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Research Article

The effect of parameter variability in the allometric projection of leaf growth rates for eelgrass (*Zostera marina* L.)

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ABSTRACT. Restoration of eelgrass *Zostera marina* meadows from harmful anthropogenic influences has made it essential to evaluate these efforts by using non-destructive assessments. Allometric methods provide a convenient framework for the derivation of reliable indirect assessments of leaf biomass and leaf growth of eelgrass. Invariance of the involved parameters could grant truly nondestructive assessments because previously fitted values could be used to produce consistent estimations. In order to explore this property we analyzed data from two natural eelgrass populations in the East Pacific (México), as well as populations in the West Pacific (two natural in Korea and one mesocosm in Japan). When we compared observed leaf growth rates with those projected allometrically by using parameter values fitted at different sites, we found that only parameter values fitted at sites within the same geographical region can produce consistent results. Therefore if this restriction holds previously fitted parameters can indeed be used to produce reliable non-destructive assessments of eelgrass leaf growth rates.

Keywords: *Zostera marina*, foliar growth, eelgrass meadow, allometric modeling, restoration evaluation.

Efectos de la variabilidad paramétrica en la obtención de tasas de crecimiento foliar en *Zostera marina* L. mediante métodos alométricos

RESUMEN. La restauración de praderas de *Zostera marina* que han sido dañadas por influencia antropogénica, ha hecho necesaria la evaluación de estos esfuerzos mediante métodos no destructivos. Las metodologías alométricas proporcionan un marco formal que favorece la obtención de estimaciones indirectas de biomasa y tasas de crecimiento foliar. La invariancia de los parámetros asociados asegura la obtención de técnicas de estimación no destructiva de gran confiabilidad, puesto que, parámetros previamente ajustados podrían usarse para producir estimaciones consistentes. Para investigar la existencia de esta propiedad se analizan datos provenientes de dos poblaciones naturales de *Z. marina* en el Pacífico oriental (México) así como también poblaciones en el Pacífico occidental (dos naturales en Corea y una cultivada en laboratorio en Japón). Al comparar valores observados de tasas de crecimiento foliar con aquellos obtenidos alométricamente, utilizando parámetros ajustados en sitios indistintos, se observó que únicamente cuando los parámetros provienen de una región geográfica equivalente se pueden producir resultados consistentes. Por lo tanto, tomando en cuenta esta restricción parámetros alométricos previamente ajustados pueden, en efecto, producir evaluaciones no destructivas y fiables de tasas de crecimiento de las hojas de *Z. marina*.

Palabras clave: *Zostera marina*, crecimiento foliar, fanerógama marina, modelación alométrica, evaluación restauración.

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INTRODUCTION

Eelgrass (*Zostera marina* L.) is an intertidal or sub-tidal marine angiosperm that grows in temperate estuaries. Eelgrass meadows are ecologically important because they provide phytoremediation of contaminated sediments (Williams *et al.*, 1994). Furthermore, since eelgrass beds are highly productive communities they also play a fundamental trophic role by provisioning the shallow-water food web with substantial organic material

(Jacobs, 1979), furnishing at the same time a structural assemblage that provides habitat or shelter for many fishes, fish larvae, attached algae and epifauna (McRoy, 1966; Thayer *et al*., 1984; Heck *et al*., 1995). Therefore the measurement of biomass and its variability is fundamental for assessing the overall values of eelgrass populations. Moreover, in restored areas, determinations of leaf-growth rates are fundamental to the assessment of the re-establishment of ecological functioning. Scaling relationships of the form

$$Y = \beta X^\alpha \qquad (1)$$

with the parameter α known as the allometric exponent and β commonly referred as the normalization constant are widely utilized in many types of biological studies (Savage *et al*., 2004; Marquet *et al*., 2005; West & Brown, 2005; Harris *et al*., 2006; Filgueira *et al*., 2008; Kaitaniemi, 2008). In functional-structural plant models, for example, they can be used for estimating unmeasured plant traits Y based on some easily measurable traits X, such as the length or diameter of structures. Duarte (1991) assembled an extensive compilation of data on architecture and growth of different seagrass species, and found consistent fittings of models of the form (1) for several pairs of representative variables X and Y. These models were used to examine the implications of differences in module size to account for differences in productivity among species. For eelgrass in particular, the results presented in McRoy (1970) and Jacobs (1979) exemplify how similar scaling equations can be used in biomass and productivity studies. Furthermore, Hamburg & Homann (1986) and Solana-Arellano *et al*. (1991, 1998, 2003) identified bivariate allometric models to represent leaf biomass in terms of leaf length and width. These results show that allometric models provide convenient non-destructive surrogates for conventional leaf-biomass assessments. Additionally the scaling of eelgrass leaf and sheath biomasses in terms of matching lengths using equation (1) led to allometric methods for the non-destructive estimation of above-ground biomass (Echavarría-Heras *et al*., 2011).

If, at a time t, eelgrass individual leaf biomass is denoted through $w(t)$ and corresponding length by means of $l(t)$ then, assuming that leaf biomass can be allometrically scaled in terms of matching length in the form given by equation (1) we will have

$$w(t) = \beta l(t)^\alpha, \qquad (2)$$

and if $w_s(t)$ denotes total blade biomass in a representative shoot s then using equation (2) to obtain the biomass of each individual leaf in the shoot, we will have

$$w_s(t) = \sum_{l(s)} \beta l(t)^\alpha, \qquad (3)$$

where $\sum_{l(s)}$ indicates summation of the leaves that the shoot, s, holds. Moreover, if Δw stand for the biomass increment gained by an individual leaf over the interval $[t, t + \Delta t]$, then denoting by means of $L_{sg}(t, \Delta t)$ the average growth rate of leaves on a shoot s over the same time interval we then have

$$L_{sg}(t, \Delta t) = \frac{\sum_{l(s)} \Delta w}{\Delta t} \qquad (4)$$

and correspondingly, if $L_g(t, \Delta t)$ denotes the linked average rate of leaf growth per shoot-day then we have

$$L_g(t, \Delta t) = \frac{\sum_{NS(t,\Delta t)} L_{sg}(t, \Delta t)}{NS(t, \Delta t)}, \qquad (5)$$

where $\sum_{NS(t,\Delta t)}$ indicates summation of the shoots collected over the marking interval $[t, t + \Delta t]$ being $NS(t, \Delta t)$ their number .

Following Echavarría-Heras *et al*. (2010), we can use equation (2) in order to derive an allometric approximation for $L_{sg}(t, \Delta t)$ which we here denote through the symbol $L_{psg}(\alpha, \beta, t, \Delta t)$ and formally expressed by

$$L_{psg}(\alpha, \beta, t, \Delta t) = \frac{\sum_s \beta l(t+\Delta t)^\alpha \delta(t, \Delta t)}{\Delta t}, \qquad (6)$$

where the factor $\delta(t, \Delta t)$ is given by

$$\delta(t, \Delta t) = \left(1 - \left(1 - \frac{\Delta l}{l(t+\Delta t)}\right)^\alpha\right). \qquad (7)$$

Similarly, if $L_{pg}(\alpha, \beta, t, \Delta t)$ denotes the associated proxy for $L_g(t, \Delta t)$ this will be given by

$$L_{pg}(\alpha, \beta, t, \Delta t) = \frac{\sum_{NS(t,\Delta t)} L_{psg}(\alpha, \beta, t, \Delta t)}{NS(t, \Delta t)}. \qquad (8)$$

The appropriateness of the allometric method of equation (8) for providing accurate and truly simplified assessments of observed leaf growth rates $L_g(t, \Delta t)$ given by equation (5) depends on a highly consistent fitting of the model of equation (2) and on the invariance of the associated parameters (Echavarría-Heras *et al*., 2010, 2011). The evaluation of the last matter is an important research problem which we address here. To that aim, we assembled data collected on four different natural eelgrass populations and a mesocosm, resulting in a total of 6751 complete leaves. All five data sets exhibited consistent fittings for the allometric scaling of leaf biomass in terms of length, and comparisons among data sets showed that the associated parameters can be considered as invariant within a given region. Moreover, allometrically projected leaf-growth rates that were obtained using equation (8) and available leaves data showed a remarkable correspondence with observed values calculated by means of equation (5) even when these proxies were obtained by using allometric parameters

fitted to data collected during a different year or at a different site within a given geographical region.

MATERIALS AND METHODS

For this study, we analyzed leaf biomass and length data from two natural eelgrass populations in the East Pacific, Punta Banda estuary and San Quintín Bay (México), and Kosung Bay and Jingdon Bay (Korea) and one mesocosm (Japan) populations in the West Pacific (Table 1). For each site, monthly means for total biomasses of complete leaves in shoots were obtained. For Punta Banda following the Kentula & McIntire (1986) technique we marked shoots at a time t and retrieved the remaining ones at a time $t + \Delta t$ with Δt fixed at two weeks. For each leaf on a sampled shoot, the corresponding length values were obtained by measuring the distance between the reference point placed at the top of the sheath and the leaf tip. The associated leaf width was obtained using the criteria in Echavarría-Heras *et al.* (2011). Leaf elongation increments $\Delta l = l(t + \Delta t) - l(t)$ were measured for each leaf; these are given by the displacement of the marking point from the reference point. The biomass Δw associated with a leaf length increment Δl, was obtained using Δl, the measured leaf width and the bivariate model of Solana-Arellano *et al.* (1998). Linked biomasses for the portions $l(t + \Delta l) - \Delta l$, were obtained in like manner. These values were used to estimate the observed overall rates of leaf growth using equation (5). The matching allometrically projected values $L_{pg}(\alpha, \beta, t, \Delta t)$ of equation (8) were also calculated.

Widely used fitting approaches utilize log-transformation of the basic model of equation (1) to obtain the allometric parameters α and β. The reluctance of many authors to acknowledge the worth of this kind of transformation is concisely described by Packard *et al.* (2010), who argued that equation (1) is intrinsically non-linear and disagreed with the logarithmic transformation approach to obtain a linearized form by pointing out that equivalent models fitted in arithmetic and logarithmic domains do not have equivalent least-square solutions. They also asserted that a lack of homoscedasticity and normality in the original data would be eliminated after logarithmic transformation and therefore, transforming data would generate a new distribution for the observed measurements. This new distribution should conform better than the original to the assumptions of parametric statistical tests, but the viability of back-transformed data it is not always possible (Glass, 1969; Packard, 2009). This back-transformation depends on the variability in the original response variable at each level

for the transformed independent variable. They claim, moreover, that small recorded deviations that do not fall on the line tend to be over weighted. *Zostera marina* shoots have leaves with large variability in length and thus of biomass. A standard shoot can have leaves measuring between 7 and 1500 mm, so dry weights can also have a wide range. Therefore, due to the observed variability on individual leaf lengths and biomasses (Table 2) following Packard *et al.* (2010) for the Baja California and mesocosm data sets, which contained measurements of both leaf length and biomass of individual leaves, we were able to fit equation (2) in its non-linear form instead of the traditional approach of linearizing the equation through a logarithmic transformation. Moreover, the logarithmic transformation approach could not be achieved for Kosung Bay and Jingdon Bay data sets, because leaf biomasses were aggregated at the shoot level, and so individual leaf dry weights were not available. Nevertheless, the dry weight of each leaf in a given shoot can be considered to be a random variable and can thus be expressed in terms of the model of equation (2) then following Echavarría-Heras *et al.* (2011) and Solana-Arellano *et al.* (2012) we fitted the several-variables version given by equation (3), and for comparison among the different sites we also used non-linear regression in these fittings. After fitting the appropriate model for each site (*i.e.*, model (2) or model (3)), we verified that the requirements of randomness normality, homoscedasticity and independence of residuals were satisfied (Seber & Wild, 1989), and then compared the obtained parameters using a Student *t* test (Table 4). Finally, to assess reproducibility of observed values through allometric proxies we used $\hat{\rho}$ values, the Concordance Correlation Coefficient (CCC) (Lin, 1989 and 1992), to evaluate the reproducibility (Table 3). In order to provide a better understanding of the CCC as a measure of reproducibility we estimated the uncertainties of the point estimates of $\hat{\rho}$ by obtaining confidence intervals. Because the point estimate of CCC is not normal, in a similar way, as in making inferences for the Pearson correlation coefficient, we normalized $\hat{\rho}$ values by using the Z-transform (inverse hyperbolic tangent) of Fisher. Also, we used the corrected formula for the variance of $\hat{\rho}$ (Steichen & Cox, 2002).

RESULTS

To facilitate recognition of the fitted parameter values we used subscripts to identify each site; for instance α_M and β_M respectively label the values of the parameters α and β fitted for the mesocosm study. In like manner, the initials of a site are used as a subscript for the corresponding parameter. The allometric parameters

Table 1. Location and sampling protocols for the *Zostera marina* leaf-length and biomass data sets used in this study.

Site	Geographic coordinates	Sampling protocol	Duration	Total leaves
Japan mesocosm	35º13.7′N, 139º43.2′W	Monthly, 3×2 m pool	Jul. 05-Sept. 06	247
Jindong Bay Korea	35º06′N, 128º32′W	Monthly, 4-6 random, 0.35 × 0.35 m quadrats	Jan. 08 -Dec. 09	917
Kosung Bay Korea	34º54′N, 128º20′W	Monthly, 4-6 random, 0.35 × 0.35 m quadrats	Jul. 07-May 08	432
Punta Banda México	31º43-46′N, 116º 37-40′W	Biweekly, 20 previously marked shoots	Jan. 99 -Dec. 99	3000
San Quintín México	30º24'-30º 37′N, 115º56'-116º01′W	Monthly, random two 20×20 cm quadrats	Nov. 92-Nov. 93	2020

Table 2. Basic statistics for observed leaf weight (w) and length (l) values.

Site	Variable	Mean	Minimum	Maximum	Variance
Japan (mesocosm)	w	162.93	1	780.2	25636.2
	l	541	17.7	1827.7	151475.8
Jindong Bay	w	1.1	0.3	2.5	0.3
	l	349.3	1	1149	52137.4
Kosung Bay	w	0.75	0.24	2.63	0.3
	l	421.9	8	1931	7733.3
Punta Banda Estuary	w	0.011	0.00005	0.06	0.0001
	l	156.8	11	540	8946.6
San Quintín Bay	w	0.015	0.00002	0.15	0.004
	l	169.9	10	976	28367.7

Table 3. Values of estimates of allometric parameters (α, β), determination coefficients of the fitting (R^2) (cf. Eqs. (2) and (3)) for different sites, and Z-transform values of the concordance correlation coefficient with 0.95 confidence interval ($\hat{\rho}$).

Samples Dates	Site	β	α	R^2	$\hat{\rho}$
Jul 2005-Sep 2006	mesocosm	$0.0001 \pm 4E\text{-}5$	1.164 ± 0.057	0.74	0.76 (0.63,0.92)
Jan 2008-Dec 2009	Jindong Bay	$0.000172 \pm 58E\text{-}6$	1.21 ± 0.054	0.77	0.83 (0.72,0.93)
Jun 2007-May 2008	Kosung Bay	$0.000087 \pm 46E\text{-}6$	1.205 ± 0.079	0.72	0.80 (0.70,0.85)
Jan 1999-Dec 1999	Punta Banda	$0.000007 \pm 4E\text{-}12$	1.43 ± 0.013	0.84	0.85 (0.75,0.97)
Nov 1992-Nov 1993	San Quintín	0.000009 ± 0.0	1.41 ± 0.012	0.92	0.90 (0.75,0.97)

Table 4. Comparisons of allometric parameter values α and β obtained at the different sites.

Site comparisons	α		β	
	t	P-level	t	P-level
Mesocosm *vs* Jingdon Bay	-0.587	0.29	-1.01	0.15
Mesocosm *vs* Kosung Bay	-0.4187	0.31	0.22	0.58
Mesocosm *vs* Punta Banda Estuary	-4.555	0.008	2.27	0.01
Mesocosm *vs* San Quintin Bay	-4.221	0.001	2.22	0.01
Jingdong Bay *vs* Kosung Bay	-0.051	0.48	1.15	0.13
Jing-dong Bay *vs* Punta Banda	-3.9609	0.005	2.847	0.003
Jindong Bay *vs* San Quintin Bay	-3.614	0.0007	2.81	0.002
Kosung Bay *vs* Punta Banda Estuary	-2.771	0.03	1.74	0.05
Kosung Bay *vs* San Quintin Bay	-2.5372	0.01	1.69	0.045
Punta Banda Estuary *vs* San Quintin Bay	-1.070	0.24	-0.71	0.39

were identified using the model of equation (2) for the mesocosm, Punta Banda and San Quintín studies, but this identification was performed by means of equation (3) for the Kosung and Jindong data. For the mesocosm data we obtained $\alpha_M = 1.16$, $\beta_M = 0.0001$ and a determination coefficient of. $R^2 = 0.74$. Although the residuals show a lack of homogeneity of variances, their normality was corroborated ($P > 0.05$). It is worth pointing out that for this data set, the log-transformation of equation (2) eliminates the lack of homogeneity of residuals, giving a larger determination coefficient ($R^2 = 0.91$) but different values than α_M and β_M for the resulting fitted parameters. For the Punta Banda data, fitted parameter values were $\alpha_{PB} = 1.43$ and $\beta_{PB} = 0.000007$ with a determination coefficient of, $R^2 = 0.84$, meanwhile for San Quintín Bay values were respectively $\alpha_{SQ} = 1.41$ and $\beta_{SQ} = 0.000009$ with $R^2 = 0.92$. A good disposition of residuals was observed for both the Punta Banda and San Quintín fittings; that is, residuals were normally distributed with a mean of zero and a constant variance ($P > 0.05$). For Jindong Bay, values of fitted parameter were $\alpha_{JB} = 1.21$ and $\beta_{JB} = 0.000172$. A determination coefficient of $R^2 = 0.77$ was obtained, and testing of residuals showed normality and homogeneity of variances ($P > 0.05$). For Kosung Bay the fitting produced $\alpha_{KB} = 1.205$ and $\beta_{KB} = 0.000087$ with a value of the coefficient of determination of $R^2 = 0.72$, and testing for normality and homogeneity of variances gave values of ($P > 0.05$) and ($P = 0.04$) respectively. The associated CCC values ($\hat{\rho}$) for reproducibility of the observed leaf biomasses by means of the applicable allometric proxies were also obtained (Table 3).

Comparisons for differences in the fitted allometric parameters between sites showed no significant differences between the mesocosm allometric exponent α_M and the value α_{JB} fitted for Jindong Bay ($P = 0.29$) (Table 4). Similarly non-significant differences between α_M and α_{KB} were found ($P = 0.31$). Likewise, the allometric exponents α_{JB} and α_{KB} were statistically equivalent ($P = 0.48$). Also, the Mexican data sets revealed no significant differences in their allometric exponents α_{PB} and α_{SQ} ($P = 0.24$). Significant differences were found in the values of α fitted for populations in Korea and Japan, relative to those values fitted for populations in Baja California ($P < 0.01$). However, significant differences were also found in the fitted values of the normalization constant β between the Eastern and Western Pacific ($P > 0.05$), while the regional values were not (β_{PB} *vs* β_{SQ} ($P = 0.39$), β_M vs β_{JB} ($P > 0.15$), β_M vs β_{KB} ($P = 0.58$) and β_{JB} vs β_{KB} ($P = 0.13$). In order to show the performance of allometric methods we provide the dynamics of observed and allometrically projected shoot biomasses

and leaf growth rates. In Fig. 1, we present comparisons of observed and allometrically projected (cf. eq. 3) monthly means for total biomasses of complete leaves in shoots for each site. For the Punta Banda data set the calculated annual average of observed monthly growth rates $L_g(t, \Delta t)$ was 0.049 g month^{-1}. We used the parameters α_{PB} and β_{PB} fitted at the site and then calculated the related projected values $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ of equation (8). The average value of projected values was 0.0452 g month^{-1}. Fig. 2 presents a comparison of observed and projected values.

To evaluate the effects of differences in allometric parameters on allometric projections of leaf growth rates we calculated projected values $L_{pg}(\alpha, \beta, t, \Delta t)$ produced by equation (8) and the Punta Banda individual leaf data but using different combinations of the values of the parameters α and β fitted in this study. In forming these parametric combinations, we also considered α_{AV} and β_{AV}, the respective averages of the presently fitted values of α and β. For the projected leaf growth rates, $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ the value of $\hat{\rho}$ was 0.79. Linear regression analysis showed, moreover, that observed rates $L_g(t, \Delta t)$ can be isometrically scaled in terms of the projected values $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ with a normalization constant of 1.05. The determination coefficient was, $R^2 = 0.97$, with good behavior of the residual. The annual average of observed $L_g(t, \Delta t)$ rates was 0.049 g month^{-1} and the annual average for the $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ values was 0.0452 g month^{-1}. Moreover, we found non-significant differences in the values of α and β fitted at Punta Banda and at San Quintín Bay (Table 4). When projected leaf growth rates using both allometric parameters were fitted at San Quintín Bay, the concordance correlation coefficient between observed $L_g(t, \Delta t)$ and projected $L_{pg}(\alpha_{SQ}, \beta_{SQ}, t, \Delta t)$ rates was of $\hat{\rho} = 0.69$ and the isometric scaling of observed versus projected values was also consistent. This produced a normalization constant of 1.05 with a value of $R^2 = 0.97$ for the determination coefficient and a fair distribution of residuals. The annual average for $L_{pg}(\alpha_{SQ}, \beta_{SQ}, t, \Delta t)$ values calculated for Punta Banda was 0.0453 g month^{-1}. No significant differences between this average, the average of $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ values and that of observed leaf growth rates $L_g(t, \Delta t)$ were found ($df_{2,33}$, $F = 0.599$, $P = 0.55$) showing that we can exchange allometric parameters between Punta Banda and San Quintín Bay to obtain consistent reproducible observed leaf growth rates by means of the related projections.

We did, however, observe significant differences between the values of α and β fitted at Punta Banda and those fitted at the Korean and mesocosm studies. We
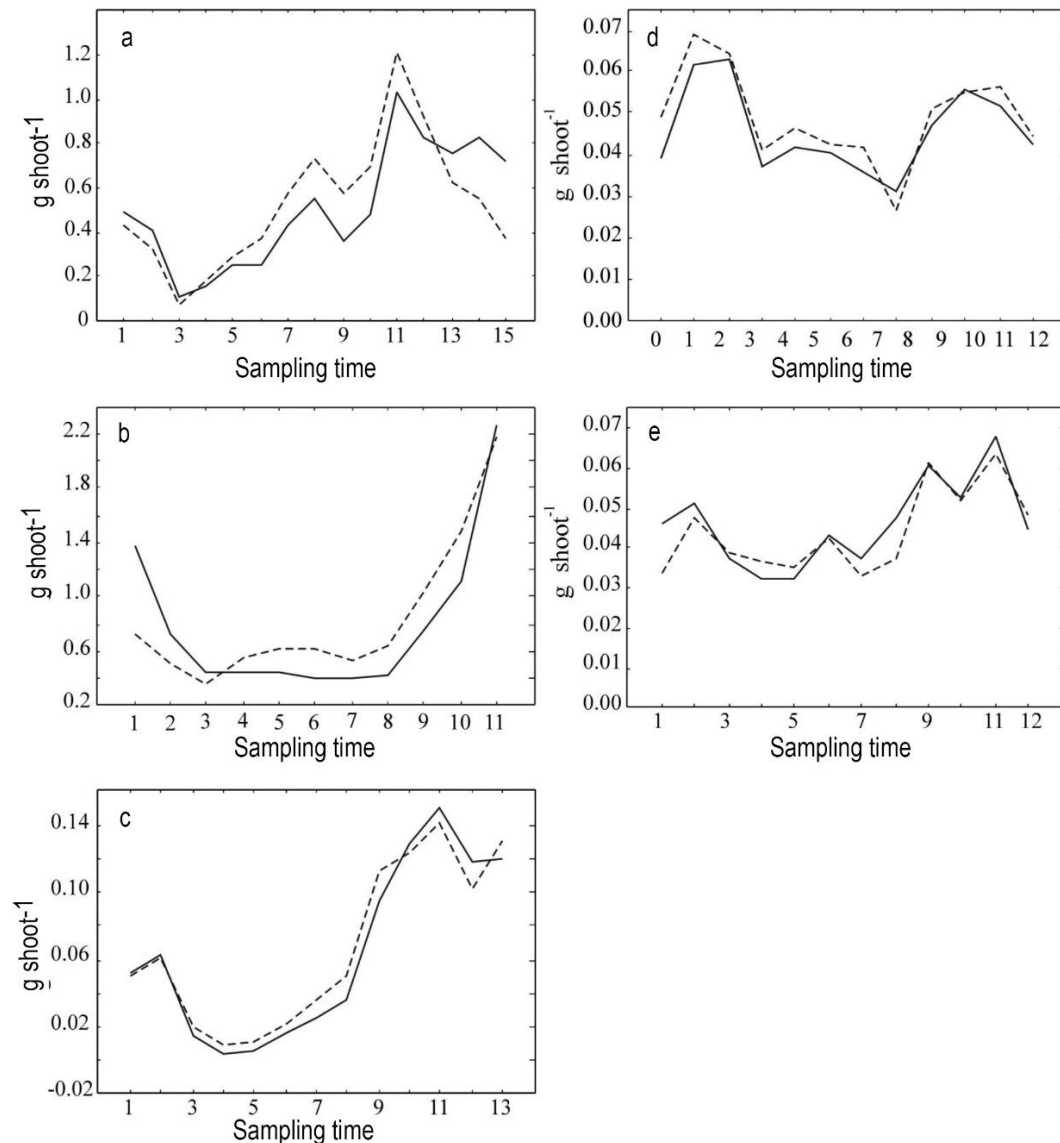
Figure 1. Observed and allometrically predicted monthly means for total biomasses of complete leaves in shoots for each site. Dashed lines for observed values and continuous line for predicted. a) Jingdon Bay, b) Kosung Bay, c) mesocosm, d) Punta Banda Estuary e) San Quintín Bay. Sampling times are months. For initial and final months of data collecting at each site the reader is referred to Table 3.

can therefore expect that projections using values of α and β fitted at these sites would induce greater deviations between observed rates than those projected using α and β fitted at San Quintín Bay. In fact the values of the concordance correlation coefficient corroborate that, among combinations formed with both allometric parameters fitted at a different site than Punta Banda estuary, the projections obtained using α and β fitted at San Quintín Bay performed relatively better for reproducing variability of observed leaf growth rates (Table 5). This points to the importance of local and regional factors in the determination of the values of the allometric parameters involved, and

consequently in the reproducibility of leaf growth rates derived through the allometric proxy considered here. For mixed parametric combinations, we can detect a penalty on the value of the concordance correlation coefficient associated with the $L_{pg}(\alpha_{PB}, \beta_{PB}, t, \Delta t)$ values, which is attributable to changes in α_{PB} and β_{PB} for projection purposes (Table 5).

DISCUSSION

The allometric exponent α (cf. eq. 1) has been the focus of theoretical and empirical studies because it often seems to have a constant value specific to a particular
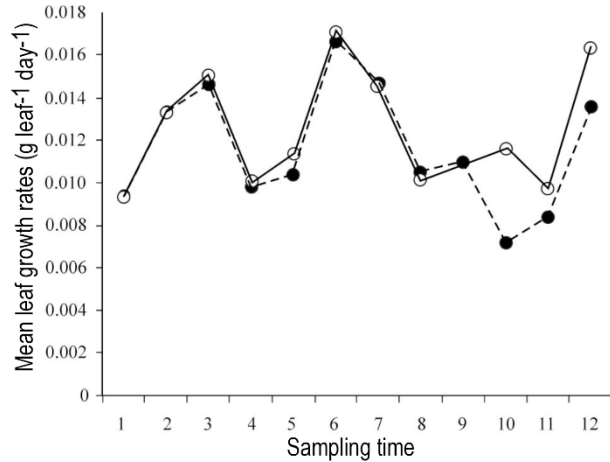
Figure 2. Projected *vs* observed leaf growth rate for Punta Banda Estuary data. In these projections we used equation (8) and the allometric parameters fitted at the site. Dashed lines for observed values and continuous line for predicted. Sampling times indicate successive biweekly intervals. For initial and final dates of collecting data the reader is referred to Table 3.

biological relationship (*e.g.*, Winter, 1976; Bernard, 1983; Jones *et al*., 1992; Enquist *et al*., 1998; Niklas & Enquist, 2001; West *et al*., 2002). On the other hand, several studies have provided evidence that support a certain variability in the exponent of allometric scaling laws (*e.g.*, Riisgård, 1998; Atanasov & Dimitrov, 2002; Bokma, 2004; Muller-Landau *et al*., 2006; Reich *et al*., 2006; White *et al*., 2006). Accordingly, the value of the normalization constant β is considered to be characteristic of species or populations (Niklas, 1994). And the variability observed in the normalization constant, moreover, has been explained as a differential response to environmental conditions (Winter, 1976; Bernard, 1983; West *et al*., 1997; Gillooly *et al*., 2001; Brown *et al*., 2002). Kaitaniemi & Lintunen (2008) demonstrated that by using a theoretically predicted fixed value for the exponent α instead of an empirical value determined by regression, it is possible to make the normalization constant β suitable for biological interpretation in terms of a dynamically changing environment. In our scaling, we observed (Table 3) deviations among numerical values of α that are comparatively smaller than those obtained for β values. We observed that absolute numerical differences between α_{PB} and each of the remaining fitted values for the allometric exponents are bounded by 0.19 α_{PB}. Meanwhile, taking now β_{PB} as a reference, the bound for the corresponding absolute deviation between this parameter and the other fitted normalization constant values increased to 23.57 β_{PB}. This could be interpreted as a result of relatively greater local influences in the determination of the β value,

these influences likely being linked to environmental factors. Interestingly, the maximum absolute deviation from β_{PB} was attained by β_{JB}. Hence our results support the view that the scaling relationships addressed here can be viewed as static relationships in which both the scaling exponent α and the normalization constant β obtain empirical values that are fixed within a single set of data (Kaitaniemi & Lintunen, 2008). Nevertheless our results suggest that values of the allometric parameters α and β can be considered statistically similar within a given geographical region. The elucidation of this hypothesis, however, requires a more extensive data set than the one used here.

Using equation (8) we projected leaf-growth rates for Punta Banda estuary using measured leaf lengths and the parameters α_{PB} and β_{PB} fitted at the site, and comparison of projected and observed values calculated using equation (5) showed consistent results (see Fig. 2).

The possibility of projecting leaf-growth rates using values of α and β that were fitted for different eelgrass populations and over different periods of time lies at the core of a universal property for the allometric parameters. But allometric scaling relationships, like that given by equation (1), are markedly sensitive to variation in the parameters involved. Although statistical differences between allometric parameters fitted at two regionally equivalent sites were not significant, there are necessarily differences in numerical values among fitted parameters. Even slight numerical differences in fitted values for these allometric parameters, along with errors of aggregation in calculating growth rates through equation (8), could induce important deviations between the observed and projected rates. This can be readily illustrated by the values of the concordance correlation coefficient (Table 5). Since a wide range of variation in $\hat{\rho}$ is observed, our results show that local influences which determine the static nature of the values of α and β are such that an invariant value for each one of these parameters would fail to consistently reproduce the dynamics of observed leaf-growth rates through the allometric device of equation (8). Moreover, among pairs of allometric parameters fitted at sites in the West Pacific, the combination formed by α_{JB} and β_{KB} recorded the smallest Euclidean distance relative to the ordered pair formed by α_{PB} and β_{PB} (see shaded row in Table 6), even though, $L_{pg}(\alpha_{JB}, \beta_{KB}, t, \Delta t)$ values produced a low concordance correlation coefficient value of $\hat{\rho} = 0.09$. Meanwhile, in Table 6 we can observe that the Euclidian distance between allometric parameters fitted at San Quintin Bay and those fitted at Punta Banda was the smallest and in correspondence

Table 5. Isometric relationship between observed growth rates and those projected allometrically through different combinations of estimated parameter. α and β stand for allometric parameters and c for the isometric normalization constant. R^2 determination coefficient, $\hat{\rho}$: Z-transformed concordance correlation coefficient of reproducibility with the corresponding 0.95 confidence interval.

α	β	c	R^2	$\hat{\rho}$
α_{PB}	β_{PB}	1.05	0.97	0.75 (0.44,0.87)
α_{SQ}	β_{SQ}	1.05	0.97	0.69 (0.35,0.80)
α_{PB}	β_{SQ}	1.12	0.98	0.70 (0.46,0.84)
α_{SQ}	β_{PB}	1.06	0.98	0.67 (0.39,0.83)
α_{KB}	β_{PB}	4.70	0.98	0.034 (0.013,0.054)
α_{AV}	β_{PB}	2.12	0.98	0.15 (0.08,0.20)
α_{M}	β_{M}	0.42	0.98	0.10 (0.08,0.14)
α_{KB}	β_{KB}	0.39	0.98	0.10 (0.06, 0.12)
α_{JB}	β_{KB}	0.37	0.97	0.09 (0.03,0.14)
α_{JB}	β_{PB}	4.60	0.97	0.034 (0.012,0.06)
α_{AV}	β_{AV}	0.2	0.95	0.03 (0.015, 0.044)
α_{JB}	β_{JB}	0.19	0.98	0.03 (0.005,0.07)
α_{M}	β_{PB}	5.90	0.97	0.024 (0.008,0.04)
α_{PB}	β_{KB}	0.11	0.98	0.017 (0.006,0.03)
α_{PB}	β_{M}	0.09	0.88	0.014 (0.004,0.023)
α_{PB}	β_{JB}	0.06	0.97	0.007 (0.002,0.013)

Table 6. Euclidian distance values $\delta_{PB}(\alpha,\beta)$ between the fixed pair (α_{PB},β_{PB}) and different combinations (α,β) obtained using the values of the allometric parameters fitted at the different site. Rounding off was avoided in order to show differences in $\delta_{PB}(\alpha,\beta)$ values.

α	β	$\delta_{PB}(\alpha,\beta)$
α_{JB}	β_{JB}	0.220
α_{M}	β_{JB}	0.266
α_{SQ}	β_{JB}	0.020
α_{KB}	β_{JB}	0.225
α_{JB}	β_{M}	0.220
α_{M}	β_{M}	0.266
α_{SQ}	β_{M}	0.020
α_{KB}	β_{M}	0.225
α_{JB}	β_{SQ}	0.220
α_{M}	β_{SQ}	0.266
α_{SQ}	β_{SQ}	0.020
α_{KB}	β_{SQ}	0.225
α_{JB}	β_{KB}	0.220
α_{M}	β_{KB}	0.266
α_{SQ}	β_{KB}	0.020
α_{KB}	β_{KB}	0.225

$L_{pg}(\alpha_{SQ},\beta_{SQ},t,\Delta t)$ values produced for $\hat{\rho}$ a consistent value of $\hat{\rho} = 0.69$ (Table 5). This suggests that, in order to obtain reasonable allometric estimations of observed leaf growth rates at a given site, the involved para-

meters should be interchangeable among regionally equivalent sites.

Our analysis showed that no universal value can be found for the allometric exponent α in equation (2). Although our results suggest that the scaling relationship addressed here might be considered static, thus implying that local factors determine the actual values of fitted parameters, we did not find significant differences at the regional level. We also analyzed data previously collected over different complete annual cycles and found time invariance of allometric parameters for the bivariate scaling of eelgrass leaf biomass in terms of length and width at San Quintín Bay (Solana-Arellano *et al.*, 1998) and for the scaling of leaf biomass and length at Jindong Bay (Echavarría-Heras *et al.*, 2011). And using data collected from April 1998 to May 1999 as well as the data set presented here, we further verified this property for the allometric parameters α and β at Punta Banda estuary. But the time-invariance property could not be tested for the present Kosung Bay or mesocosm studies because we lack data for different annual cycles. If time invariance of the allometric parameters can be statistically demonstrated for eelgrass at a given site, this implies that values previously fitted there -or at a regionally equivalent site- can be expected to produce accurate non-destructive leaf-biomass assessments by means of equation (8). We must, however, stress that only when data on leaf lengths and leaf-length increments over

marking intervals can be obtained without removing shoots, previously fitted and time-invariant values for the allometric parameters α and β can be used to produce, through equation (8), reliable cost-effective and non-destructive assessments of leaf growth rates.

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