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Environmental tolerances of free-living coralline algae (maerl): implications for European marine conservation

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Abstract

Maerl is a general term used for loose-lying subtidal beds of nodular coralline red algae. Maerl beds support high associated invertebrate and algal biodiversity, and are subject to European and UK conservation legislation. Previous investigations have shown European maerl to be ecologically fragile due to growth rates of approximately 1 mm per year. However, these very slow growth rates have hampered attempts to determine the key ecological requirements and sensitivity characteristics of living maerl. In this study, photosynthetic capacity determined by pulse amplitude modulated (PAM) fluorometry was used as a diagnostic of stress caused by various environmental conditions. Maerl species were exposed to a range of temperatures, salinities and light levels and to burial, fragmentation, desiccation and heavy metal treatment. Maerl was not as susceptible as previously assumed to extremes of salinity, temperature and heavy metal pollution, but burial, especially in fine or anoxic sediments, was lethal or caused significant stress. These data indicate that the main anthropogenic hazard for live maerl and the rich communities that depend on them is smothering by fine sediment, such as that produced by trawling or maerl extraction, from sewage discharges or shellfish and fish farm waste, and sedimentation resulting from disruption to tidal flow.

Keywords: Maerl; Pulse amplitude modulated fluorometry; Temperature; Light; Salinity; Fragmentation; Burial; Metal contamination; Conservation; Sewage discharge; Fish farm waste

1. Introduction

1.1. Maerl beds

The term maerl refers to calcified marine algae (Rhodophyta, Corallinales) that grow unattached and usually lack articulated joints. Maerl (Fig. 1) can accumulate to form large beds of live and dead material under favourable conditions in temperate and tropical waters. The branched twig-like thalli lock together into a lattice which provides ecological niches for a diverse range of seaweed and invertebrate species, some of which may be confined to the maerl habitat (Keegan, 1974; Bosence, 1976; Foster, 2001; Steller et al., 2003).

Maerl has traditionally been used an a soil conditioner on a small scale in Galicia (Spain), Brittany (France) and western Ireland and Scotland, but more recently it has been industrially extracted by dredging in Brittany, Cornwall (England) and Bantry Bay, Ireland (Grall and Hall-Spencer, 2003). Legislation has been proposed to minimize the destruction of maerl beds (reviewed by Donnan and Moore, 2003). Two maerl-forming species, *Phymatolithon calcareum* and *Lithothamnion corallioides*, are included in Annex V of the EC Habitats Directive (Council Directive 92/43/EEC), as species of community interest whose taking in the wild and exploitation may be subject to management measures. Within the UK, maerl biotopes are protected as a key habitat under the Joint Nature Conservation Committee's interpretation of the Habitats Directive, in the category 'sand banks slightly covered by seawater at all times'. The UK Biodiversity Action Plan (BAP) includes a habitat action plan for maerl beds (UK Biodiversity Group, 1999). Four of the UK's candidate

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Fig. 1. Thalli of *P. calcareum* maerl from Strangford Lough, Northern Ireland, labelled with beads attached by fishing line.

marine Special Areas of Conservation (SACs) include maerl and one, the Sound of Arisaig, was designated primarily for its maerl beds. In France, maerl occurs in 14 out of 28 Natura 2000 sites proposed along the Breton coast (Grall and Hall-Spencer, 2003).

Maerl is considered a non-renewable resource due to its very slow growth rate, approximately 1 mm (0.5–1.5 mm) per year (Bosence and Wilson, 2003; Blake and Maggs, 2003), compared to harvests of thousands of tonnes per annum in Brittany (Briand, 1991). Extensive dead maerl beds in Atlantic European waters suggest that environmental conditions may previously have been more suitable for maerl growth. The most common and widespread species in Europe, *P. calcareum*, is nevertheless assumed to have fairly narrow ecological requirements, judged by its distribution in relation to controlling environmental factors (Bosence, 1976). Although the most significant threats to the functionally diverse and species-rich infaunal communities of maerl beds have been evaluated (Barbera et al., 2003; Hall-Spencer et al., 2003), ecological sensitivies of the maerl species themselves are very poorly known due to the difficulties of working with these slow-growing calcified algae (Birkett et al., 1998). Living maerl is essential to the survival of maerl beds and their associated high biodiversity (Barbera et al., 2003), so it is important to understand the environmental factors that permit maerl thalli to survive and grow.

The aim of the present study was to fill gaps in the understanding of key ecological requirements and sensitivity characteristics of living maerl that is needed for compliance with the Habitats Directive, to meet the UK BAP, to inform conservation management of SACs and for the Oslo and Paris Convention (protecting the marine environment of the north-east Atlantic). We review the most important environmental factors and potential threats influencing survival and growth of maerl. We present Pulse Amplitude Modulated (PAM) fluorometry data for photosynthetic capacity, a proxy for stress levels, of maerl species under a wide range of laboratory conditions. Finally, we evaluate the implications of these data for maerl bed conservation in Europe.

1.2. Environmental tolerances of maerl

1.2.1. Temperature

Temperature has long been known to be the primary determinant of geographical distribution, because the boundaries of biogeographical regions are associated with isotherms (Lüning, 1990). Maerl biotopes occur in a wide range of temperature regimes, from the tropics to northern Norway, but the species composition of the maerl beds is greatly influenced by temperature. Adey and Adey (1973) showed that the distribution of coralline algal species in the North Atlantic could be correlated with temperature/habitat boundaries. An obvious temperature-related maerl phenomenon in the UK is the absence of *Lithothamnion corallioides* from Scotland, either because winter temperatures occasionally drop below the minimum survival temperature of this species (between 2 and 5 °C) or because temperatures do not remain high enough for long enough to support sufficient annual growth (Adey and McKibbin, 1970). Laboratory studies on Spanish maerl showed that *P. calcareum* survived down to 2 °C, dying at 0.4 °C, and the optimum temperature for growth was 15 °C (Adey and McKibbin, 1970). *L. corallioides* had a higher minimum survival temperature, dying at 2 °C and surviving without growth at 5 °C (Adey and McKibbin, 1970). Temperature appears to confine *Lithothamnion glaciale* to northern parts of the British Isles, possibly because reproductive conceptacles are only produced in winter when water temperatures are below 9 °C (Hall-Spencer, 1994), although maerl species are known to propagate mainly by fragmentation.

1.2.2. Salinity

Maerl beds were previously thought to occur where salinity was depressed (Joubin, 1910), but although the surface salinity in the vicinity of maerl beds in France and Ireland is often low, the bottom water is generally fully saline (Bosence, 1976). King and Schramm (1982) found that growth of *P. calcareum* is impaired at salinities below 24 (practical salinity scale) whereas *L. glaciale* is presumed to be tolerant of low salinities because it occurs in Scottish sealochs (Connor et al., 1997).

1.2.3. Irradiance

Irradiance requirements of maerl species are not known (Birkett et al., 1998). Many coralline algae are low-light-adapted. This holds both for jointed, erect species (Hader et al., 1996) and for crustose forms (Kuhl et al., 2001; Roberts et al., 2002). For example, in the

crustose Arctic species *Phymatolithon foecundum*, the compensation irradiance was only 1.6 μmol photons $m^{-2} s^{-1}$, and photosynthetic saturation, determined using PAM techniques, was at about 40 μmol photons $m^{-2} s^{-1}$(Kuhl et al., 2001). By contrast, however, the tropical reef species *Hydrolithon onkodes* had saturating irradiance values of 200–600 μmol photons $m^{-2} s^{-1}$, depending on the degree of photoacclimation, and photoinhibition was observed only at 1600 μmol photons $m^{-2} s^{-1}$ (Payri et al., 2001).

1.2.4. Desiccation

Beds containing live thalli are found only below astronomical low water mark, so maerl is presumed to be highly sensitive to desiccation and unable to withstand emersion (Birkett et al., 1998).

1.2.5. Burial and sedimentation

Water movement is a key factor concerning the distribution of maerl. Extensive maerl beds are found in areas where there are moderate to strong seabed currents, or in relatively open, yet sheltered, areas with sufficient wave action, such as coastal bays or inlets (Bosence, 1979). It has therefore been suggested that maerl beds require both shelter from wave action to prevent burial of thalli and enough water movement to prevent smothering with silt (Hall-Spencer, 1998).

Scallop dredging in Scotland buried maerl up to 8 cm deep and later a plume of sediment settled out on the substratum (Hall-Spencer and Moore, 2000). Dredging reduced the number of live maerl thalli by more than 70%, with no sign of recovery after four years. Commercial extraction of maerl likewise releases fine particles that settle on the communities causing degradation (De Grave and Whitaker, 1999; Grall and Hall-Spencer, 2003). The loss of live maerl following burial or sedimentation is thought to result from its death due to lack of light when buried (Steller and Foster, 1995; Hall-Spencer and Moore, 2000), but actual experimental data are lacking. Organic input and heavy metal pollution from sewage discharges in Brittany have reportedly reduced the thickness of, or entirely killed, live maerl cover (Grall and Hall-Spencer, 2003).

1.2.6. Fragmentation

Demersal fishing practices, such as scallop dredging, can crush the open lattice structure of maerl beds, leaving less interstitial space (Hall-Spencer and Moore, 2000). In the Firth of Clyde, Scotland, maerl beds subject to scallop dredging over the past century consist of significantly smaller thalli than adjacent control beds (Hall-Spencer and Moore, 2000).

1.2.7. Heavy metal contamination

There have been no previous studies on the response of maerl to heavy metal exposure; we are not aware of any experiments on other coralline algae. Elevated concentrations of heavy metals are quite common in many coastal habitats where maerl is found (Cullinane and Whelan, 1982; Rainbow et al., 2002), and thus likely to be a relevant environmental stress.

1.3. Measurement of stress in marine algae

Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists (Maxwell and Johnson, 2000). PAM technology is now used widely in studies of photosynthetic rates in marine algae, particularly for investigations of stress conditions (Hader et al., 1996; Beer et al., 2000; Parkhill et al., 2001; Kuhl et al., 2001; Ivorra et al., 2002). Compared with traditional measurements of photosynthetic rates, it is a non-invasive, non-destructive technique, so although it only yields relative photosynthetic rates it is widely applicable and versatile. The principle of chlorophyll fluorescence analysis is that light energy absorbed by chlorophyll molecules can be used to drive photosynthesis (photochemistry), dissipated as heat (non-photochemical quenching) or be re-emitted as light (fluorescence) (Schreiber et al., 1986, 1994). The three processes occur in competition with each other, so chlorophyll fluorescence yields information about changes in photochemistry and heat dissipation (Maxwell and Johnson, 2000).

The optimal quantum yield of photosystem II (PSII) is the ratio of variable to maximal fluorescence (F_v/F_m). The variable fluorescence (F_v) represents the difference between the maximal fluorescence (F_m), which is the fluorescence from the sample when all PSII reaction centres are reduced (and thus active), and the initial fluorescence (F_0), when all the PSII reaction centres are oxidized (or closed) (Buchel and Wilhelm, 1993; Hanelt, 1998; Gomez et al., 2001). A ratio of the variable to maximal fluorescence represents the quantum efficiency of that plant, which can then be compared to other readings. The F_v/F_m ratio can be used to evaluate reductions in PSII activity caused by stresses such as high temperature or by photoinhibition (Ludlow, 1987; Demmig and Bjorkman, 1987; Schreiber and Bigler, 1987). A decrease in the F_v/F_m value represents a reduction in the potential for additional photochemistry.

2. Materials and methods

2.1. Collection and acclimation of maerl

Samples of coralline algae were obtained from subtidal locations in Strangford Lough, Northern Ireland, at least four weeks before their use in experiments. *P. calcareum* was collected at a depth of 10–12 m by SCUBA diving in Castleward Bay on 4 October 2001

and 20 May 2002. *L. glaciale* was collected on stones at 3–4 m depth at Rainey Island on 15 June 2002. Conspicuous macrofauna was removed. Thalli were then held either in running seawater at ambient temperature at the Queen's University Marine Laboratory, Portaferry (in 2001), or in 10 litre tanks of aerated seawater in a constant temperature of 9 °C, under a photoperiod of 16 h light:8 h dark, at a photon irradiance of ca. 13 μmol photons m^{-2} s^{-1} (in 2002). The seawater was sourced from an intake in the Strangford Lough Narrows near the Marine Laboratory, where salinity is approximately 33 at sites monitored by Agriculture and Food Sciences Northern Ireland (AFSNI; http://www.afsni.ac.uk/services/coastalmonitoring/default.htm). Seawater was sand-filtered and UV-treated to eliminate toxins and unicellular organisms associated with seawater. Salinity and temperature were monitored regularly and kept constant. *P. calcareum* was tested under all environmental conditions described below. *L. glaciale* was used only in salinity experiments.

2.2. Experimental conditions and procedures

2.2.1. Temperature

The effects of temperature on photosynthetic capacity were investigated in two sets of experiments. All treatments were at a photoperiod of 16 h light:8 h dark, under a photon irradiance of ca. 13 μmol photons m^{-2} s^{-1}. In 2001, control thalli were maintained at 9 °C, and ten thalli were transferred to 25 °C for 5 weeks. In 2002, we focussed on comparing photosynthetic capacity at realistic winter (9 °C) and summer (17 °C) temperatures determined at sites near local *P. calcareum* beds monitored by AFSNI. Temperature was controlled constantly using thermostats (Digitherm, Algarde, Pinxton, Nottinghamshire) and 50 W aquarium heaters (Eco-therm, Aquarium systems, Padova, Italy), checked regularly and monitored with maximum-minimum thermometers. Initial PAM measurements were taken after acclimation but prior to the treatments, and thereafter measurements were made weekly for 4 or 5 weeks. In short-term experiments carried out at 40 °C, PAM measurements were made at the start and every 15 min up to 90 min.

2.2.2. Salinity

Tanks were set up at salinities of 3, 15, 33 and 40. The reduced salinity conditions were obtained by adding distilled water to the tanks containing seawater, and increased salinity was obtained by adding sea salt to normal seawater. Salinity within the tanks was measured using a hand-held refractometer cross-checked with a conductivity meter. The aerated tanks were tightly sealed with electrical tape, and regularly monitored to prevent unwanted salinity changes. Both *P. calcareum* and *L. glaciale* were subjected to salinity treatments. PAM was measured every hour for the first 12 h in each salinity condition, and thereafter every week for 5 weeks. Results were evaluated relative to the control condition (salinity of 33).

2.2.3. Irradiance

Three irradiance conditions were set up: (1) complete darkness, (2) "bright light" of 140 μmol photons m^{-2} s^{-1} (16 h light:8 h dark) and (3) control "ambient light" of 13 μmol photons m^{-2} s^{-1} (16 h light:8 h dark). PAM measurements were made after 12 and 48 h, and thereafter every week for 5 weeks.

2.2.4. Desiccation

Fifteen thalli were removed from holding tanks and placed uncovered at room temperature and ambient humidity. PAM measurements were taken after 5, 10, 15 and 30 min, and at 24 h.

2.2.5. Burial

Treatments involved burial of maerl at three depths in three different sediment types: (1) coarse clean maerl gravel (diameter 1–10 mm) collected from a maerl beach at Carraroe, Co. Galway, Ireland and washed carefully in seawater before use; (2) coarse clean sand (diameter 0.3–1.0 mm; Play Sand, Early Learning Centre, Swindon, England); and (3) muddy sand from below the redox layer, black in colour and smelling of hydrogen sulphide, collected at Dundrum Beach, Co. Down, Northern Ireland. In each condition the replicates were all kept in the same tank and the sediment was assumed to be homogeneous in composition.

Groups of ten thalli were strung on line and their F_v/F_m values recorded before burial. Ten thalli were placed at each of 0, 4 and 8 cm depths in separate tanks of maerl gravel and clean sand. In the muddy sand treatment, ten thalli were placed on the surface and at depths of 0.25 and 2 cm. Ten control thalli for each treatment were kept in separate tanks without sediment because thalli on the sediment surface became covered with a fine layer of sand due to aeration of the water. Maerl thalli were buried at the specified depths for a total of 5 weeks (2 weeks for the muddy sand treatment). At the end of the treatment, thalli were carefully removed and PAM measurements were made.

2.2.6. Fragmentation

The treatments were (1) breaking maerl thalli in half or (2) breaking off one branch from a thallus. Only one PAM measurement was made on each fragment of the maerl due to their small size. PAM measurements were taken weekly for 2 weeks.

2.2.7. Heavy metal contamination

Treatments were set up using a standard model effluent solution (Mellor, 2002) in 5 litre glass containers.

Zn:Pb:Ni:Cu:Cd were provided in the ratio 37:16:14:11:1, at a range of concentrations with Cd varying from 0.174 to 174 ppb. At a concentration with Cd at 1.74 ppb this solution is considered to be that of a standard industrial effluent. Ten thalli of *P. calcareum*, weighing approximately 10 g, were exposed to each treatment. PAM measurements were taken daily over a 7-day period.

2.3. Pulse amplitude modulated fluorometry

All measurements were made in a temperature-controlled room at 9 °C, and maerl was dark-adapted for 30 min prior to measurement. Chlorophyll fluorescence was measured with a PAM-2000 portable pulse modulation fluorometer (Heinz Walz, Germany), attached to a computer which displayed the F_v/F_m value. A decrease in F_v/F_m values indicates a reduction in the PSII activity; if this decrease is a response to an environmental factor, it can be assumed that this factor had a negative effect on the photosynthetic apparatus of the sample (Genty et al., 1989). Changes in the efficiency of heat dissipation also occur (non-photochemical quenching), in addition to changes in efficiency of photochemistry. These changes are reflected as F_m variation which cannot be inhibited totally, and all estimations of non-photochemical quenching are strictly relative to some dark-adapted point. Therefore a dark-adapted period was designed into PAM experiments.

The fibre optic bundle of the PAM was placed on the sample at an angle so the alga was not shaded, and after dark adaptation PAM reading were taken immediately in ambient light. The length of the saturating pulses was 0.6 s. Initial investigations showed that the tips of the thalli gave more repeatable readings than the centres or branches of the thalli, presumably because the more even surface for measurement provides greater accuracy. Thereafter, readings were obtained only from the tip areas of the thalli. Observations such as changes in the colour of the thallus were also noted as the F_v/F_m values were recorded. Any colour changes could be associated with the loss of accessory pigments, indicating a negative response in the photosynthetic apparatus of the thallus. For each thallus three measurements taken on different tips were considered replicates. The emersion time was minimised to ensure that the F_v/F_m values were not affected by desiccation, and the measurement only lasted around 40 s.

2.4. Statistical analysis

Data were analysed using various statistical tests. Two way Analysis of Variance (ANOVA), incorporating normality tests, was performed with SigmaStat (for Windows, v 2.03, SPSS Inc. Chicago) to identify significant differences in F_v/F_m values between conditions. Significance level of all tests was set at $\alpha = 0.05$. Tukey's test was used for multiple comparisons within each of the conditions, to isolate those conditions that were significantly different from each other. Tukey's test determines if there is a significant difference between two results based upon differences in the least square means. Error bars presented are standard errors.

3. Results

3.1. PAM values of controls

F_v/F_m values for controls of about 0.5 ± 0.1 were obtained for both maerl species. Under these conditions, there was good consistency between replicate measurements, and between 9 °C controls in 2001 and 2002 (Fig. 2(a)). In general, as more stressful conditions were applied, variability became much greater.

3.2. Experimental treatments

3.2.1. Temperature

For *P. calcareum*, there were no significant differences in F_v/F_m values between the 9 °C controls and either the 17 °C or 25 °C temperature treatments over periods of 4 or 5 weeks. There was no significant change at any temperature over the course of the experiments (Fig. 2(a)). At 40 °C, F_v/F_m values dropped significantly within 15 min and continued to decline until at 90 min the mean was <0.003, and the thalli were judged to be dead by comparison with results of the desiccation experiment (Fig. 3(b)).

3.2.2. Salinity

For *P. calcareum* (Fig. 2(b)), F_v/F_m values at 40 psu were not significantly different from those of the 33 psu controls throughout the experiment. At 15 psu, F_v/F_m values dropped significantly lower than the control during the first week ($p < 0.05$), but then recovered and were not significantly different from the control from week 2 onwards. Values for the 3 psu treatment showed a significant drop in F_v/F_m values within the first week, as in the 15 psu treatment, but failed to recover. They remained significantly lower than the control throughout the experiment, although all thalli remained alive during the 5 week period, with an F_v/F_m value of above 0.25.

For *L. glaciale* (Fig. 2(c)), the F_v/F_m values for the 3 psu treatment dropped steadily for the first 3 weeks, and were significantly lower than those of the control for the entire 5-week period of the experiment. As with *P. calcareum*, all thalli remained alive. None of the other treatments showed significantly different values from the control over the 5-week period, except for the 15 psu condition which produced significantly lower values ($p < 0.05$) than the control in week 5 (Fig. 2(c)).

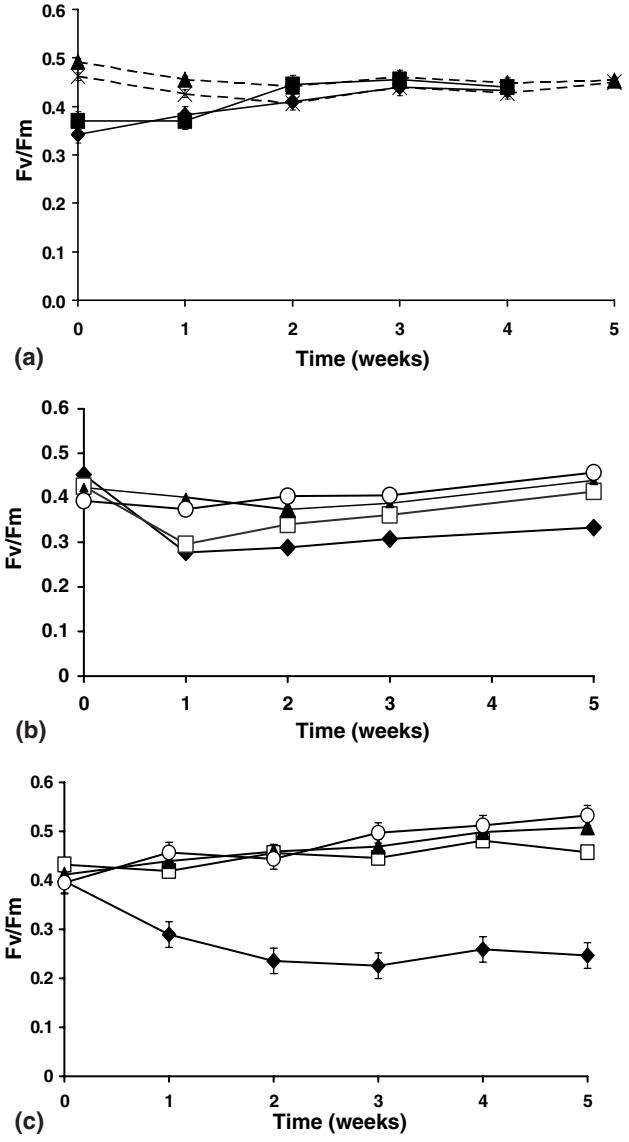

Fig. 2. (a)–(c) The effect of temperature and salinity on photosynthetic capacity of maerl. (a) The effect of temperature on F_v/F_m values of *P. calcareum* grown in aerated tanks, at a photoperiod of 16 h light:8 h dark, under a photon irradiance of ca. 13 $\mu mol\,m^{-2}\,s^{-1}$. The 25 °C treatment and its 9 °C control, both shown with dashed lines, were carried out in 2001, and the 17 °C treatment and its 9 °C control in 2002. Symbols represent: diamonds, 9 °C control for 17 °C treatment; squares, 17 °C treatment; triangles, 9 °C control for 25 °C treatment; ×, 25 °C treatment. All values are means and SEs of three PAM measurements on each of ten thalli. (b) The effect of salinity (diamonds, 3; open squares, 15; triangle, 33; open circles, 40) on F_v/F_m values of *P. calcareum* grown at 9 °C, with other conditions as described previously. (c) The effect of salinity (diamonds, 3; open squares, 15; triangle, 33; open circles, 40) on F_v/F_m values of *L. glaciale* grown at 9 °C, with other conditions as described for (a).

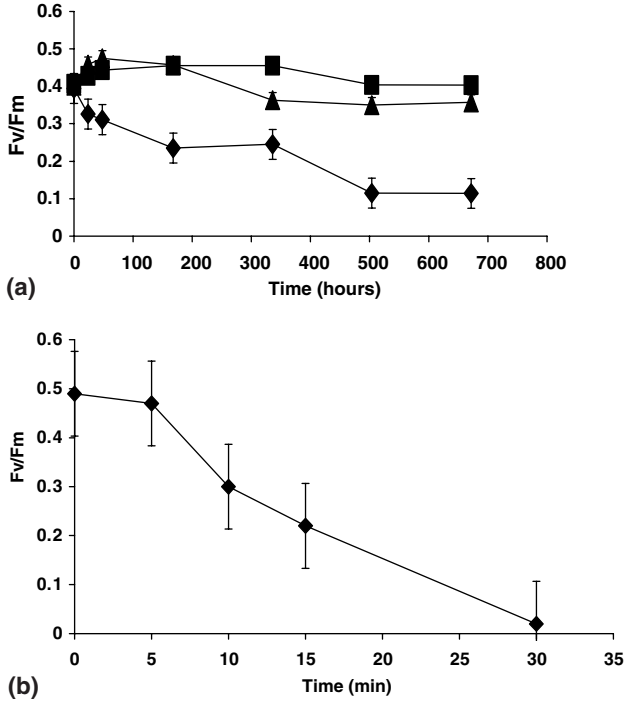

Fig. 3. (a) and (b) The effect of irradiance and desiccation treatments on the photosynthetic capacity of *P. calcareum*. (a) The effect of photon irradiance levels on F_v/F_m in maerl grown at 9 °C, with other conditions as described for Fig. 2a. "Bright" (diamonds) was 140 $\mu mol\,m^{-2}\,s^{-1}$ (16 h light:8 h dark); "control" (squares) was 13 $\mu mol\,m^{-2}\,s^{-1}$ (16 h light:8 h dark) as provided during the pre-experimental acclimation period, and "dark" treatments (triangles) were kept aerated in the dark. (b) The effect of desiccation on F_v/F_m values (diamonds) of *P. calcareum* removed from tanks and kept uncovered at room temperature and ambient humidity.

3.2.3. Irradiance

At 140 $\mu mol\,m^{-2}\,s^{-1}$ ("bright" conditions) the F_v/F_m values for *P. calcareum* were significantly lower than in the control after 24 h, and they continued to drop throughout the 4-week period. The F_v/F_m values in the dark treatment were significantly lower than for the 13 $\mu mol\,m^{-2}\,s^{-1}$control after 336 h (2 weeks), and remained significantly reduced for the duration of the 4-week experiment (Fig. 3(a)). Values for the dark treatment nevertheless were significantly higher than for the "bright" conditions.

3.2.4. Desiccation

There was a significant decrease in the mean F_v/F_m values of the *P. calcareum* maerl when the thalli had been out of water for longer than 5 min ($p < 0.005$). The F_v/F_m values steadily declined over a 30-min period (Fig. 3(b)), to a point where the thalli were determined to be dead, because no further change occurred when the desiccation period was extended to 24 h (not shown; F_v/F_m values were 0.001–0.008).

3.2.5. Burial

The F_v/F_m values of *P. calcareum* thalli on the surface and buried at both depths in both the gravel and sand conditions were significantly lower than those of the sediment-free controls ($p < 0.001$ in all instances) (Fig. 4(c)). In the gravel treatments, after 4 weeks there

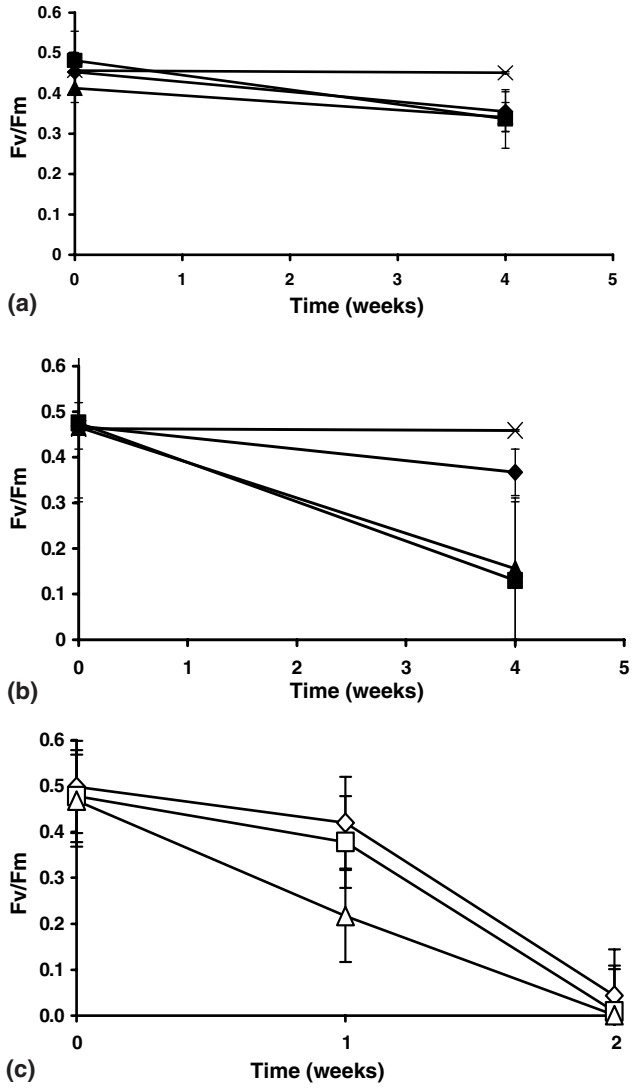

Fig. 4. (a)–(c) The effect on F_v/F_m values of *P. calcareum* of burial under different conditions. (a) The effect of burial at three depths in maerl gravel (diamonds, 0 cm depth but partially covered by gravel as a result of water motion in the tanks; squares, 4 cm; triangles, 8 cm; controls, ×) in tanks at 9 °C, with other conditions as described in Fig. 2(a). Controls were kept in a separate tank. (b) The effect of burial at different depths in coarse clean sand on the F_v/F_m of *P. calcareum*; (diamonds, 0 cm depth but partially covered by sand as a result of water motion in the tanks; squares, 4 cm; triangles, 8 cm; controls, ×) in tanks at 9 °C, with other conditions as described in Fig. 2(a). Controls were kept in a separate tank. (c) The effect of burial in anoxic muddy sand at depths of 0.25 cm (open squares), 2 cm (open triangles), and 0 cm but covered with scattered sediment by the water motion (open diamonds); all other conditions as described for Fig. 2(a).

was no significant difference between the thalli on the surface of the sediment, which had become lightly covered by fine gravel, and those at depths of 4 and 8 cm. In the sand treatments, after 4 weeks there was no significant difference between the thalli at depths of 4 and 8 cm, but thalli on the surface of the substratum had significantly higher F_v/F_m values ($p < 0.05$). All thalli in both treatments remained alive.

In the muddy sand treatments, after one week the thalli buried at 2 cm were a very pale pink while the thalli on the sediment surface and those buried under 0.25 cm were a stronger pink. All thalli had become white by the end of the second week, when they were dead as judged by PAM values (Fig. 4(c)).

3.2.6. Fragmentation

Fragmentation had no significant effect on F_v/F_m of maerl (Fig. 5).

3.2.7. Heavy metals

A significant decrease in F_v/F_m compared to the control was observed in all treatments after 24, 48 and 72 h. The most marked drop was at the highest metal concentrations (120 $ml\,l^{-1}$). Recovery then occurred, and none of the values was significantly lower than the controls after 1 week (Fig. 6).

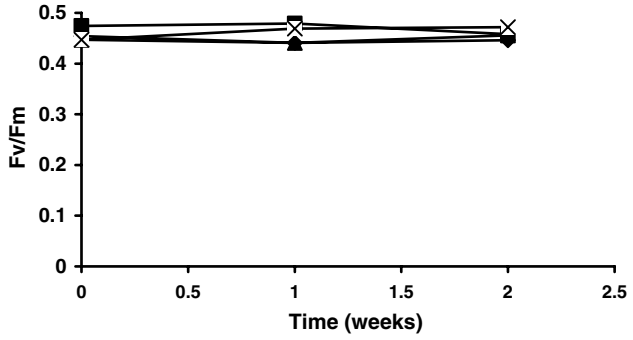

Fig. 5. The effect of fragmentation of maerl thalli into halves (triangles, ×) or by breaking off branches (''twigs'', squares) from thalli (diamonds) on the F_v/F_m of *Phymatolithon calcareum.* All other conditions were as described for Fig. 2(a).

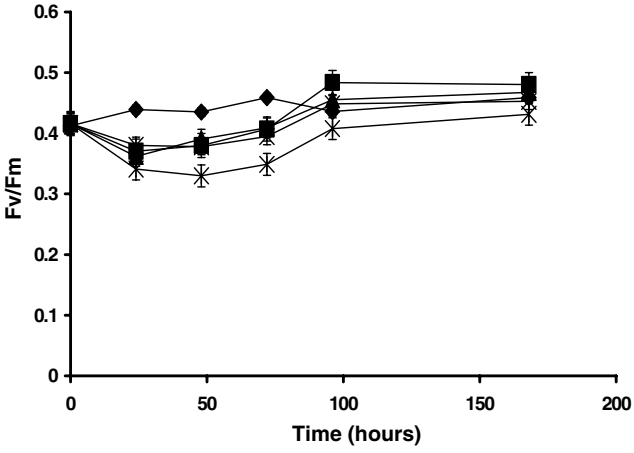

Fig. 6. The effect of various concentrations of a heavy metal model effluent (expressed as ml of effluent per litre of seawater: diamonds, control; squares, 0.12; triangles, 1.2; ×, 12; star, 120) upon the F_v/F_m of *P. calcareum* at 9 °C; all other conditions as described for Fig. 2(a).

4. Discussion

4.1. Evaluation of photosynthetic capacity in maerl using PAM

The optimal quantum yield differs between taxa. Expected F_v/F_m for red algae is normally considered to be around 0.5–0.6 (Dring et al., 1996), so control values of 0.6 were anticipated. However, our control results for maerl were consistently in the range 0.4–0.5, even when thalli had been very carefully protected from excessive irradiance or other stress and had been acclimated to controlled temperature conditions for four weeks. It is possible that the calcareous matrix of maerl affects optimum quantum yields, but this was not investigated further since we were interested only in the values obtained during stress treatments compared to their controls.

4.2. Evaluation of physiological stress in maerl using PAM

4.2.1. Temperature

Previous studies of temperature effects on maerl have examined growth over long periods necessitated by the slow rates. In *P. calcareum* the optimal temperature for growth was inferred to be 12–13 °C (Adey and McKibbin, 1970). The rapid lethal effects of the 40 °C treatment show how effective the PAM system is for determining maximum survival temperatures. The demonstration here that F_v/F_m values did not differ between 9 °C ("winter") and 17 °C ("summer") are in accord with our previous findings that temperature had no detectable effect on *P. calcareum* in comparisons of growth rates at 10, 14 and 18 °C (Blake and Maggs, 2003). However, an acclimation period of up to 5 months may be necessary after temperature shifts (Adey and McKibbin, 1970), so it is possible that changes in optimal quantum yield would be detected after longer periods of acclimation. The seasonality of growth will also be influenced by day length, fouling and shading by other species in addition to temperature.

The lack of detectable stress at 25 °C indicates that *P. calcareum* tolerates high temperatures better than most subtidal red algae from the temperate zone, many of which die above 23 °C (Lüning, 1990; table 7.1). *P. calcareum* is widely distributed in Europe, from northern Norway to northern Spain and the Mediterranean (Irvine and Chamberlain, 1994). Temperature survival responses are likely to be more significant near the edges of its range, but biogeographic limits are not set by requirements for reproduction as this species reproduces principally by fragmentation (Irvine and Chamberlain, 1994).

4.2.2. Salinity

Both maerl species were tolerant of increased salinity up to 40 psu, showing no decrease in photosynthetic capacity. Although most subtidal seaweeds can survive up to 50 psu, and some tropical crustose coralline algae can grow at even higher salinities (Barry and Woelkerling, 1995), photosynthetic rates drop slightly above 30 psu (Lüning, 1990; p. 336).

The difference between the two maerl species in their responses to depression of salinity was contrary to predictions based on field observations (Birkett et al., 1998). Whereas it had previously been suggested that *L. glaciale* was more likely to be tolerant of low salinity than other maerl species (Birkett et al., 1998), it was significantly less tolerant of both the 3 psu and 15 psu conditions than *P. calcareum*. Although the photosynthetic capacity of *P. calcareum* was depressed initially in both low salinity treatments, acclimation to control values occurred at 15 psu. This salinity is at the lower extreme of tolerances observed in other subtidal algae (Lüning, 1990), and is lower than salinities previously seen to impair growth (King and Schramm, 1982). Maerl beds often occur in the vicinity of estuaries (Joubin, 1910) so tolerance to reduced salinity may be required under some hydrodynamic conditions.

4.2.3. Irradiance

The reduction in the ability of *P. calcareum* maerl to perform additional photochemistry under high irradiance conditions shows that this species, like many other corallines, is adapted to low photon irradiances. Crustose species such as *Phymatolithon foecundum* and *Phymatolithon tenue* showed effective adaptation to low irradiance under sea ice (Kuhl et al., 2001; Roberts et al., 2002). Various north-west Atlantic coralline algae grew best under low irradiance conditions of 0.7–20 $\mu mol\,m^{-2}\,s^{-1}$ (Johansen, 1981). The erect jointed corallines *Corallina mediterranea* and *Jania rubens* are also adapted to low irradiance levels and Hader et al. (1996) showed that photosynthesis was inhibited by exposure to excessive radiation. The extent of photoinhibition can also be related to whether the alga has experienced shade or sun exposure (Payri et al., 2001), so the prior acclimation of maerl to low irradiance may have affected the degree of stress observed. A longer experiment would be required to determine whether *P. calcareum* can acclimate to high irradiance.

Maerl showed little stress after being kept in the dark for 4 weeks. The maintenance of optimal quantum yields over an extended period of darkness has also been observed in the Antarctic red seaweed *Palmaria decipiens* during a 6-month simulated winter in darkness (Lüder et al., 2002). F_v/F_m values for *P. decipiens* remained high for the first 2 months in the dark before dropping suddenly and then decreasing more gradually to less than 0.05. Photosynthetic capacity recovered very

rapidly following illumination in the artificial spring (Lüder et al., 2002). Although we investigated a relatively short dark period, it is very likely that maerl can survive several months of darkness without deleterious effects.

4.2.4. Desiccation

In general coralline algae are not tolerant of desiccation and rarely occur in areas which are exposed at low tide (Johansen, 1981). Expectations that maerl would be intolerant of desiccation were borne out by our experiments. The very rapid lethal effects of desiccation on maerl may be related to its low water content and lack of a protective mucilage coating as found in many other marine algae

4.2.5. Burial

We examined thalli subjected to periods of darkness as well as to burial in coarse, fine and organic-rich muddy sediment. Darkness alone or burial in coarse sediment had less severe effects on the algae than periods of burial in fine sediment. In the fine sediments, reduction of water movement around the thalli probably limits gaseous exchange with detrimental effects on the algae. Chapman and Fletcher (2002) considered that the effects on *Fucus* embryos of burial in sediments probably result from the accumulation of metabolic products around the algae due to reduced diffusion. These data are consistent with the distribution of maerl beds in areas of high current or tidal flow, such as the mouths of sea-lochs, or headlands, where there is reduced sedimentation (Birkett et al., 1998).

Burial by a shallow layer of muddy sand containing hydrogen sulphide was quickly detrimental, and even maerl on the surface of the sediment was dead within two weeks. Sediment chemistry, particularly the level of hydrogen sulphide, has recently been found to be paramount in determining the survival of buried *Fucus* embryos (Chapman and Fletcher, 2002). A 1 mm layer of organically rich biodeposits killed >90% of *Fucus* embryos even when the overlying seawater was oxygenated. The toxic effects on maerl of fine organic sediment and associated hydrogen sulphide explain the observed detrimental effects of *Crepidula fornicata* in Brittany. Faeces and pseudofaeces produced by high densities of these invasive gastropods have damaged Breton maerl beds (Chauvaud et al., 2000; Grall and Hall-Spencer, 2003). Similarly, sewage outfalls and aquaculture rafts and cages can also heavily impact maerl beds by smothering with organic-rich discharges, faeces and unused fish food (Grall and Hall-Spencer, 2003).

Burial of maerl thalli in fine sediment by scallop dredging has previously been identified as a severe threat in the Firth of Clyde, Scotland, by Hall-Spencer and Moore (2000). However, death of buried maerl was interpreted as resulting from a lack of light (Hall-Spencer and Moore, 2000), whereas our data indicate that it is more likely to be primarily due to other physical and chemical effects of the sediment.

4.2.6. Fragmentation

This had no effect on the photosynthetic capacity of maerl, as expected because maerl propagation in the British Isles is primarily by fragmentation (Irvine and Chamberlain, 1994). Although trawling breaks the maerl thalli (Hall-Spencer and Moore, 2000), subsequent death of maerl is more likely to be due to reduction of water movement around the maerl caused by compaction or sedimentation.

4.2.7. Heavy metal contamination

There have been no previous studies on heavy metal contamination of maerl or other coralline algae. In this closed system experiment the algae were subjected to one dose of pollutants and monitored for a week afterwards. At all concentrations the algae were initially negatively affected, but after one week they appeared to have assimilated all the contamination and recovered normal photosynthetic values. Recovery is probably due to the absorption of heavy metals onto the wall matrix or some other non-metabolic component of the algae. Sequestration of metals by algae is a well-known phenomenon, with algae being widely used for biomonitoring and bioremediation of metals (Hamdy, 2000; Stirk and Van Staden, 2002). Metals are taken up both passively and actively by algae (Lobban and Harrison, 1994). In the natural environment, however, the algae might be affected worse by chronic pollution than by a single event. The lowest concentration of effluent used appeared to increase photosynthesis of the thalli after one week, suggesting that, at this level, added metals had some nutritional benefit.

5. Conclusions

We have shown that PAM fluorometry can be used as a non-invasive, rapid, repeatable measure of stress levels in maerl coralline algae. It is particularly useful in this group because growth rates of mature thalli are so slow as to preclude short-term experiments involving growth. Our results will contribute to meeting the requirement of the UK BAP to make provision by 2005 for "the maintenance of the extent and health of maerl bed communities in management plans for SACs" (UK Biodiversity Group, 1999).

These laboratory studies have confirmed that *P. calcareum*, the most abundant maerl species in Europe, is extremely intolerant of desiccation. It is also deleteriously affected by high irradiances. *P. calcareum* is, however, unexpectedly tolerant of rapid changes in

salinity, being better able to acclimate to both high and very low values than *L. glaciale*. The photosynthetic capacity of *P. calcareum* was maintained over a wide temperature range, 9–25 °C. This species was not affected by treatment with a high dose of simulated industrial effluent, but the presence of fine sediment with a high organic load and hydrogen sulphide content was rapidly lethal.

This investigation of a wide range of threats to maerl has demonstrated that the main anthropogenic hazard for maerl algae and the rich communities that depend on them is smothering by fine sediment, produced by trawling or maerl extraction, or resulting from barriers to normal tidal flow, such as causeways and barrages in Western Scotland (UK Biodiversity Group, 1999). Sewage discharges, and shellfish and finfish farm waste are likely to be particularly damaging due to the increased oxygen demand. Our study confirms the recommendation in the Habitat Action Plan for maerl beds (UK Biodiversity Group, 1999) that coastal zone management plans involving maerl beds should focus on preventing activities that would cause their smothering or eutrophication.

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