SHORT RESEARCH NOTE

Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in Southern Europe (Ria Formosa, Portugal)

S. I. Massa · S. Arnaud-Haond · G. A. Pearson · E. A. Serrão

Abstract The dwarf seagrass *Zostera noltii* is an important primary producer in Atlantic coastal ecosystems from Mauritania to southern Norway and the Mediterranean Sea. Sessile intertidal organisms existing at the interface between marine and terrestrial environments may be particularly vulnerable to environmental change. In this study, we asked how near to thermal tolerance limits natural populations of *Z. noltii* are in the Ria Formosa coastal lagoon system in southern Portugal. We recorded the maximum temperatures in the Ria Formosa during the 2007 summer, and conducted experiments to determine the sub-lethal temperature of *Z. noltii* shoots sampled at two sites located at different tidal heights. Mortality rates and photosynthetic performance were recorded within a range of heat shock temperatures between 35 and 41°C. Survival was recorded ≤37°C, while higher temperatures led to a sudden drop in photosynthetic capacity followed by mortality (shoot loss) that occurred more rapidly with increasing temperatures. At 39°C and above, the rate of shoot mortality in both sites was close to 100%, occurring between 5 and 13 days after the heat shock. Survival was ca. 95 and 90% at 35 and 37°C, respectively. From these results for *Z. noltii* populations in the Ria Formosa we estimated sub-lethal temperature to be approximately 38°C for *Z. noltii*, close to the maximum of 36°C recorded in the summer 2007. Considering predicted trajectories in the coming decades, these results raise concern as to the future viability of intertidal *Z. noltii* populations near the southernmost edge of their distribution.

Keywords Seagrass · Heat shock · Global warming · *Zostera noltii* · Photosynthesis · Chlorophyll fluorescence

S. I. Massa · S. Arnaud-Haond · G. A. Pearson · E. A. Serrão (✉)
CCMAR-CIMAR, Universidade do Algarve, Gambelas, 8005-139 Faro, Portugal
e-mail: eserrao@ualg.pt

S. Arnaud-Haond
IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France

Predictions for climate change scenarios along temperate regions of the North Atlantic forecast sea surface temperature (SST) to increase by 0.2°C per decade (IPCC, 2007), with temperatures increasing more rapidly near coastal areas than in the open ocean. The impact of such changes in marine ecosystems is, however, likely to be magnified in intertidal habitats (Helmuth et al., 2006), where shifts in range distributions have already been reported (Zacherl et al., 2003; Mieszkowska et al., 2006). Complex patterns of temperature variation may occur in the intertidal, and lethal temperatures for some organisms may be

reached during low tide periods (Wethey, 2002). Climate change will have major impacts when affecting species that play key roles in ecosystem function, as is the case for seagrasses (Duarte, 2002). The seagrass *Zostera noltii* is one such key player in ecosystem functioning, in sheltered soft bottom habitats along the Eastern North Atlantic.

Seagrasses are key-species in coastal systems, contributing most of the primary production, while also providing nursery areas and food for many invertebrate and fish species. In intertidal ecosystems, cycles of immersion and emersion mean that seagrass meadows are exposed to a wide range of environmental variation in, e.g. temperature, desiccation and solar radiation. Water temperature in small isolated pools during low tide is primarily a function of air temperature and irradiance, and can therefore, reach extremely high values, especially during summer.

In the Ria Formosa, a coastal lagoon on the South coast of Portugal, *Z. noltii* dominates the intertidal zone (Silva et al., 2005) and is one of the main primary producers in this area. For the sister species *Zostera marina* in the Ria Formosa local population extinction has been observed during the past 5 years (EAS, personal observation) in two meadows previously studied (Billingham et al., 2003, 2007). The factors responsible for this local disappearance are unknown, but it correlates with the warmest summers experienced throughout Europe, including Southern Portugal, and which have strongly affected other *Z. marina* populations (Reusch et al., 2005; Reusch, 2006). In common with *Z. marina* at higher latitudes (Reusch et al., 2005), individual growth, maximal shoot density and reproductive output of *Z. noltii* in the Ria Formosa occurs mostly during the summer (Alexandre et al., 2005; Peralta et al., 2005), in the same window of time when patches of local mortality are commonly observed (J. Silva, personal communication), coinciding with sites most exposed to desiccation during the warmest annual temperatures. There is therefore concern for persistence of the species in these habitats in the context of predicted climate change.

Photosynthesis is a heat-sensitive process, thought to be primarily due to heat labile components associated with PSII (Berry & Bjorkman, 1980; Weis & Berry, 1987; Rokka et al., 2000), and can provide evidence of heat stress before other symptoms are detected. Chlorophyll fluorescence has been shown to

be a reliable method of determining the physiological condition of photosynthetic organisms (Beer et al., 1998; Silva & Santos, 2004), since its relationship with photosynthetic activity was first observed (Kautsky & Hirsch, 1931). Rapid, non-invasive measurements using Pulse Amplitude Modulated (PAM) fluorometry have been successfully applied in several studies of seagrass species (Beer et al., 1998; Bjork et al., 1999; Schwarz et al., 2000; Schwarz & Hellblom, 2002) and PAM fluorometry has been shown to be a valid proxy for photosynthetic production of *Z. noltii* except at photoinhibitory irradiances, where photorespiration is likely responsible for deviations from expectations based on gas exchange (Beer et al., 1998; Silva & Santos, 2004). The available evidence for various seagrass species suggests that capacity to tolerate light and/or temperature stressors may be more important in setting vertical distributional limits on the shore than desiccation tolerance (Bjork et al., 1999; Ralph, 1999).

Here, we report the capacity of *Z. noltii* to resist and recover from heat shock during simulated low tide exposures in one of the warmest parts of its range, the Ria Formosa Natural Park in southern Portugal. Experimental stress exposures were based on some sparse water temperature data (unpublished) available for the Ria Formosa, and confirmed later on with daily records in natural *Z. noltii* stands. Shoot mortality rates and photosynthetic performance were monitored over a time course of several days following the stress. The natural thermal environment during summer was recorded by logging the temperature in an intertidal *Z. noltii* meadow in the Ria Formosa, at three different depth levels, over a period of ca. 7 weeks in order to record the maximum temperatures *Z. noltii* currently experiences.

In situ temperature profiles

Temperature loggers (DS1921G Thermochron® iButton®, ±1°C accuracy, 0.5°C resolution) were placed at three heights from the upper to lower limits in natural *Z. noltii* meadows at Ramalhete (Ria Formosa) to record summer temperatures. At each of the three heights, two iButtons® were placed 2 cm below the surface (in the sediment) and two above surface (near *Z. noltii* leaves). Therefore, four replicate measurements were planned per tidal height.

However, some iButtons® were lost during the course of the experiment such that in the sediment at medium and low tide levels, respectively, only two and one data series could eventually be collected. Temperature data were recorded every 60 min from 30th of July to the 12th of September 2007, which spans the period when temperatures usually reach their annual maxima (Instituto de Meteorologia, Lisbon, Portugal).

Heat shock experiments

Zostera noltii plants were collected in sets of several shoots connected by one rhizome in April 2007 at two sites in the Ria Formosa: Ramalhete (37°00′18″N, 7°58′01″W) and Praia de Faro (37°00′15″N, 7°59′16″W). Since the size of *Z. noltii* clones in the Ria Formosa may reach several meters (Diekmann et al., 2005), plants were collected haphazardly across the meadow with a minimum distance of 10 m between samples in order to minimise clonal repetition. The populations sampled were representative of a range of tidal exposure conditions that can be met throughout the Ria Formosa. The meadow from Ramalhete is exposed to air for up to 6 h during spring low tides, whereas Praia de Faro is lower in the intertidal, and plants do not become completely dry, but remain covered by residual, very shallow water during daily low tide periods.

Samples of *Z. noltii* were placed in small plastic aquaria (approx. 2.5 l) re-planted in approximately 10 cm sediment from the Ria Formosa, in an outdoor tank (1.5 × 1.5 m, maximum seawater volume 225 l) with continuous seawater flow and artificially simulated tides. Plants were acclimated for approximately 1 month before heat shock tests were performed at four temperatures between 35 and 41°C. For each temperature treatment, 20 individual shoots from each site were transferred into one individual small plastic aquarium (approx. 2.5 l) and re-planted in sediment, under approximately 5 cm aerated seawater. Additionally, one aquarium per site was similarly prepared with 20 individual shoots, to act as a control for the temperature stress. Both control aquaria were kept in the tank with continuous water flow and simulated tides during the course of the experiments. The heat shock was applied for 3 h, to mimic a low tide exposure, by warming the water with thermostatic heaters until the temperatures to be tested (35, 37, 39 or 41°C) were reached. Temperatures were chosen on the basis of unpublished data, and compared afterwards with data obtained from iButtons® showing 36°C as maximum temperature in 2007 (here detailed) and 38°C as maximal temperature in July 2008 in order to ensure that they were appropriate proxies for predicted increases due to climate change. After the heat shock, ambient seawater was gradually added (approx. flow rate 0.3 l/min) to the small aquaria to return the temperature to its initial level. The small aquaria with the shoots were then transferred back to the large tank with simulated tides.

The physiological effects of heat shock were assessed from chlorophyll fluorescence measurements of F_v/F_m, the maximum quantum yield of photosystem II (PSII), using a portable fluorometer (FMS2, Hansatech, UK). Measurements were performed over time for each shoot in the four temperature treatments and in both controls. Prior to fluorescence measurements, leaves were dark-adapted for a minimum of 10 min, after which the minimum (F_o) and maximum (F_m) fluorescence yield of open and closed PSII reaction centres before and after a saturating light pulse, respectively, were determined. F_v/F_m was measured periodically after the heat shock in all shoots, until they had recovered or died (determined by the brownish-black discoloration of the leaves or loss of them). The survey was stopped in the treatments that still had surviving shoots after approximately 3 weeks, when significant mortality was no longer occurring and most of the remaining shoots had reached F_v/F_m values that did not differ significantly from those in controls.

Mean and SE of the F_v/F_m values for every available shoot were estimated for each time step, treatment and population. We performed a factorial ANOVA (STATISTICA 7.0© StatSoft, Inc.) to address three specific questions at particular points in time: (i) are any differences detectable between the initial F_v/F_m ratio of plants from both sites (by comparing values before experiments, at $t = 0$, performing an ANOVA among treatments and sites); (ii) How is photosynthetic efficiency affected by heat-shock conditions (by comparing values before mortality peaks, at $t = 24$ h, performing an ANOVA among treatments and sites); (iii) Is survival different among treatments, and do the survivors recover the same photosynthetic efficiency

(comparing percentage survival and F_v/F_m values of survivors, controls and sites, using ANOVA at the end of the experiment).

In both populations, an increase in water temperature led to a decrease in all fluorescence parameters, suggesting a high thermal sensitivity of primary photochemical processes in *Z. noltii* shoots. At 41°C, in both populations, all shoots survived the first 3 days of experiment, but after that there was a clear drop in survival eventually leading to 100% shoot mortality for both sites (Fig. 1a, b). At 39°C, for both populations, shoot survival was >95% until the fifth day, at the seventh day there was a sudden drop and mortality reached 25% (Fig. 1a, b). Over 90% of the shoots were dead by the 17th day and by the end of the survey there was only one surviving shoot from Praia de Faro (Fig. 1a, b) in the 39°C temperature treatment. Shoots from Praia de Faro had a survival rate of at least 95% at both 35 and 37°C (Fig. 1a), similar to values for Ramalhete (94 and 80% survival at 35 and 37°C, respectively) (Fig. 1b).

The initial mean ratio of shoot F_v/F_m was not significantly different between Ramalhete and Praia de Faro [factorial ANOVA, $P > 0.05$, $n = 20$ (except for treatments Praia de Faro control, Praia de Faro at 41°C and Ramalhete at 41°C, for which $n = 19$)], and ranged from 0.692 to 0.775 in Ramalhete, and from 0.741 to 0.799 in Praia de Faro (Fig. 1c, d). Twenty-four hours after the heat shock, F_v/F_m was significantly different between treatments (factorial

ANOVA, $P = 0.000$). The lowest F_v/F_m values were recorded on the third day for Praia de Faro (0.575) (Fig. 1c) and on the seventh day for Ramalhete (0.665) (Fig. 1d), followed by a gradual increase of F_v/F_m in surviving shoots to their initial level, despite the fact that shoot mortality was still occurring (Fig. 1a, b).

The sensitivity of shoots to a single short exposure of >37°C can be compared with the maximum temperature observed and predicted in this part of the species range. Temperatures in natural stands recorded from July 30th to September 12th, 2007 at Ramalhete are shown in Fig. 2. Tidal heights for that period are also shown in Fig. 2. Temperature in intertidal meadows in Ria Formosa usually ranges from 15 to 35°C (Alexandre et al., 2004); the data collected this summer shows that sediment/surface temperatures in *Z. noltii* stands routinely reached 30°C at low tide in August, with even higher temperatures during spring tides (Fig. 2). However, the most exposed plants are more subject to air than to water temperature during low tide, and the plants covered by a thin layer of water may be exposed to even higher temperatures linked to the effect of irradiance (cf. Fig. 2a, b), with temperatures measured in the canopy of *Z. noltii* reaching a maximum of 36°C, while upper sediment maxima were on average 5°C lower. It is noteworthy that temperature regimes in these intertidal ecosystems vary not only temporally, but also between sites within the same

Fig. 1 Survival rate **a**, **b** and F_v/F_m **c**, **d** for *Z. noltii* shoots from the two sampled sites, Praia de Faro and Ramalhete, at 35, 37, 39 and 41°C, and in controls (no heat shock applied). Values for F_v/F_m are means ± SE (initial $n = 20$)
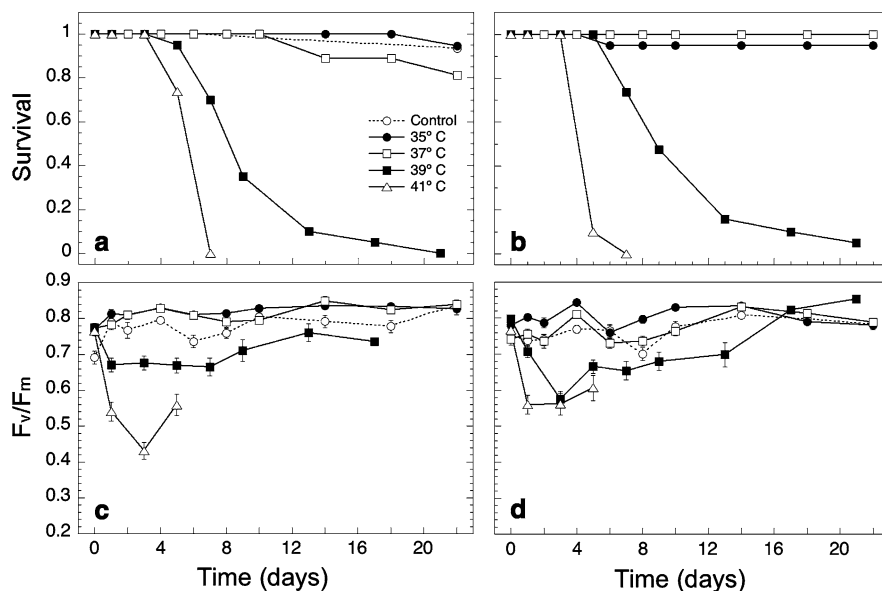
habitat and even between different parts of the same 'individual' (i.e. clone).

Changes in global average surface warming due to rising greenhouse gas concentrations lead to expect an increase of at least 2–4.5°C to be reached within the next 100 years for air temperature, and 1–3°C for SST (IPCC, 2007). Although no data are available for coastal and lagoon seawater, intertidal organisms that are exposed in air or covered by shallow films of water at low tide are likely to be more influenced by air than sea temperature changes. This suggests that in the next century the physiological tolerance of Z. noltii may be reached in the warmest habitats of its range, such as southern shallow coastal lagoons, where they are exposed to high air temperatures at low tide. This is particularly true as we applied a single heat shock of 3 h, whereas predicted increases in temperature would imply longer, and multiple, exposures to extreme temperatures. Although the Ria Formosa is not at the edges of the distribution for this species, it might be one of the most extreme environments this plant has to cope with. This shallow coastal lagoon indeed reaches extreme temperatures and irradiances during the summer (Silva & Santos, 2003; Alexandre et al., 2004) and the greatest mortality for Z. noltii has been recorded in the summer (J. Silva, personal communication), coinciding with the season for greatest shoot production and reproductive output (Alexandre et al., 2005), as for the sister species Z. marina during this season (Reusch et al., 2005). It is thus during the main growth and reproductive season of Z. noltii that events of local mortality occur in sites under most extreme tidal exposure conditions, suggesting that the present temperatures occurring in the Ria Formosa during summer are already close to the physiological limits of this species. Therefore, the predicted increase of about 0.2°C per decade in the global temperature for the next two decades (IPCC, 2007) may be especially harmful for Z. noltii in many habitats and may affect its distributional range. Local extinction of this species in the Ria Formosa would have a considerably negative effect on the ecosystem, with potential consequences on the very economically important bivalve production in this coastal lagoon.

Our results demonstrate the sensitivity of Z. noltii to slight increases in water temperature as it approaches its physiological limits, as even an increase of 2°C causes an increase in shoot mortality from 5–20% to almost 100%. The limiting temperature for Z. noltii shoots in the Ria Formosa for a 3 h heat shock seems to be slightly above 37°C, as mortality was, respectively, 0% and approx. 19% of all shoots used in this temperature treatment in Praia de Faro and Ramalhete. Also, at the end of the experiment photosynthetic efficiency of the surviving plants did not differ among treatments (factorial ANOVA on F_v/F_m values, $P > 0.05$, either using all surviving shoots or using the same sample size for all treatments, i.e. $n = 12$), but differed significantly between populations (factorial ANOVA, $P = 0.000$, $n = 12$), revealing a difference in the physiology of plants from different sites after a heat shock and 2 months in common-garden culture conditions. The observed population differences in photosynthetic efficiencies following recovery suggest that there may be local adaptation or phenotypic acclimation to specific sites within the coastal lagoon. One month acclimation period in "common-garden" conditions should have limited/buffered short-term phenotypic differences, and a significant difference between populations after this period, plus about one extra month in common experimental conditions therefore suggests a genetically based effect in the ability to recover from the stress. This would support the hypothesis of local adaptation in these populations. Despite the small distance scales separating populations inside the Ria Formosa, outbreeding depression, one possible consequence of local adaptation, has been reported between populations of the congeneric species Zostera marina also within this coastal lagoon (Billingham et al., 2007). In the Z. noltii populations studied here, the most obvious difference in selective pressure between sites is the level and duration of exposure at each site during low tide. Accordingly, plants at Praia de Faro are exposed to emersion-stress less often (and for shorter periods) and may therefore be expected to experience lower selective pressure for temperature stress resistance than plants at Ramalhete. It remains to be seen if other factors (e.g. population size, genetic diversity) may play a role in generating the observed differences. Whatever the ultimate cause, this difference raises interesting questions about the possible roles of genetic composition, phenotypic plasticity, demographic factors and/or selective processes in shaping the diversity and distribution of Z. noltii along its distribution range, and particularly in extreme conditions such as those encountered in the Ria Formosa.

Fig. 2 Temperature data recorded in the *Z. noltii* meadow in Ramalhete, once every 60 min, from 30/07/2007 to 12/09/2007: **a** upper intertidal canopy, **b** upper intertidal sediment, **c** mid intertidal sediment and **d** lower intertidal sediment. Replicate measurements were recorded at each tidal height/microhabitat (full and dotted lines), with the exception of the lower intertidal sediment. Panel **e** shows tide heights for Barra Faro-Olhão for the period between 30/07/2007 and 12/09/2007 (data from Instituto Hidrográfico). Maximum temperature registered each day is shown in three ranges in the colour bar below the graph (<30°C in white, 30–33°C in grey, >33°C in black), as well as the number of hours it reached temperatures above 30°C
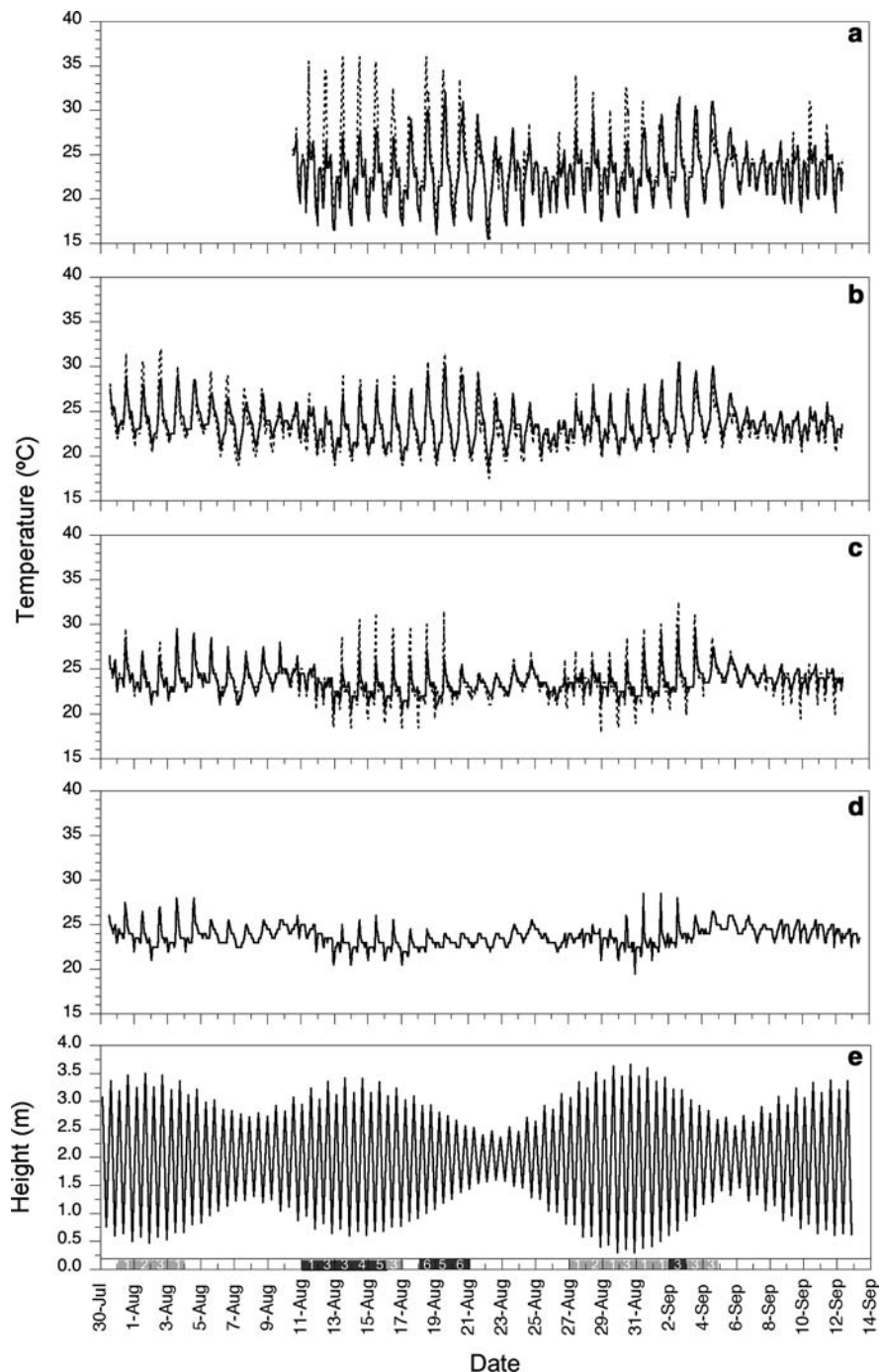
According to various models and scenarios, global temperature is expected to increase between 2.0 and 4.5°C, with a best estimate of about 3°C, by the year 2100 (IPCC, 2007). This study suggests that this predicted increase may have consequences on the persistence of *Z. noltii*, and on the ecosystem based on this habitat structuring species, in the warmer habitats of their ranges, as shown here in a shallow coastal lagoon in southern Europe.

References

Alexandre, A., J. Silva & R. Santos, 2004. The maximum nitrate reductase activity of the seagrass *Zostera noltii* (Hornem.) varies along its vertical distribution. Journal of Experimental Marine Biology and Ecology 307: 127–135.

Alexandre, A., R. Santos & E. Serrao, 2005. Effects of clam harvesting on sexual reproduction of the seagrass *Zostera noltii*. Marine Ecology Progress Series 298: 115–122.

Beer, S., B. Vilenkin, A. Weil, M. Veste, L. Susel & A. Eshel, 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. Marine Ecology Progress Series 174: 293–300.

Berry, J. & O. Bjorkman, 1980. Photosynthetic response and adaptation to temperature in higher-plants. Annual Review of Plant Physiology and Plant Molecular Biology 31: 491–543.

Billingham, M. R., T. B. H. Reusch, F. Alberto & E. A. Serrao, 2003. Is asexual reproduction more important at geographical limits? A genetic study of the seagrass *Zostera marina* in the Ria Formosa, Portugal. Marine Ecology Progress Series 265: 77–83.

Billingham, M. R., T. Simoes, T. B. H. Reusch & E. A. Serrao, 2007. Genetic sub-structure and intermediate optimal outcrossing distance in the marine angiosperm *Zostera marina*. Marine Biology 152: 793–801.

Bjork, M., J. Uku, A. Weil & S. Beer, 1999. Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. Marine Ecology Progress Series 191: 121–126.

Diekmann, O. E., J. A. Coyer, J. Ferreira, J. L. Olsen, W. T. Stam, G. A. Pearson & E. A. Serrao, 2005. Population genetics of *Zostera noltii* along the west Iberian coast: consequences of small population size, habitat discontinuity and near-shore currents. Marine Ecology Progress Series 290: 89–96.

Duarte, C. M., 2002. The future of seagrass meadows. Environmental Conservation 29: 192–206.

Helmuth, B., N. Mieszkowska, P. Moore & S. J. Hawkins, 2006. Living on the edge of two changing worlds: forecasting the responses of rocky intertidal ecosystems to climate change. Annual Review of Ecology Evolution and Systematics 37: 373–404.

Instituto de Meteorologia, Lisbon, Portugal. Normais Climatológicas 1961–1990. www.meteo.pt.

Instituto Hidrográfico, Previsão das Marés, www.hidrografico.pt.

IPCC, 2007. Climate Change 2007: The Physical Science Basis. Summary for Policymakers: 1–21.

Kautsky, H. & A. Hirsch, 1931. Neue Versuche zur Kohlenstoffassimilation. Naturwissenschaften 19: 964.

Mieszkowska, N., M. A. Kendall, S. J. Hawkins, R. Leaper, P. Williamson, N. J. Hardman-Mountford & A. J. Southward, 2006. Changes in the range of some common rocky shore species. A response to climate change? Hydrobiologia 555: 241–251.

Peralta, G., F. G. Brun, I. Hernandez, J. J. Vergara & J. L. Perez-Llorens, 2005. Morphometric variations as acclimation mechanisms in *Zostera noltii* beds. Estuarine Coastal and Shelf Science 64: 347–356.

Ralph, P. J., 1999. Photosynthetic response of Halophila ovalis (R-Br.) Hook. f. to combined environmental stress. Aquatic Botany 65: 83–96.

Reusch, T. B. H., 2006. Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. Molecular Ecology 15: 277–286.

Reusch, T. B. H., A. Ehlers, A. Hammerli & B. Worm, 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences of the United States of America 102: 2826–2831.

Rokka, A., E. M. Aro, R. G. Herrmann, B. Andersson & A. V. Vener, 2000. Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature. Plant Physiology 123: 1525–1535.

Schwarz, A. M. & F. Hellblom, 2002. The photosynthetic light response of Halophila stipulacea growing along a depth gradient in the Gulf of Aqaba, the Red Sea. Aquatic Botany 74: 263–272.

Schwarz, A. M., M. Bjork, T. Buluda, H. Mtolera & S. Beer, 2000. Photosynthetic utilisation of carbon and light by two tropical seagrass species as measured in situ. Marine Biology 137: 755–761.

Silva, J. & R. Santos, 2003. Daily variation patterns in seagrass photosynthesis along a vertical gradient. Marine Ecology Progress Series 257: 37–44.

Silva, J. & R. Santos, 2004. Can chlorophyll fluorescence be used to estimate photosynthetic production in the seagrass *Zostera noltii*? Journal of Experimental Marine Biology and Ecology 307: 207–216.

Silva, J., R. Santos, M. L. Calleja & C. M. Duarte, 2005. Submerged versus air-exposed intertidal macrophyte productivity: from physiological to community-level assessments. Journal of Experimental Marine Biology and Ecology 317: 87–95.

Weis, E. & J. A. Berry, 1987. Quantum efficiency of photosystem-II in relation to energy-dependent quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta 894: 198–208.

Wethey, D. S., 2002. Biogeography, competition, and microclimate: the barnacle Chthamalus fragilis in New England. Integrative and Comparative Biology 42: 872–880.

Zacherl, D., S. D. Gaines & S. I. Lonhart, 2003. The limits to biogeographical distributions: insights from the northward range extension of the marine snail, Kelletia kelletii (Forbes, 1852). Journal of Biogeography 30: 913–924.