Increasing temperature induces shorter leaf life span in an aquatic plant

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Because leaf life span (LLS) is related to cost–benefit balances (such as maximal net gain or efficiency of net gain per individual leaf), factors associated with cost–benefit balances in individual leaves may control LLS. In seagrasses, water temperature and irradiance strongly affect metabolism, and epiphytes can attenuate irradiance reaching the leaves. Therefore, we predicted that seagrass LLS is largely controlled by water temperature, irradiance and epiphyte biomass on seagrass leaves. In the present study, we investigated the relationship between LLS of eelgrass *Zostera marina* and these parameters over the course of one year. LLS ranged from 34.9 to 89.6 days and was negatively related to water temperature but not related to irradiance, while epiphyte biomass was strongly related to water temperature. Furthermore, path analysis supported a much stronger relationship between LLS and temperature than that between LLS and epiphyte biomass. This sensitivity of eelgrass leaves to increasing water temperature has apparently resulted in a substantial range in LLS and therefore a substantial range in leaf number. Our study indicates that because high water temperature reduces eelgrass LLS, eelgrass and similar plants may acclimate poorly to any future increases in global water temperature.

Plants can shed leaves based on cost–benefit balances such as maximal net gain or efficiency of net gain per individual leaf, and this suggests that factors associated with cost–benefit balances in the individual leaf may control leaf life span (LLS) (Kikuzawa 1991, Ackerly 1999). Previous studies indicated that LLS of terrestrial plants is long when irradiance and nutrient supply are low (Shaver 1981, Sterck 1999, Balster and Marshall 2000, Reich et al. 2004). These relationships may be controlled by balances of carbon and nutrients in individual leaves; that is, a resource-limited plant may slow its metabolism and increase its LLS in order to increase the quantity of resource gained by individual leaves (Kikuzawa 2005).

Seagrass LLS is lower than LLS of terrestrial herbaceous plants, shrubs and trees (Hemminga et al. 1999) and varies seasonally by 2- to 3-fold (Nakaoka et al. 2003, Lee et al. 2005), suggesting that seagrass LLS may be sensitive to changes in intrinsic and/or extrinsic factors. Because seagrass metabolism, including photosynthesis and respiration, is affected by water temperature and irradiance (Marsh et al. 1986, Dennison 1987, Zimmerman et al. 1989), we predict that seagrass LLS will respond to changes in irradiance and/or water temperature. In addition, because epiphytes on seagrass leaves greatly reduce irradiance reaching the leaf (for example, 5–74% of attenuation in irradiance by 2 mg DW cm \(^{-2}\) in epiphyte biomass, and 9–83% by 3 mg DW cm \(^{-2}\); Brush and Nixon 2002), we predict that attachment of epiphytes on seagrass leaves will also affect seagrass LLS. Furthermore, the effect of epiphyte attachment on seagrass leaves may be related to irradiance and water temperature because these factors affect epiphyte abundance (Borowitzka et al. 2006).

In this study, we focused our attention on seasonal variations of LLS of eelgrass, *Zostera marina*, an evergreen seagrass. To test our predictions, we examined the relationship between eelgrass LLS and irradiance, water temperature, and epiphyte biomass over the course of one year.

**Material and methods**

**Design of mesocosm study**

Using a mesocosm, we maintained calm conditions in an enclosed system to exclude the effects of physical disturbance on LLS. The study was conducted in an indoor experimental pool housed in a shed with a glass roof and walls at the Port and Airport Research Institute located on Kurihama Bay at the mouth of Tokyo Bay (35°13′N, 139°43′W). The mesocosm pool is 3 m long and 2 m wide with sandy sediments (0.13 mm median diameter) placed to a depth of 0.4 m at the bottom. Seawater (salinity 29–35, pH 7.8–8.3) was obtained from Kurihama Bay and was introduced without treatment twice each day with every semi-diurnal tide; the water exchange rate was 24% per tide. Spores of epiphytes entered the mesocosm with untreated
Sampling and data analyses

Fifteen vegetative shoots were tracked to measure the number of leaves, leaf plastochrone interval \((P_L)\); interval between formation of new leaves), and export interval \((E_I)\; (sheding interval of oldest leaf) from August 2005 to September 2006, with a sampling interval from 6 to 15 days. When the tracked vegetative shoots were lost or changed into reproductive shoots, alternate vegetative shoots were assigned. \(P_L\) and \(E_I\) were determined using the leaf marking technique of Zieman (1974). Briefly, a needle was used to form a hole at the sheath top of all leaves, and new leaves could be identified and counted because they lacked holes. \(P_L\) and \(E_I\) were calculated according to the following equation (Jacobs 1979, Kentula and McIntire 1986).

\[
P_L = \frac{n \times \Delta t}{\sum_{n} N}
\]

where \(n\) is the number of shoots, \(\Delta t\) is the sampling interval (d), and \(N\) is the number of new leaves formed or old leaves shed on a shoot in the sampling interval. LLS was calculated according to King (1994),

\[
LLS = 2 \times \overline{NL}/(P_L^{-1} + E_I^{-1})
\]

where \(\overline{NL}\) is the average number of leaves.

To quantify specific leaf area (SLA) of the eelgrass and the biomass of epiphytes on eelgrass leaves, five leaf bundles were sampled, and four younger leaves were collected from each leaf bundle every month. Salt and any remaining debris on the leaves were then removed by rinsing with distilled water. The leaf area was measured using image analysis software (HANAKO ver. 13, Just System) after the leaves were photographed on graph paper using a digital camera (EOS Kiss digital, Canon). Following this, leaves (and associated epiphytes) were freeze-dried, and the dry weight of the leaves plus epiphytes was determined. The epiphytes, which had naturally attached to and grown on the eelgrass leaves, were then removed, and the dried leaves without epiphytes were weighed. The dry weight of epiphytes was determined by subtracting leaf weight without epiphytes from leaf weight with epiphytes. Finally, SLA and epiphyte biomass per eelgrass leaf area were calculated for each leaf age, which was defined as the relative rank of a leaf in a leaf bundle; for example, the youngest leaf and second youngest leaf were labeled Leaf 1 and Leaf 2, respectively. Here, epiphyte biomass on Leaf 3 was selected as the representative value because eelgrass leaf number varies within samples and plants did not always have four leaves; data for epiphyte biomass on Leaf 4 was thus insufficient for statistical analysis.

Water temperature in the mesocosm pool was measured on weekdays only using a water quality meter (U-21XD, HORIBA). Irradiance (290–3000 nm) reaching the ground surface was collected every ten seconds using an irradiance meter by the Japan Meteorological Agency from the meteorological observatory in Tokyo (53 km from Kurihama Bay), and converted into daily irradiance (MJ m\(^{-2}\) day\(^{-1}\)).

Statistical analyses

First, we examined the effects of water temperature and irradiance on LLS but excluded the effect of epiphyte biomass as an explanatory variable to avoid the possibility of multicollinearity with these environmental factors. We then tested the effects of water temperature and irradiance on epiphyte biomass on eelgrass leaves. In these examinations, we used the generalized linear model. A priori selection of candidate models was based on the principle of parsimony and scientific plausibility (Burnham and Anderson 2002). The global model was fitted with explanatory variables (water temperature and irradiance in all analyses, and leaf age in the analysis of epiphyte biomass on leaf) and second order interactions. In these analyses, we used log-transformed epiphyte biomass on eelgrass leaves and monthly averaged irradiance because daily irradiance was too variable.

Akaike’s information criterion (AIC) was used to compare the fits of candidate models. When the ratio of the sample size to number of predictor variables in the global model was less than 40, the bias-corrected AIC (AIC\(_C\)) was used (Hurvich and Tsai 1989). The best fitting model has the smallest AIC (AIC\(_\text{min}\)), and the difference in AIC values between models indicates the relative support for the models. Therefore, for model i, the AIC difference \((\Delta)\) was calculated as: AIC\(_i\)–AIC\(_\text{min}\). Because the best fitting model is often highly variable and thus has uncertainty, inference based on a single best fitting model can cause bias. To reduce model selection bias, multimodel inference was implemented using a confidence set of models (Burnham and Anderson 2002). A confidence set was selected to include only the candidate models with \(\Delta < 4\), because models with \(\Delta > 4\) are considerably less likely to approximate the true model (Burnham and Anderson 2002).

To interpret the relative likelihood of a model in confidence sets, an Akaike weight \((w_i)\) was calculated according to Burnham and Anderson (2002); \(w_i\) is the probability that model i would be selected as the best fitting model in the confidence set. Selection probability for predictor variables was calculated by summing \(w_i\) across all the models in the confidence set. Such a model averaging technique, weighted by \(w_i\), was also used for coefficient estimates. For coefficient \(\beta_j\), the model-averaged estimate \(\bar{\beta}_j\) was calculated as:
\[ \hat{\beta}_j = \sum_{i=1}^{R} w_i \hat{\beta}_{ij} \]  

where \( \hat{\beta}_{ij} \) is the estimate of \( \beta_j \) if predictor \( j \) is included in model \( k_i \), or is zero otherwise (Burnham and Anderson 2002). The standard error of \( \hat{\beta}_j \) was calculated as:

\[ \text{SE}(\hat{\beta}_j) = \sum_{i=1}^{R} w_i \sqrt{\text{SE}(\hat{\beta}_{ij})^2 + (\hat{\beta}_j - \hat{\beta}_{ij})^2} \]  

The bias on coefficient estimates was estimated as: 

\[ \text{Bias} = \frac{\text{SE}(\hat{\beta}_j)}{\hat{\beta}_j}. \]  

In the present study, the predictor variables that meet both the selection probability > 0.8 and the bias on coefficient estimates <1.5 are considered to be strongly supported by the models.

If the same explanatory variables were selected in models for both LLS and epiphyte biomass, we constructed a path diagram for LLS, which included the explanatory variables as direct effects and also included indirect effects via epiphyte biomass and latent variables; contributions of these effects on LLS were tested using structural equation modeling (SEM). Because epiphyte biomass on eelgrass leaves was measured monthly, this analysis used a data set consisting of monthly averages for epiphyte biomass on eelgrass leaves, LLS, and water temperature. In addition, we assumed that epiphyte biomass on Leaf 3 (the mature leaf stage), was a representative value of epiphyte biomass on eelgrass leaves and used log-transformed epiphyte biomass on Leaf 3 for the path analysis.

These statistical analyses were performed using R ver. 2.4.1, and path analysis was run using the SEM library of the R (Fox 2006).

**Results**

Seasonal variation in water temperature was substantial, ranging from 9.2°C in January 2006 to 28.0°C in September 2006. Surface irradiance had a different seasonality than water temperature (Fig. 1a).

After eelgrass was transplanted into the mesocosm pool in February 2004, the number of eelgrass shoots increased, and the growth pattern was the same as for eelgrass in natural habitats on Japanese coasts (data not shown). The number of leaves per shoot varied seasonally from approximately three between July and October to five between December and March (Fig. 1b). LLS values were higher in January and February than in August and September, with a greater than two-fold range (from 34.9 to 89.6 days with a mean value of 60.0 day) within the year (Fig. 1c). Model selection for LLS supported a strong negative relationship with water temperature (Table 1; \( \hat{\beta} \) was negative, selection probability was 1.00, bias was 0.11; Fig. 2) but did not support a relationship with irradiance (Table 1; selection probability was 0.30, bias was 2.58). SLA on Leaf 3 ranged from 204 to 390 cm² g⁻¹.

The epiphytes were mainly crustose coralline algae. Epiphyte biomass showed an inverse seasonal variation in relation to the number of leaves (Fig. 1b) and, while almost

![Graph](image-url)

**Figure 1.** Seasonal variations of (a) irradiance and water temperature, (b) number of leaves per eelgrass shoot and epiphyte biomass on eelgrass Leaf 3, and (c) leaf life span. Error bars indicate SD.

| Table 1. Model selection result for leaf life span (LLS) in eelgrass (n = 38). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | WT              | Irrad           | WT × Irrad      | AICc            | \( \Delta_i \)  | wi              |
| AIC best       | ×               | ×               | ×               | 131.7           | 0.00            | 0.70            |
| ×               | ×               | ×               | 134.2           | 2.50            | 0.20            |
| ×               | ×               | ×               | 135.6           | 3.90            | 0.10            |
| Selection probability | 1.00          | 0.30            | 0.10            |
| \( \hat{\beta} \) | −2.53          | −0.14           | 0.01            |
| SE(\( \hat{\beta} \)) | 0.28           | 0.35            | 0.02            |
| Bias           | 0.11            | 2.58            | 2.20            |

The explanatory variables are water temperature (WT), irradiance (Irrad), and interaction between these parameters.

The predictor variable included in the selected models is shown as ×.

\( \Delta_i \), AIC difference calculated as AIC_i − AIC best; \( w_i \), Akaike weight showing the probability that model i would be selected as the best fitting model in the confidence set; \( \hat{\beta} \), model-averaged coefficient estimate; SE(\( \hat{\beta} \)), standard error of \( \hat{\beta} \); Bias, bias on coefficient estimate calculated as SE(\( \hat{\beta} \))/\( \hat{\beta} \).
undetectable on Leaf 1, biomass gradually increased with leaf age (Fig. 3). The model selection result supported that epiphyte was strongly related to the interaction between water temperature and leaf age, and to leaf age and water temperature (Table 2; selection probability 1.00, bias 0.11–1.36; Fig. 3).

From these results, which were obtained using generalized linear models, we constructed a path diagram for LLS with a direct path from water temperature and an indirect path from water temperature via epiphyte biomass on Leaf 3 (Fig. 4). This model structure was well supported by these data ($\chi^2 = 1.9 \times 10^{-14}$, DF = 0 (saturated model)). The path coefficient was much higher for the direct path than for the indirect path from water temperature on LLS (Fig. 4). The determination coefficient for the indirect path (via epiphyte biomass) to water temperature was slight, and the determination coefficient was much smaller for latent variables than for the direct path.

### Discussion

The model selection results strongly supported a negative relationship between eelgrass LLS and temperature. To our knowledge, there have been very few detailed studies measuring temporal variation in seagrass LLS (Nakaoka et al. 2003, Lee et al. 2005), and this study is the first to show the negative impact of water temperature. Because the seasonal pattern of eelgrass LLS was similar to LLS in *Zostera caulescens* in temperate coastal areas of Japan (Nakaoka et al. 2003), we suggest that the negative impact of temperature on LLS may be common in aquatic plants.

The greater determination coefficient in the direct path from temperature to eelgrass LLS indicates that temperature may be a strong explanatory for LLS regardless of epiphyte biomass. Although epiphytes may affect eelgrass LLS to a greater or lesser extent due to attenuation of irradiance (Brush and Nixon 2002), a low determination coefficient in the indirect path via epiphyte biomass means that the effect of epiphytes was camouflaged by the effect of temperature. In addition, because epiphyte biomass per leaf remains low until leaves become older (Fig. 3), we suspect that the effect of epiphytes on LLS might be conditional depending on leaf age.

Damage due to free-radical byproducts of photosynthesis has also been considered as a factor affecting LLS (Bowler et al. 1992). Photosynthetic capacity generally declines with aging (Hensel et al. 1993, Kitajima et al. 1997), and Hensel et al. (1993) suggested that the decline is caused by an imbalance between free-radical effects and the maintenance and repair of the photosynthetic apparatus. Since photosynthetic capacity of eelgrass has been shown to increase with increasing temperature (Marsh et al. 1986, Dennison 1987, Zimmerman et al. 1989), leaf deterioration in eelgrass may thus be caused by the damaging effects of free-radicals which are generated as a result of increased photosynthetic activity at elevated temperatures. These components of leaf metabolism could explain the strong relationship between LLS and temperature.

We additionally predicted that irradiance would have a substantial effect on eelgrass LLS, but model selection results did not support this. It is possible that, despite
seasonal variation in irradiance, light levels do not fall sufficiently low to disrupt resource balances such as carbon (Zimmerman et al. 1989). Physical disturbance and nutrients are also candidates for affecting LLS (Shaver 1981, Balster and Marshall 2000, Ryser and Urban 2000, Reich et al. 2004). However, the effect of physical disturbance on LLS has been excluded by use of the mesocosm, and water column nutrients in Kurihama Bay, from which seawater is introduced to the mesocosm pool, show no relationship with water temperature (T. Hibino pers. comm.). Therefore, we conclude that these factors do not affect variation in LLS, and that the effect of temperature provides the best explanation.

By comparison with the LLS and SLA of terrestrial plants (Reich et al. 1992, Poorter and Bongers 2006), values for eelgrass are relatively low and high, respectively. This means that eelgrass produces thin and wide and therefore fragile leaves (rather than thick and tough leaves), supporting a suggestion of growth–survival trade-off (physiologically active leaves have high growth but low survival; Reich et al. 1992, Poorter and Bongers 2006). The production of thin and wide leaves could result from selection to increase photosynthesis (Reich et al. 1992, Poorter and Bongers 2006) because aquatic macrophytes may profit by producing longer leaves that extend toward the water surface and that therefore obtain more light (Goldsbrough and Kemp 1987, Krause-Jensen et al. 2000).

The production of fragile leaves (thin leaves with a large surface area) results in the substantial range observed for eelgrass LLS. In turn, the variability of LLS may result in variability in nutrient use efficiency (Berendse and Aerts 1987, Eckstein et al. 1999, Kazakou 2007) and total photosynthetic rate in a shoot may also be affected through reduction in number of leaves, which determines leaf area for photosynthesis. In addition, water temperature affects eelgrass shoot survival by influencing the meristematic oxygen balances in eelgrass (Greve et al. 2003). These negative impacts of water temperature might affect eelgrass aclimatisation to global increases in water temperature in the future.

In conclusion, our results indicate that temperature is a strong explanatory for LLS variation in an aquatic plant regardless of epiphyte biomass. Temperature probably induces complex mechanism responses in eelgrass metabolism, all of which can affect LLS. This suggests that a global increase in water temperature in the future could affect eelgrass ecology, its importance for coastal ecosystem functioning and production.

Acknowledgements — We thank E. Miyoshi and S. Konuma for the installation and operation of the mesocosm facilities and R. Kimura, S. Sato, K. Kamio, and H. Murakami for help during work. We also thank T. Hibino for his offer of water quality data for Kurihama Bay and M. Nakaoka and the member of Graduate School of Science of Chiba Univ. and three anonymous reviewers for valuable comments. This research was supported by a grant to our research group from the Port and Airport Research Institute.

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