China is on the track tackling *Enteromorpha* spp forming green tide

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Summary

Green tide management is supposed to be a long term fight rather than an episode during the 29th Olympic Games for China, since it has been gaining in scale and frequency during the past 3 decades in both marine and estuary environment all over the world. A number of rapid-responding studies including oceanographic comprehensive surveys along the coastline have been conducted during the bloom and post-bloom periods in 2008 by Chinese marine scientists. The preliminary results are as below: (1) phylogenetic analysis indicates that the bloom forming alga forms a clade with representatives of the green seaweed *Enteromorpha linza*, though, the alga has been identified as *E. proliera* by means of morphological; (2) the present data suggest that the bloom was originated from south of Yellow Sea, but not the severely affected area near Qingdao City; (3) pathways of reproduction for *E. prolifera* have approved to be multifarious, including sexual, asexual and vegetative propagation; (4) somatic cells may act as a propagule bank, which is supposed to be a very dangerous transmitting way for its marked movability, adaptability and viability; (5) pyrolysis of the alga showed that three stages appeared during the process, which are dehydration (18–200℃), main devolatilization (200–450℃) and residual decomposition (450–750℃), and activation energy of the alga was determined at 237.23 KJ•mol^{-1}. Although the scarce knowlegde on *E. prolifera* not yet allow a fully understanding of the green tide, some of the results suggests possible directions in further green tide research and management.

Species identification

Algae of *Enteromorpha* are common elements on sandy or muddy intertidal flats along the coastline of China (Tseng 1984), but have never occurred in thick mats before 2007. *Enteromorpha* bloomed for the first time in the middle area of Yellow Sea in 2007. A large-scale

Enteromorpha spp forming green tide broke out in the middle and south area of Yellow Sea in late May, 2008 (*eg*. Ye et al. 2008). By July 16, 1 million tonnes of algae had been cleared from one of the severely affected area, Qingdao City, China Sun et al. 2008). The green tide algae have been classified as *Enteromorpha* genera from their morphological characteristics, but their taxonomy at species level was uncertain and became the most controversial issue.

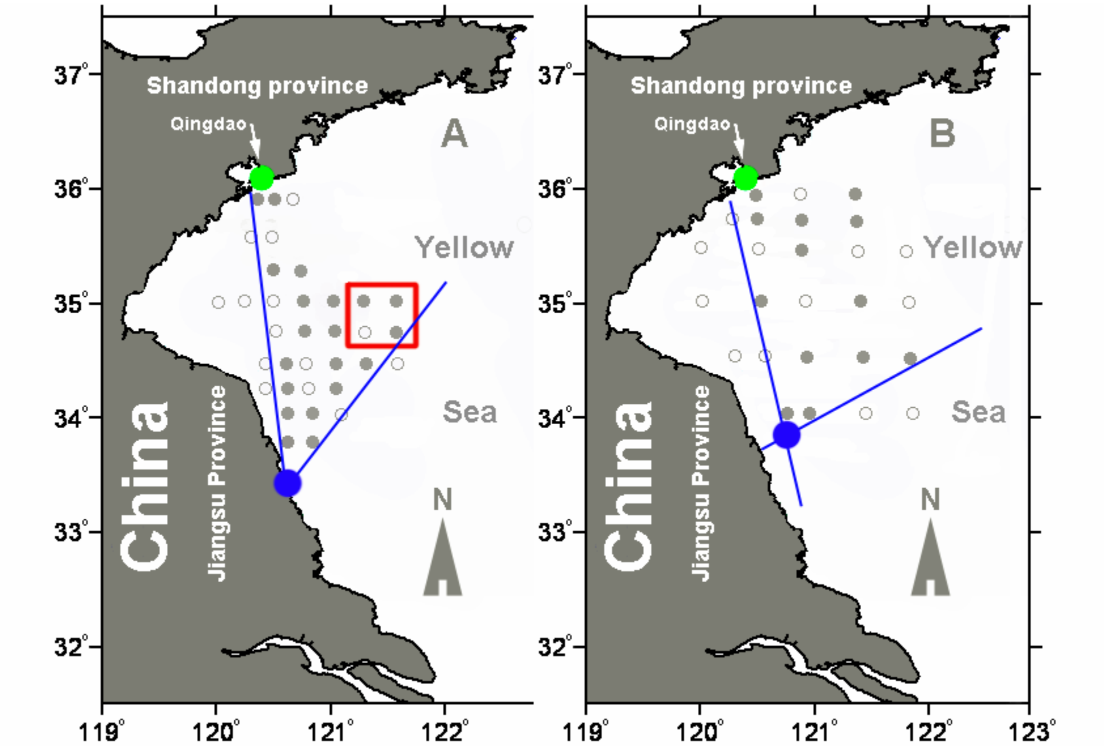

Fig. 1. Map showing locations in Yellow Sea investigated by 'Beidou' Marine Scientific Research Vessel. (A). Locations of the first survey conducted during June 9–16, 2008. Solid circles represent the sites where floating samples were fetched. The red box indicates the area that the green tide was first dectected on May 15, where is about 150 km southeast from Qingdao City. (B). Locations of the second survey conducted during June 9–16, 2008. Solid circles represent the sites where sedimental samples were obtained. Blue solid circles susggest the area where the bloom most likely originated.

In our study, molecular data were used in parallel with morphological characters to resolve the taxonomic problems. 22 samples from 20 localities in Yellow Sea and one free-floating sample from Zhanqiao of Qingdao have been studied (Fig. 1A). Thalli are characterized by unattached, highly branched narrow tubes, one layer of cells (Fig. 2), and fall within the morphological limits of *Enteromorpha prolifera* O.F. Müller (Tseng 1984).

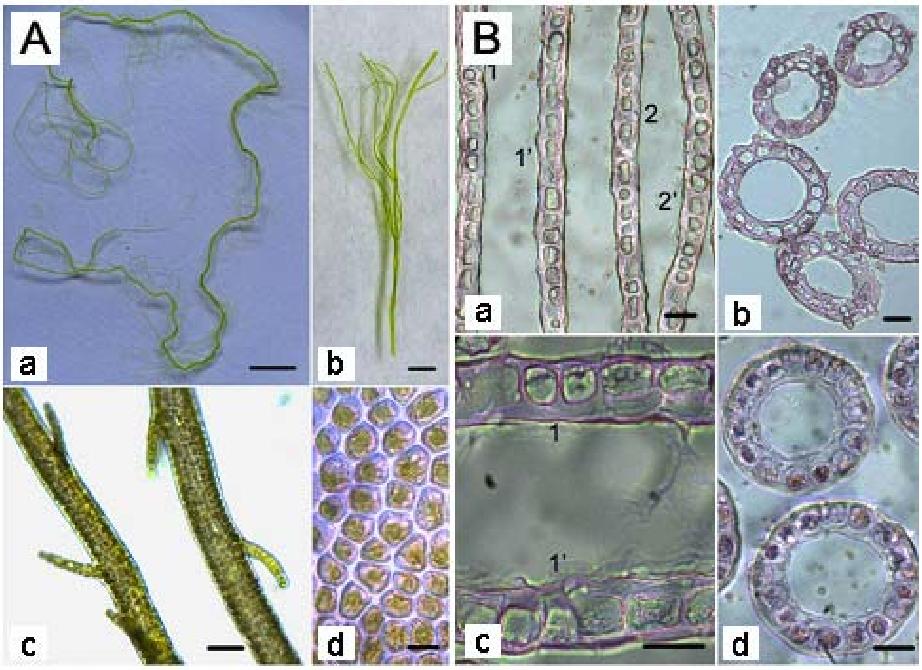

Fig. 2. Morphologic and Section view of *Enteromorpha* spp thallus.

(A). Morphologic characteristic of the thallus. (a) A complete thallus of *Enteromorpha prolifera* with slender brances, bar=1cm; (b) One segment with multi branches, bar=500μm; (c) One segment with incipient tiny branches, bar=100 μm; (d) Surface view showing irregular cells, bar=20 μm. (B). Section view of the thallus. (a) and (c) Longitudinal section view, 1 and 1' show cell walls of one thallus, and 2 and 2' show another, bar=500 μm for a and 50 μm for c; (b) and (d) Transverse section view, bar=500 μm for b and 50 μm for d.

Phylogenetic analysis of sequences of the internal transcribed spacers ITS1 and ITS2 and the 5.8S rDNA gene show that all the 23 *Enteromorpha prolifera* samples belong to the same specie, which is assembled with *E. linza* (Fig. 3).

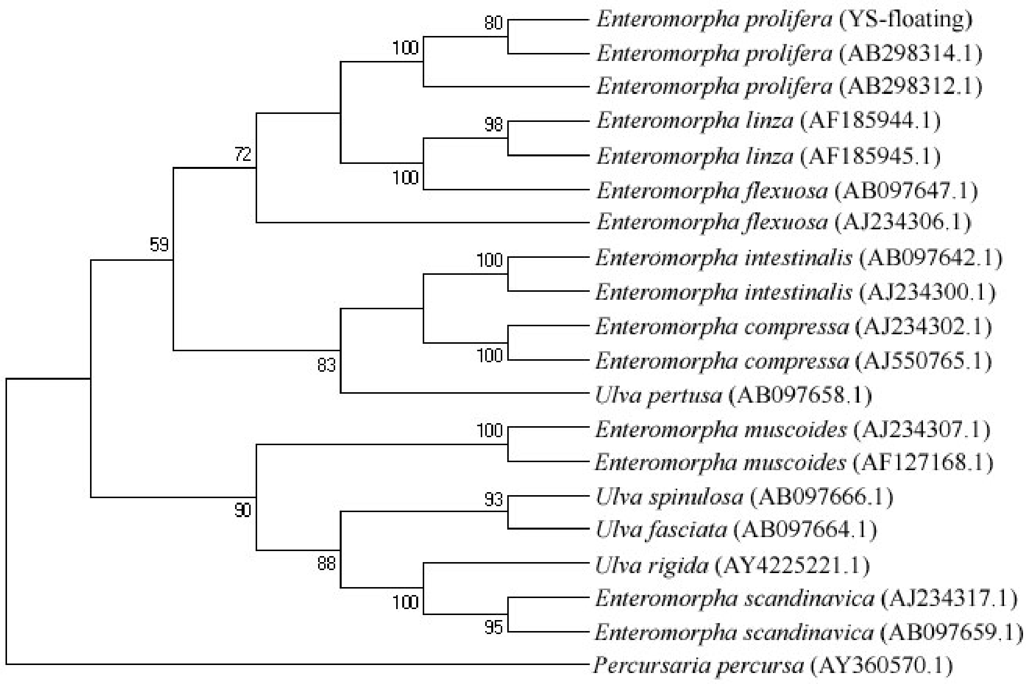

Fig.3. Phylogenetic tree of ITS sequences constructed by NJ method.

YS-floating represents free-floating samples in Yellow Sea. Numbers at nodes represent bootstrap values from 1,000 replications.

Bloom site retrospection

The first survey was conducted during the period when the green tide covered the largest area and the alga was the most prosperous. 20 floating samples were collected but no sediment alga was found during the voyage (Fig. 1A). The second survey performed in August was during the time when most of the floating algae had disappeared and the remains showed themselves in obvious senescence. The investigation obtained massive sediment samples in 13 sites that covered the investigated area using Agassiz trawl or stainless steel corer (Fig. 1B).

E. prolifera is a benthic species and widely distributed in intertidal zones of most global oceans, especially in the subtropical area (*eg*. Tseng, 1984). The massive green algae bloom happened in Yellow Sea in the year of 2008 was not orignated from the coastal area of Qingdao, but originated from an offshore area 150 km southeast of Qingdao, then difted to the destination by seasonal south wind and currents. Both of the survey suggest that the origin area lies in between 33º–34 º N along the coastline of Yellow Sea (Fig. 1A).

Function of somatic cells regeneration

Eutrophication is the primary reason and may explain the extensive growth of opportunistic macroalgal species which are able to take advantages of such conditions (Fletcher 1996, Morand and Briand 1996, Schores et al. 2000, Largo et al. 2004, Wang et al. 2008). However, little is konwn about the survival and successful development of green macroalgae, which may play in regulating the seasonal metagenesis. Potentially, the free-living green alga mat is commonly achieved by (1) from overwintering adult plant which persist partially embedded in the sediment and start growing in the following spring (Kamermans et al. 1998) or (2) from settled spores, vegetative fragments, or other microscopic forms of the life cycle which remain dormant or survive with little growth until enviromental conditions become favorable (Santelices 1990, Hoffmann and santelices 1991, Schores 1995, Worm et al. 2001). 'Bank of microscopic froms' (Chapman 1986) surviving long periods of unfavorable environmental conditions and to recover following severe disturbances is crucial to the persistence of algae populations occurring in temporally variable environments (Carney and Edwards 2006). *Enteromorpha* have developed life histories in which the microscopic stages suspend growth during unfavorable conditions and then re-establish activity when conditions improve (Schories and Reise 1993, Schores 1995, Kolwalkar et al. 2007). Although previous studies have revealed that the reproduction of *Enteromorpha* is mainly due to the asexual zoospores that derived from distal end of the thalli (Nienburg 1927, Callow 1996, Worm et al. 2001, Eriksson and Johansson 2005), pathways of reproduction for E. prolifera are approved to be multifarious, including sexual, asexual and vegetative propagation (Dan et al. 1997, Ye et al. 2008).

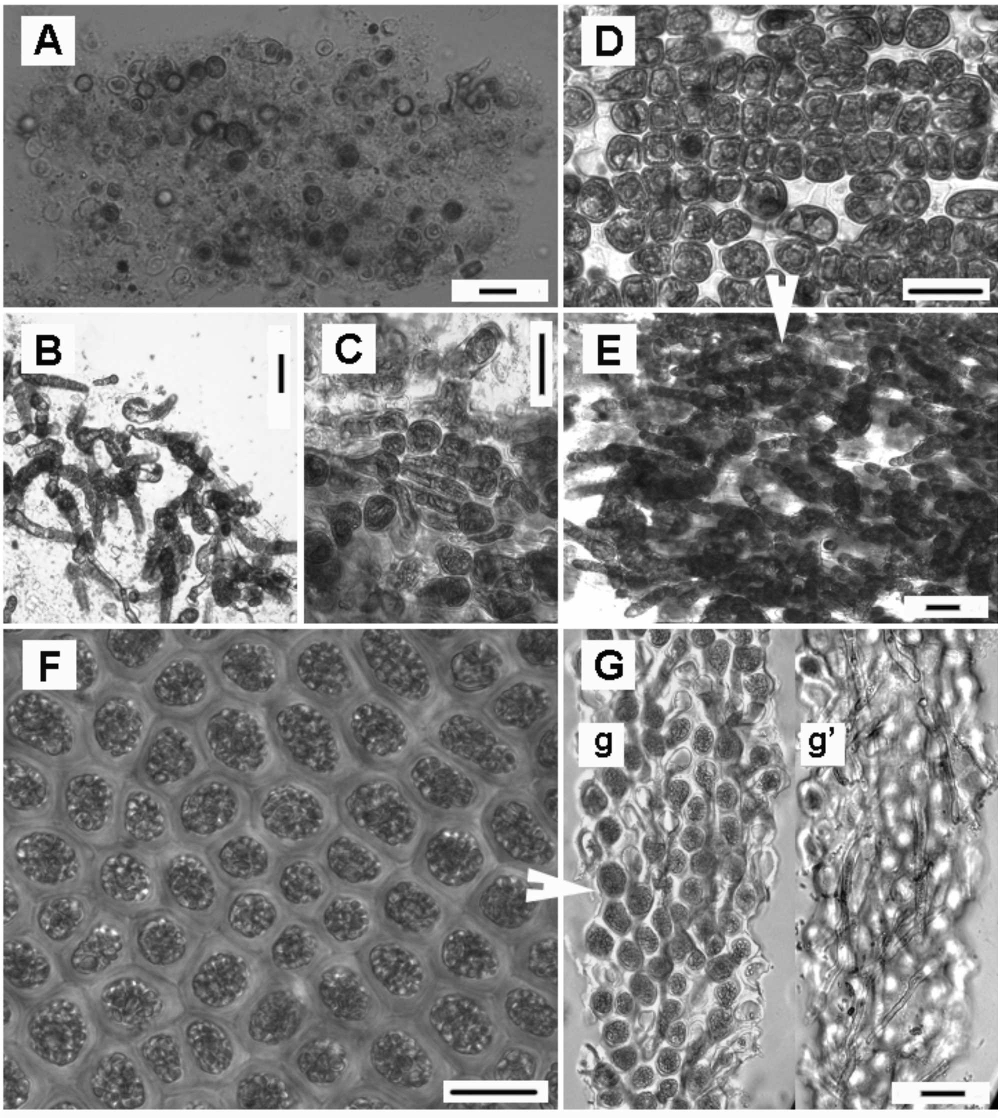

Fig. 4. The regeration of somatic cells cultured under different temperatures.

A–D, and F show the status of somatic cells after two months of culture under 0, 5, 15, 20, and 30°C, separately; E and G are the status of somatic cells that resumed growth after one month of culture under normal conditions, which had been cultured under 5 and 0°C respectively for two month previously. In G, g and g' are the same cell mass with different foci, the former showing the mother cells and the latter showing newly germinated thalli.

Arrows show the shift of the cultured conditions from stress to normal.

Laboratory experiments demonstrated that somatic regeneration was one of the most imporant approaches for the successful colonization and flourish of *E. prolifera* (Fig. 4). The culture showed that at least 19.32% somatic cells of the filaments segments could survive 2 months under ranges of different temperatures (0, 5, 10, 15, 20 and 30°C at the irradiance of 60 μmol photons m^{-2} s^{-1}) and light densities (darkness, 5 10, 15, 20 and 30 μmol photons m^{-2} s^{-1} at the temperature of 20°C). More than 35.85% somatic cells could survive the lower temperature (0°C) or darkness for 2 months (Fig. 5).

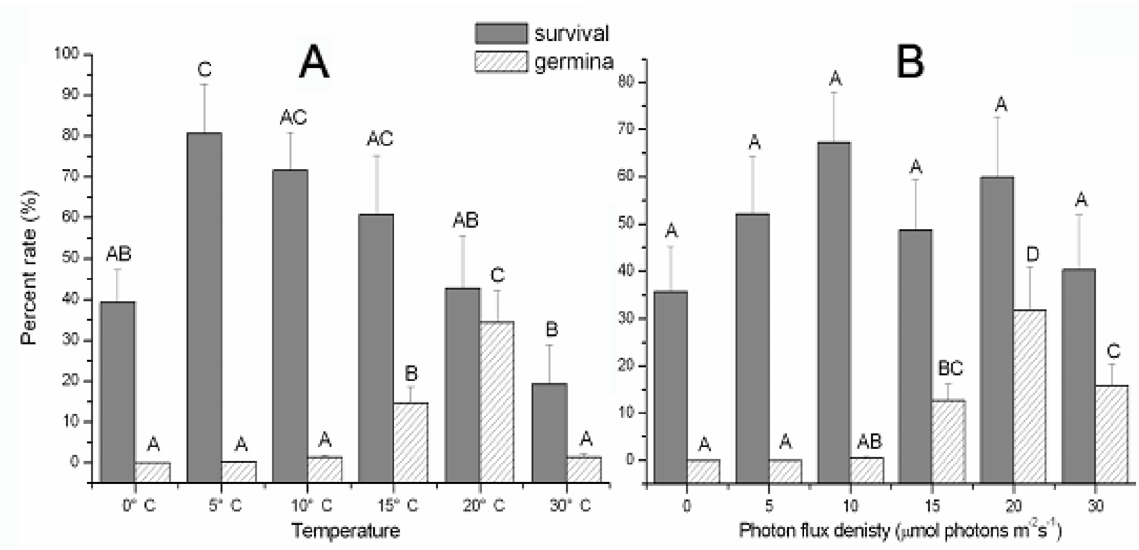

Fig. 5. The survival rate and germination rate of somatic cells cultured under different temperatures and linght densities.

(A) The survival rate and germination rate of somatic cells cultured under different temperatures. (B) The survival rate and germination rate of somatic cells cultured under different light densities.Values are means ± S.D. In each graph, statistically significant difference (ANOVA, DNMR) are indicated by capital letter superscripts ($P < 0.01$).

No less than 15.99% of them resumed growth when the temperature and photon flux denstiy were adjusted to the normal levels (20°C and 60 μmol photons m^{-2} s^{-1}) (Fig. 6). Results of field surveys showed that viable *E. prolifera* was widely and massively presented in the sediment of the Yellow Sea when the macroalga was absent (Fig. 1B). The present results suggest that somatic cells may acts as a overwintering stage for the annual spring bloom of *E. prolifera*. Floating filaments of the green alga carrying prolific cells can reach everwhere driven by the dynamics of seasonal wind and current and can survied various of stessful environment as our results shown.

So, somatic cells regeneration is supposed to be a very dangerous transmitting way for its marked movability and adaptability. By this way, *E. prolifera* spread generations to more and more sea areas with the passage of time. This is suggested as a mechanistic explanation of the the scale of green tide in Yellow Sea 2008 that is much bigger than the one happened last year at the same place.

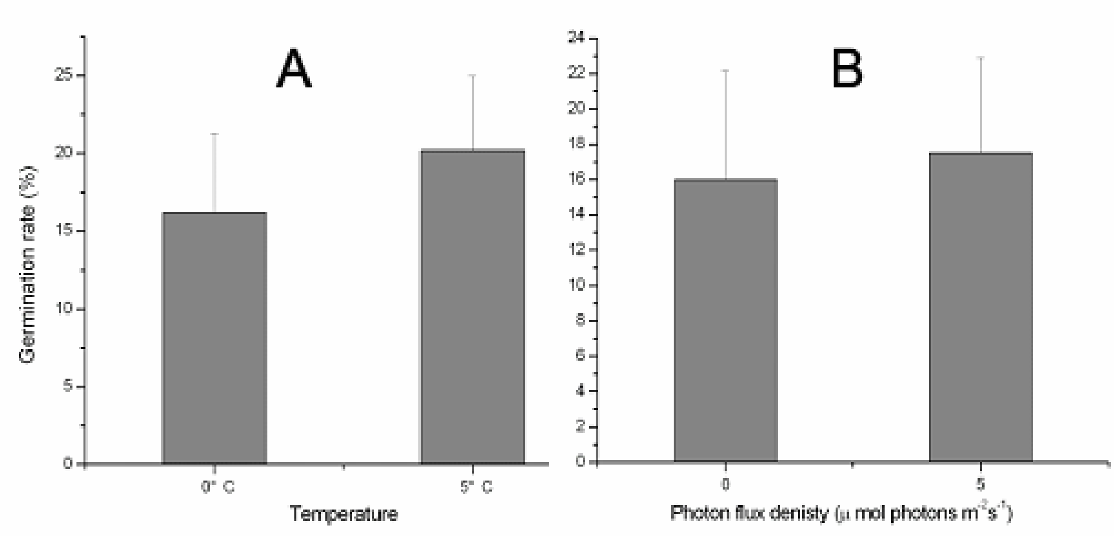

Fig. 6. The germination rate of somatic cells tranfered from stress to normal conditions (Values are means ± S.D).

(A) The germination rate of somatic cells tranfered from 0 and 5°C to normal conditions. (B) The germination rate of somatic cells tranfered from 0 and 5 μmol photons m^{-2} s^{-1} to normal conditions.

Pyrolytic characteristics of the alga

The pyrolytic characteristics of the aglae were studied at heating rates of 10, 20 and 50℃, separately. And the activation energy was calculated using Flynn-Wall-Ozawa method. The results indicated that three stages appeared during pyrolysis, which were dehydration (18–200℃), main devolatilization (200–450℃) and residual decomposition (450–750℃). Different heating rates had considerable influence on the pyrolysis of *Enteromorpha* spp. The initial temperature of pyrolysis, temperature at the maximum weight loss and the maximum weight loss increased with the increasing of heating rates. The activation energy of the alga was determined at 237.23 KJ•mol^{-1}.

It is critical that leaders take the threat from the green tide seriously. In the Mediterranean, for instance, government officials essentially ignored the problem when *Caulerpa brachypus*'s cousin, *Caulerpa taxifolia*, was first found there until it was too late to control. Thousands upon thousands of acres of reef have now been destroyed and billions of dollars worth of damage done. Chinese government had paided much attention to this problem and thus it becames an important scientific research topic. So far, several projects have been launched and a 1.5 million RMB project named 'Application and Research of the emergency treatment technoloy tackling the bloom of *Enteromorpha* spp forming green tide' has been authorized by the Science and Technology Department of China.

References

Callow, M. E. 1996. Ship-fouling: the problem and methods of control. Biodeterioration Abstracts 10:411–421.

Carney, L. T., and Edwards, M. S. 2006. Cryptic process in the sea: a review of delayed development in the microscopic life stages of marine macroalgae. Algae 21:161–168.

Chapman, A. R. O. 1986. Population and community ecology of seaweeds. Pages 23:1–161 in Blaxter, J. H. S., and A. J. Southwood, editors. Advances in marine ecology. Academic Press, London, United Kingdom.

Dan, A., Ohno, M., and Matuoka, M. 1997.Cultivation of the green alga *Enteromorpha prolifera* using chopped tissue for artificial seeding. Suisan Zoshoku 45: 5–8.

Eriksson, B.K., and Johansson, G. 2005. Effects of sedimentation on macroalgae: species-specific responses are related to reproductive traits. Oecologia 143:438–448.

Fletcher, R. L. 1996. The occurrence of 'green tides': a review. Pages 7–43 in W. Schramm and P. H. Nienhuis, editors. Marine Benthic Vegetation: Recent Changes and the Effects of Eutrophication. Springer, Berlin, Germany.

Hoffmann, A. J., and Santelices, B. 1991. Banks of algal microscopic forms: hypotheses on their functioning and comparisons with seed banks. Marine Ecology Progress Series 79:185–194.

Kamermans, P., E. J. Malta, J. M. Verschuure, L. F. Lentz, and L. Schrijvers. 1998. Role of cold resistance and burial for winter survival and spring initiation of an *Ulva* spp. (Chlorophyta) bloom in a eutrophic lagoon (Veerse Meer lagoon, The Netherlands). Marine Biology 131: 45–51

Kolwalkar, J.P., Sawant, S.S., and Dhargalkar, V. K. 2007. Fate of *Enteromorpha flexuosa* (Wulfen) J. Agardh and its spores in darkness: Implications for ballast water management. Aquatic Botany 86:86–88.

Largo, D. B., Sembrano, J., Hiraoka, M. and Ohno, M. 2004. Taxonomic and ecological profile of "green tide" species of Ulva (Ulvales, Chlorophyta) in central Philippines. Hydrobiologia 512: 247–253.

Liang, Z., Lin, X., Ma, M., Zhang, J., Yan X., and Liu, T. 2008. A Preliminary Study of the *Enteromorpha prolifera* Drift Gathering Causing the GreenTide Phenomenon. Periodical of Ocean University of China 38:601–604.

Morand, P., and Briand, X. 1996. Excessive growth of macroalgae: a symptom of environmental disturbance. Botanica Marina 39:491–516.

Nienburg, W. 1927. Zur Ökologie der Flora des Wattenmeeres. 1. Teil. Oer KÖnigshafen bei List auf Sylt. Wiss. Meeresunters. (Abt. Kiel) 20:146–196.

Santelices, B. 1990. Patterns of reproduction, dispersal and recruitment in seaweeds. Oceanography and Marine Biology: An Annual Review 28:177–276.

Schories, D. 1995. Sporulation of *Enteromorpha* spp. (Chlorophyta) and overwintering of spores in sediments of the Wadden Sea, Island Sylt, North Sea. Journal of Aquatic Ecology 29:341–347.

Schories, D., and K. Reise. 1993. Germination and anchorage of *Enteromorpha* spp. in sediments of the Wadden Sea. Helgoländer Meeresuntersuchungen 47:275–285

Schories, D., Anibal, J., Chapman, A., Herre, E., Isaksson, I., Lillebo, A. I., Pihl, L., Reise, K., Sprung, M., and Thiel, M. 2000. Flagging greens: hydrobiid snails as substrata for the development of green algal mats (*Enteromorpha* spp.) on tidal flats of North Atlantic coasts. Marine Ecology Progress Series 199:127–136.

Sun, S., Wang, F., Li, C., Qin, S., Zhou, M., Ding, L., Pang, S., Duan, D., Wang, G., Yin, B., Yu, R., Jiang, P., Liu, Z., Zhang, G., Fei, X., and Zhou, M. 2008. Emerging challenges: Massive green algae blooms in the Yellow Sea. Nature precedings. hdl:10101/npre.2008.2266.1.

Tseng, C. K. 1984. Common seaweeds of China. Science Press, Beijing, China.127 pp.

Wang, S. F., Tang, D. L., He, F. L., Fukuyo, Y. S., and Azanza, R. V. 2008. Occurrences of harmful algal blooms (HABs) associated with ocean environments in the South China Sea. Hydrobiologia 596:79–93.

Worm, B., Heike, K., and Sommer. U. 2001. Algal propagules banks modify competition, consumer and resource control on Baltic rocky shores. Oecologia 128:281–293.

Ye, N., Zhang, X., Mao, Y., Zhuang, Z., and Wang, Q. 2008. Life history of *Enteromorpha prolifera* under laboratory conditions. Journal of Fishery Sciences of China 15:853–859.