Biotechnology Letters (2005) 27: 915–918
DOI 10.1007/s10529-005-7182-8
© Springer 2005

Regulation of gamete release in the economic brown seaweed *Hizikia fusiforme* (Phaeophyta)

Dinghui Zou[1,*] & Kunshan Gao[1,2]
[1]*Marine Biology Institute, Science Center, Shantou University, 515063, Guangdong, China*
[2]*Institute of Hydrobiology, The Chinese Academy of Sciences, 430072, Wuhan, Hubei, China*
**Author for correspondence (Fax: 86-754-2903977; E-mail: dhzou@stu.edu.cn)*

Received 13 January 2005; Revisions requested 27 January 2005 and 5 April 2005; Revisions received 24 March 2005 and 3 May 2005; Accepted 5 May 2005

Key words: gamete release, *Hizikia fusiforme*, receptacles, regulation

Abstract

Gamete release is an essential event in artificial seeding of the economic brown seaweed, *Hizikia fusiforme*. Mass egg release occurred in the dark, with few eggs being discharged in the light. Release of eggs was elicited with eight practical salinity units (one PSU ≡ 1 g sea salts l^{-1}) and was inhibited by salinity levels > 32 PSU. Egg release was optimal at 23 °C, and was decreased by 72% in agitated seawater compared to unstirred seawater. Inhibitors of photosynthesis and ions channels suppressed egg release, indicating that this process was physiologically associated with photosynthetic activity and ion transport.

Introduction

Hizikia fusiforme (Harvey) Okamura (Sargassaceae, Phaeophyta) is an important economic brown seaweed, being used as a healthy foodstuff, medical herb and marine vegetable in Southeast Asia, China, Japan and Korea. Population of this species grows attached on lower intertidal or upper subtidal rocks, gravel or shells (Zou & Gao 2004). In China, *Hizikia fusiforme* has been experimentally cultivated since 1981 in Shandong and Zhejiang provinces, using a rope technique. However, complete artificial seedling of *Hizikia* has not yet been developed. Every year, at the beginning of cultivation period (November–March next year), a large number of young fronds of *Hizika* (3–10 cm in length) are collected from the natural beds by seed collectors for cultivation. As a consequence, it is now difficult to find this alga in many costal waters (Ruan & Xu 2001). Therefore, complete artificial seedling of *Hizikia* is essential in both conserving the natural resource and further promoting cultivation development for this economic important species.

The culture of seedlings via sexual reproduction was considered as a feasible approach, and synchronous massive release of gametes from its receptacles must be achieved artificially for the seed production of *Hizikia* (Li 2001). However, little is known about the influences of environmental factors on the gamete release process of this species. The present work aims to investigate the impacts of key environmental factors on the egg release of *H. fusiforme*.

Materials and methods

Healthy fertile fronds of *Hizikia fusiforme* were collected at low tide during May, 2003, from the natural populations at Yunao Bay, Nanao Island, Shantou, China, and were immediately transported to Marine Biology Station of Shantou University located 10 km away. The female receptacles (about 0.6 cm length) were cut off

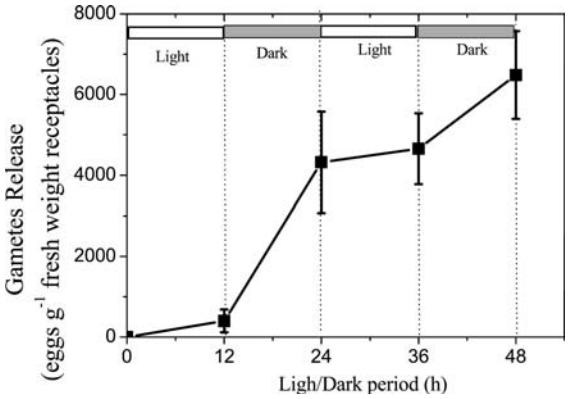

Fig. 1. Egg release from the receptacles of *Hizikia fusiforme* incubated under 12:12 h light-dark regime. Receptacles were incubated at 23 °C in unstirred seawater with a salinity of 32 practical salinity units (one PSU ≡ 1 g sea salts l^{-1}). Accumulated gametes released were counted at the end of each light and dark period. The error bars represent mean standard deviations for the data points.

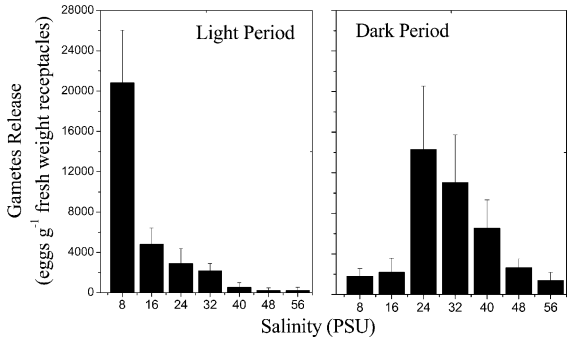

Fig. 2. Effects of salinity on egg release from the receptacles of *Hizikia fusiforme*. Salinity of 32 practical salinity units (PSU) represents the control. Receptacles were incubated at 23 °C in unstirred seawater with varied salinity. Released eggs were counted at the end of light period and at the end of the subsequent dark period. The error bars represent mean standard deviations for the data points.

and used for experiments immediately or within 1 week of storage at 5 °C. They were then incubated in 50 ml filtered seawater in 100 ml flasks under different experimental conditions. Incubations were usually at 23 °C with about 70 μmol photons m^{-2} s^{-1} (12 h light/12 dark) in agitated or unstirred seawater. Salinity levels of the seawater were set from 8 to 56 practical salinity units (one PSU ≡ 1 g sea salts l^{-1}). Stirring (~60 rpm) was provided by a magnetic follower. Released eggs were counted under a dissecting microscope. Each experiment was repeated three times. Data were tested with one-way analysis of variance (ANOVA) or *t*-test, and the significance level was set at 0.05. The error bars in the Figures indicate mean standard deviations for the data points.

Results

Only a few eggs were discharged from the female receptacles of *H. fusiforme* during the light period of cultivation. In contrast, massive egg release occurred in the dark (Figure 1). Salinity significantly ($p < 0.01$) affected egg release, with a large discharge of eggs occurring at 8 PSU in the light period and at 24 PSU in the dark (Figure 2). The maximal number of eggs released under the light period was 46% higher than that under the dark. The eggs released during the dark decreased by 41%, 76% and 88% with the salinities of 40, 48 and 56 PSU, respectively, compared to those with salinity of 32 PSU (Figure 2). Maximum egg release occurred at 23 °C (Figure 3), which is the ambient temperature of seawater during the sexual reproductive period of *H. fusiforme* in the field. Water motion inhibited the egg release by 72% compared to unstirred conditions (Figure 4). Various inhibitors significantly ($p < 0.01$) depressed the egg release (Figure 5). Compared

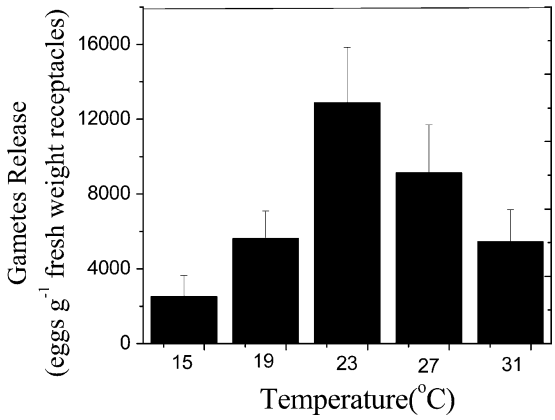

Fig. 3. Effects of temperature on egg release from the receptacles of *Hizikia fusiforme*. Receptacles were incubated at various temperatures in unstirred seawater with a salinity of 32 practical salinity units (PSU). Released eggs were counted at the end of one light-dark period (12 h light plus 12 h darkness). The error bars represent mean standard deviations for the data points.

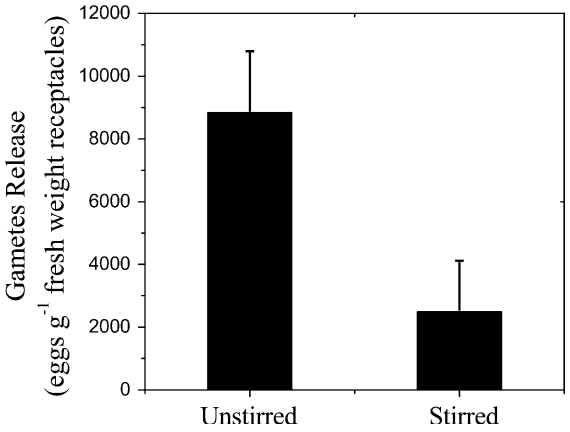

Fig. 4. Effects of water motion on egg release from the receptacles of *Hizikia fusiforme*. Receptacles were incubated at 23 °C in stirred or unstirred seawater with a salinity of 32 practical salinity units (PSU). Released eggs were counted at the end of one light-dark period (12 h light plus 12 h darkness). The error bars represent mean standard deviations for the data points.

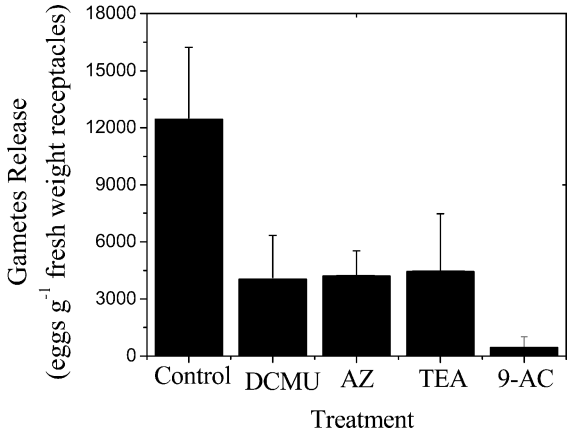

Fig. 5. Effects of 3-(3,4-dichlorophenyl)−1,1-dimethylurea (DCMU), acetazolamide (AZ), tetraethylammonium ion (TEA^+) and anthracene-9-carboxylic acid (9-AC) on egg release from the receptacles of *Hizikia fusiforme*. DCMU was dissolved in 95% (v/v) ethanol and used at 15 μM. AZ was dissolved in 0.05 M NaOH and used at 200 μM. TEA^+ was dissolved in seawater and used at 100 mM. 9-AC was dissolved in ethanol/DMSO (95:5 v/v) and used at 1 mM. Receptacles were incubated at 23 °C in unstirred seawater with a salinity of 32 practical salinity units (PSU). Released eggs were counted at the end of one light-dark period (12 h light plus 12 h darkness). The error bars represent mean standard deviations for the data points.

to the control, DCMU, AZ, TEA^+ or 9-AC decreased the numbers of discharged eggs by 68%, 66%, 64% and 96%, respectively.

Discussion

Optimal egg release from the female receptacles of *H. fusiforme* was in seawater of 24 PSU at 23 °C in the darkness following a light period. A low salinity of 8 PSU in the light gave the highest release but these discharged eggs quickly lost viability.

Santelices (1990) predicted that a higher release of algal propagules would take place in the dark or at low irradiance. The present results also shows that more eggs were released from *H. fusiforme* in the dark than in the light. However, a greater release of spores occurred at higher levels of irradiance (140 μmol photons m^{-2} s^{-1}) in *Gracilaria* spp. (Kain & Destombe 1995, Garza-Sanchez *et al.* 2000). Changes of salinity can directly affect the cellular water potential (Kirst 1989) which thereby influence the turgor pressure and swelling of mucilage within the receptacles of *H. fusiforme* and will, in turn, impact on the release of eggs. A salinity of 8 PSU immediately stimulated egg release; however, these discharged eggs bleached quickly, suggesting a loss of viability. Agitation of seawater adversely affected the egg release in *H. fusiforme*, which might be due to specific mechanical sensitivity to agitation via stretch-activated channels, as suggested by Serrão *et al.* (1996) and Pearson *et al.* (1998). Egg release in *H. fusiforme* was associated with photosynthetic performance since the photosynthetic electron-transport inhibitor, DCMU, and the CO_2-uptake-inhibitor, AZ, both suppressed the egg discharge process. Inhibition of egg release by the ion channel blockers, TEA^+ and 9-AC, suggested that uptake of K^+ and slow-type anion channel played a role in controlling egg expulsion in *H. fusiforme*, as reported in *Pelvetia compressa* (Pearson & Brawley 1998).

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30470343 and 90411018), the Chinese "863" project (No. 2002AA628090), Guangdong Science Technology Bureau (No. 2002C32705) and Guangdong Natural Science Foundation (No. 04010990 and 032048).

References

Garza-Sanchez F, Zertuche-Gonzalez JA, Chapman DJ (2000) Effects of temperature and irradiance on release, attachment and survival of spores of *Gracilaria pacifica* Abbott (Rhotophyta). *Bot. Mar.* **43**: 205–212.

Kain JM, Destombe C (1995) A reviewer of the life history, reproduction and phenology of *Gracilaria*. *J. Appl. Phycol.* **7**: 269–281.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **40**: 21–53.

Li S (2001) Studies on technique of industrial raising seedlings of *Sargassum fusiforme*. *J. Zhejiang Ocea. Univ.* **20**: 251–265 (in Chinese with English summary).

Pearson GA, Brawley SH (1998) A model for signal transduction during gamete release in the fucoid alga *Pelvetia compressa*. *Plant Physiol.* **118**: 305–313.

Pearson GA, Serrão EA, Brawley SH (1998) Sensing hydrodynamic conditions via carbon acquisition: control of gamete release in fucoid algae. *Ecology* **79**: 1725–1739.

Ruan J, Xu L (2001) A preliminary study on the reproductive and developmental biology of *Sargassum fusiforme*. *J. Zhejiang Univ.* **28**: 315–320 (in Chinese with English abstract).

Santelices B (1990) Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanogr. Mar. Biol. Annu. Rev.* **28**: 177–276.

Serrão EA, Pearson GA, Kautsky L, Brawley SH (1996) Successful external fertilization in turbulent environments. *Proc. Natl. Acad. Sci. USA* **93**: 5286–5290.

Zou D, Gao K (2004) Comparative mechanisms of photosynthetic carbon acquisition in *Hizikia fusiforme* under submersed and emersed conditions. *Acta Bot. Sinica* **46**: 1201–1208.