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The Effects of Genotype, Density and Irradiance on the Growth and Mortality of the Brown Seeweed *Fucus serratus* Germlings

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Effects of genotype, settlement density and irradiance on the growth, mortality and population structure of *Fucus serratus* germlings were examined in the laboratory. The growth of *F. serratus* germlings was influenced by genotype of parent plants, which is likely to occur by microclimatic differentiation of substrata. The growth of germlings were greater at lower densities than higher densities indicating that intraspecific competition between germlings occurred within three weeks. Also, the growth of *F. serratus* germlings was better at 120 μmol m^{-2} s^{-1} than at 60 μmol m^{-2} s^{-1}. However, mortality of germlings was determined by settlement density rather than irradiance. In population structure, skewness was increased at low irradiance and high density indicating that population structure could be changed by intraspecific competition. Thus, it is concluded that genotypic differentiation of *F. serratus* germlings occurred in a microgeographic scale, and the experimental density and irradiance level affected the growth of germlings.

On rocky shores, seaweeds may colonize new areas and maintain their populations with propagules or zygotes, which have different genotypes from their parent plants. The dispersal of propagules is closely related to the patterns of gene dispersion within and among populations (Kendrick and Walker, 1991). The dispersal distance of algal propagules is from 1 to 30 m for *Sargassum* spp. (Deysher and Norton, 1982; Kendrick and Walker, 1991) and ca. 5 m for *Fucus serratus* (Arrontes, 1993; Chapman, 1995). Generally, long-distance dispersal decreases genetic differentiation among populations (Innes and Yarish, 1984; Kendrick and Walker, 1991).

After settlement, the germination and growth of germlings depend on their genotype (De Silva and Burrows, 1973; Sideman and Mathieson, 1983). When genetic differentiation between populations occurs, the growth of germlings (and older plants) originating from different parents (or populations) is different even if they are cultured under the same environmental conditions (Innes, 1987, 1988). Genotypic variation in higher plant populations has been reported frequently (Ellstrand and Roose, 1987) and it occurs within small areas (Bullock et al. 1994). In seaweeds, however, the effects of the genotype on the survival and growth of germlings have received relatively little attention (Sideman and Mathieson, 1983; Innes, 1988). Studies on genetic differentiation have been performed for *Enteromorpha linza* (Innes, 1988) and *Fucus distichus* (Sideman and Mathieson, 1983). Both found genetic differentiation between plants of the same species inhabiting the high and low intertidal zones and speculated that it occurred in the process of adaptation to different environments.

On the shore, propagule settlement has been observed near parent plants or in areas where the canopy has been cleared (Deysher and Norton, 1982; Kendrick and Walker, 1991). After settlement, propagules may interact with cohorts (germling-germling) and their parents. A canopy may inhibit the growth and survivorship of germlings by reducing light by 60-98% (Figueiredo et al., 1996). On the shore, the relative effects of light level on germlings can be examined by its manipulation, but it is nearly impossible to investigate the combined effects of irradiance and density because the density of germlings may be readily altered during the experiment by other environmental factors (i.e. wave movement and grazing). Therefore, intraspecific competition between germlings and the effect of light on this process should be studied in laboratory cultures.

Vadas et al. (1992) suggested, on the basis of the survivorship data of visible juveniles, that competitive interactions between germlings occur before the plants become visible. Intraspecific competition between germlings is density-dependent: the greater the density, the stronger the competitive stresses and the lower the resultant mean germling length and survivorship. Density-dependent mortality and growth occur between germlings in both culture and the field for fucoids

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(Kendrick, 1994; Creed et al., 1996b, 1997).

Fucus serratus is a canopy-forming species growing at low levels of most rocky shores in the Isle of Man (Jenkins et al., 1999) whose dispersal distance of propagules is about 5 m (Arrontes, 1993; Chapman, 1995). On the shore, the occurrence of dense monospecific stands of *F. serratus* juveniles resulting from high settlement densities of propagules (Creed et al., 1996a) indicates that they experience density effects. This species grows faster than any other fucoids, indicating that intraspecific competition can be readily detected. Thus, *Fucus serratus* has been chosen to test the following hypotheses: 1) Because of the dispersal of propagules, genetic variations are unlikely to occur between germlings originating from different parent plants grown in the upper and lower regions of the *F. serratus* zone. 2) At the early stage, settlement density and irradiance determine the growth and mortality of germlings.

Materials and Methods

Two different experiments were carried out to examine the effects of genotype, density and irradiance on the survivorship and growth of germlings in the laboratory. For both experiments, fertile plants of *Fucus serratus* were collected from Port St. Mary ledges (54°0'N, 4°44' W) during a low tide. On the shore, *F. serratus* occupied a vertical zone below mean low water neaps and 2.0-2.6 m above Lowest Astronomical Tide (LAT). The collected plants were transported to the laboratory where receptacles were excised, rinsed in cold tap water, wiped with paper towels and washed in filtered seawater. Propagule release and the preparation of zygote suspensions were achieved as described by Creed et al. (1996b).

To investigate genotype effects on germlings, 2 horizontal lines in the upper and lower regions of the *Fucus serratus* zone and 1 vertical line crossing the horizontal lines were established. The distance between horizontal zones was 6 m and vertical height between the two shore levels was 1 m. For each zone, 5 fertile female plants were collected from within 3 m (horizontal distance) of each other, plus 1 male plant from the low level on 27th December 1999.

Genotypic variation between parent plants or between populations has been examined by the physiological or growth responses of plants or propagules to environmental factors in controlled culture rooms (Dawes et al., 1988). In the present study, genetic variation was tested by the growth responses of germlings originating from each parent plant collected from the upper and lower regions of the *F. serratus* zone. Eggs from each female plant were released in different beakers and fertilized from a solution of antherozoids that originated from a single male plant.

Propagules were settled on cut glass slides (2.5×3.0 cm), which had been numbered on edges to distinguish treatments before inoculation with propagules. Uniform settlement density of propagules was obtained by inoculating 5 ml of zygote suspensions of 300 zygotes ml^{-1} for each treatment. After 24 h, slides on which germlings were most evenly distributed were chosen. For each shore level, 4 Petri dishes each containing 50 ml of culture medium (Kain, 1964) and 5 slides bearing germlings originating from different parent plants were prepared. Additional 3 slides for each treatment were set aside to determine the initial settlement density.

Cultures were carried out on a shelf of at constant temperature of 10 ± 1°C, photoperiod of 16:8 h LD (light: dark), and irradiance of 120 μmol m^{-2} s^{-1}. In order to variation minimize and in culture conditions along and across the constant temperature room shelf, the positions of Petri dishes were systematically changed every week. The culture medium was weekly changed.

To determine the initial settlement density, counts were made of the number of propagules in 4 random 6 mm^2 areas of each slide. Three slides for each treatment were used. At the start of the experiment, settlement density of propagules was about 30 zygotes cm^{-2}.

The lengths of a total 25 germlings were measured on each of 4 replicate slides for each treatment after 4 wk. Percentage mortality of germlings was also monitored at the end of experiment. A total of 100 germlings (alive and dead) on each slide was counted to determine the mortality of germlings.

To examine the effects of density and irradiance on the performance of germlings, 10 fertile female plants and 1 male were collected from the Port St. Mary ledges on 23rd November 1999. All receptacles of 10 female plants were put in a plastic tank (4 L) and the zygote suspension was mixed well to reduce the effects of genetic variation.

With the zygote suspension, 4 different settlement densities of propagules were made. Eight Petri dishes each containing 4 slides each bearing the different density and 50 ml of culture medium were prepared. Germlings were cultured at 10 ± 1°C, 16:8 h LD and 120 μmol m^{-2} s^{-1} or 60 μmol m^{-2} s^{-1} during a period of 3 weeks. A half of the Petri dishes was covered with black chiffon to reduce the level of light.

The settlement density, mortality and the length of germlings were measured as described above and 4 replicates were used to determine settlement density. The initial densities chosen were low [142.19 ± 7.38 germlings cm^{-2} (mean ± SE)], medium-low (393.0 ± 15.52), medium-high (909.38 ± 17.02), and high (2346.88 ± 21.12).

Statistical analyses utilized STATISTICA version 5.0. Homogeneity of variance was tested by Cochrans test (Underwood, 1997). Analyses of variance (ANOVA) were used for mean comparisons between treatments. When significant differences between means were detected, the Tukey HSD test was applied. Where necessary, data were transformed to meet the assumptions of the parametric statistics.

Using the lengths of germlings, three population measures were calculated in order to describe the popula-

tions in the density and light experiment. Skewness coefficient was used to quantify the relationship between the number of large and small plants within each population. Skewness, ranging from negative to positive, gives more information on whether the inequality in the size distribution is due to the presence of more large plants and fewer small ones (negative skewness) or more small plants and fewer large ones (positive skewness) (Ang and De Wreede, 1992). Coefficient of variation (CV) was used to calculate variability in individual plant sizes and the Gini coefficient was used as a measure of hierarchy of plant sizes (Bendel et al., 1989). The coefficient of variation and skewness were calculated as described by Zar (1984) and the Gini coefficient by Dixon (1993).

Results

Genotype effects

Genetic variation of 10 female parent plants collected from the upper and lower regions of the *F. serratus* zone was examined by the growth of their progeny. Here, regions and parent plants refer to the origin of germlings. Initial length (or diameter) of propagules ranged between 82.97-86.57 μm (n = 30). After four weeks, the lengths of germlings ranged from 0.89 ± 0.03 mm (mean ± SE, n = 25) to 1.04 ± 0.02 mm for the upper region and from 0.92 ± 0.04 mm to 1.09 ± 0.03 mm for the lower region (Fig. 1a). The mean lengths of germlings were significantly different among parent plants of the upper region ($F_{4,15}$ = 4.60, P < 0.05) and of the lower region ($F_{4,15}$ = 3.30, P < 0.05). To compare the growth of germlings between the regions, the five germling- populations of each region were pooled. Mean lengths of germlings were 0.95 ± 0.03 mm (mean ± SE, n = 5) for the upper region and 1.03 ± 0.03 mm for the lower region. Results of one-way ANOVA showed no significant difference between the upper and lower regions ($F_{1,8}$ = 3.20, P = 0.11).

Mortality of germlings ranged between 5.61-11.11% for the germlings from the upper region of the *F. serratus* zone and between 5.69-16.69% from the lower region (Fig. 1b). There were no significant differences in mortality of germlings (arcsine square root transformed) among parent plants from the upper region ($F_{4,15}$ = 1.42, P = 0.28), but significant differences were found from the lower region of the *F. serratus* zone ($F_{4,15}$ = 11.76, P < 0.001). For the germlings from the lower region, the Tukey HSD test revealed that the mortality of germlings originating from three parent plants was significantly higher than that of the other plants (Fig. 1b).

To compare the mortality of germlings between upper and lower region parents, five plants collected from each region were pooled. The mortality of germlings was 7.95 ± 0.95% (mean ± SE) and 10.38 ± 2.08% for the upper and lower regions, respectively. ANOVA test showed that no significant difference in mortality between the regions ($F_{1,8}$ =1.11, P = 0.32).

Effects of density and irradiance on germlings

Density and light influenced the *F. serratus* germlings (Fig. 2a). After three weeks, the lengths of germlings ranged

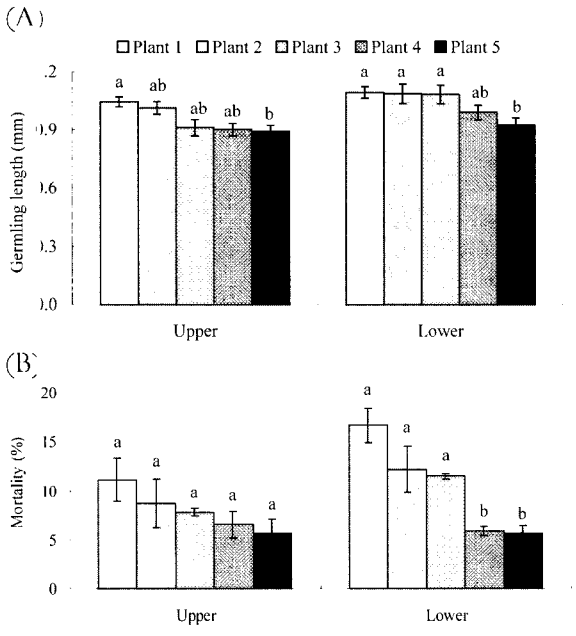

Fig. 1. Effects of genotype on the growth (A) and mortality (B) of germlings originating from different female plants taken from the upper and lower *Fucus serratus* zones after four weeks in culture. Bars show standard errors (n = 4 replicates). Letters a and b indicate significant group of means found with the Tukey HSD tests.

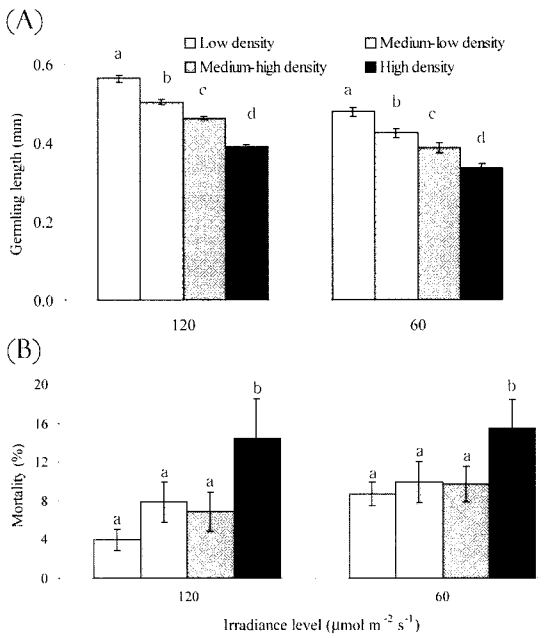

Fig. 2. The effects of density and irradiance level on the (A) growth and (B) mortality of *Fucus serratus* germlings after three weeks in culture. Bars show standard errors (n = 4 replicates). Letters a to d indicate significant group of means found with the Tukey HSD tests.

Table 1. Results of ANOVA for the effects of density and irradiance on the growth of *Fucus serratus* germlings

Factor	DF	MS	F	P
Irradiance	1	0.043	113.54	<0.001
Density	3	0.036	95.81	<0.001
Interaction	3	0.0004	0.95	0.43
Residuals	24	0.0004		

between 0.39-0.56 mm at 120 μmol m^{-2} s^{-1} and between 0.34-0.48 mm at 60 μmol m^{-2} s^{-1}. Their growth was significantly greater at 120 than 60 μmol m^{-2} s^{-1} (Table 1). Maximal growth occurred at the lowest density at both irradiances and the growth of germlings was significantly retarded with increasing settlement density (Table 1). A Tukey HSD test revealed significant differences among densities (Fig. 2). However, no interac-tion between light level and density level was found.

Mortality of germlings ranged between 3.92-15.48% after three weeks in culture (Fig. 2b). Two-way ANOVA showed that the mortality of germlings (arcsine square root transformed) was mainly determined by settlement density ($F_{3,24}$ = 5.20, P < 0.01) and not irradiance ($F_{1,24}$ = 2.61, P = 0.12). No interactions between light level and density were found ($F_{3,24}$ = 0.24, P = 0.87). Mortal ty was significantly higher at the highest density but no significant differences were found among the three lower densities (Tukey HSD test).

Table 2. Parameters in length of *Fucus serratus* populations grown for three weeks post settlement under various conditions of irradiance and four densities

Irradiance (μmol m^{-2} s^{-1})	Density level	Skewness coefficient	Coefficient of variation	Gini coefficient
120	Low	0.16	0.15	0.085
	Medium-low	0.40	0.15	0.087
	Medium-high	0.37	0.16	0.091
	High	0.71	0.16	0.087
60	Low	0.98	0.15	0.085
	Medium-low	0.69	0.16	0.091
	Medium-high	0.68	0.16	0.088
	High	0.87	0.16	0.093

Population structure was influenced by density of germlings and irradiance. The distribution of germling length in all treatments was positively skewed (more small plants and fewer large ones). Even after only three weeks of settlement important differences had taken place in the four density populations under 60 and

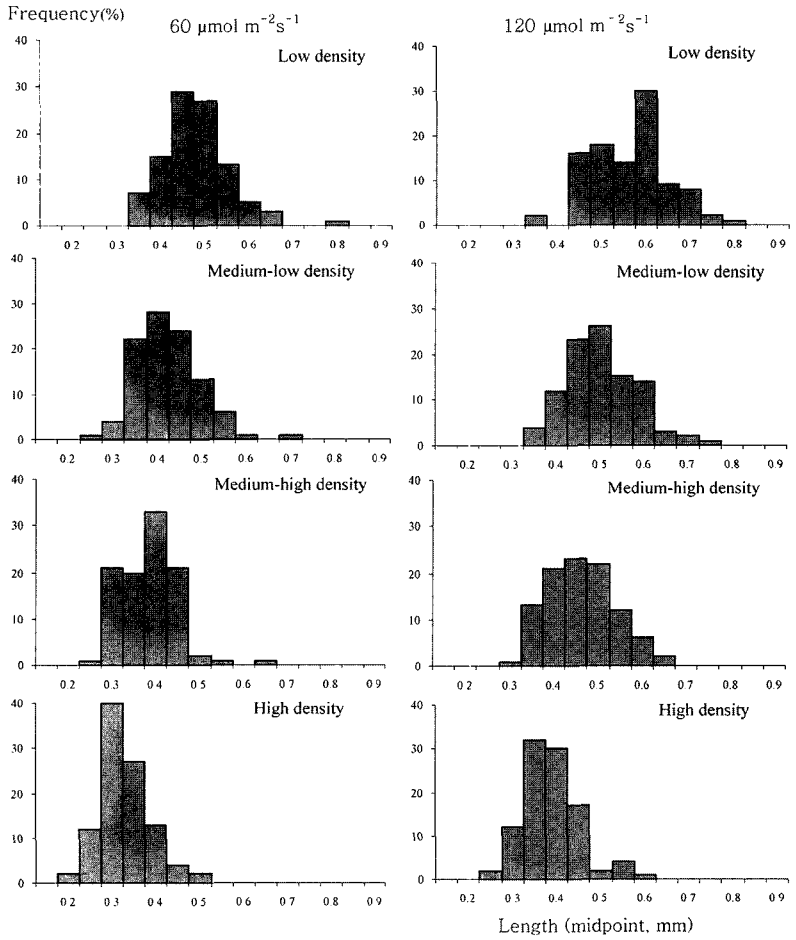

Fig. 3. The population structure of *Fucus serratus* germlings cultured for 3 weeks at two irradiance levels and four densities (r = 100).

120 μmol m^{-2} s^{-1} (Fig. 3). Skewness was greater under 60 than 120 μmol m^{-2} s^{-1} and increased with increasing density. Coefficient of variance and inequality (Gini coefficient) generally increased with density at both levels of irradiance (Table 2). The Gini coefficient has a minimum value of 0 when all individuals are the same size and a theoretical maximum of 1.

Discussion

The genetic differentiation within and among populations generally decreases with dispersal distance of seeds or propagules (Innes and Yarish, 1984; Loveless and Hamrick, 1984; Kendrick and Walker, 1991). In a population of an intertidal alga, *F. distichus,* its genotype was different between two shore levels (Sideman and Mathieson, 1983). However, my results indicated that plants of *F. serratus* living within 3 m of each other (irrespective of vertical level within the *F. serratus* zone) are probably genotypically different. The limited dispersal range of *F. serratus* propagules (Arrontes, 1993; Chapman, 1995) may foster the genotypic differentiation of plants.

A steep environmental gradient, such as that on the shore, is also known to encourage genetic differentiation (Dawes et al., 1988; Innes, 1988), as found in *F. distichus* populations, which are distributed over a wide range from the mid-eulittoral down to the upper sublittoral zone (Sideman and Mathieson, 1983). However, for *F. serratus,* which inhabits the comparatively benign lower shore, differences in microclimate may be equally or even more important than the level on the shore. Innes (1987) reported that genetic differentiation of *Enteromorpha linza* occurs on a microgeographic scale and at the spore stage. In my collection area, *F. serratus* grows on rock covered by rough-surfaced crustose coralline algae, *Phymatolithon* spp. (Figueiredo et al., 1996) and *Cladophora rupestris*. *C. rupestris* is a turf-forming alga that accumulates sediment (Jenkins et al., 1999). These communities may be enough to make microclimatic differentiation, which could select propagules and result in different genotypes succeeding.

Whatever the causes, in seaweed populations genetic differentiation can occur on a microgeographic scale and one might expect that it would be more common in populations of upper shore algae because they grow in a highly stressful environment (Sideman and Mathieson, 1983; Dawes et al., 1988). Thus, it is suggested that the genetic structure of an algal population should be considered in studies of seaweed ecology because the performance of plants depends on their genotypes (Sideman and Mathieson, 1983; Loveless and Hamrick, 1984). For instance, if the aim of study is to test the effects of temperature on the growth of germlings, propagules may be released from a single parent plant to reduce confounding effects between temperature and genotype. However, when many parent plants are used to provide propagules, we can more realistically estimate the overall growth range of the species, and by following the performance of the progeny from each parent some indication of genetic variability may also be gained.

Density also influences the performance of higher plants (Law and Watkinson, 1987; Bullock et al., 1994) and seaweeds (Creed et al., 1996b, 1997). In seaweeds, density-dependent competition is found not just in adult plants, but also in populations of germlings and juveniles (Knight and Parke, 1950; Creed et al., 1997). The present results show that *F. serratus* germlings grow faster at low density than at high density in laboratory culture. This indicates that intraspecific competition occurs between germlings within only three weeks, which is much earlier than the results of previous studies and settlement density resulting in nutrient deficiency and light reduction is an important factor determining their performance.

On the shore, propagules of *F. serratus* settle beneath the canopy where irradiance is severely reduced up to 98% (Figueiredo et al., 1996) and survive for long periods as juveniles do (Jenkins et al., 1999) even though their survival rate is still unknown. Thus, the growth of *F. serratus* germlings was mainly affected by the irradiance levels of 60 and 120 μmol m^{-2} s^{-1} because their growth is saturated at 135~180 μmol m^{-2} s^{-1} in adult plant (Stromgren and Nielsen, 1986). However, their mortality was not significantly influenced at the irradiance levels tested in the present study. It is suggested that *Fucus* germlings could survive at 60 μmol m^{-2} s^{-1} because they grow in low intertidal zone.

In conclusion, the growth of *F. serratus* germlings was influenced by genotype of parent plants, which is likely to occur by microclimatic differentiation of substrata. Also, intraspecific competition affecting the mortality, growth and population structure of germlings was found within three weeks, The growth of *F. serratus* germlings was also enhanced with increasing irradiance.

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References

Ang PO, Jr, and DeWreede RE (1992) Density-dependence in a population of *Fucus distichus*. *Mar Ecol Prog Ser* 90: 169-181.

Arrontes J (1993) The nature of the distributional boundary of *Fucus serratus* on the north shore of Spain. *Mar Ecol Prog Ser* 93: 183-193.

Bendel RB, Higgins SS, Teberg JE, and Pyke DA (1989) Comparison of skewness coefficient, coefficient of variation, and Gini coefficient as in equality measures within populations. *Oecologia* 78: 394-400.

Bullock JM, Mortimer AM, and Begon M (1994) The effect of clipping on interclonal competition in the grass *Holcus lanatus*-

a response surface analysis. *J Ecol* 82: 259-270.
Chapman ARO (1995) Functional ecology of fucoid algae: twenty-three years of progress. *Phycologia* 34: 1-32.
Creed JC, Norton TA, and Harding SP (1996a) The development of size structure in a young *Fucus serratus* population. *Eur J Phycol* 31: 203-209.
Creed JC, Norton TA, and Kain JM (1996b) Are neighbours harmful or helpful in *Fucus vesiculosus* populations? *Mar Ecol Prog Ser* 133: 191-201.
Creed JC, Norton TA, and Kain JM (1997) Intraspecific competition in *Fucus serratus* germlings: The interaction of light, nutrients and density. *J Exp Mar Biol Ecol* 212: 211-223.
Dawes CM, Bird K, and Hanisak MD (1988) Physiological responses of transplanted populations of *Sargassum pteropleuron* Grunow in Florida. *Aquat Bot* 31: 107-123.
De Silva MWRN and Burrows EM (1973). An experimental assessment of the status of the species *Enteromorpha intestinalis* (L.) Link and *Enteromorpha compressa* (L.) Grev. *J Mar Biol Ass UK* 53: 895-904.
Deysher LE and Dean TA (1986) *In situ* recruitment of sporophytes of the giant kelp, *Macrocystis pyrifera* (L.) C.A. Agardh: effects of physical factors. *J Exp Mar Biol Ecol* 103: 41-63.
Dixon PM (1993) The bootstrap and jackknife: describing the precision of ecological indices. In: Scheiner SS and Gurevitch J (eds.), Design and Analysis of Ecological Experiments, Chapman and Hall, New York, pp 290-318.
Ellstrand NC and Roose ML (1987) Patterns of genotypic diversity in clonal plant species. *Am J Bot* 74: 1371-1380.
Figueiredo MAdeO, Kain JM, and Norton TA (1996) Biotic interactions in the colonization of crustose coralline algae by epiphytes. *J Exp Mar Biol Ecol* 199: 303-318.
Innes DJ (1987) Genetic structure of asexually reproducing *Enteromorpha linza* (Ulvales: Chlorophyta) in Long Island Sound. *Mar Biol* 94: 456-467.
Innes DJ (1988) Genetic differentiation in the intertidal zone in populations of the alga *Enteromorpha linza* (Ulvales: Chlorophyta). *Mar Biol* 97: 9-16.
Innes DJ and Yarish D (1984) Genetic evidence for the occurrence of asexual reproduction in populations of *Enteromorpha linza* (L.) J. Ag. (Chlorophyta, Ulvales) from Long Island Sound. *Phycologia* 23: 311-320.
Jenkins SR, Hawkins SJ, and Norton TA (1999b) Interaction between a fucoid canopy and limpet grazing in structuring a low shore intertidal community. *J Exp Mar Biol Ecol* 233: 41-63.
Kain (Jones) JM (1964) Aspects of the biology of *Laminaria hyperborea* III. Survival and growth of gametophytes. *J Mar Biol Ass UK* 44: 415-433.
Kendrick GA (1994) Effects of propagule settlement density and adult canopy on survival of recruits of *Sargassum* spp. (Sargassaceae: Phaeophyta). *Mar Ecol Prog Ser* 103: 129-140.
Kendrick GA and Walker DI (1991) Dispersal distance for propagules of *Sargassum spinuligerum* (Sargassaceae: Phaeophyta) measured directly by vital staining and venturi suction sampling. *Mar Ecol Prog Ser* 79: 133-138.
Knight M and Parke M (1950) A biological study of *Fucus vesiculosus* L. and *F. serratus* L. *J Mar Biol Ass UK* 29: 439-514.
Law R and Watkinson AR (1987) Response surface analysis of two species competition: an experiment on *Phleum arenarium* and *Vulpia fasciculata*. *J Ecol* 75: 871-886.
Loveless MD and Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Ann Rev Ecol System* 15: 65-95.
Sideman EJ and Mathieson AC (1983) Ecological and genecological distinctions of a high intertidal dwarf form of *Fucus disticus* (L.) Powell in New England. *J Exp Mar. Biol Ecol* 72: 171-188.
Underwood AJ (1997) Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge.
Vadas RL, Johnson S, and Norton TA (1992) Recruitment and mortality of early post-settlement stages of benthic algae. *Br Phycol J* 27: 331-351.
Zar JH (1984) Biostatistical Analysis. 2nd Ed. Prentice-Hall, London.

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