and the potential blade area (A_p) that would be attained in the more crowded cultures in the absence of density-dependent mortality (self-thinning). Starting with actual initial densities in the medium- and high-density groups ($D_{a\text{ init}}$), potential density (D_p), a density that would be attained in a crowded culture in the absence of density-dependent mortality, was the product $D_p = S_p D_{a\text{ init}}$. Likewise, the actual mean blade area attained in the low-density group over a given period was considered to be the potential mean blade area (A_p) for the medium- and high-density groups (in which actual blade area was smaller due to crowding). For a given period, hypothetical potential yield (Y_p) in the medium and high density cultures was the product $Y_p = D_p A_p$. Potential yield was compared over the same period with actual yield (Y_a), the product of actual mean blade area and blade density in the medium- and high-density groups. The RCD index was the difference between actual (Y_a) and potential (Y_p) yield, divided by whichever term was greater (Y_{rel}), i.e., $RCD = (Y_a - Y_p) / Y_{rel}$. The RCD index ranges between -1 and 1: the sign indicates a positive or negative effect of density on overall yield, and the absolute magnitude indicates the strength of the effect (Steen and Scrosati 2004). Only data from sampling dates when the RCD index was negative (indicating competition) were included in the linear regression analyses.

Regression analyses were conducted on each density group using the R function **lmodel2** (available at <http://cran.fhcrc.org/>). Strength of the association between density and mean blade area was evaluated with the correlation coefficient r , and significance of r was indicated by a one-tailed parametric p-value. The ranged major axis (RMA) slope and intercept and 95% confidence intervals were provided in the **lmodel2** output, and their significance was indicated by a one-tailed permutation p-value (Legendre 2008). To determine differences among groups in model parameters, 95% confidence intervals were compared.

Substrate experiment

Six materials were tested for attachment and germination of conchospores: double and single strands of Japanese

nori net, nylon plankton net of mesh size 60, 20, and 10 μm , and plain glass microscope slides. All materials were sterilized in boiling water, except the double nori net strand, which was treated with hot (65–70°C) tap water only because it tended to unravel in boiling water. After sterilization, nine pieces of each material were placed into each of two 2.4-l containers. Substrates in each container were seeded with 0.25 g fresh weight of *Pyropia torta* conchocelis. Cultures were grown for 3 weeks in conchospore-releasing conditions (8:16 L:D, 50–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 11°C) in sterile seawater enriched with Guillard's f/2 (Guillard and Ryther 1962), and with water motion provided by 3-D rotary platforms at 30 rpm. Conchospore release began within 4 days and was 90–95% complete after 10 days. After 17 days, the majority of conchospores had attached to the substrate and begun to germinate. After 3 weeks, the 18 substrate pieces per substrate type with attached spores and germlings were transferred to individual culture dishes and placed in growth conditions (16:8 L:D, 75–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 11°C) in enriched sterile seawater. After 3 days, attached spores and blades were counted in six randomly selected fields of view (2.54 mm^2) in each of 12 cultures randomly selected from the 18 total cultures per substrate type. Counts were normalized to number mm^{-2} (attached spores and germlings). The surface area of the nori net strands was estimated as half of the cylindrical surface, $0.5\pi dl$ (d =strand diameter; l =strand length). Differences in density of settled spores and blades between substrate types were evaluated using one-way analysis of variance. Data were \log_e transformed to meet the assumptions for one-way analysis of variance. Multiple comparison tests were conducted using the Tukey method (S-Plus 4 1997, TIBCO Software, Inc., Palo Alto, CA, USA).

Results

Density effects experiment

Settlement density, 3 weeks from initiation of these cultures, increased with conchocelis seeding weight

($p < 0.001$) and differed significantly among the three treatment groups (Table 1). Germling density also increased with conchocelis weight ($p < 0.001$), but the difference between medium- and high-density groups was not significant. Germination rate was significantly higher in the medium density group than in the high- and low-density groups ($p < 0.01$; Table 1).

Changes in density and blade size showed pronounced differences over time among the three initial density groups (Figure 1). In the high-density group, density declined sharply following germination and continued to decline through most of the experimental period. In the medium- and low-density groups, a steep decline in density during the 2 weeks after germination was followed by much less decline over the remainder of the experiment (Figure 1A). The growth trajectory was similar among all three groups during the first 4 weeks of the experiment (Figure 1B), although blades in the high- and medium-density groups attained slightly larger sizes, on average. Overall, however, blade growth followed a nearly exponential trajectory in the low-density group throughout the experimental period but substantially slowed or stopped after the first 4 weeks in the medium- and high- density groups. The relationship between density and blade growth was also substantially different among the three groups (Figure 2). Self-thinning (i.e., decrease in density) occurred in all three groups, but in the low-density group, self-thinning eventually stopped, and blades continued to grow. In contrast, in the medium- and high-density groups, self-thinning increased sharply and was accompanied by a decline or cessation of growth. The point at which density, or crowding, was considered to inhibit growth and lead to self-thinning was measured with relative competition-density (RCD) indices (Table 2). Positive RCD index values during the first part of the experimental period indicated that actual yield (product of blade size and numbers) at those density and blade size combinations was better than potential yield in a hypothetical uncrowded culture. This suggests positive density-dependence or facilitative interaction among germlings and small blades during early growth. However, as the experiment progressed, increasingly negative RCD

Table 1 *Pyropia torta* group means and ANOVA results for a) settlement density, b) germling density, and c) germination rate, in cultures seeded with 0.2 g (high), 0.02 g (medium), and 0.002 g (low) conchocelis.

Density group	High	Medium	Low
a. Settlement density (total spores and germlings $\text{cm}^{-2} \pm \text{SE}$)	325 (± 33) ^a	75 (± 11) ^b	29 (± 7) ^c
b. Germling density (germlings $\text{cm}^{-2} \pm \text{SE}$)	89 (± 11) ^a	45 (± 5) ^a	5 (± 1) ^b
c. Germination rate (germlings: total spores+germlings $\pm \text{SE}$)	0.286 (± 0.027) ^a	0.520 (± 0.031) ^b	0.186 (± 0.037) ^a

^{a,b,c}Different letters indicate significant differences between group means at $p < 0.01$.

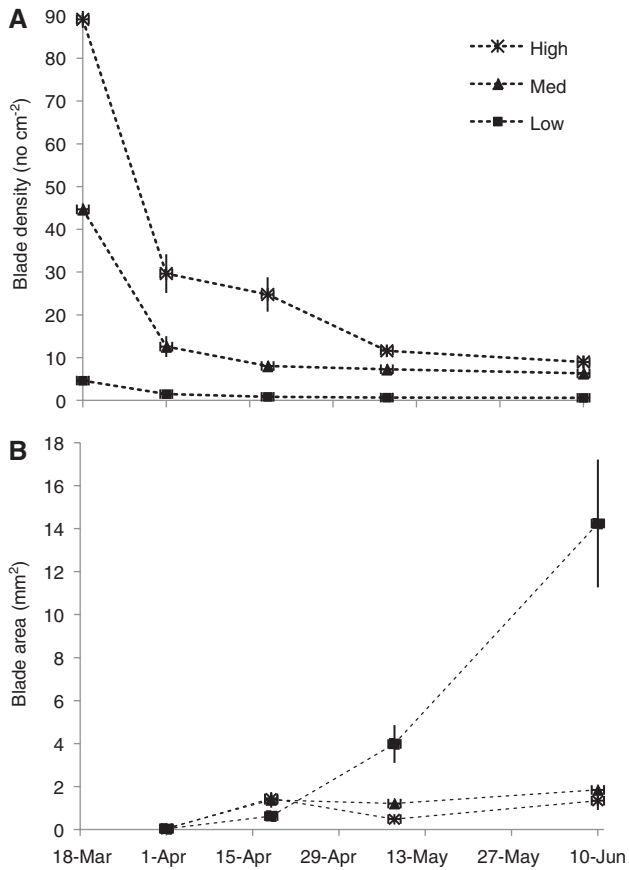


Figure 1 *Pyropia torta* time trajectories of (A) density (number cm⁻²) and (B) area (mm²), with standard errors.

indices suggest competitive interaction resulting in self-thinning and reduced growth. The more negative indices in the high-density group indicated stronger density-dependent effects in the more crowded cultures.

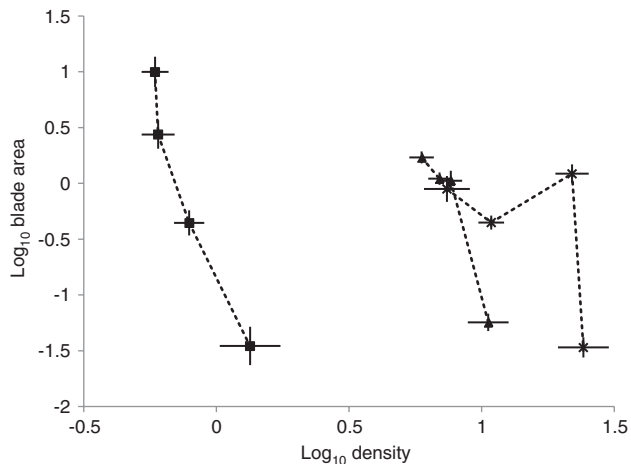


Figure 2 *Pyropia torta* blade area versus density trajectory (both variables log₁₀ transformed) in low-, medium-, and high-density cultures, with standard errors in both density and blade area. Symbols as in Figure 1.

Table 2 *Pyropia torta* cultures: relative competition-density (RCD) indices, comparing yields (i.e., product of density and blade size) in crowded cultures, over time, with potential yields in an uncrowded culture.

Group	Date	D_p^1	Y_p^2	Y_a^3	RCD ⁴	Indicates:
High	18-Apr	14.21	8.94	36.09	0.75	Facilitation
	8-May	5.22	20.81	5.71	-0.73	Competition
	10-Jun	3.77	53.67	12.10	-0.77	Competition
Medium	18-Apr	4.63	2.91	11.16	0.74	Facilitation
	8-May	3.28	13.07	8.85	-0.32	Competition
	10-Jun	2.66	37.83	11.68	-0.69	Competition

A positive RCD value indicates higher than expected yields at high densities (suggesting facilitation or positive interaction among crowded individuals), and a negative RCD value indicates lower than expected yields at high densities (suggesting competition or negative interaction). Data from the low-density group was used to represent potential survival and blade growth in uncrowded conditions.

¹Potential density: product of survival in low-density group and density in high-density group. ²Potential yield: product of D_p and mean blade area in low-density group. ³Actual yield: product of actual density and mean blade area in the high-density group. ⁴Relative competition-density index: $(Y_a - Y_p) Y_{rel}^{-1}$ (Y_{rel} is the larger of Y_a or Y_p , indicated in bold).

Using only data from periods with a negative RCD index, a model II regression was fitted to 24 data points from two sampling dates each in the medium- and high-density groups. The model was also fitted to the entire data set (48 data points from four sampling dates) in the low-density group. In all three groups, negative slopes (Figure 3) and negative and significant correlation coefficients (r) indicated a significant association between density and blade size (Table 3). The r -value in the high density group (-0.871) indicated the strongest association among the

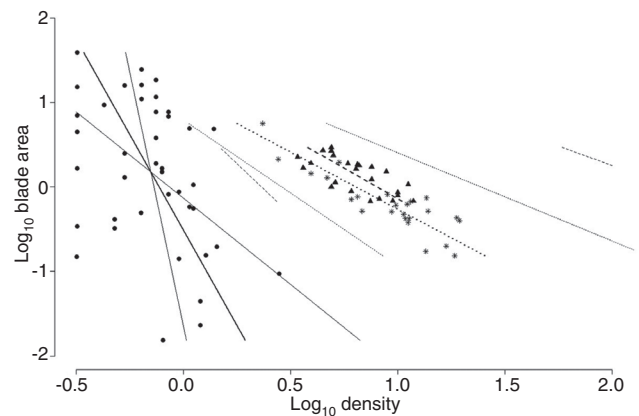


Figure 3 *Pyropia torta* self-thinning model II regression lines fit to data (log₁₀ blade area vs. log₁₀ density) for blades grown in culture at low (•; solid line), medium (▲; dashed line) and high (*; dotted line) initial densities. Lighter weight lines represent 95% confidence interval bounds corresponding to each regression line.

Table 3 *Pyropia torta* self-thinning model II regression parameters and statistics for \log_{10} blade area versus \log_{10} density in cultures seeded at high, medium, and low densities.

Density group	Slope	95% CI (slope)		Intercept	95% CI (intercept)		Correlation coefficient (<i>r</i>)	p-Value
		Lower	Upper		Lower	Upper		
High (n=24)	-1.35	-1.73	-1.04	1.09	0.79	1.45	-0.87	<0.001
Medium (n=24)	-1.44	-2.39	-0.91	1.30	0.87	2.07	-0.69	<0.001
Low (n=48)	-4.52	-11.99	-2.04	-0.51	-1.66	-0.13	-0.40	0.01

Significance (p-value) of the regression was determined by a permutation test.

three groups. Progressively less negative *r*-values found in the medium (-0.691)- and low (-0.395)-density groups indicate a weaker association. Slopes of -1.35, -1.44, and -4.52 in the high-, medium-, and low-density groups (Table 3) were all significant. However, the 95% confidence interval for the slope was very wide in the low-density group indicating a large amount of uncertainty in the model for this group. The high- and medium-density groups had similar slopes (Figure 3) and largely overlapping confidence intervals for both slope and intercept (Table 3), indicating no significant difference between them. The confidence intervals overlapped much less between the high- and low-density groups and the medium- and low-density groups, indicating significant differences between these two pairs.

Substrate experiment

The number of settled spores per unit area varied with substrate type in the following order: 60- μ m mesh nylon net > 20- μ m mesh nylon net > double-strand nori net twine > 10- μ m mesh nylon net > glass slides > single strand nori net twine (Figure 4). Settlement was significantly greater on the 60- μ m mesh nylon net, and significantly less on the glass slides and single-strand twine, than on the other substrates. Recruitment was best on those materials with interstitial spaces large enough to accommodate several conchospores (which are about 10 μ m in diameter) and worst on substrates with no interstitial spaces or spaces less than or equal to the diameter of one conchospore.

Discussion

The intention of these experiments was to determine optimum and limiting initial conditions for the cultivation of *Pyropia torta*. Understanding how to control and optimize growth in culture is necessary before attempting

to seed nets and conduct mariculture trials. We found strong evidence for density-dependent growth and mortality at the earliest stages of blade growth in our experimental cultures. Achieving an optimal initial seeding density appears to be very important to the success of later growth and the ultimate yield of *Py. torta* grown in culture. Knowledge of optimum initial densities in laboratory cultures will help determine realistic amounts of conchocele material and target density ranges for seeding nets in mariculture trials.

The higher germination rate observed at moderate density suggests that germination may be facilitated in some way by the presence of other individuals, but at higher densities, such beneficial effects of neighbors may be outweighed by competitive or inhibitory interactions. Positive density dependence in germination is known in terrestrial plants and may result from chemical (e.g.,

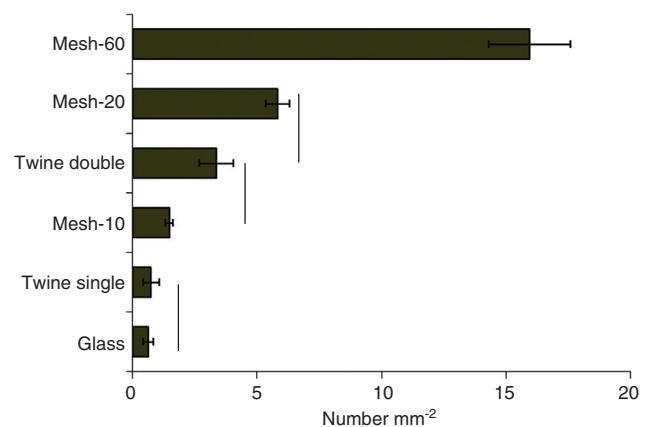


Figure 4 *Pyropia torta* recruitment densities of germlings on six different substrate materials: nylon net with 60-, 20- or 10- μ m mesh (mesh-60, -20, -10); traditional nori net twine with 1 or 2 strands (twine double, single); and glass slides (glass). Conchospores were allowed to settle over these substrates, and those that successfully attached to the substrate and germinated were counted after 3.5 weeks. Bars indicate mean counts (number mm⁻²) \pm SE from n=12 cultures per substrate type. Lines adjacent to pairs of bars indicate densities that were not significantly different (Tukey method, $p=0.05$).

release of germination stimulators, such as respiratory CO₂) (Harper 1977) or mechanical (e.g., causing changes in water movement and ion and water absorption) (Antonovics and Levin 1980) mediation. Positive density dependence in germination and in the initial growth of germlings has been observed in other seaweeds, mainly through protection from desiccation, photoinhibition, herbivory, and wave battering (Schiel and Choat 1980, Ang and DeWreede 1992, Scrosati and DeWreede 1998). In some brown algae, a minimum spore density appears to be necessary for successful recruitment (Reed 1990). High densities may also protect germlings against infestations by diatoms or other microorganisms (Karez 2003). These facilitative or protective advantages represent a trade-off in early survival versus intense intraspecific competition later as blades mature.

Density dependence due to competitive effects was manifested by both reduced growth and increased mortality, with clear differences among the three density levels. Our results were consistent with the concept of dynamic self-thinning relationships, in which the size-density relationship is not described by a fixed boundary but varies according to environmental conditions (Weller 1987, 1991, Karez 2003, Scrosati 2005). The only environmental condition that we varied in this experiment was initial density. We know very little about the conditions affecting recruitment and early growth in *Py. torta* gametophytes in their natural habitat, in part because of the difficulty in observing microscopic spores and blades on exposed rocky shores during winter. However, given the combination of nonmotile spores, turbulent water, and highly uneven substrates in nature, initial settlement densities in nature must be extremely variable. Conchospores of *Py. torta* are released during midwinter months when storms are frequent (Conway and Cole 1977, Lindstrom and Cole 1993) and, like all red algal spores, are nonmotile and drift passively as they slowly settle. Initial attachment to the rock substratum is probably aided by both the mucilage coating of the spores themselves and by crevices and depressions in the substratum (Kain and Norton 1990). Undoubtedly, these conditions, with minute differences in substrata and microhabitats, result in uneven distribution of recruits and wide local variation in density. For growing fronds of *Py. torta*, this environment is characterized by intense intraspecific competition, on one hand, and exposure to extreme physical conditions, on the other. Mature blades form dense, monospecific bands in the high intertidal zone of exposed rocky shores, where they are subjected alternately to heavy wave action and tidal currents then freezing and desiccation. Those individuals that manage to survive and, due either to chance of settlement location

or to self-thinning in their proximity, are not subject to crowding, would have ample light and nutrients and be able to grow rapidly. Field observations of *Py. torta* and the closely related *Pyropia abbottiae* indicate that mature stands have self-thinned and comprise individuals of large and fairly uniform sizes (Conitz 1999). Our experiment lasted for about 12 weeks, covering the period from initial attachment and germination of spores through to growth of blades to macroscopic sizes, up to about 1.0 cm in length. During this period, the largest blades in the low-density group attained average sizes more than 10 times greater than those in the high-density cultures, which grew very little, if at all, after the first 4–5 weeks. In Japanese *Pyropia tenera* (as *Porphyra tenera*) overall yield (total dry weight per unit length of *hibi* string) was independent of density (Yoshida 1972). In other words, more crowded cultures produced smaller blades, an effect commonly observed in terrestrial plants as well (Harper and White 1974, Harper 1977). Environmental stressors, largely absent in a culture environment, may help structure natural stands and favor faster-growing individuals through selective mortality and adaptation. Natural and simulated stressors have sometimes been provided to cultures in the development of *Porphyra* and *Pyropia* mariculture (Blouin et al. 2011). *Porphyra* in Japan was originally cultivated in the intertidal because growers thought periodic exposure and drying were necessary (Mumford and Miura 1988). *Porphyra* species from higher tidal elevations have been demonstrated to have greater adaptation to emersion; they more quickly recovered nitrogen and phosphate uptake and assimilation and maintained higher tissue nitrogen content (Kim et al. 2008). In experimental cultivation in tanks and ponds, *Porphyra linearis* attained maximal growth with a few hours of daily air exposure, giving it a competitive advantage by simulating its natural habitat in the upper intertidal zone (Friedlander 2008). Natural or simulated water motion or current may provide similar advantages.

Clearly, initial recruitment densities are affected by substrate type. Our experimental glass microscope slides did not resemble any natural substrate, and performed worst, in terms of successful recruitment, than all other substrate materials tested. They were used in the density experiment to facilitate counting and measurement of settled spores and germlings under the microscope, but may not have produced the same levels of density or growth as natural substrate. Materials with interstitial spaces somewhat larger than the diameter of a *Py. torta* spore (roughly 10 µm) held larger numbers of spores and may have provided more natural small-scale spatial variation in density. These materials could produce different

patterns of thinning in crowded cultures, by creating more variable local densities on the substrate and possibly providing some blades with a competitive advantage. Other advantages conferred by materials with interstitial spaces could include more favorable conditions for initial adhesion of spores, better attachment of rhizoids, and protection of small blades against desiccation, bleaching, and fouling.

In laboratory studies including this one, we have successfully controlled the life cycle of *Py. torta* and related Alaskan species such as *Py. abbottiae* through reproduction in both sporophyte and gametophyte phases. Conditions for maintenance and propagation of conchocelis (sporophyte stage) and recruitment and early growth of gametophytes have been identified (Conitz 1999, Lin 1999, Stekoll et al. 1999, Lin et al. 2008, Lindstrom et al. 2008). An extended study of density effects through the entire blade growth period from germination to maturity would be useful for mariculture development, as would a

comparison of survival, growth, and final yield between natural and cultivated populations. Other important conditions to consider include the role of water motion and the relationship between water motion and localized depletion of nutrients, the effect of periodic air exposure, and relationships between substrate, density, and growth.

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