

CONTRASTING PATTERNS OF ALLOMETRY AND REALIZED PLASTICITY IN THE SISTER SPECIES *MAZZAELLA SPLENDENS* AND *MAZZAELLA LINEARIS* (RHODOPHYTA)¹

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Phenotypic differences between the low wave exposure *Mazzaella splendens* (Setchell et Gardner) Hommersand and the high exposure *Mazzaella linearis* (Setchell et Gardner) Fredericq could be due to plasticity or genetic differentiation. Common gardens were used to assess their levels of plasticity after describing allometric relationships. As thalli lengthened, stipe development for *M. splendens* almost ceased even though blades continued to expand, but *M. linearis* formed a larger stipe before developing a blade that continued to stay narrow at longer thallus lengths. Common gardens demonstrated that *M. splendens* regrown in the site of *M. linearis* produced a wider blade than *M. splendens* regrown in its natural low energy site and that *M. linearis* regrown in low wave energy either could not form a wider blade or became narrower than thalli from its high energy site. Tetrasporophytes of *M. splendens* produced a longer and thicker stipe in the high energy site, but the larger *M. linearis* stipe was not realized because its wider blades made it vulnerable to hydrodynamic removal. *Mazzaella splendens* therefore had low survivorship in the high wave energy site, and survivors were not long enough to reproduce. Survivorship and reproduction of *M. linearis* was similar in both environments. Some of the *M. splendens* and *M. linearis* characters are plastic, but this plasticity was insufficient for convergence of phenotypes, and blade width plasticity was maladaptive at least for *M. splendens*. Developmental systems producing the stipe and blade phenotypes of each species have undergone genetic differentiation.

Key index words: allometry; genetic differentiation; *Mazzaella*; phenotypic plasticity; reproduction; survivorship

Understanding how much of the phenotypic variation within or between sister species is due to plasticity and genetic differentiation is important for discerning how species survive and reproduce and for reconstructing microevolutionary events and evaluating the reliability of taxonomic characters. Phenotypic

plasticity is the potential of the genotype and developmental system of an organism to produce a range of phenotypes in response to varying environmental conditions (Scheiner 1993, Morales et al. 2002). Plasticity is genetically controlled by either regulatory plasticity genes that develop distinct phenotypes in response to a change in environment, such as in the case of the submerged and emerged leaf morphs produced by some aquatic plants, or allelic sensitivity that produces a continuous range of variation along an environmental gradient (Schlichting and Pigliucci 1998). Both genetic mechanisms rely on the capacity of the organism to detect changing environmental signals. Phenotypic plasticity is heritable and is considered by many to be an adaptation to environmental heterogeneity (Williams 1960, Bradshaw 1965, Jain 1978, Sultan 1987, Schlichting and Pigliucci 1998, Morales et al. 2002). For example, plasticity is adaptive in the intertidal red algae *Gelidium coulteri* Harvey and *Corallina vancouveriensis* Yendo. These species have few branches and high estimated net photosynthetic rates when growing in humid microclimates, whereas thalli of these species occurring in drier microclimates are densely branched, which minimizes desiccation stress but results in low photosynthetic rates (Taylor and Hay 1984, Taylor 1985).

The degree of environmental heterogeneity encountered by an alga through time in one location, or by propagules dispersed out of this location, may be beyond its capacity to buffer by phenotypic plasticity, in which case either local extinction or selection for new genotypes, sometimes recognized as ecotypes, may occur (Dixon 1963, Bradshaw 1972, Russell 1986, Innes 1984, Schlichting and Pigliucci 1998). Within the Gulf of Maine, the vertical intertidal and the horizontal wave energy gradients, respectively, for *Fucus distichus* (L.) Powell and *Chondrus crispus* (L.) Lyngb. have apparently been too extreme to be buffered by plasticity. Differences in thallus height and reproductive phenology between low intertidal *Fucus distichus* ssp. *edentatus* (De la Pylaie) Powell and a high intertidal nontide pool member of the *F. distichus* complex were maintained when zygotes were outplanted to a common garden (Sideman and Mathieson 1983). Similarly, reciprocal transplants of *C. crispus* exposed to either high or low wave energy, respectively, maintained their narrow or wide branching patterns when young fronds grew to adult sizes (Floc'h 1969).

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Red algae in the family Gigartinales are good candidates for comparative ecological and evolutionary studies because some genera, particularly *Mazzaella* G. DeToni f. and *Chondracanthus* Kützinger, are speciose, and taxa occur in distinctive habitats. Studies using gene sequences have provided consistent support for most clades within the family (Hommersand et al. 1994, 1999), but there remains a taxonomic need to know if morphological characters used to identify these taxa are reliable. *Mazzaella splendens* (Setchell et Gardner) Hommersand and *Mazzaella linearis* (Setchell et Gardner) Fredericq, which are the focus of the present study, are a case in point and are hypothesized to be sister species based on morphological, reproductive, and gene data (Abbott 1971, Kim 1976, Hommersand et al. 1993, 1994, 1999, Hughey et al. 2001, Ross et al. 2003). These two species as well as others in the Gigartinales have a convoluted taxonomic history that has been summarized by several investigators (Shaughnessy 1996, Hughey et al. 2001, Ross et al. 2003). *Mazzaella linearis* occurs in high wave impact sections of the rocky intertidal and is characterized by a long thick stipe with a narrow blade, whereas *M. splendens*, which occurs at sites with low or intermediate wave energy, has a shorter thinner stipe with a wider blade (Abbott 1971, Shaughnessy 1996). Thalli of *M. splendens* from intermediately exposed sites have the same short thin stipe as thalli from low exposure sites but narrower blades and thus are considered to be a different morphology of *M. splendens* (Shaughnessy 1996). This hypothesis is supported by sequence data from the internal transcribed spacer region of the ribosomal cistron (Ross et al. 2003).

Despite the strong support for the distinctiveness of these two sister species, there is no reason to assume that the stipe and blade morphologies are due to genetic differentiation rather than phenotypic plasticity. DNA sequences used in phylogenetic studies could be taken from genes directly involved in development but are more often from genes that are indirectly involved in development or not at all because it may be important for a sequence to come from a noncoding region of the genome. In addition, the process of developing particular attributes of an overall phenotype involves many genes (Schlichting and Pigliucci 1998). Because the relationship between the morphology of *M. splendens* and *M. linearis* and water motion has been described (Shaughnessy 1996) and the functional significance of the morphological differences between the species examined (Shaughnessy et al. 1996, 2001), the objective of this study was to determine whether stipe and blade differences between the species are due to genetic differentiation or to phenotypic plasticity.

A field experimental approach was used to address this objective. Reciprocal common gardens were established where all blades were removed from holdfasts and the latter were divided in half so that control and experimental treatments contained matching genotypes. Gametophyte and tetrasporophyte phases of

each species were evaluated separately because phases of *M. splendens* are not morphologically identical (Shaughnessy 1996), and there is the potential for the diploid tetrasporophyte to demonstrate greater plasticity than gametophytes because the former could use allelic sensitivity (*sensu* Schlichting and Pigliucci 1998) as a mechanism for producing a range of phenotypes. At the conclusion of the experiment 13 months later, it became apparent that treatments had a significant effect on thallus length and that a nonlinear allometric relationship existed between thallus length and the various stipe and blade characters. Thus, assessing patterns of plasticity by comparing treatment means, each with a different developmental stage, was not as useful as contrasting the allometric curves of control and experimental treatments. The present study therefore uses a descriptive approach to compare allometric relationships for these species and phases first and then in a separate experimental analysis contrasts levels of plasticity in all treatment groups following a period of growth in common gardens.

MATERIALS AND METHODS

Site description. The low wave energy Wizard Rocks and high wave energy Prasiola Point in Barkley Sound, British Columbia (48° 50'N, 125° 10'W) were sites for common gardens where *M. splendens* and *M. linearis*, respectively, occur (Fig. 1). Thalli at these low intertidal sites represented the extremes of stipe and blade variation between the two species in Barkley Sound; an intermediate wave energy site was not included in this study. Physical and biological features of these sites, as well as *Mazzaella* thallus morphologies, are similar to the low wave energy Second Beach site (site 3) and the high exposure site of Nudibranch Point (site 10) used by Shaughnessy (1996). Common gardens were situated within naturally occurring ranges of each species, and the surrounding community was only disturbed enough to attach transplants. The *M. splendens* common garden at Wizard Rocks was located between 1.01 and 0.69 m above lowest normal tide (LNT; Canadian Chart Datum). The qualitatively most abundant perennial macrophytes at the natural upper extremity of the *M. splendens* zone (i.e. 1.01 m LNT) were the algae *Egregia menziesii* (Turner) Areschoug and *Fucus gardneri* Silva. *Chondracanthus corymbiferus* (Kützinger) Guiry and *Laminaria setchellii* Silva dominated the lowest elevation of the *M. splendens* zone (i.e. 0.69 m LNT). The *M. linearis* common garden at Prasiola Point was located between 1.31 and 1.01 m above LNT. The alga *Lessoniopsis littoralis* (Tilden) Reinke, barnacles *Pollicipes polymerus* Sowerby and *Semibalanus cariosus* Pallas, and mussel *Mytilus californianus* Conrad dominated at the natural upper extremity of the *M. linearis* zone, whereas only *L. littoralis* dominated the lowest elevation.

Experimental design. During May 1991 thalli of each species were collected from Wizard Rocks and Prasiola Point and brought back to the Bamfield Marine Station where they were kept in flowing seawater. All stipes arising from each holdfast were removed, air dried, and bagged for later descriptions of morphology, phase, and reproductive status. Because the experimental design was a reciprocal common garden, each holdfast was divided in half so that control (i.e. natural site) and experimental (i.e. site of the other species) treatments started with a matching set of genotypes. Holdfast halves were randomly assigned to either the control or experimental treatment and then placed into tight twists of polypropylene rope that was attached to vinyl covered metal

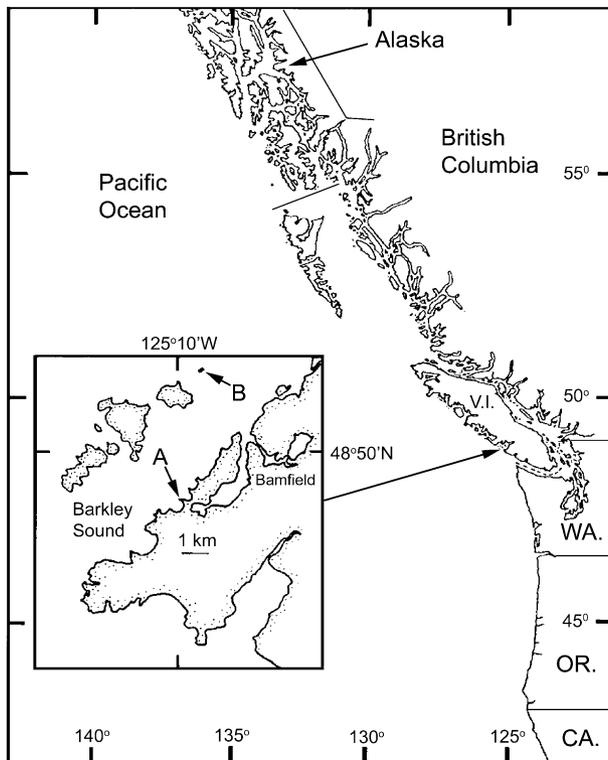


FIG. 1. The location of common gardens in Barkley Sound, British Columbia. The high wave energy common garden was located at Prasiola Point (A), where *M. linearis* naturally occurs, and the low wave energy common garden was located at Wizard Rocks (B), where *M. splendens* occurs.

grids. There were similar numbers of gametophytes and tetrasporophytes within and among grids. Four grids were created for each treatment, with 32 bare holdfast halves in each grid. Within a site, treatment grids were randomly assigned to one of eight possible intertidal positions. Grids were attached to the rock substratum using anchor bolts, fender washers, and nuts. These steps were completed 24 h after the thalli were collected. Further details and an illustration of this method are described by Shaughnessy and De Wreede (2001). The experiment was terminated during June 1992 after 13 months of growth, and all thalli were collected and air dried for description.

Thallus description. Collected thalli that had been air dried at the beginning of the experiment in 1991 and the end of the experiment in 1992 were rehydrated in seawater for 15 min, after which several morphological variables were enumerated. Stipe length was from the base of the stipe to where it was the same thickness as the blade base (i.e. apophysis). For *M. splendens*, about 75% of this length was terete in cross-section with the remainder being slightly canalliculate as it graded into the apophysis, whereas 100% of the *M. linearis* stipe was terete. Stipe and blade thickness was measured with vernier calipers, and then blades were placed between two Plexiglas sheets for photocopying. Blade lengths, blade widths, and angles of blade bases (i.e. base angle) were measured from the photocopies. Details and an illustration of these characters are in Shaughnessy (1996). Assessing blade length accurately was important because thallus length was the combination of blade (apophysis included) and stipe lengths, and thallus length was used as the independent variable for describing allometric relationships.

Blade length was simple to determine in most cases because blade margins were complete and clearly formed an apex. This was almost always the case for the 1991 thalli, but approximately 5% of the 1992 thalli, particularly for experimental *M. splendens*, were broken blades that were also wide. Without a correction for blade length, it would appear as if short thalli were very wide and the wrong developmental trajectory would be portrayed. If the blade was broken, actual blade length was estimated by drawing the missing part of the blade on the photocopy. The curvature on the sides of the blade was used to approximate where the apex would be.

Several other response variables were calculated so that the relationship between levels of plasticity and fitness could be examined. Percent survivorship, calculated for each wire grid, was the number of each phase remaining divided by the starting number of that phase. The percent of survivors that were reproductive was calculated by dividing the number of survivors of a phase by the number of survivors in each possible reproductive condition for that phase. Reproductive condition (i.e. vegetative gametophyte, spermatangial, carposporangial, vegetative tetrasporophyte, tetrasporangial) was determined from reproductive structures or if vegetative by the resorcinol method as described by Garbary and De Wreede (1988). Maximum thallus length was a character that only included the longest blade from each holdfast in contrast to the thallus lengths used to examine allometry where all blades from each holdfast were used. Maximum thallus length gave an indication of how large a thallus could get in a specific treatment. Thallus wet weight, which included all blades on a holdfast as well as the holdfast itself, was the blotted weight taken after 15 min of rehydration.

Data analysis. All the 1991 thalli were used in the first part of the study to describe the allometric relationships between thallus length and the development of the stipe and blade. These 1991 data, rather than the control 1992 data, were used because the former data set was larger. Allometric curves can be linear, curvilinear, or nonlinear, and variables may or may not be log transformed. The choice of the regression model is guided by biological reality, the ease with which the curve or coefficients in the model can be interpreted (Schlichting and Pigliucci 1998), as well as the quality of the fit (Ryan 1997). TableCurve 2D (1996), software that allows a wide variety of regression models to be fit and compared, was used to choose appropriate models. The intercept was not forced through zero because there were many short (minimum length, 0.4 cm) thalli in the sample, and only extreme outliers (<0.02% of sample) were dropped. Equations 1 and 2, which are power relationships, were used to describe allometric relationships for all stipe and blade characters except blade width, which was best fit by the sigmoid model shown in Equation 3. The regression models were as follows:

$$y = ax^b \quad (1)$$

where y is a morphological variable, x is thallus length, and a and b are coefficients, and

$$y = a + bx^c \quad (2)$$

where y is a morphological variable, x is thallus length, and a – c are coefficients, and

$$y = a + b / (1 + \exp^{-(x-c)/d}) \quad (3)$$

where y is blade width, x is thallus length, and a – d are coefficients. These equations were fit to untransformed data because log-log transformations did not completely straighten the point scatter, and untransformed curves that directly portrayed how thalli were developing were more easily interpreted. Confidence limits (95%) for curves were calculated

by TableCurve 2D and presented graphically; all graphs were created in SigmaPlot 2000 (SPSS Inc., Chicago, IL, USA).

The same type of power and sigmoid models were used in the second experimental part of the study when comparing allometric trajectories that occurred after 13 months of growth in the common garden treatments (i.e. the 1992 data set). Because control and experimental treatments started with matching holdfasts, the strongest test of whether or not plasticity was occurring would have been to restrict the fitting of curves to only those thalli that could be matched 13 months later. However, due to differential survivorship among treatments, this restriction lowered sample sizes, so curves were also fit to all of the blades produced by all holdfasts. The latter curves are the only ones presented, but trajectories for the matched data set were similar except that confidence intervals were wider. For the curves presented, the percent of holdfast matching between treatments, calculated as the number of holdfasts matched in control and experimental treatments divided by the total holdfasts in control plus total holdfasts in experimental treatments, was the following for each species and phase: *M. splendens* gametophytes, 16.7%; *M. splendens* tetrasporophytes, 19.1%; *M. linearis* gametophytes, 11.1%; *M. linearis* tetrasporophytes, 18.2%.

A fixed effects analysis of variance (ANOVA) was used to compare the fitness response variables of percent survivorship, thallus length, and genet weight measured from the common garden experiment. Reproductive condition could not be compared using ANOVA because there were not enough degrees of freedom. Effects in the model included species (*M. splendens*, *M. linearis*), phase (gametophyte, tetrasporophyte), and environment (control, experimental). Interaction terms were included because of the potential of the phases of one species to be more isomorphic than those of the other species (e.g. species \times phase) or for the possibility that only one phase (e.g. phase \times environment) or one species (e.g. species \times environment) might demonstrate plasticity. Normal probability plots and the distribution of residuals were used to check, respectively, the assumptions of normality and homogeneity of variances; it was necessary to log transform maximum thallus length and thallus wet weight. These analyses were done using SYSTAT 10 (2000, SPSS Inc.).

RESULTS

Allometric relationships. The Eq. 1 power model was most effective at fitting the relationships between thallus length and almost all the blade and stipe characters, except for blade width (Table 1), which had a sigmoid relationship with thallus length. Stipes of *M. linearis* were about twice as long and thick as those of *M. splendens* when thalli were 5.0 cm long, and beyond 10.0 cm of thallus length, stipe development slowed more for *M. splendens* than for *M. linearis* (Fig. 2, A and B). Gametophytes and tetrasporophytes of *M. splendens* had similar developmental curves for stipe length and thickness, but for *M. linearis*, gametophyte stipes became longer and thicker than tetrasporophyte stipes at long thallus lengths. Both phases of *M. splendens* had an almost linear, or isometric, relationship between thallus length and blade width, with tetrasporophyte blades beginning to develop a narrower blade than gametophytes when thalli were about 5.0 cm in length (Fig. 2C). *Mazzaella linearis* formed narrower blades than those of *M. splendens*, and trajectories for *M. linearis* were more sigmoid (Table 1). In contrast to the gametophytes, tetrasporophyte blades of *M. linearis* ceased expanding after they became about 13.0 cm in length. Patterns of base angle development demonstrated a power rather than a sigmoidal relationship (Fig. 2D). Gametophytes of *M. splendens*, which had the widest blades, also had the greatest base angles, which continued to expand as thalli became longer. However, tetrasporophytes of *M. splendens* and gametophytes of *M. linearis* had the same base angle trajectories, and there was no relationship between thallus length and base angle for tetrasporophytes of *M. linearis*. Blade thickness also demonstrated a

TABLE 1. Relationships between thallus length (x) and the morphological characters (y) for the phases of each species.

Character	Group	Model	n	a	b	c	d	R^2	Fit SE	F statistic
Stipe length	<i>Mazzaella splendens</i> gam.	$y = ax^b$	228	0.26	0.46			0.54	0.29	267.75
	<i>M. splendens</i> tet.		166	0.36	0.40			0.45	0.31	137.45
	<i>Mazzaella linearis</i> gam.		186	0.80	0.32			0.28	0.59	75.38
	<i>M. linearis</i> tet.		153	0.89	0.24			0.20	0.48	40.76
Stipe thickness	<i>M. splendens</i> gam.	$y = ax^b$	229	0.55	0.17			0.34	0.18	120.15
	<i>M. splendens</i> tet.		169	0.63	0.13			0.17	0.21	36.02
	<i>M. linearis</i> gam.		184	1.02	0.19			0.26	0.35	66.32
	<i>M. linearis</i> tet.		154	1.19	0.10			0.09	0.30	17.79
Blade width	<i>M. splendens</i> gam.	$y = a + b/(1 + \exp^{-(x-c)/d})$	227	-10.03	39.08	15.92	14.04	0.94	1.59	1102.22
	<i>M. splendens</i> tet.		167	-3.20	22.75	16.51	9.17	0.95	0.81	1179.87
	<i>M. linearis</i> gam.		183	-45.38	53.55	-26.93	16.20	0.84	0.70	321.16
	<i>M. linearis</i> tet.		156	-0.07	3.99	6.64	2.71	0.81	0.58	229.53
Base angle	<i>M. splendens</i> gam.	$y = ax^b$	227	23.49	0.47			0.63	22.02	386.83
	<i>M. splendens</i> tet.		168	22.21	0.37			0.30	20.65	72.96
	<i>M. linearis</i> gam.		187	19.84	0.36			0.20	21.89	47.90
	<i>M. linearis</i> tet.		156	36.40	-33.68	-5.16		0.00	20.19	0.74
Blade thickness	<i>M. splendens</i> gam.	$y = ax^b$	229	0.16	0.20			0.36	0.06	130.66
	<i>M. splendens</i> tet.		169	0.16	0.23			0.38	0.06	107.70
	<i>M. linearis</i> gam.		185	0.21	0.25			0.49	0.06	182.17
	<i>M. linearis</i> tet.		153	0.23	0.21			0.37	0.06	93.51

All fits were significant ($P < 0.000$) except for the base angle fit for tetrasporophytes of *M. linearis*. $a-d$, coefficients; n , sample size; gam., gametophyte; R^2 , adjusted degrees of freedom; tet., all tetrasporophytes.

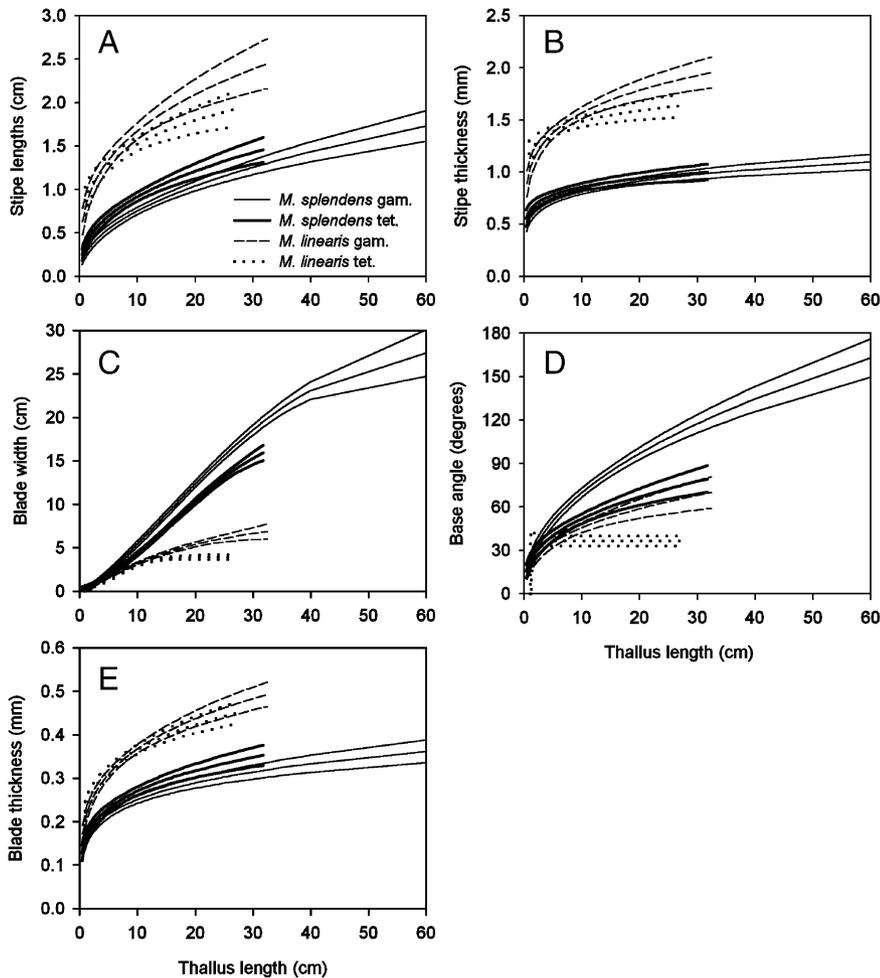


FIG. 2. Allometric relationships between thallus length and stipe length (A), stipe thickness (B), blade width (C), base angle (D), and blade thickness (E) based on the 1991 sample taken at the beginning of the common garden experiment. Power or sigmoid regression models were fit to the gametophytes (gam.) and all tetrasporophytes (tet.) of *M. splendens* and *M. linearis*. In each cluster of three curves, the middle one is the model's prediction, which is surrounded by the upper and lower 95% confidence limits. Information about each model fit is in Table 1.

power relationship, with thickness increasing beyond 10.0 cm in length (Fig. 2E). *Mazzaella linearis* blades were always thicker than those of *M. splendens*, and phases within a species had similar trajectories.

Common garden responses. The stipe length trajectory for gametophytes of *M. splendens* grown in the high wave energy (i.e. experimental) environment of *M. linearis* had the same trajectory as both phases in the low energy (i.e. control) site, but experimental tetrasporophytes of *M. splendens* developed stipes that were slightly longer (Fig. 3A). The trajectory for the experimental low wave energy thalli of *M. linearis* was to form a shorter stipe relative to control gametophytes, but this distinction was not apparent until thalli were about 10.0 cm in length (Fig. 3B). Stipe thickness responses (data not presented) paralleled those of stipe length for both species. Even though a correction was made for the thallus lengths of broken blades, both phases of experimental *M. splendens* started growing wider blades than controls and then began to slow blade width development, unlike for controls of this species (Fig. 3C). Experimental gametophytes of *M. linearis* had the same blade width trajectories as for control thalli, but experimental tetrasporophytes of *M. linearis* devel-

oped blades narrower than control thalli (Fig. 3D). Developmental patterns for base angle were similar to those of blade width for both species, but 95% confidence intervals were wider than for any other character (data not presented). Blade thickness was the same between control and experimental environments for both species (Fig. 3, E and F).

The percent survivorship of control *M. splendens* thalli, of both phases, was significantly greater than for experimental thalli of this species, but survivorship for *M. linearis* was unaffected by environment (Table 2, Fig. 4A). Maximum thallus lengths achieved by survivors also showed an affect of environment on *M. splendens*, with the tetrasporophytes of *M. splendens* being significantly longer than gametophytes (Table 2, Fig. 4B). Environment had no effect on maximum thallus length of *M. linearis*, and the phases of this species were also not different between control and experimental environments (Table 2, Fig. 4B). Despite the fact that all blades from each holdfast were included in the measurement of wet weight, the response of this variable was similar to that of maximum thallus length, with *M. splendens* biomass being more affected by environment than *M. linearis* (Table 2, Fig. 4C). From 35% to 40% of control *M. splendens* survivors became

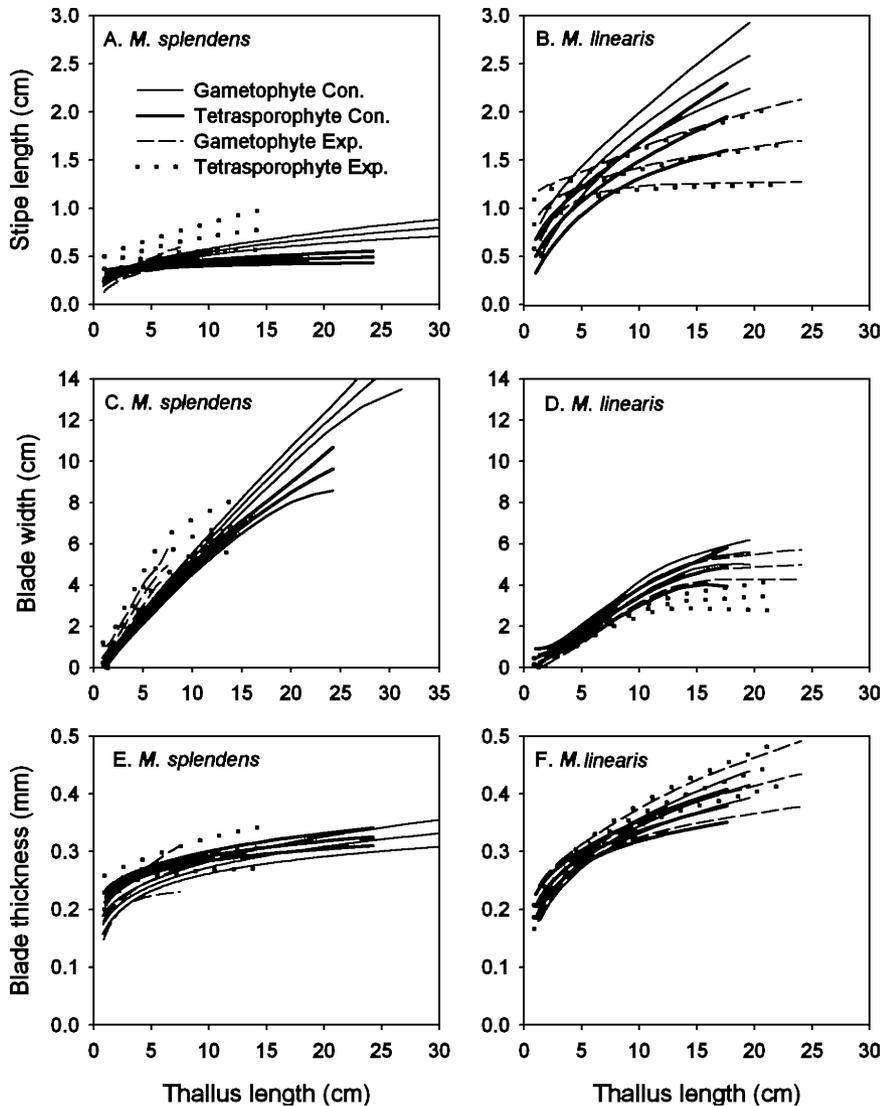


FIG. 3. Allometric responses for phases of *M. splendens* and *M. linearis* after 13 months of growth in control (cont.) and experimental (exp.) common garden sites. Only stipe length (A and B), blade width (C and D), and blade thickness (E and F) responses are presented because stipe thickness and base angle, respectively, paralleled stipe length and blade width. In each cluster of three curves, the middle one is the model's prediction, which is surrounded by the upper and lower 95% confidence limits.

spermatangial, carposporangial, or tetrasporangial, but none of the experimental *M. splendens* thalli became reproductive (Fig. 5). All reproductive categories were evident in experimental thalli of *M. linearis*, and the percent reproductive was similar between control and experimental treatments.

DISCUSSION

Understanding the degree of phenotypic plasticity that seaweeds can demonstrate is not only important for identifying stable taxonomic characters, it also improves understanding of how thalli survive and reproduce across an environmental gradient, such as wave energy in the case of *M. splendens* and *M. linearis*. The blade and stipe differences between these two species, which are taxonomically and functionally important, could be due to plasticity or genetic differentiation. To determine which of these hypotheses was best supported, common garden responses were examined by comparing

allometric relationships of thalli from different environments (i.e. control, experimental), rather than mean character dimensions, because there was a wide range of thallus lengths, and there is a nonlinear relationship between thallus length and several of the stipe and blade variables. However, because the rigor of these comparisons at the end of the common garden experiment is affected by sample sizes of surviving thalli, the present study first described the allometric curves for the larger sample of thalli taken at the beginning of the experiment and qualitatively compared them with the control curves at the end of the experiment.

Comparisons of these allometric relationships from the beginning of the experiment show that the two species differ with respect to when the same character is formed as well as whether growth continues or almost ceases at longer thallus lengths. Blade formation for *M. splendens* begins at the same time as the stipe, and both the stipe and blade have almost reached their characteristic thickness by 10.0 cm in length. Stipes of

TABLE 2. ANOVA comparisons of response variables measured at the termination of the reciprocal common garden experiment in June 1992.

Response variable	Source	SS	df	MS	F-ratio	P
% Survivorship	Species	1630.096	1	1630.096	8.206	0.009
	Phase	420.018	1	420.018	2.114	0.160
	Env.	5436.654	1	5436.654	27.367	0.000
	Species × Phase	101.072	1	101.072	0.509	0.483
	Species × Env.	6211.532	1	6211.532	31.268	0.000
	Phase × Env.	3.043	1	3.043	0.015	0.903
	Species × Phase × Env.	123.119	1	123.119	0.620	0.440
	Error	4370.409	22	198.655		
Maximum thallus length	Species	0.009	1	0.009	0.091	0.764
	Phase	0.014	1	0.014	0.144	0.705
	Env.	1.421	1	1.421	14.867	0.000
	Species × Phase	0.527	1	0.527	5.517	0.020
	Species × Env.	2.178	1	2.178	22.786	0.000
	Phase × Env.	0.020	1	0.020	0.208	0.649
	Species × Phase × Env.	0.316	1	0.316	3.310	0.070
	Error	19.884	208	0.096		
Wet weight	Species	0.197	1	0.197	1.800	0.181
	Phase	0.003	1	0.003	0.027	0.869
	Env.	2.271	1	2.271	20.765	0.000
	Species × Phase	0.390	1	0.390	3.566	0.060
	Species × Env.	1.739	1	1.739	15.905	0.000
	Phase × Env.	0.095	1	0.095	0.868	0.353
	Species × Phase × Env.	0.079	1	0.079	0.725	0.395
	Error	22.526	206	0.109		

Terms in the ANOVA model include Species (*M. splendens*, *M. linearis*), Phase (gametophyte, tetrasporophyte), and Environment (Env. control, experimental). Percent survivorship is the number of thalli (i.e., holdfasts) present at the beginning of the experiment in 1991 divided by the number remaining in 1992. Maximum thallus length is the longest blade plus stipe on each holdfast, and wet weight is the blotted weight of each holdfast plus all blades from that holdfast after rehydration in seawater for 15 min. Log transformations were used for maximum thallus length and wet weight to produce a more homogeneous distribution of residuals. df, degrees of freedom; MS, mean squares; SS, sum-of-squares.

M. splendens lengthen and thicken as blades expand, until thalli become long, at which point stipe development slows but blade expansion remains almost linear. In contrast, *M. linearis* blade development is delayed and does not accelerate until after stipes have achieved most of their thickness. Another difference is that at longer thallus lengths, stipes of *M. linearis* continue to lengthen and thicken and blades thicken, even though expansion of gametophyte and tetrasporophyte blades either slows or ceases, respectively. *Mazzaella splendens* therefore maximizes the production of reproductive surface area but minimizes structural support, whereas *M. linearis* has a greater degree of structural support much of which is formed before developing a reduced reproductive surface area.

These different developmental strategies, along with previous morphological and biomechanical studies of these species and their phases (Shaughnessy 1996, Shaughnessy et al. 1996), expand on what is known about how their form and function change as they develop. For *M. splendens*, short thalli of each phase have stipes with similar junction cross-sectional areas as well as similar stipe thicknesses and lengths, which results in similar junction (i.e. where the stipe meets the holdfast) break forces. As thalli lengthen, the junction cross-sectional areas and junction break forces for both phases also increase, but even so stipe development does not keep pace with the almost linear expansion of the blade. The phases of *M. splendens*

become morphologically different beyond 5.0 cm in length, when tetrasporophytes are narrower and have a lower planform area than gametophytes. Phases of *M. linearis* also start out with similar morphologies, but as thalli lengthen the stipes as well as the blades of the two phases diverge. Short thalli of each phase have similar thick long stipes, and junction break forces are already as great as those demonstrated by long thalli of this species. As *M. linearis* thalli lengthen, gametophyte stipes become thicker than those of tetrasporophytes, and the junction cross-sectional area and the junction break force of gametophytes are also greater than that of the tetrasporophytes. Gametophyte blades also become wider than those of the tetrasporophytes, but not until thalli are at least 15.0 cm in length. As a consequence of the morphological and biomechanical attributes of each species, transplants of holdfasts with intact blades of *M. splendens* survive better and can only reach lengths necessary for sexual reproduction in their sheltered home site, whereas the low surface area of even long intact transplants of *M. linearis* thalli allows them to survive and reproduce in its wave impacted habitat (Shaughnessy and De Wreede 2001).

The phenotypes of *M. splendens* and *M. linearis* and their phases could be the result of plasticity, but there are several methodological points to consider about common gardens before drawing conclusions from the results of the present experiment. First, the shapes of the allometric curves from the starting 1991 data set

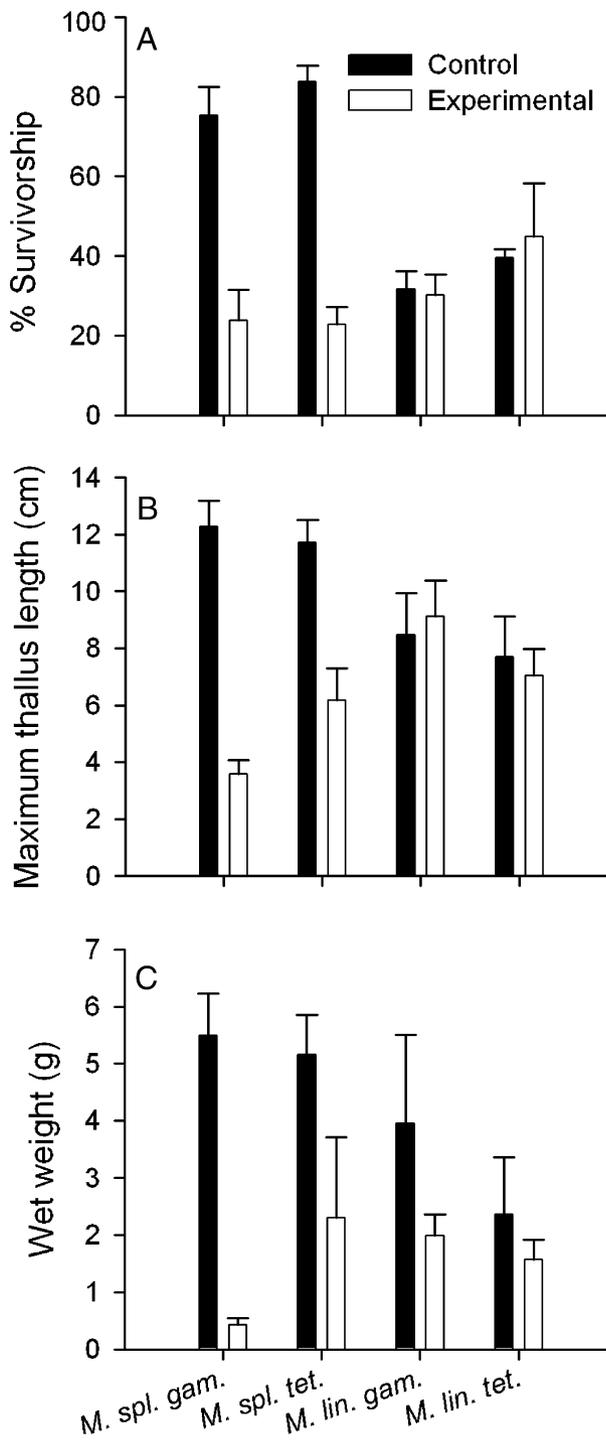


FIG. 4. The survivorship (A), maximum thallus length (B), and wet weight (C) of the gametophytes (gam.) and all tetrasporophytes (tet.) of *M. splendens* (*M. spl.*) and *M. linearis* (*M. lin.*) from control and experimental treatments at the completion of the common garden experiment in 1992. Error bars are SE of the mean.

are very similar to those of the control thalli at the end of the experiment (note difference in the *x*-axis scale in Figs. 2 and 3), indicating that the 1992 control and experimental curves could be compared with confi-

dence. Second, a comparison of blade shapes from nonremoved versus transplanted thalli was not attempted because it would have been confounded by genotype. Effects of initial holdfast removal on subsequent morphology were probably minimal because almost all the holdfast could be removed, and its polarity was maintained when placed into the rope. The similarity of the 1991 and control 1992 curves supports this conclusion. A third methodological consideration comes from the suggestion that the extent of plasticity demonstrated by a transplanted adult, as apposed to a spore or zygote, might be constrained by historical environmental signals (Chapman 1974, Russell 1978, Foster 1982). However, there are many seaweed examples where adult stages demonstrated blade plasticity (Sundene 1962, 1964, Druehl and Kemp 1982) or plasticity varied among characters within an adult thallus (Chapman 1974). The potential for plasticity to occur should depend more on the length of time relevant meristems are active in transplant environments rather than on whether young or adult stages are transplanted. It should be maladaptive for a perennial organism to have its phenotype set by an environmental signal received early in life (Bradshaw 1965, Schlichting and Pigliucci 1998, Morales et al. 2002). The hydrodynamic, light, nutrient, and herbivore environment will change over the lifetime of a single *Mazzaella* blade and potentially even more so over the lifetime of a holdfast. Historical constraints are also unlikely in the present study because not only were initial blades removed, but the blades harvested in June 1992 were likely the second cohort produced, because holdfasts put out May 1991 produced a set of blades, many of which should have been lost over the winter.

Given these methodological points, the reciprocal common garden experiment demonstrated either a complete lack of plasticity, as in the case of blade thickness, or stipe differences between the two species that might have converged did not do so because of maladaptive plasticity by blade characters. The lack of developmental coordination between the moderate amounts of plasticity displayed by these characters is an indication that the developmental systems of the two species have genetically diverged. More specifically, *M. splendens* could not thicken its blade in the high wave energy environment of *M. linearis*, and *M. linearis* could not make a thinner blade, thus improving its surface area-to-volume ratio in the low energy environment of *M. splendens*. Tetrasporophytes of *M. splendens* were capable of moderate increases in stipe length and thickness, whereas both phases of *M. linearis* eventually showed the capacity to decrease stipe dimensions. Both responses could be considered adaptive because they would, respectively, make stipes stronger and make resources available for other aspects of growth or reproduction. Although *M. splendens* shows some potential to produce the long thick stipe of *M. linearis*, this convergence by *M. splendens* is never realized in the high energy environment because widened

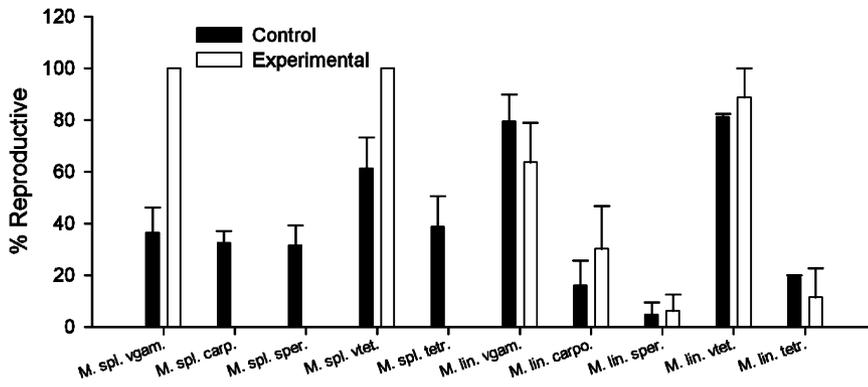


FIG. 5. The % of survivors of each phase and species that had at least one reproductive blade at the end of the experiment. Error bars are SE of the mean. M. spl., *M. splendens*; M. lin., *M. linearis*; vgam., vegetative gametophyte; carp., carposporangial; sper., spermatangial; vtet., vegetative tetrasporophyte; tetr., tetrasporangial.

blades resulted in blade breakage or removal of the entire upright thallus. *Mazzaella linearis*, despite its ability to decrease stipe dimensions at the wave sheltered site, did not show the capacity to make a wider blade, and tetrasporophytes made a narrower blade. The maladaptive plastic expansion of blades demonstrated by experimental thalli of *M. splendens* may have occurred in response to being in a more physiologically optimal habitat (e.g. less desiccation relative to control), whereas experimental thalli of *M. linearis* may have been more physiologically limited (e.g. more desiccation relative to control).

The fact that plasticity did not result in abrupt changes in phenotype suggests that the genetic mechanism for the plasticity in these taxa is likely to be allelic sensitivity rather than regulatory genes, and the former mechanism could have resulted in the inconsistent patterns of plasticity observed. For example, *M. splendens*, in which only the tetrasporophyte demonstrated moderate levels of stipe plasticity, may be more heterozygous than *M. linearis* for the genes involved in stipe development. In a situation where heterozygosity is low, both phases could have similar levels of plasticity because even though gametophytes and tetrasporophytes of these taxa are, respectively, haploid and diploid, they are also multinucleate and homologous cells of the two phases have the same amounts of DNA (Cole 1990, Goff and Coleman 1990). As a consequence, phases could be producing similar amounts of the same gene products. In contrast, the similar levels of blade width plasticity demonstrated by the *M. splendens* phases could be because it is less heterozygous than *M. linearis* for the genes involved in blade development. In red algae, the potential for plasticity may not be limited by the level of heterozygosity in a nucleus because genetic polymorphism at the level of the thallus could be raised by coalescence of sporelings with different genotypes to form a chimera (Maggs and Cheney 1990, Santelices et al. 1996, 1999, Santelices 1999). A field study of *Mazzaella laminarioides* (Bory) Fredericq suggested that a chimera only exists for this taxon in coalesced holdfasts and lower stipes but not the upright blades, whereas the chimeric organization does extend into the upright thalli of laboratory grown *Gracilaria chilensis* Bird, McLachlan

et Oliveira (Santelices et al. 1996, 2003). For *G. chilensis*, coalescence could therefore result in the functional equivalent of allelic sensitivity, thus increasing the potential for phenotypic plasticity in the upright portions of the thallus.

The lack of plasticity for some characters and the maladaptive plasticity of other characters resulted, at least for *M. splendens*, in lowered fitness. *Mazzaella splendens* regrown in the high energy habitat had low survivorship and a length too short for sexual reproduction. Survivorship was lowest for this treatment because hydrodynamic forces completely removed many thalli from the metal grid. Thalli of experimental *M. splendens* that did survive, although they were dark purple with active marginal cell divisions, had the shortest maximum thallus lengths and margins on the apical end of the blade were broken. This hydrodynamic cropping effect also resulted in the wide-bladed gametophytes of experimental *M. splendens* being significantly shorter than the narrow-bladed tetrasporophytes. Size limitation by wave-induced forces has also been demonstrated for a wide variety of seaweeds (Carrington 1990, Dudgeon and Johnson 1992, Gaylord et al. 1994, Blanchette 1997, Milligan and De Wree 2000, Blanchette et al. 2002, Duggins et al. 2003). In the absence of the ability to change blade shape, it might have been possible for *M. splendens* to produce reproductive structures by forming more blades per holdfast and reducing the thallus length necessary to produce these reproductive structures. Neither of these options occurred. The wet weight for experimental *M. splendens* thalli was also lower than for control thalli, and none of these thalli became reproductive in contrast to the carposporangial, spermatangial, and tetrasporangial thalli present in its natural control site.

In contrast to *M. splendens*, *M. linearis* had similar levels of survivorship between control and experimental treatments. Even though the probability of mortality from hydrodynamic forces in a low wave energy site is reduced for *M. linearis* (Shaughnessy et al. 1996), it has been hypothesized that this is offset by novel sources of mortality such as desiccation (Shaughnessy and De Wree 2001). The low wave energy thalli of *M. linearis* reached a weight and maximum length

similar to those in the control site, and the lengths were sufficient for all possible reproductive categories to occur. This reproductive response contradicts an earlier finding by Shaughnessy and DeWreede (2001), who used the same reciprocal common garden design but with intact blades on holdfasts and found that almost none of the experimental *M. linearis* thalli (new blades) harvested after 17 months had reproductive structures. The present 13-month study where all blades were initially removed had similar levels of reproductive thalli between control and experimental treatments. Developmental systems responsible for sexual reproduction in this species of *Mazzaella* may be less flexible in reacting to environmental signals than systems responsible for vegetative growth.

This study in combination with others continues to support the hypothesis that *M. splendens* and *M. linearis* are distinct species. Multiple individuals of each reputed species form sister clades when either the plastid encoded *rbcL* gene or the internal transcribed spacer unit of the ribosomal cistron are used (Hommersand et al. 1994, 1999, Hughey et al. 2001, Ross et al. 2003), there are differences in vegetative and reproductive morphology (Abbott 1971, Kim 1976, Hommersand et al. 1993, Shaughnessy 1996), they have different allometric patterns when developing stipes and blades, and they occur at different positions along a gradient of wave energy (Shaughnessy 1996). Shaughnessy (1996) and Ross et al. (2003) recommend, respectively, that field and herbarium identification of these taxa be made more on the basis of stipe differences between the species because blade width variation demonstrates a continuous cline, and phases differ for this character as well. Based on this common garden experiment, stipe characters are still more useful because although stipes of *M. splendens* tetrasporophytes can become slightly longer and thicker, a convergence in stipe morphology between the two species is not realized in Barkley Sound because the inability of *M. splendens* to narrow its blade results in the death of these thalli by hydrodynamic removal. Stipe dimensions of *M. linearis* can decrease, but they are still greater than those of *M. splendens*, and even if they did converge completely, the *M. linearis* blade would still be distinctive because it can only form a very narrow morphology. The present study did not transplant the intermediate form of *M. splendens*, which in Barkley Sound has the same short thin stipe as the wide-bladed form but a blade of intermediate width. Although the intermediate form occurs midway along the wave energy gradient, stipe plasticity may not occur because blade surface area has been reduced. Further work may show that this intermediate form of *M. splendens* is an ecotype, because morphological and sequence data reject the hypothesis that this intermediate form in Barkley Sound, British Columbia is a hybrid (Shaughnessy 1996, Ross et al. 2003).

Mazzaella splendens and *M. linearis* have different developmental strategies for producing their respective stipe and blade phenotypes, and in Barkley Sound the

latter are due to genetic differentiation rather than phenotypic plasticity. The moderate levels of plasticity demonstrated by some characters does not result in phenotypes converging in common gardens either because the developmental response is in the wrong direction, or it is maladaptive and so thalli die before convergence is realized. Results of this study also continue to support the usefulness of stipe characters in delineating *M. splendens* and *M. linearis*.

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