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CONTRIBUTION OF TEMPORAL AND SPATIAL COMPONENTS TO MORPHOLOGICAL VARIATION IN THE KELP *ECKLONIA* (LAMINARIALES)[1]

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Environmental conditions that are known to cause morphological variation in algae (e.g., wave exposure) often vary in both space and time and are superimposed onto the distinct seasonal growth cycles of most temperate macroalgae. We tested the hypothesis that the morphology of the small kelp *Ecklonia radiata* (C. Agardh) J. Agardh is the product of an interaction between site (five reefs of different wave exposure) and the time of year that sampling occurs (summer vs. winter 2004). We determined that wave exposure had a strong directional effect on kelp morphology, with "Reefs" accounting for up to 43.4% of variation in individual morphological characters. "Times" had a narrowly nonsignificant effect on overall morphology but accounted for up to 31% of variation in individual characters. Many characters were affected by wave exposure, whereas only a few were (strongly) affected by time (e.g., thallus biomass). Interactive effects between "Reefs" and "Times" were generally small, accounting for 15.8% of variation in lamina thickness, but much less for most other characters. We conclude that wave exposure exerts a strong control over the morphology of *E. radiata*, but that the nature of the effect depends on the magnitude of wave exposure. We also conclude that most of the effects of wave exposure are consistent through time and do not interact with cycles of growth and pruning in any major way.

Key index words: **Australia; *Ecklonia radiata*; kelp; morphology; temporal and spatial variation**

Many macroalgae exhibit great intraspecies morphological variation, and this has important physiological and ecological implications for the algae themselves and for other organisms living in their proximity (Gerard and Mann 1979, Velimirov and Griffiths 1979, Blanchette et al. 2002, Fowler-Walker et al. 2005b). Morphological variation is generally believed to increase the fitness and survival of algae (Friedland and Denny 1995, Blanchette et al. 2002, Duggins et al. 2003, Roberson and Coyer 2004). Broadly, four classes of morphological variation, can be distinguished: (i) ontogenetic variation, (ii) genotypic variation, (iii) phenotypic variation, and (iv) imposed morphological variation. Ontogenetic and genotypic variation are autogenic and reflect the genetic makeup of the algae, either as a genetically predetermined progression through morphologically dissimilar life stages (Kirkman 1981, Chapman 1986) or as the expression of fixed characters that have been selected for over generations (Serisawa et al. 2003, Roberson and Coyer 2004). In contrast, phenotypic and imposed variations are exogenic, respectively originating as a response of the alga to environmental cues (e.g., light: Hymanson et al. 1990) and as a condition imposed on the alga by its surroundings (e.g., grazing: Kalvas and Kautsky 1993; tattering: Blanchette 1997). These causes of morphological variation are not necessarily independent, and in reality, they are hard to separate because phenotypic plasticity, or the susceptibility to environmental pressures, may depend on the life stage or the genotype of the alga. Moreover, some environmental pressures may cause morphological responses through different processes across these broad classes of variation: wave exposure, for example, can promote certain genotypes by causing selection for specific hydrodynamically advantageous blade morphologies (Roberson and Coyer 2004), or it can impose morphological differences by causing shifts in age structure (Kalvas and Kautsky 1993) or tattering (Blanchette 1997). Clearly, the underlying nature of morphological variation is not always straightforward (see Wernberg and Thomsen 2005, Matson and Edwards 2006 for examples of complexity).

Complicating matters, many of the environmental conditions, processes, and biological interactions that drive morphological differentiation vary in time as well as in space. A prime example is wave exposure; in most temperate coastal systems, where large macroalgae dominate, a seasonal cycle with increased wave activity in winter (Lemm et al. 1999) is superimposed on spatial gradients caused by varia-

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tion in coastal topography (depth, headlands, reefs, etc.) (Graham et al. 1997, Fowler-Walker et al. 2006). At the same time, many algae including kelps and fucoids have distinct seasonal growth patterns (Kirkman 1989, Wernberg et al. 2001, Tala and Edding 2005). Previous studies of macroalgae (op. cit.) have almost entirely focused on how processes that vary in space cause morphological variation, with little attention to the influence of temporal variability. Yet, the concatenation of spatial and temporal variation in factors that are known to cause morphological variation (wave exposure in particular) and growth patterns, where algae build up or shed thallus tissue, present a strong possibility for spatial and temporal interactions.

Understanding how spatial and temporal factors interact to affect the morphology of canopy-forming macroalgae is important because morphology is a key feature in defining the ecological function of macroalgae as foundation species on reefs. Two of the main ways in which macroalgae influence reef-associated biota are by providing habitat, physical three-dimensional structure (Wernberg et al. 2004, Tuya et al. 2008), and by modifying local environmental conditions (light, sediments, scour, Kennelly 1989, Wernberg et al. 2005). Both of these functions depend on the morphology of the algae, and temporal variation in both has been associated with effects on benthic and phytal communities (Gagnon et al. 2003, Wernberg et al. 2004).

The main research question we examine in this study is whether spatial variation in morphology is consistent in time. We addressed this question by testing for an interaction between site (reefs of different wave exposure) and the time of year that sampling occurs (summer vs. winter 2004, contrasting seasons in temperate environments). In testing this hypothesis, we detail the nature of spatial and temporal variation in individual morphological characters of the kelp *E. radiata*, and we estimate the magnitude of temporal variation relative to spatial variation across a gradient in exposure to oceanic swell.

E. radiata is a small kelp that dominates many temperate reefs in Australia and New Zealand (Wernberg et al. 2003b). Morphological differences among populations across >4,000 km of coastline between Kalbarri in Western Australia and Adelaide in South Australia are relatively small compared to variation among populations on reefs <1 km apart (Wernberg et al. 2003a, Fowler-Walker et al. 2005a). Differences in wave exposure is one of the most likely contributors to this small-scale variation (Wernberg and Thomsen 2005, Fowler-Walker et al. 2006), but other sources that also vary across similar spatial scales, such as canopy composition (monospecific *E. radiata* vs. *E. radiata* mixed with fucoids, Fowler-Walker et al. 2005a) and the spatial arrangement of individuals within the canopy (solitary vs. aggregated, Wernberg 2005), also affect the morphology of *E. radiata*. Moreover, *E. radiata* has a clear seasonal growth pattern with high growth rates and increasing biomass in spring (September–October) to a summer biomass maximum (December–January), followed by biomass loss over autumn (April–May) to a winter low (June–August) (Kirkman 1989, Wernberg and Goldberg 2008). Collectively, these factors imply a primary importance of environmental drivers in determining the morphology of *E. radiata* and therefore also a potential for complex temporal effects as the growth cycle interacts with seasonal cycles in environmental conditions such as wave exposure.

MATERIALS AND METHODS

Our study included five limestone reefs 8–13 km off the coast of Fremantle on the west coast of Western Australia (Table 1). These reefs are structurally complex ridges (vertical relief >2 m) varying in depth from 6 to 12 m. Kelps are abundant on most of these reefs, and kelp density is not related to the wave-exposure gradient (Wernberg 2009). Kelps were collected haphazardly on each reef. To avoid confounding spatial and temporal effects by ontogenetic variation during the juvenile sporophyte stages (life stages 1 and 2, Kirkman 1981), we only sampled grown kelp thalli that were fully differentiated with third-order laterals (life stage 3, Kirkman 1981). Once the kelp reaches this stage, the morphology no longer changes ontogenetically but is influenced by the seasonal growth and erosion of the thallus. We collected 10 separate kelp thalli from each reef three times in the austral summer 2004 (December 2003 and January and February 2004) and three times in winter 2004 (June, August, and September), with each sampling date >1 month apart. The kelps were stored frozen (−18°C) until processing, where 14 morphological characters (cf. Fig. 1, Table 2) were measured on each thallus. These methods and morphological characters are described in previous papers (Wernberg et al. 2003a, Wernberg 2005, Wernberg and Thomsen 2005).

The wave exposure of each reef was calculated as Baardseth's index (Baardseth 1970), modified to suit an environment with multiple submerged reefs. This index takes into account wave influences from multiple directions, such as swell and diurnally shifting sea and land breezes. Moreover, Baardseth's index has

TABLE 1. Geocoordinates and wave exposure of the five reefs sampled. Wave exposure was calculated from marine charts as a modified Baardseth's index.

	Mewstone Rock	Roarers	Straggler Rocks	Seaward Reef	Casuarina Shoals
GPS coordinates	S32°05.101 E115°39.420	S32°06.231 E115°38.789	S32°03.972 E115°38.549	S32°07.044 E115°36.215	S32°08.971 E115°36.243
Wave exposure	58	72	77	98	110

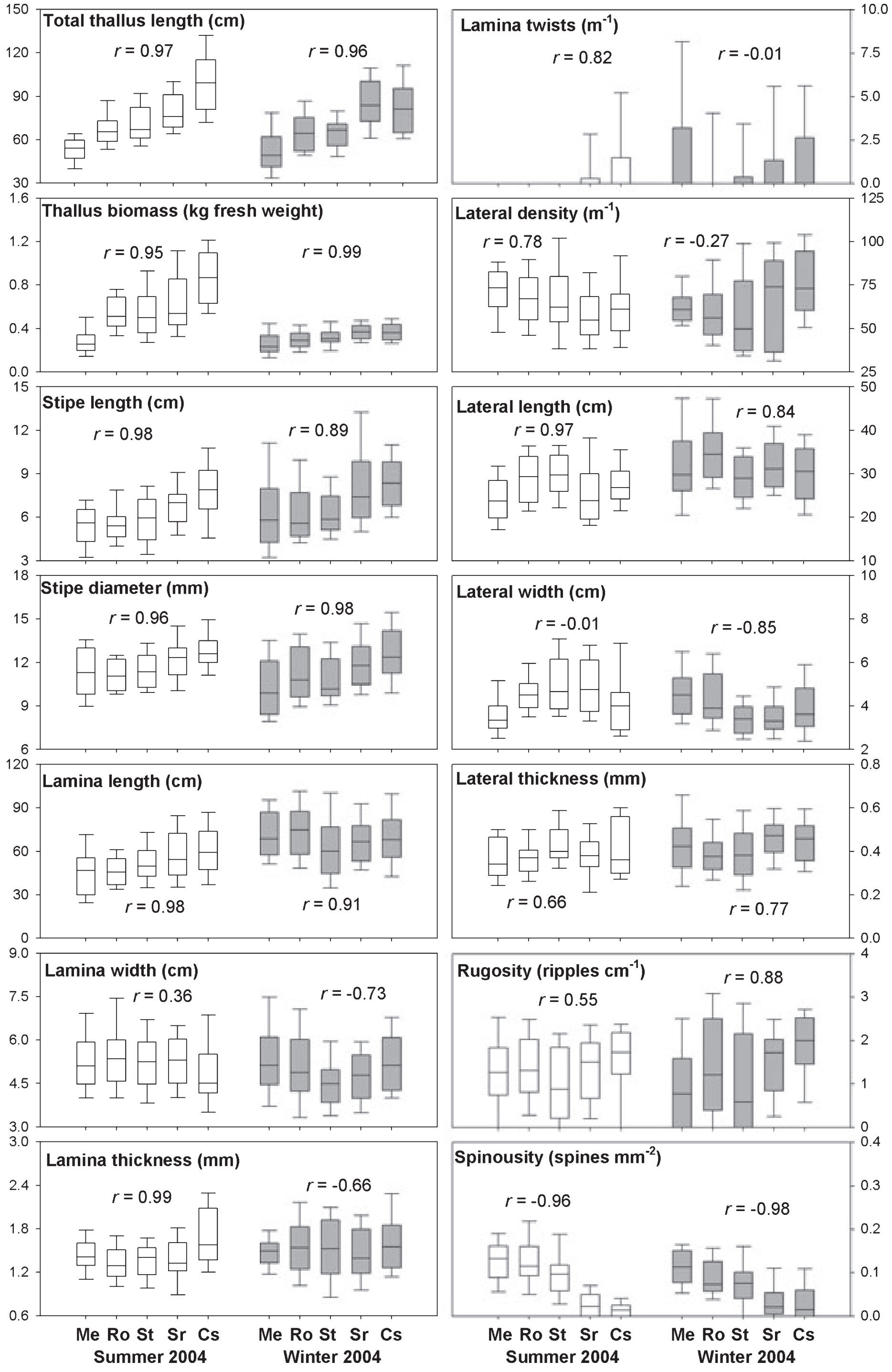

FIG. 1. Box plot showing the variation in morphological characters of *Ecklonia radiata* at each of the five reefs sampled (in order of increasing wave exposure) in summer 2004 (white boxes) and winter 2004 (gray boxes). Data were pooled across sample dates within times for each reef; boxes represent $n = 30$ kelp individuals, the line is the median value, and whiskers are 5th/95th percentiles. The *r*-values are product moment correlation coefficients between the mean value or each character and the rank wave exposure (cf. Table 1). Me, Mewstone Rock; St, Straggler Rocks; Ro, Roarers; Sr, Seaward Reef; Cs, Casuarina Shoals. Statistical analyses of individual morphological characters can be found in Table 4.

previously been used for studies on similar reefs, where it was also demonstrated to agree well with measurements of wave height and maximum water velocity (Wernberg and Thomsen 2005, Fowler-Walker et al. 2006). The surroundings of each reef were divided into 40 sectors of 9°, and a fetch of 7.5 km examined on a 1:75,000 nautical chart (WA001: Ocean Reef to Cape Peron, Maritime Cartographical Services, Perth). Each sector was weighted according to the shallowest obstacle to the fetch within that sector (emergent reefs = 0; 0–2 m = 1; 2–5 m = 2; 5–10 m = 3; >10 m = 4), and the values were summed. The resulting index ranged between 0 for a completely protected site, surrounded by emergent reefs and 160 for a completely exposed site with no protection.

All statistical analyses of morphological variables were performed using PRIMER 6 with PERMANOVA+. The routines mentioned refer to this program and are described in Clarke and Gorley (2006) and Anderson et al. (2008). Data for all morphological variables were first normalized to convert all measures to a commensurable scale. Subsequently, differences among individual kelps were calculated as Euclidian distances,

TABLE 2. PERMANOVA testing for morphological differences among kelps, based on 14 morphological characters. Data were normalized, and dissimilarities calculated as Euclidian distances. *P*-values were calculated from 999 permutations of the residuals under the reduced model (Anderson et al. 2008). The effect size was calculated as the relative contribution of each factor to the components of variation.

Source of variation	df	MS	Pseudo-*F*	*P*(perm)	Effect size (%)	Unique permutations
Reef	4	150.99	7.5	0.001	14.1	999
Time	1	222.99	1.9	0.068	4.6	999
Reef × Time	4	27.26	1.4	0.092	13.9	998
Date (Time)	4	117.02	12.8	0.001	1.5	997
Reef × Date (Time)	16	20.13	2.2	0.001	7.1	993
Residual	270	8.11			58.8	

PERMANOVA, analysis of variance by permutation.

and the multivariate patterns illustrated by metric ordination (PCO). Analysis of variance by permutation (PERMANOVA) tested effects on multivariate patterns and individual morphological characters in accordance with a three-factor mixed model ANOVA design with the orthogonal factors Reef (fixed, five levels) and Time (fixed, two levels), and sampling date nested within time (random, three levels). For each factor in the experimental design, effect sizes were determined as the percentage of ''components of variation,'' a term that includes both variation due to fixed and random effects (Anderson et al. 2008, see also Graham and Edwards 2001). Significance levels were calculated from 999 permutations of the residuals under the reduced model. Reef, the only significant fixed factor in the multivariate analysis, was tested for differences in multivariate dispersion (PERMDISP), and post hoc pair-wise comparisons were used to distinguish which reefs differed from one another ($P < 0.05$). Correlation analyses were used to relate the wave exposure of each site to the degree of multivariate morphological variation (RELATE) and variation in each individual character (product moment correlation on mean values, $n = 5$ reefs). SIMPER analyses tested the relative contribution of each morphological character to the separation of reef groups along the gradient in wave exposure.

RESULTS

The overall morphology of *E. radiata* showed complex variation across most spatiotemporal scales considered in this study. Unexplained variation among replicate individuals from each reef at each sampling date (=residual) accounted for 58.8% of the total multivariate morphological variation, and variation associated with the sampling date [i.e., ''Date (Time)'' and ''Reef × Date (Time)''] an additional 8.6% (Table 2). Nevertheless, above this random variation, there was significant variation among reefs, but not between times (Fig. 1, Table 2). The *P*-values for effects of ''Time'' and ''Reef × Time'' were, however, small [*P*(perm) <0.092], and together these two fixed factors accounted for 18.8% of total multivariate variation, corresponding to ~60% of what was explained by all fixed factors (i.e., ''Reef,'' ''Time,'' ''Reef × Time''; 32.6% of total variation). Multivariate dispersion was equal at all reefs except Casuarina Shoals (Mewstone Rock = Straggler Rocks = Roarers = Seaward Reef <Casuarina), so with the potential exception of this reef, differences detected in the PERMANOVA were due to differences in location of centroids (i.e., ''mean overall morphology'') rather than differences in the degree of variability among individual kelps). Post hoc pair-wise comparisons among reefs revealed three groups of centroids (Mewstone Rock ≠ Roarers = Straggler Rocks ≠ Seaward Reef = Casuarina Shoals). SIMPER analyses showed a relatively even contribution of ~5% (range: 2.8%–10.5%) of all morphological characters to the differences between reef groups, with no single character standing out as particularly important in any comparison (Table 3). There was a very strong correlation between multivariate differences in morphology and differences in wave-exposure rank of reefs [RELATE: $\rho = 0.909$, *P*(perm) = 0.016]. The strong multivariate correlation with wave exposure, and the near-significant effect of season, was evident in the ordination as a clear unidirectional and sequential separation of reef centroids according to wave exposure along PCO1, and a separation of times along PCO2 (Fig. 2). The pattern of sequential change across reef groups was virtually parallel at the two times, although differences within the reef groups ''Straggler Rocks–Roarers'' and ''Seaward Reef–Casuarina Shoals'' were markedly greater in summer than in winter 2004. Most individual characters correlated strongly with wave exposure ($|r| > 0.55$ for 24 of 28 correlations; Fig. 1) and the direction of correlations was mostly consistent for each character at each time (10 of 14 characters). Analyses of individual morphological characters also showed very large variation associated with the random factors at the lowest levels of analysis for most characters (Table 4), with the fixed factor effects accounting for a maximum of ~60% of the variation for thallus biomass and considerably less for most other characters (Fig. 3). Variation due to ''Reef'' contributed most to the variation of fixed effects in 50.0% of the characters, ''Time'' in 35.7%, and ''Reef × Time'' in 14.3% of the characters.

DISCUSSION

Exposure-effect relations. The morphology of the small kelp *E. radiata* varied considerably between discrete reefs and correlated strongly ($\rho = 0.91$) with the magnitude of wave exposure. Morphological

Table 3. SIMPER analyses showing the relative contribution (%) of each morphological character to multivariate differences between reef groups.

Rank	Mewstone Rock vs. Roarers and Straggler Rocks (ASD = 22.38)	Mewstone Rock vs. Seaward Reef and Casuarina Shoals (ASD = 36.40)	Roarers and Straggler Rocks vs. Seaward Reef and Casuarina Shoals (ASD = 27.99)	Summer vs. Winter (ASD = 26.47)
1	Rugosity (9.4%)	Thallus length (10.5%)	Spinousity (8.8%)	Lateral width (9.9%)
2	Lateral thickness (9.2%)	Lamina length (9.2%)	Lamina thickness (8.4%)	Thallus biomass (9.3%)
3	Lamina twists (8.8%)	Spinousity (8.9%)	Thallus length (7.8%)	Lamina thickness (8.2%)
4	Lamina width (8.7%)	Lateral length (8.6%)	Lamina length (7.7%)	Lamina width (8.0%)
5	Stipe diameter (8.38%)	Thallus biomass (7.7%)	Stipe length (7.4%)	Lateral density (7.94%)
6	Lateral density (7.6%)	Stipe diameter (7.6%)	Rugosity (7.3%)	Lateral thickness (7.3%)
7	Spinousity (7.2%)	Lamina twists (7.6%)	Lateral density (7.2%)	Rugosity (7.3%)
8	Stipe length (7.0%)	Stipe length (7.5%)	Lateral length (7.2%)	Lamina twists (7.0%)
9	Lateral width (7.0%)	Lamina thickness (7.3%)	Lamina width (7.2%)	Stipe length (6.6%)
10	Lateral length (6.8%)	Lateral thickness (5.9%)	Stipe diameter (7.1%)	Lateral length (6.5%)
11	Lamina length (6.02%)	Lateral density (5.7%)	Lateral thickness (6.6%)	Stipe diameter (6.4%)
12	Thallus biomass (5.6%)	Rugosity (5.5%)	Lamina twists (6.3%)	Lamina length (5.9%)
13	Thallus length (5.6%)	Lamina width (4.8%)	Lateral width (5.7%)	Thallus length (5.0%)
14	Lamina thickness (2.8%)	Lateral width (3.2%)	Thallus biomass (5.2%)	Spinousity (4.8%)

ASD, average squared distance.

differences between summer and winter 2004 kelps were less pronounced for both multivariate morphology (4.6% of variation) and most individual morphological characters (0%–31% of variation). Interactions between variation due to Reef and Time were relatively small (0%–15.8% of variation), suggesting that temporal effects were mostly consistent across reefs of different wave exposure.

We found a strong progressive change in morphology with increasing wave exposure, and this is not surprising given the rife literature documenting effects of wave exposure on macroalgal morphology (Back 1993, Sjøtun and Fredriksen 1995, Roberson and Coyer 2004, Fowler-Walker et al. 2006). Previous studies have also found wave exposure to affect the morphology of *E. radiata* (Wernberg and Thomsen 2005, Fowler-Walker et al. 2006), although for some morphological characters (e.g., thallus biomass), the direction of effects were opposite between studies. When contrasting morphologies between ''exposed'' and ''sheltered'' sites across widely separated areas (10s–100s km), Wernberg and Thomsen (2005) observed large inconsistencies in what morphological characters were driving overall differences. The present study reconciles these discordant findings by demonstrating that the importance (rank order of contribution to overall morphological difference) of individual morphological characters differed depending on the magnitudes of ''sheltered'' and ''exposed'' conditions. In other words, the assumption that the response of morphology to wave exposure is monotonic across all characters is invalid, and the relative magnitude of difference may dictate the direction of response (see discussion in Wernberg and Thomsen 2005).

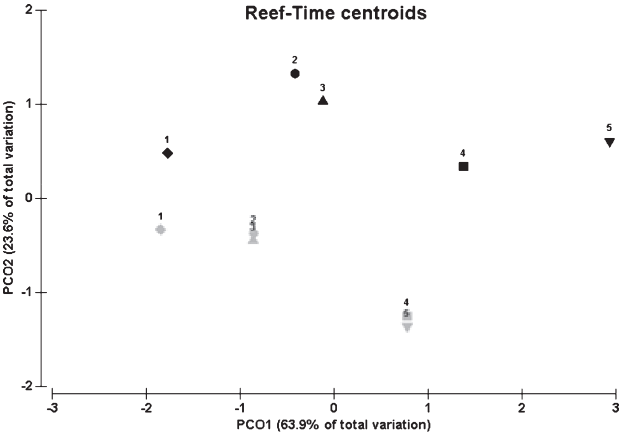

Fig. 2. Ordination (PCO) of Reef-Season centroids. Summer 2004 (black) and winter 2004 (gray). Mewstone Rocks (◇), Roarers (○), Straggler Rocks (△), Seaward Reef (□), and Casuarina Shoal (▽). Numbers indicate the rank exposure of each reef.

Responses of individual morphological characters. In accordance with the findings of Wernberg and Thomsen (2005), characters associated with overall size increased with increasing wave exposure (e.g., thallus length, biomass, stipe length). Similar effects have also been seen in other kelps (Sjøtun et al. 1998). The increase in length-related characters is potentially a biomechanical adaptation to ''go with the flow'' in wave-exposed environments where a long flexible thallus will follow the orbital water flow, only being fully extended toward the end of the wave cycle, thus reducing the absolute force on the thallus from the surge (Koehl 1986). It is also possible that there is a positive scaling effect of growing large; both attachment force and drag increase with increasing size (Thomsen and Wernberg 2005), but the net effect may be positive because laterals rearrange in flow, compacting the thallus and reducing the effective area subjected to drag (Armstrong 1989, Wernberg 2005).

Strength-related characters (particularly stipe diameter) were found to increase with increasing

TABLE 4. PERMANOVA testing for differences in individual morphological characters among Reef (fixed factor), Time (fixed factor), and Date (Time) (random factor). Each variable was transformed to minimize heterogeneity of variances (lowest possible Cochran's *C*). Analyses were based on Euclidian distances. *P*-values were calculated from 999 permutations of the residuals under the reduced model (Anderson et al. 2008). The effect size was calculated as the relative contribution of each factor to the components of variation. Negative components of variation were set to 0 (Graham and Edwards 2001).

Source of variation	df	MS	Pseudo-*F*	*P*(perm)	Effect size	MS	Pseudo-*F*	*P*(perm)	Effect size
		Thallus length				Thallus biomass			
Transformation and Cochran's *C*		None; $C = 0.1062$*				LN(x); $C = 0.0890$ ns			
Reef	4	12,550	22.9	0.001	40.1	4.9266	24.7	0.001	21.7
Time	1	1,226.1	0.5	0.536	0.0	18.118	14.2	0.021	31.0
Reef × Time	4	1,018.3	1.9	0.165	3.1	1.0997	5.5	0.01	8.3
Date (Time)	4	2,426.5	11.9	0.001	8.9	1.2721	11.7	0.001	6.4
Reef × Date (Time)	16	548.7	2.7	0.001	6.9	0.19942	1.8	0.029	2.5
Residual	270	204.53			41.0	0.10888			30.1
		Stipe length				Stipe diameter			
Transformation and Cochran's *C*		LN(x); $C = 0.0914$ ns				None; $C = 0.0733$ ns			
Reef	4	1.5821	8.2009	0.001	15.7	32.452	5.3821	0.009	12.3
Time	1	1.079	1.1544	0.348	0.7	6.2179	0.4692	0.555	0.0
Reef× Time	4	0.0161	0.0832	0.985	0.0	2.0722	0.3437	0.843	0.0
Date (Time)	4	0.93475	9.6659	0.001	11.4	13.253	5.1473	0.001	6.0
Reef × Date (Time)	16	0.19292	1.9949	0.018	6.5	6.0296	2.3419	0.004	9.7
Residual	270	0.0967			65.7	2.5747			72.0
		Lamina length				Lamina width			
Transformation and Cochran's *C*		None; $C = 0.0832$ ns				LN(x); $C = 0.0914$ ns			
Reef	4	8,898.3	14.042	0.001	29.2	0.12429	1.8059	0.187	1.7
Time	1	1,999.5	0.75968	0.444	0.0	0.63131	10.625	0.041	6.9
Reef × Time	4	1,065.7	1.6817	0.221	3.0	0.13502	1.9618	0.139	4.0
Date (Time)	4	2,632	11.335	0.001	10.2	0.0594	1.3107	0.263	0.5
Reef × Date (Time)	16	633.71	2.7292	0.001	8.5	0.0688	1.5182	0.09	4.3
Residual	270	232.2			49.1	0.0453			82.6
		Lamina thickness				Lamina twists			
Transformation and Cochran's *C*		None; $C = 0.0972$*				LN($x + 1$); $C = 0.1156$**			
Reef	4	0.30089	1.2773	0.335	0.6	0.0011	3.5792	0.035	3.3
Time	1	0.50348	0.23263	0.635	0.0	0.0027	5.5904	0.068	3.8
Reef × Time	4	1.0511	4.462	0.009	15.8	0.0003	0.9905	0.461	0.0
Date (Time)	4	2.1643	24.608	0.001	24.1	0.000	1.3355	0.251	0.6
Reef × Date (Time)	16	0.23558	2.6785	0.001	8.6	0.0003	0.8399	0.649	0.0
Residual	270	0.080			51.0	0.0004			92.3
		Lateral density				Lateral length			
Transformation and Cochran's *C*		SQRT($x + 1$); $C = 0.0875$ ns				LN(x); $C = 0.0813$ ns			
Reef	4	0.46592	0.15766	0.955	0.0	1.0245	5.6018	0.005	20.5
Time	1	12.998	0.29242	0.618	0.0	0.13889	0.66368	0.471	0.0
Reef × Time	4	3.6333	1.2294	0.337	1.2	0.0714	0.3905	0.815	0.0
Date (Time)	4	44.448	52.538	0.001	44.7	0.20927	5.7922	0.001	5.1
Reef × Date (Time)	16	2.9553	3.4932	0.001	10.8	0.18289	5.062	0.001	21.5
Residual	270	0.84602			43.4	0.0361			52.9
		Lateral width				Lateral thickness			
Transformation and Cochran's *C*		LN(x); $C = 0.0722$ ns				LN($x + 1$); $C = 0.1133$**			
Reef	4	12,550	22.872	0.001	0.5	0.0346	2.1089	0.111	3.5
Time	1	1,226.1	0.5053	0.536	35.7	0.17386	2.0376	0.208	6.9
Reef × Time	4	1,018.3	1.8559	0.165	0.0	0.00161	0.0981	0.976	0.0
Date (Time)	4	2,426.5	11.864	0.001	18.2	0.0853	17.219	0.001	18.7
Reef × Date (Time)	16	548.7	2.6828	0.001	3.7	0.01642	3.3148	0.001	13.3
Residual	270	204.53			41.9	0.00496			57.6
		Rugosity				Spinousity			
Transformation and Cochran's *C*		None; $C = 0.0843$ ns				None; $C = 0.0766$ ns			
Reef	4	4.8005	2.5117	0.081	4.6	0.11048	51.118	0.001	43.4
Time	1	0.1373	0.00718	0.949	0.0	0.00606	0.3963	0.526	0.0
Reef × Time	4	0.89299	0.46724	0.771	0.0	0.00557	2.577	0.084	2.7
Date (Time)	4	19.125	38.76	0.001	35.3	0.01529	7.8434	0.001	6.4
Reef × Date (Time)	16	1.9112	3.8733	0.001	13.4	0.00216	1.109	0.338	0.5
Residual	270	0.49343			46.7	0.00195			46.9

PERMANOVA, analysis of variance by permutation. ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$.

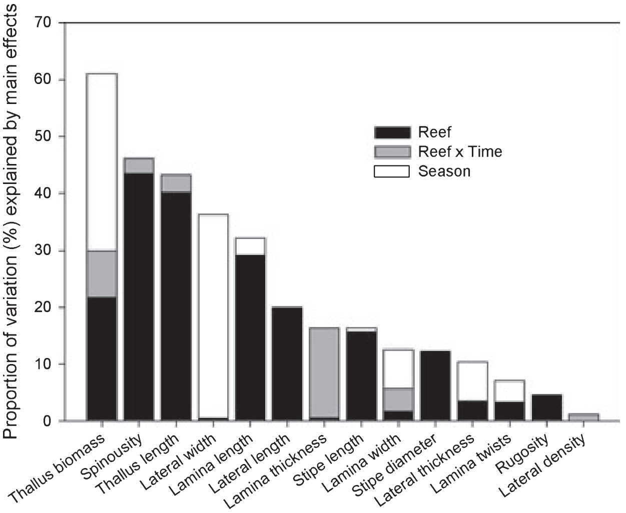

FIG. 3. Proportions of variation explained by the fixed factor effects for all measured morphological characters. Full analysis (ANOVA) tables can be found in Table 4.

wave exposure, whereas drag-increasing characters (e.g., spinousity) decreased. Previous studies of *E. radiata* (Wernberg and Thomsen 2005, Fowler-Walker et al. 2006), other small understory kelps (Duggins et al. 2003), and red algae (Jackelman and Bolton 1990) have reported similar effects across a suite of morphological characters with either strength-related (e.g., holdfast size, tissue toughness) or drag-related (e.g., blade width) properties, indicating the ubiquity of the pattern.

Spinousity is an interesting morphological character because it was the defining character for the previously recognized entity *E. radiata* forma *biruncinata* (Bolton and Anderson 1994). Spinousity decreased with increasing wave exposure, a pattern that has also been found for a *Gigartina* species in South Africa (Jackelman and Bolton 1990). The ecological significance of the densely packed surface spines remains unknown. Wernberg et al. (2003a) speculated that it could be a protection against herbivores. Incidentally, sheltered reefs also have the highest abundances of sea urchins (Vanderklift and Wernberg 2008), but spinousity cannot be a response to urchin grazing because these urchins are passive drift feeders (Vanderklift and Kendrick 2005) that consume spinous and smooth kelps at equal rates (Vanderklift and Wernberg 2008). Although sporadic in the region, a recent study suggests that fish grazing might be higher on inshore sheltered reefs compared to offshore exposed reefs (Vanderklift et al. 2009). Similarly, sheltered reefs generally have more small herbivores than exposed reefs (e.g., meso-gastropods <2 cm, Tuya et al. 2008). Herbivory from both fish and small invertebrates could be promoting this character, but experimental tests are required to establish this conclusively. That spinousity serves to break down the boundary layer and create turbulent flow over the blade during periods of low water movement to increase nutrient uptake and waste removal is a less likely alternative because, in our experience, the water never really is quiescent at any of these reefs.

Thallus twists only occurred at the two most exposed reefs in summer 2004 and at most reefs in winter 2004 (season of high wave activity), strongly suggesting that this morphological character is a short-term (i.e., associated with seasonal growth) phenotypic response to wave activity. Presumably, the twisted thallus acts like a spring, slowly twisting and extending the thallus as the drag increases over the wave cycle, thus abating transfer of force from the water to the holdfast. Presumably, thalli then ''straighten'' again toward the following summer through combined erosion and shedding as the wave climate becomes increasingly benign.

Temporal effects. It is well established that the overall size and appearance of *E. radiata* undergo seasonal change (Kirkman 1989). This was also evident in our data by the fact that effects associated with temporal variation (summer 2004 vs. winter 2004) contributed ~20% of variation to overall multivariate morphology and significant variation in several individual morphological variables; however, it was also clear that these differences were driven largely by strong variation in a few characters such as thallus biomass.

The strong temporal response of thallus biomass likely reflects seasonal variation in growth and pruning rates (Kirkman 1989, Tala and Edding 2005). The general lack of significant interactions suggests that these temporal cycles do not influence effects of wave exposure in any major way. Nevertheless, the significant ''Reef × Time'' interaction for biomass, and the relatively small *P*-value and large effect size for the multivariate ''Reef × Time'' interaction, indicates that kelp morphology responds more strongly to wave exposure in summer, when the thallus is fully developed, than in winter, when the thallus is reduced (cf. Fig. 2: separation of ''SR-CS'' and ''R-ST'' in summer but not in winter 2004). The interpretation of this could be that imposed changes, such as pruning of laterals during high wave activity, overpower other sources of variation to make morphologies converge in winter, whereas morphologies diverge in spring and summer when growth allows geno- or phenotypic expressions to manifest. Some caution is, however, warranted when interpreting the generality of these ''seasonal'' effects because we surveyed each season in a single year only, implying that our findings are specific to that year (i.e., 2004). Variation among years, for example, in storminess during winter, might introduce interannual variation in seasonal patterns.

There was considerable random, short-term temporal variation, as demonstrated by the ''Date (Time)'' being significant for multivariate morphology and for most individual morphological characters. This finding perhaps is suggestive of a response to discrete short-term events such as peak wind waves. In contrast to swells, wind waves can originate from all directions depending on prevailing wind directions and thus cause exposure events that are seemingly incongruent to an average exposure classification such as Baardseth's index. The particularly large effect size of ''Date (Time)'' for lateral density (44.7%) suggests that pruning (an imposed effect), where laterals are torn off the main lamina (only 10.2% of variation), is a key process behind this random morphological variation.

The nature of morphological variation in E. radiata. *E. radiata* predominantly occurs in environments where grown thalli routinely experience water velocities and drag forces that match or exceed attachment strength (Thomsen et al. 2004). Morphological changes to reduce drag are therefore critical to persistence in wave-exposed environments; dislodgment is usually fatal to kelps, and, presumably, these environments would exert a strong selective pressure toward exposed (or sheltered) genotypes. To this end, Roberson and Coyer (2004) found genetically, morphologically, and ecologically distinct genotypes of *Eisenia arborea*, a genetically and morphologically very similar species to *E. radiata* (Lane et al. 2006), in sheltered and exposed environments. Although environmental genotypes are known for other *Ecklonia* species (*E. cava*, Serisawa et al. 2003) and close relatives (*Pelagophycus porra, E. arborea*: Miller et al. 2000, Roberson and Coyer 2004), all current evidence suggests that morphological variation in *E. radiata* is under environmental (phenotypic and imposed) control rather than genetic control. This evidence includes experiments, where reciprocal transplants attained the morphology of resident individuals (Fowler-Walker et al. 2006), and field surveys documenting no morphological differences between populations separated by thousands of kilometers (Wernberg et al. 2003a, Fowler-Walker et al. 2005a), but large differences among populations in different hydrodynamic environments just a few kilometers apart (Wernberg and Thomsen 2005, this study).

We conclude that wave exposure exerts a strong control over the morphology of *E. radiata*, but that the nature of the effect depends on the magnitude of wave exposure. We also conclude that most of the effects of wave exposure are consistent through time and do not interact with seasonal cycles of growth and pruning in any major way.

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