Journal of Coastal Life Medicine

Journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.201414J5 

# Antioxidant response of the brown algae *Dictyota dichotoma* epiphytized by the invasive red macroalgae *Lophocladia lallemandii*

Silvia Tejada[1], Antoni Sureda[2*]

[1]*Experimental Laboratory, Research Unit, Son Llàtzer Hospital, IUNICS, Ctra. Manacor km 4, E-07198 Palma de Mallorca, Balearic Islands, Spain.*

[2]*Research Group on Community Nutrition and Oxidative Stress, University of Balearic Islands, E-07122 Palma de Mallorca, Balearic Islands, Spain, and CIBERobn CB12/03/30038, Instituto de Salud Carlos III (ISCIII), Spain*

PEER REVIEW

**Peer reviewer**

Dr. Comte Katia, MNHN, UMR7245, 12, rue Buffon-CP39, 75231 Paris Cedex 05 France.
Fax: +33 0 140 793594
E-mail: kcomte@mnhn.fr

**Comments**

The authors have shown in the present work, a significant lipid peroxidation (MDA) and scavenging enzyme activity (SOD, CAT, GRD, GPX) in the brown algae when exposed to the epiphytic algae, in comparison to the control algae (*i.e.* without epiphytes). This tends to suggest an oxidative stress response of the host-plant due to the presence of algal invaders.
Details on Page 365

ABSTRACT

**Objective:** To evaluate the response of the brown alga *Dictyota dichotoma* (*D. dichotoma*) epiphytized by the red alga *Lophocladia lallemandii* in Mallorca coastal waters (Balearic Islands) by means of biomarker measures.
**Methods:** Samples of epiphytized and non-epiphytized *D. dichotoma* were collected in Cala Morlanda (East Mallorca, Balearic Islands). Markers of lipid peroxidation and activities of antioxidant enzymes were measured in *D. dichotoma*.
**Results:** Lipid peroxidation measured as malondialdehyde and all the antioxidant activities measured were significantly higher in the epiphytized brown algae when compared with the control algae.
**Conclusions:** In conclusion, the invasive algae *Lophocladia lallemandii* seems to produce a more oxidized status in the epiphytized *D. dichotoma* and cellular damage that could induce increased mortality.

KEYWORDS
Mediterranean Sea, Alien species, Oxidative stress, Antioxidants

## 1. Introduction

Invasive macroalgae are a current problem around all coastal waters in the Western Mediterranean. The red alga *Lophocladia lallemandii* (Montagne) F. Schmitz (*L. lallemandii*) is considered as an alien species in the Mediterranean Sea and it was probably introduced via the Suez Channel[1]. This alga is very aggressive invasive species and it settles over all types of substrates such as bare bedrocks, macroalgae on rocky bottoms, *Posidonia oceanica* seagrass meadows, and coralligenus communities.

During normal cellular activities, the organelles (chloroplast, mitochondrium, peroxisome) suffer various processes inside the cells that produce reactive oxygen species (ROS), since they present a highly oxidizing metabolic activity or due to the photosynthetic electron

*Corresponding author: Dr. Antoni Sureda, Research Group on Community Nutrition and Oxidative Stress, University of Balearic Islands, Ctra. Valldemossa, km 7.5, E-07122 - Palma de Mallorca, Balearic Islands, Spain.
Tel: +34-971173142.
Fax: +34-971173184
E-mail: tosugo@hotmail.com

Foundation Project: Supported by the Spanish Ministry of Health and Consumption Affairs with grant No. CIBERobn CB12/03/30038, and Balearic Island Government and FEDER funds with grant No. 23/2012.

Article history:
Received 20 Jan 2014
Received in revised form 2 Feb, 2nd revised form 19 Feb, 3rd revised form 5 Mar 2014
Accepted 20 May 2014
Available online 28 May 2014

transport chains[2]. Excess of ROS leads to the oxidation of biological macromolecules such as nucleic acids, proteins, carbohydrates and lipids which results in oxidative stress and cellular damage. Cells contain a complex network of antioxidant defences that scavenge or prevent the generation of ROS, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductasa (GRD). In order to protect the cell from the oxidative damage, the free radical $O_2^{\bullet-}$ is metabolised to hydrogen peroxide by SOD, then the hydrogen peroxide is decomposed to water and molecular oxygen by GP and CAT preventing the generation of hydroxy radicals, the most reactive species derivated from oxygen[3,4]. The epiphytism of *L. lallemandii* over algaes reduces light availability and hampers water movement; these circumstances enhance organic and nutrient enrichment and oxygen consumption resulting in a stressful situation. Moreover, *L. lallemandii* are a source of lophocladines, alkaloid molecules with cytotoxic effects[5] and consequently, can negatively affect the development of other macroalgaes[6]. *Dictyota dichotoma* (Hudson) J.V. Lamouroux (*D. dichotoma*) is a brown algae present in all the oceans and in the Mediterranean Sea. In fact, it has been defined as the only cosmopolitan species of the genus by some authors[7]. Moreover, biological studies have shown a significant number of dictyota secondary metabolites to possess cytotoxic, anti-bacterial, ichthyotoxic and anti-feedant activities[8-10]. However, to our knowledge, oxidative stress studies in *D. dichotoma* are lacking. In accordance, the aim of the present work was to evaluate the antioxidant response of the brown alga *D. dichotoma* under stress due to the epiphytism of *L. lallemandii* by means of the existence of oxidative lipid damage and the response of the CAT, SOD, GPX and GR activities.

## 2. Materials and methods

### 2.1. Algae sampling and processing

All samples were carried out in Cala Morlanda (Mallorca, Balearic Islands, western Mediterranean; Figure 1; between 39°33′26.11″N, 3° 22′9.77″E and 39°33′26.68″N, 3°22′14.04″E) the same day during summer time (August 2013). Twelve individuals of *D. dichotoma* (Hudson) Lamouroux were collected the same day in the following conditions: (1) *D. dichotoma* epiphytized by *L. lallemandii* ($n$=6) and (2) *D. dichotoma* not epiphytized by *L. lallemandii* ($n$=6). All *D. dichotoma* samples were collected at a similar depth (1-2 m) by expert apnea divers. After collection, algae were transported to the laboratory in a cooler at 5-7 °C. Once in the laboratory, algae were carefully separated from epiphytes and the dead matrix by scratching its surface, using a dull scalpel, in running water and rinsed several times in distilled water. Then, samples of Dictyota were immediately stored at −75 °C until biochemical analysis.

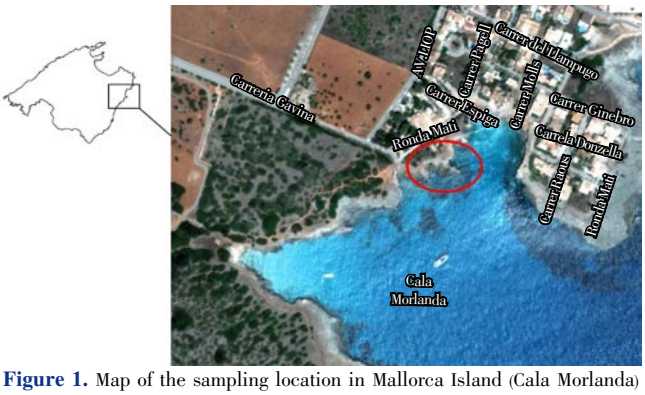


**Figure 1.** Map of the sampling location in Mallorca Island (Cala Morlanda) (www.maps.google.com).

Six replicates of *D. dichotoma* samples were homogenised with a Potter-Elvehjem glass/Teflon (Anorsa, Barcelona, Spain) homogeniser in five volumes (w/v) of 100 mmol/L Tris-HCl buffer at pH 7.5. Each homogenate was centrifuged at 8 000 r/min at 4 °C for 10 min. After centrifugation, supernatants were collected and immediately used for biochemical assays. All assays were performed in duplicate and results were corrected to the total protein content of the samples (Biorad Protein Assay, Biorad Laboratories, Madrid, Spain), using bovine serum albumin as standard.

### 2.2. Antioxidant enzyme activities

CAT activity (K/s/mg protein) was measured by the method based on the decomposition of $H_2O_2$[11]. SOD (pmol/min/mg protein) activity was determined by the degree of inhibition of the reduction of cytochrome C by the superoxide anion generated by the xanthine oxidase/hypoxanthine system. The activity was recorded at a wavelength of 550 nm[12]. GPX activity (nmol/min/mg protein) was measured using $H_2O_2$ as substrate[13]. The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to $NADP_+$ was indicative of the enzyme activity. GR activity (nmol/min/mg protein) was measured by the rate of conversion of oxidized glutathione to reduced glutathione estimated by monitoring oxidation of NADPH in the assay system at 340 nm[14]. All antioxidant enzyme activities were determined with a ShimadzuUV-2100 spectrophotometer at 25 °C.

### 2.3. Malondialdehyde (MDA) determination

The concentration of MDA (mmol/mg protein), as a marker of lipid peroxidation, was analysed by a specific colorimetric assay kit for MDA determination (Calbiochem®, San Diego, CA, USA), following the manufacturer's instructions. Briefly,

samples or standard were placed in glass tubes containing *n*-methyl-2-phenylindole in acetonitrile:methanol (3:1). HCl was added and samples were incubated for 1 h at 45 °C. Absorbance was measured at 586 nm and the concentration of MDA was calculated using a standard curve of known concentrations.

### 2.4. Statistical analysis

Statistical analysis was carried out using a statistical package (SPSS® v. 19.0 for Windows®). The homogeneity of the variance was assessed by the Kolmogorov-Smirnov test. Statistical significance of the data was assessed by independent samples *t*-test. Results were expressed as mean±SEM (Standard Error of the Mean) and $P<0.05$ was considered statistically significant.

## 3. Results

There was no presence of the invasive algae *L. lallemandii* in *D. dichotoma* collected at the control site. No significant differences were evidenced in the protein content between control samples and the samples in the areas where the epiphytism of *L. lallemandii* over algae *D. dichotoma* was present [(0.185±0.009) mg/mL in control *vs.* (0.181±0.006) mg/mL in epiphytized].

Lipid peroxidation was measured by the amount of MDA, as the marker of lipid damage. This measure is shown in Figure 2. MDA values were significantly increased in the epiphytized *D. dichotoma* samples when compared with the control algae ($P<0.05$).

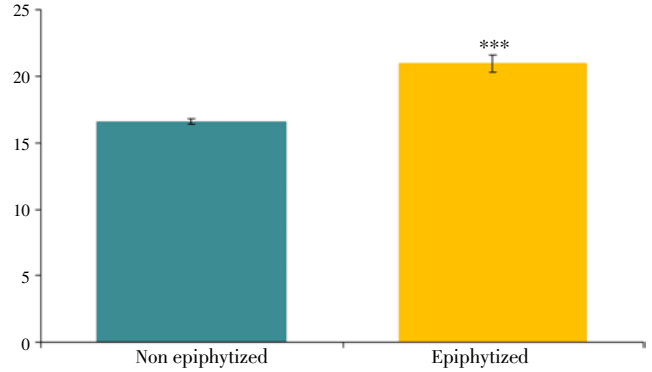


**Figure 2.** MDA (mmol/mg protein) determined in epiphytized and non-epiphytized *D. dichotoma*.
Statistically significant differences between epiphytized and non-epiphytized algae were reported: $^{***}P<0.001$ (One-way ANOVA). Values are expressed as mean±SEM.

The antioxidant enzymes activities are shown in Figure 3. A significant increased in all enzymatic activities (CAT, SOD, GRD and GPX) were observed in the epiphytized algae when compared with the non-epiphytized ones ($P<0.05$).

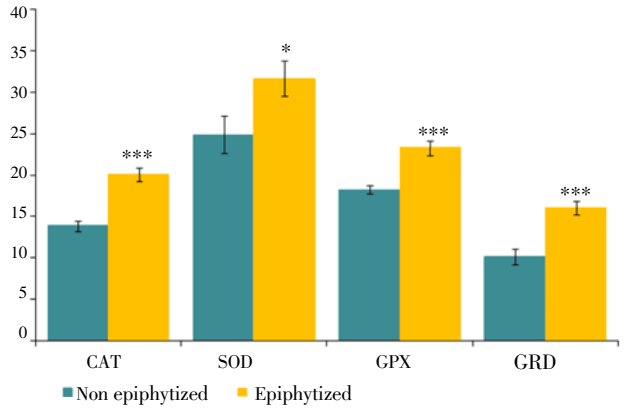


**Figure 3.** Antioxidant enzyme activities in *D. dichotoma* samples.
CAT (K/s/mg protein); SOD (pmol/min/mg protein); GPX (nmol/min/mg protein) and GRD (nmol/min/mg protein) were determined in epiphytized and non-epiphytized *D. dichotoma*. Statistically significant differences between epiphytized and non-epiphytized algae were reported: $^{*}P<0.05$, $^{***}P<0.001$ (One-way ANOVA). Values are expressed as mean±SEM.

## 4. Discussion

The the epiphytic growth of *L. lallemandi* over the *D. dichotoma* algae in Cala Morlanda waters (Mediterranean Sea) could induce an stressful situation by altering the adequate oxygenation and reducing the irradiance reaching the algae. No significant differences were evidenced in the protein content between control and epiphytized areas. In consequence, the data describing changes in MDA and antioxidant enzyme activities are not a consequence of a reduction in protein content in *D. dichotoma* epiphytized by *L. lallemandii*.

*L. lallemandii* is a red filamentous alga which usually appears as a mat of red filaments intertwined with themselves or with other algae. The epiphytism of *L. lallemandii* over *D. dichotoma* is mainly observed in the summer and autumn due to the tropical affinities of genus; in fact, *L. lallemandii* better develop with higher summer temperatures[15]. Due to its high invasive potential, *L. lallemandii* is able to cover most kinds of substrate, such as algae communities, resulting in a reduction of density and growth of these algae that can lead to stressful situation and death for native species[1,16,17].

Cellular antioxidant status is used to evaluate the ability of organisms to resist an environmental stress situation[18]. Lipid peroxidation, measured by the amount of MDA, and the antioxidant enzymes which play an important role in protecting from oxidative damage, are both biomarkers of oxidative stress[19]. *D. dichotoma* epiphytized by *L. lallemandii* appeared to undergo an oxidative stress, since a significant increase in MDA concentration was observed. The antioxidant defence system seemed to have been overwhelmed, since the antioxidant enzyme activities were not strong enough to prevent membrane lipid peroxidation.

It has been evidenced that algae in order to defence against herbivore pressure produce higher concentrations of defensive compounds[20]. Moreover, the production of ROS has been shown to have a significant contribution towards the survival of algae against pathogens[21]. It has been suggested that the release of $H_2O_2$ may act as a chemical defence against herbivores and epiphytes or as an allochemical in direct competition with other algal species[22]. $H_2O_2$ has also been reported to act as cellular messenger for the induction of the antioxidant defence system in response to an oxidative stress situation[23]. In accordance, our group has previously evidenced an increased $H_2O_2$ production in epiphyted *Posidonia oceanica* suggesting that oxidative stress is involved in the interaction of the invasive *L. lallemandii* and the seagrass[24].

The increase of antioxidant enzyme activities is related to the higher production of ROS, which will be detoxified as result of the antioxidant reactions. However, in the present study, the MDA level in epiphytized *D. dichotoma* was increased indicating that this species is very susceptible to suffer from oxidative stress induced by *L. lallemandii*. The current results are in accordance with previous studies that reported an increase in antioxidant defences of several organisms affected by *L. lallemandii* epiphytism. The invasion of *Posidonia oceanica* meadows by *L. lallemandii* and the growing of this alga on the endemic bivalve *Pinna nobilis* and on the bryozoan *Reteporella grimaldii* induced oxidative stress in these organisms as evidenced by increased levels of oxidative stress markers and in the antioxidant defences[24-26]. *Caulerpa taxifolia* epiphytized by *L. lallemandii* also responded by increasing the production of the toxic metabolite caulerpenyne and $H_2O_2$ and increasing the antioxidant enzymes activities as a defensive mechanism[6]. In another study, sea urchins fed during three months with *L. lallemandii* responded with an increased antioxidant response enough to avoid oxidative damage[27].

In conclusion, the present results reported that the interaction of the native *D. dichotoma* with invasive species of macroalgae such as *L. lallemandii* could alter the normal environmental conditions surrounding the algae. The epiphytism of *L. lallemandii* over *D. dichotoma* constitutes a new impact to the algae, resulting in oxidative stress evidenced with an increased antioxidant enzyme activities and lipid peroxidation that could alter the growth or physiology of the native species. Further studies are necessary to elucidate if this algae interaction would result in a decrease of the *D. dichotoma* abundance.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

This work was supported by the Spanish Ministry of Health and Consumption Affairs with grant No. CIBERobn CB12/03/30038, and Balearic Island Government and FEDER funds with grant No. 23/2012.

## Comments

### *Background*

Interactions between native and introduced algal species represent a threat to biodiversity and ecosystem functioning, especially in enclosed Mediterranean Sea. The red algae *L. lallemandii* is able to cover most kinds of substrates including macroalgae and becoming epiphytic. The invasive species can be a cause for the progressive regression of seagrasses.

### *Research frontiers*

This study was carried out to determine some stress responses and negative effect of epiphytic invasive *L. lallemandii* on the native brown algae: *D. dichotoma*, by focusing on ROS scavenging enzymes and lipid peroxidation (in comparison to a no-stressed algae: *i.e.* without epiphytes).

### *Related reports*

The invasive *L. lallemandii* is commonly investigated in many ecological studies as it is widespread through tropical and temperate areas in many oceans, leading to a loss of biodiversity. However, not many studies reported their potential negative physiological effects on the plants or algae they invaded.

### *Innovations and breakthroughs*

The brown algae, host-plant, was commonly known to produce many secondary metabolites including anti-bacterial activities. However, no previous work was assessed on the oxidative stress of this brown algae in response to an epiphytic algae invader (*i.e.* biotic interaction).

### *Applications*

The invasive species are often reported in the literature as inducing deleterious effects on native species in marine and ecosystems. This study supports these findings by focusing on biomarker (*i.e.* antioxidant enzymes and lipid peroxidation) levels in the detection of increased ROS under oxidative stress conditions.

### *Peer review*

The authors have shown in the present work, a significant lipid peroxidation (MDA) and scavenging enzyme activity

(SOD, CAT, GRD, GPX) in the brown algae when exposed to the epiphytic algae, in comparison to the control algae (*i.e.* without epiphytes). This tends to suggest an oxidative stress response of the host-plant due to the presence of algal invaders.

## References


[1] Boudouresque CF, Verlaque M. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Mar Pollut Bull* 2002; **44**: 32-38.

[2] Tripathy BC, Oelmuller R. Reactive oxygen species generation and signaling in plants. *Plant Signal Behav* 2012; **7**: 1621-1633.

[3] Cheeseman KH, Slater TF. An introduction to free radical biochemistry. *Br Med Bull* 1993; **49**: 481-493.

[4] Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; **92**: 3007-3017.

[5] Gross H, Goeger DE, Hills P, Mooberry SL, Ballantine DL, Murray TF, et al. Lophocladines, bioactive alkaloids from the red alga *Lophocladia* sp. *J Nat Prod* 2006; **69**: 640-644.

[6] Box Centeno A, Sureda A, Terrados J, Pons A, Deudero S. Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae *Lophocladia lallemandii* (Montagne). *J Exp Mar Biol Ecol* 2008; **364**: 24-28.

[7] Laneuville-Texeira V, Da Silva-Almeida SA, Kelecom A. Chemosystematic and biogeographic studies of the diterpenes from the marine brown alga *Dictyota dichotoma*. *Biochem Sys Ecol* 1990; **18**: 87-92.

[8] Rabanal M, Ponce NM, Navarro DA, Gomez RM, Stortz CA. The system of fucoidans from the brown seaweed *Dictyota dichotoma*: chemical analysis and antiviral activity. *Carbohydr Polym* 2014; **101**: 804-811.

[9] Abou-El-Wafa GS, Shaaban M, Shaaban KA, El-Naggar ME, Maier A, Fiebig HH, et al. Pachydictyols B and C: new diterpenes from *Dictyota dichotoma* Hudson. *Mar Drugs* 2013; **11**: 3109-3123.

[10] Ayyad SE, Makki MS, Al-Kayal NS, Basaif SA, El-Foty KO, Asiri AM, et al. Cytotoxic and protective DNA damage of three new diterpenoids from the brown alga *Dictoyota dichotoma*. *Eur J Med Chem* 2011; **46**: 175-182.

[11] Aebi HE. Catalase. In: Bergmeyer HU, editor. *Methods in enzymatic analysis*. Basel: Verlag Chemie; 1983, p. 273-286.

[12] Flohe L, Otting F. Superoxide dismutase assays. *Methods Enzymol* 1984; **105**: 93-104.

[13] Flohe L, Gunzler WA. Assays for glutathione peroxidase. *Methods Enzymol* 1984; **105**: 114-121.

[14] Goldberg DM, Spooner RJ. Glutathione reductase. In: Bergmeyer HU, editor. *Methods in enzymatic analysis*. Basel: Verlag Chemie; 1983, p. 258-265.

[15] Ballesteros E, Cebrian E, Alcoverro T. Mortality of shoots of *Posidionia oceanica* following meadow invasion by the red alga *Lophociadia lallemandii*. *Bot Mar* 2007; **50**: 8-13.

[16] Scheibling RE, Gagnon P. Competitive interactions between the invasive green alga *Codium fragile* ssp. tomentosoides and native canopy-forming seaweeds in Nova Scotia (Canada). *Mar Ecol Prog Ser* 2006; **325**: 1-14.

[17] Piazzi L, Balata D. The spread of *Caulerpa racemosa* var *cylindracea* in the Mediterranean Sea: an exemple of how biological invasions can influence beta diversity. *Mar Environ Res* 2008; **65**: 50-61.

[18] Welker AF, Moreira DC, Campos EG, Hermes-Lima M. Role of redox metabolism for adaptation of aquatic animals to drastic changes in oxygen availability. *Comp Biochem Physiol A Mol Integr Physiol* 2013; **165**: 384-404.

[19] Sureda A, Box A, Tejada S, Blanco A, Caixach J, Deudero S. Biochemical responses of *Mytilus galloprovincialis* as biomarkers of acute environmental pollution caused by the Don Pedro oil spill (Eivissa Island, Spain). *Aquat Toxicol* 2011; **101**: 540-549.

[20] Paul VJ, Hay ME. Seaweed susceptibility to herbivory-chemical and morphological correlates. *Mar Ecol Prog Ser* 1986; **33**: 255-264.

[21] Egan S, Fernandes ND, Kumar V, Gardiner M, Thomas T. Bacterial pathogens, virulence mechanism and host defence in marine macroalgae. *Environ Microbiol* 2013; doi: 10.1111/1462-2920.12288.

[22] Choo KS, Nilsson J, Pedersen M, Snoeijs P. Photosynthesis, carbon uptake and antioxidant defence in two coexisting filamentous green algae under different stress conditions. *Mar Ecol Prog Ser* 2005; **292**: 127-138.

[23] Wang X, Fang H, Huang Z, Shang W, Hou T, Cheng A, et al. Imaging ROS signaling in cells and animals. *J Mol Med (Berl)* 2013; **91**: 917-927.

[24] Sureda A, Box A, Terrados J, Deudero S, Pons A. Antioxidant response of the seagrass *Posidonia oceanica* when epiphytized by the invasive macroalgae *Lophocladia lallemandii*. *Mar Environ Res* 2008; **66**: 359-363.

[25] Box A, Sureda A, Deudero S. Antioxidant response of the bivalve Pinna nobilis colonised by invasive red macroalgae *Lophocladia lallemandii*. *Comp Biochem Physiol C Toxicol Pharmacol* 2009; **149**: 456-460.

[26] Deudero S, Blanco A, Box A, Mateu-Vicens G, Cabanellas-Reboredo M, Sureda A. Interaction between the invasive macroalga *Lophocladia lallemandii* and the bryozoan *Reteporella grimaldii* at seagrass meadows: density and physiological responses. *Biol Invasions* 2010; **12**: 41-52.

[27] Tejada S, Deudero S, Box A, Sureda A. Physiological response of the sea urchin *Paracentrotus lividus* fed with the seagrass *Posidonia oceanica* and the alien algae *Caulerpa racemosa* and *Lophocladia lallemandii*. *Mar Environ Res* 2013; **83**: 48-53.