Marine Macroalgae: Prospective Hitchhikers of Ship Ballast

Temjensangba Imchen^{1,*}

¹Biofouling and Bioinvasion Division, CSIR-National Institute of Oceanography, Dona Paula 403004, Goa, India

*Corresponding author: timchen@nio.org, temjen.imchen@gmail.com

KEYWORDS

Ballast tank
Caulerpa taxifolia
Invasive species
Macroalgae
Ulva
Zoospore

ABSTRACT Invasive alien species, on successful establishment, can displace native species. The threat of invasive species arises in view of their ability to outcompete and destabilize native biodiversity. Invasive species are found across all taxonomic groups of plants, animals and microorganisms. The green macroalga *Ulva flexuosa* has a potential to become invasive and this species was investigated for its hitchhiking potential under laboratory conditions. Zoospores of *U. flexuosa* were maintained at 4°C for nearly 10 months in the dark. Recruitment potential of zoospores after dark stress was tested in a modified Provasoli medium under optimal laboratory conditions. The success rate of zoospore recruitment was 61%. The paper describes the transfer potential through shipping activities by correlating the *Ulva* zoospores recruitment potential and survivability.

© The Author(s) 2018. This article is distributed under a Creative Commons Attribution-ShareAlike 4.0 International license.

1. INTRODUCTION

Invasive alien species (IAS) are non-native introduced species which on successful establishment become a threat to native biodiversity. IAS is considered to be a main cause of biodiversity loss across the world (Meinesz 1999; Pimentel et al. 2001; Thomson & McGlathery 2006). Plants, animals and microorganisms all have invasive representatives. The threat and impact of an invasive species depend on their ability to out-compete native species and destabilize the native biota of the ecosystem. IAS can cause huge economic damage due to loss of crops, diminished fisheries, fouling of ships' hulls and other marine infrastructure, as well as forest and environmental damages. It is conservatively estimated to cost many thousands and possibly a trillion dollars annually due to loss of crops, forest and other environmental damages, according to Convention of Biological Diversity (CBD) report (Pimentel et al. 2001). Preventing unwanted invasive alien species from crossing biogeographic borders is possibly the most effective strategy, because once an alien species has established itself in marine habitat it becomes nearly impossible to eliminate them (Thresher & Kuris 2004). This situation is further exacerbated due to climate change and other anthropogenic activities that are detrimental to ecosystem's health.

Invasive macroalgal species on successful establishment in introduced ecosystems bring about unprecedented changes due to their ability to alter and bio-engineer the ecosystem. For instance, the nursery habitats of fish and invertebrates are affected, reduces light penetration; change biogeochemical cycles and economically important shellfish are being destroyed (Chapman 1999; Wallentinus 2002; Rueness 2005; Thomson & McGlathery 2006; Molnar et al. 2008). Many species of macroalgae have become invasive on crossing the boundary of their native ecosystems.

A list of bioinvasive macroalgae with the potential of becoming invasive on crossing its biogeographical boundaries is given in Table 1. *Caulerpa taxifolia*, a native of the Indian Ocean is one species that has caused drastic change in the ecosystem in the Mediterranean Sea by preventing native species to grow (Gambi & Terlizzi 1988; Buia et al. 2001; Piazzzi, Ceccherelli & Cinelli 2001). *Kappaphycus alvarezii*, a fast growing red alga and a source of carrageenan, was earlier introduced from the Philippines for aquaculture has become invasive India, and it is now a threat to the coral reefs in the Gulf of Mannar Marine Biosphere Reserve along the coast of Tamil Nadu, India (Bagla 2008).

Many species belonging to the green algal genus *Ulva* are also reportedly invasive with fouling potential (Beach et al. 1995) and *U. flexuosa* is one among them. *U. flexuosa* Wulfen is a fast growing, opportunistic invasive green alga found in the littoral zone. The alga has a highly efficient rate of multiplication due to its ability to reproduce asexually. There is evidence of a co-existence of both C3 and C4 pathways in *U. prolifera*, and this was attributed for its successive intense bloom (Hu et al. 2010). Under favourable conditions, zoospores are released daily and are a potent cause of green tide or blooms in eutrophic conditions.

Aquaculture activities, aquarium trade and shipping are major means of introduction of aquatic invasive species. The transport can take place through ship hull fouling, ballast water tanks or through other parts like propellers and anchors (Ribera Siguán 2003; Flagella, Soria & Buia 2006; Molnar et al. 2008; Gaonkar et al. 2010). It is estimated that ca. 3000-4000 species are being transported daily throughout the world in the holds of ships (Carlton and Geller 1993). Many harmful and invasive species were introduced through ship ballast, e.g., the zebra mussels *Dreissena polymorpha* and the Asian clam *Potamocorbula amurensis* in the USA; the comb jelly *Mnemi-*

Table 1. List of invasive marine macroalgae in the world, compiled from various sources, including Hawai'i invasive algae database (Bishop Museum, <http://data.bishopmuseum.org/algae/results1.php>); Thomsen & McGlathery (2006); Piazzzi & Cinelli (2000); Boudouresque et al. (2002); Verlaque et al. (2003).

Name of species	Algal group
<i>Acanthophora spicifera</i> (Vahl) Borgesen	Green
<i>Avrainvillea amdelfa</i> (Mont.) A. Gepp & E. Gepp	Green
<i>Bryopsis pennata</i> Lamour.	Green
<i>Caulerpa racemosa</i> var. <i>cylindracea</i> (Sonder) V, H & B	Green
<i>Caulerpa taxifolia</i> (M.Vahl) C.Agardh	Green
<i>Cladophora laetevirens</i> (Dillwyn) Kutz.	Green
<i>Cladophora sericea</i> (Huds.) Kutz.	Green
<i>Cladophora socialis</i> Kutz.	Green
<i>Cladophora vagabunda</i> (L.) Hoek.	Green
<i>Codium fragile</i> ssp. <i>tomentosoides</i> (van Goor) Silva	Green
<i>Colpomenia sinuosa</i> (Mert. ex Roth) Derbes & Solier	Green
<i>Ulva flexuosa</i> (Wulfen) J Agardh	Green
<i>Ulva fasciata</i> Delile	Green
<i>Ulva reticulata</i> Forssk.	Green
<i>Dictyosphaeria cavernosa</i> (Forssk.) Børgesen	Green
<i>Hinckia mitchelliae</i> (Harvey) Silva	Brown
<i>Sargassum muticum</i> (Yendo) Fensholt,	Brown
<i>Turbinaria ornata</i> (Turner) J Agardh	Brown
<i>Undaria pinnatifida</i> (Harvey) Suringar,	Brown
<i>Acrothamnion preissii</i> (Sonder) Wollaston	Red
<i>Asparagopsis armata</i> Harvey	Red
<i>Bonnemaisonia hamifera</i> Hariot	Red
<i>Gracilaria vermiculophylla</i> (Ohmi) Papenfuss	Red
<i>Gracilaria salicornia</i> (C.Agardh)	Red
<i>Hypnea musciformis</i> (Wulfen) J.V. Lamour	Red
<i>Hypnea spinella</i> (C.Agardh) Kutz.	Red
<i>Kappaphycus alvarezii</i> (Doty) Doty ex Silva	Red
<i>Neosiphonia tongatensis</i> (Harvey ex Kutz.) Kim & Lee	Red

opsis leidyi in the Black and Azov Seas; the toxic dinoflagellate *Gymnodinium catenatum* and the northern Pacific seastar *Asterias amurensis* in Australia (CSIRO, 2001). Flaggella et al. (2006) suggested that ballast water can play an important role in spreading the organisms. A study done in the aftermath of massive *U. prolifera* bloom in the Yellow Sea, China found that the cause of bloom is due to the aquaculture of *Porphyra yezoensis* (nori) along the 200 km shoreline of Subei bank north of Yangtse river mouth (Hu et al. 2010). The nets used for nori culture also act as a substratum for *Ulva*, which become a propagule bank resulting in algae blooms every year. Keeping this background in mind, the ability of *U. flexuosa* zoospores to survive dark stress was examined in this present study. The scenario of ballast water intake during a macroalgal bloom, which may serve as a potential zoospore bank for future recruitment, was also taken into account. The transfer potential of macroalgal zoospores in ship ballast, and as a potential zoospore bank for future recruitment, were examined by correlating the survivability of the zoospores under simulated dark conditions of ballast tanks.

2. MATERIALS AND METHODS

Zoospores of *Ulva flexuosa* were obtained through the dehydration method (Shin 2008; Imchen 2012). These were stored in the dark at 4°C for nearly 10 months by wrapping cultures in black polythene bags. Thereafter, the zoospores were cultured in a modified Provasoli medium (West & McBride 1999). The seawater used was filtered through 0.22 µm filter paper (Millipore). The ex-

perimental set up was a two-factorial viz: Salinity (15–35 psu) x Nitrate (20–150 µM) (S x N) with 8 µM as constant; Salinity (25–35 psu) x Phosphate (4–15 µM) (S x P); Light (20–80 µmol. m⁻² s⁻¹) x Salinity (15–35 psu) supplemented by Nitrate (80 µM) and Phosphate (8 µM) as constant, and Light (20–80 µmol. m⁻² s⁻¹) x Phosphate (4–15 µM) (L x P) with 80 µM nitrate as constant. Germanium dioxide (0.5 mg.L⁻¹) was incorporated into the culture medium to control diatom growth. The culture was maintained under optimal laboratory conditions supported by white fluorescent light (40 µmol m⁻² s⁻¹) at room temperature of 24±1°C with a day-night cycle of 14/10 h. A set of six culture vessels was maintained as replicates. Percentage recovery and recruitment were scored and data were transformed, when required, for subsequent analysis which was performed using Statistica 6 software.

3. RESULTS AND DISCUSSION

The ability of plants to survive unfavorable environmental condition is an inherent trait. It remains in a suspended or reduced metabolic activity by undergoing dormancy in the form of seeds, spores, zoospores and cysts, etc. Macroalgae do not produce seed to undergo dormancy like terrestrial plants but exhibit morphological variation that apparently make them more tolerant to physical damage (Edwards 2000). This characteristic is developed in a single life cycle through phenotypic plasticity (Richardson 1981; Mathieson 1982; Edwards 2000) or through heteromorphic life history (Lubchenco and Cubit 1980; Slocum 1980; Edwards 2000).

It is generally believed that the microscopic forms of macroalgae persist better during unfavorable periods than larger morphologies (Anderson 1982; Foster 1982; Klinger 1984; Blanchette 1996; Edward 2000), but due to difficulties in observing them in the field, little is known about their persistence and survival (Dayton 1985; Santelices et al. 1995; Edwards 1999). The results showed that zoospores survived unfavorable conditions and they can serve as a potential propagule source or bank for recruitment. The subsequent resumption of growth was recorded when the zoospores were provided with optimal growth conditions. The percentage of recruitment was highest (81%) in S x N growth condition followed by L x S growth condition (63%), and L x P (58%). The overall recovery rate or the percentage of recruitment was about 61% (Figure 1). Recruitment was significantly affected by the factors, and the effect of S x P growth condition on recruitment was significantly different (one-way ANOVA, P<0.05) from other combinations. The rate of recovery indicated that factors like light, nitrate and phosphate supplemented by 25 psu salinity condition may play a significant role in the recruitment process. Amongst these, the results also showed that light and nitrate could be more significant. The results further showed that 20 µM of nitrate achieved ca 80% of recruitment. High nitrate concentration may facilitate high biomass growth of macroalgae, but it may be inferred from this result that nitrate concentration for recruitment need not necessarily be high. It is also apparent that the transfer to ambient growth conditions breaks the cold and dark induced suspended metabolic activity of the zoospores.

U. flexuosa zoospores could be recruited after 10 months of cold and dark storage on return to ambient environmental conditions (Imchen 2012). However, a similar study on *Eichhornia crassipes*, a highly invasive

Figure 1. Percentage of recruitment of *Ulva flexuosa* under optimal laboratory conditions. The experimental set up was a two-factorial viz: Light ($80 \mu\text{mol. m}^{-2} \text{ s}^{-1}$) x Salinity (25) with Nitrate ($80 \mu\text{M}$) and Phosphate ($8 \mu\text{M}$) as constant (L x S); Light ($80 \mu\text{mol. m}^{-2} \text{ s}^{-1}$) x Phosphate ($8 \mu\text{M}$) while Nitrate ($80 \mu\text{M}$) and Salinity (25 psu) as constant (L x P); Salinity (25 psu) x Nitrate ($20 \mu\text{M}$) with Phosphate ($8 \mu\text{M}$) as constant (S x N); Salinity (25 psu) x Phosphate ($8 \mu\text{M}$) with Nitrate ($80 \mu\text{M}$) as constant (S x P). Effect of S x P was significantly different from other growth conditions. The culture was maintained under optimal laboratory conditions supported by white fluorescent light at room temperature of $24 \pm 1^\circ\text{C}$ with a day-night cycle of 14/10 hours. A set of six culture vessels was maintained as replicates.

aquatic weed called “terror of Bengal” in India, showed that by the 11th day the plant is highly decomposed (Imchen et al. 2017) under dark conditions. This indicated that it requires a direct human-mediated intervention to introduce this plant. Not all invasive species are resistant to physiological stresses, but any superior performance of invasive species over native algae was attributed to the microhabitat where stresses are minimal (Thomsen and McGlathery 2006). In a similar study, regeneration of new fronds was recorded from the holdfast region of *U. fasciata* and *Cladophora antennina* after a period of exposure to dark stress conditions (pers. obs.).

According to Burrow (1991), each cell of the monostromatic hollow thallus of *Ulva*, except for the rhizoidal region, has the potential to produce eight, sometimes 16, zoospores or gametes. The distal end of the thallus is the region of sporogenesis where maximum number of zoospores are released 3–5 d before each spring tide (Christie and Evans 1962). *Ulva linza*, having a monostromatic thallus, contains ca 5×10^5 cells cm^{-2} which roughly equals to 4×10^6 zoospores cm^{-2} considering each cell produces eight zoospores (Callow and Callow 2000). So, a typical 1 cm^{-2} of *Ulva* thallus has the potential of releasing zoospores at the rate of 5×10^5 per plant per day. We can infer the huge zoospore load it will generate, for the recruitment potential, by extrapolating this ratio. Since the species of *Ulva* can reproduce through sexual, asexual and vegetative means, a fragment of the thallus is a potential propagule bank for perpetuation of its life cycle. In the brown alga *Desmarestia* the microscopic stages are the sole source for recruitment of the larger form ‘sporophyte’ and survive in the field for at least three months (Edward 2000). Similarly, the gametophyte of *Macrocystis pyrifera* survived for many years in the laboratory (tom Dieck, 1993). Therefore, if ballast water is taken from a bloom occurring area/region, a huge zoospore load is expected to find its way to a new frontier.

Coastal areas across the world are highly disturbed due to various anthropogenic activities. Such conditions provide an ideal condition to the opportunistic and invasive species to thrive. Enabled by increased nutrient load, tenfold increase in toxic *Karenia brevis* blooms on the

west Florida shelf was recorded in recent years (Brand & Compton 2007). *Ulva flexuosa* ssp. *pilifera*, a fresh-water variant, was also reported to occur in a region of high nutrient concentration (Messiasz et al., 2013).

Considering the proportion of sporulation and the zoospore load that will be generated, the rate of recovery and the percentage of recruitment in the study is realistic. Review of literature and the present study indicate that *Ulva* and other macroalgae are physiologically capable of long-term survival with tolerance to wide range of salinity. Therefore, there is high probability of dissemination through ship ballast between regions of similar environmental conditions. *U. flexuosa* is, therefore, an ideal candidate to be transported either in ship ballast or as ship hull fouling, or as stowaways in ship’s hull. The threats become more real as there is less resident time between ports, due to improved and better vessels. For instance, a ship from Australia takes less than three weeks to arrive at the port of Naples/Salerno, Italy in the Mediterranean (Flagella et al. 2006).

ACKNOWLEDGMENTS

The contents of this manuscript were first presented at the ASEAN-India international conference on the extent of transfer of alien invasive organisms in South/South-east Asia region by shipping, held in Chiang Mai, Thailand from 26 to 28 November 2013. I wish to thank the ASEAN Committee for Science and Technology (COST) and Ministry of External Affairs, India for their generous support. I am also grateful to Director, CSIR-National Institute of Oceanography, Dona Paula, Goa, India and Dr. AC Anil for their support and encouragement.

REFERENCES

- Anderson RJ. 1982. The life history of *Desmarestia firma* (C. Ag.) Skottsb. (Phaeophyceae, Desmarestiales). *Phycologia* 21:316–322.
- Bagla P. 2008. Ecology: seaweed invader elicit angst in India. *Science* 320:1271.
- Beach KS, Smith CM, Michael T, Shin HW. 1995. Photosynthesis in reproductive unicells of *Ulva fasciata* and *Enteromorpha flexuosa*: implications for ecological success. *Marine Ecology Progress Series* 125:229–237.
- Bishop Museum. n.d. Invasive algae database. Honolulu: Bishop Museum; [accessed 2013 Apr 12]. <http://data.bishopmuseum.org/algae/results1.php>
- Blanchette CA. 1996. Seasonal patterns of disturbance influence recruitment of the sea palm, *Postelsia palmaeformis*. *Journal of Experimental Marine Biology and Ecology* 197:1–14.
- Boudouresque CF, Verlaque M. 2002. Assessing scale and impact of ship-transported alien macrophytes in the Mediterranean Sea. In: Briand F, editor. *Alien marine organism introduced by ships in the Mediterranean and Black Seas*. Monaco: CIESM. CIESM Workshop Monographs 20:53–61.
- Brand L, Compton A. 2007. Long term increase in *Karenia brevis* abundance along the southwest Florida Coast. *Harmful Algae* 6:232–252.
- Buia MC, Gambi MC, Terlizzi A, Mazzella L. 2001. Colonization of *C. racemosa* along the southern Italian coast: I. distribution, phenological variability and ecological role. In: Gravez V, Ruitton S, Boudouresque CF, Le Direac’h L, Meinesz A, Scabbia G, Verlaque M, editors. *Proceedings of the 4th Inter-*

- national Workshop on *Caulerpa taxifolia*. Marseille: GIS Posidonie. p. 352–360.
- Burrow EM. 1991 Seaweeds of the British Isles - volume 2: Chlorophyta. London: Natural History Museum Publications.
- Callow ME, Callow JA. 2000. Substratum location and zoospore behaviour in the fouling alga *Enteromorpha*. *Biofouling* 15(1–3):49–56.
- Carlton J, Geller R. 1993. Ecological roulette: the global transport of non-indigenous marine organisms. *Science* 261:s78–82.
- Chapman AS. 1999. From introduced species to invader: what determines the success of *Codium fragile* ssp. *tomentosoides* (Chlorophyta) in the North Atlantic Ocean? *Helgoländer Meeresuntersuchungen* 52:277–289.
- Christie AO, Evans LV. 1962. Periodicity in the liberation of gametes and zoospores of *Enteromorpha intestinalis* Link. *Nature London* 193:193–194.
- CSIRO. 2001. Marine research: marine pest information sheet ballast water. Canberra: CSIRO; [accessed 2013 Jul 31]. http://www.marine.csiro.au/crimp/Reports/Infosht2_Ballastwater_A3S.pdf.
- Dayton PK. 1985. Ecology of kelp communities. *Annual Review of Ecology and Systematics* 16:215–245.
- [DAISIE] Delivering Alien Invasive Species Inventory for Europe. c2006. Species factsheet: *Caulerpa racemosa*. DAISIE; [accessed 2013 Jul 31]. <http://www.europe-aliens.org/speciesFactsheet.do?speciesId=53253>.
- Edwards MS. 1999. Using in situ substrate sterilization and fluorescence microscopy in studies of microscopic stages of marine macroalgae. *Hydrobiologia* 398/399:253–259.
- Edwards MS. 2000. The role of alternate life-history stages of a marine alga: a seed bank analogue. *Ecology* 81(9):2404–2415.
- Flagella MM, Soria A, Buia MC. 2006. Shipping traffic and introduction of non-indigenous organisms: study case in two Italian harbours. *Ocean and Coastal Management* 49:947–960.
- Foster MS. 1982. The regulation of macroalgal associations in kelp forests. In: Strivastava LM, editor. *Synthetic and degradative processes in marine macrophytes*. New York: Walter de Gruyter & Company. p. 185–203.
- Gambi MC, Terlizzi A. 1998. Record of a large population of *Caulerpa racemosa* (Forsskål) J. Agardh (Chlorophyceae) in the Gulf of Salerno (Southern Tyrrhenian Sea, Italy). *Biologia Marina Mediterranea* 5(1):553–556.
- Gaonkar CA, Sawant SS, Anil AC, Venkat K, Harkantra SN. 2010. Mumbai harbor, India: gateway for introduction of marine organisms. *Environmental Monitoring and Assessment* 163:583–589.
- Hu C, Li D, Chen C, Ge J, Muller-Karger FE, Liu J, Yu F, He MX. 2010. On the recurrent *Ulva prolifera* blooms in the Yellow Sea and East China Sea. *Journal of Geophysical Research: Oceans* (1978–2012) 115:C05017.
- Imchen T. 2012. Recruitment potential of a green alga *Ulva flexuosa* Wulfen dark preserved zoospore and its development. *PLoS ONE* 7(3):e32651.
- Imchen T, Sawant SS, Ezaz W. 2017. Fate of *Eichhornia crassipes* (Mart.) Solms on exposure to saltwater and its implications. *Current Science* 113:439–443.
- Klinger T. 1984. Allocation of the blade surface area to meiospore production in annual and perennial representatives of the genus *Laminaria* [thesis]. Vancouver: University of British Columbia.
- Lubchenco J, Cubit J. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology* 61:676–687.
- Mathieson AC. 1982. Field ecology of the brown alga *Phaeostrophion irregulare* Setchell et Gardner. *Botanica Marina* 25:67–85.
- Meinesz A. 1999. Killer algae – the true tale of a biological invasion. Chicago: The University of Chicago Press.
- Messyasz B, Czerwik-Marcinkowska J, Massalski A, Uher B, Rybak A, Szendzina L, Pikosz M. 2013. Morphological and ultrastructural studies on *Ulva flexuosa* subsp. *pilifera* (Chlorophyta) from Poland. *Acta Societatis Botanicorum Poloniae* 82(2):157–163.
- Molnar JL, Gamboa RL, Revenga C, Spalding MD. 2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* 6:485–492.
- Piazzi L, Cinelli F. 2000. Effects de l'expansion des Rhodophyceae introduites *Acrothamnion preissii* et *Womersleyella setacea* sur les communautés algales des rhizomes de *Posidonia oceanica* de Méditerranée occidentale. *Cryptogamie Algologie* 21:291–300.
- Piazzi L, Ceccherelli G, Cinelli F. 2001. Threat to macroalgal diversity: effects of the introduced green alga *Caulerpa racemosa* in the Mediterranean Sea. *Marine Ecology Progress Series* 210:149–159.
- Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agricultural Ecosystems and Environment* 84:1–20.
- Reef Resilience Network. c2013. Coral reefs – a model of the reef resilience toolkit. Washington: The Nature Conservancy; [accessed 2013 Jul 31]. http://www.reefresilience.org/Toolkit_Coral/Stressors/Stressors_Invasive_Algae.html.
- Ribera Siguan MA. 2003. Pathways of biological invasions of marine plants. In: Ruiz GM, Carlton J, editors. *Invasive species vectors and management strategies*. Washington: Island Press. p. 183–226.
- Richardson JP. 1981. Persistence and development of *Dasya baillouviana* (Gmelin) Montagne (Rhodophyceae, Dasyaceae) in North Carolina. *Phycologia* 20(4):385–391.
- Rueness J. 2005. Life history and molecular sequences of *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta), a new introduction to European waters. *Phycologia* 44:120–128.
- Santelices B, Hoffman AJ, Aedo D, Otaíza R. 1995. A bank of microscopic forms on disturbed boulders and stones in tide pools. *Marine Ecology Progress Series* 129:215–228.
- Shin HW. 2008. Rapid attachment of spores of the fouling alga *Ulva fasciata* on biofilms. *Journal of Environmental Biology* 29(4):613–619.
- Slocum CJ. 1980. Differential susceptibility to grazers in two phases of an intertidal alga: advantages of heteromorphic generations. *Journal of Experimental Marine Biology and Ecology* 46:99–110.
- Thomsen MS, McGlathery KJ. 2006. Stress tolerance of the invasive macroalgae *Codium fragile* and *Gracilaria vermiculophylla* in a soft-bottom turbid lagoon. *Biological Invasions* 9:499–513.
- Thresher RE, Kuris AM. 2004. Options for managing invasive marine species. *Biological Invasions* 6:295–300.
- tom Dieck (Bartsch) I. 1993. Temperature tolerance and sur-

- vival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Marine Ecology Progress Series 100:253–264.
- Verlaque M, Durand C, Huisman JM, Boudouresque CF, Le Parco Y. 2003. On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpaceae, Chlorophyta). European Journal of Phycology 38:325–339.
- Wallentinus I. 2002. Introduced marine algae and vascular plants in European aquatic environment. In: Lepakoski E, Olenin S, Gollasch S, editors. Invasive aquatic species of Europe: distribution, impacts and Management. Dordrecht: Kluwer Academic Publishing. p. 27–54 (+1 App). <http://www.ku.lt/nemo/EuroAqualInvaders.htm>.
- West JA, McBride DL. 1999. Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). Hydrobiologia 298/299:101–113.