

Seasonal pattern of reproduction of *Hizikia fusiformis* (Sargassaceae, Phaeophyta) from Nanao Island, Shantou, China

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Abstract The maturation pattern of sexual reproduction in *Hizikia fusiformis* (Harvey) Okamura (Sargassaceae, Phaeophyta) was examined in 2003 at Yunao Bay, Nanao Island, Shantou, China. Maturation began in mid-April (seawater temperature 19–21 °C), reached the peak in mid-May (maturation rate ca. 70%, and seawater temperature 23.5–25 °C) and finished in late-June (seawater temperature 27.5–30 °C). The *Hizikia* plants continued to gain the length from the beginning of maturation season to reach a maximum mean length of 34.8 cm in mid-May, after which the mean length was reduced drastically due to the senescence and rupture of the larger plants in size. The major portion of the mature plants belonged to the larger plants between April and May, but to the smaller ones in June. It is suggested that the plant must achieve a critical size before reproductive maturation occurred. There was a positive relationship between the number of receptacles (NR), as well as the reproductive allocation (RA), and the plant size of *Hizikia* population, with the recorded

maximum values of NR and RA being 1220 and 64.3% respectively, for a single plant.

Keywords *Hizikia fusiformis* · Maturation · Growth · Reproductive allocation · Receptacle

Introduction

Hizikia fusiformis (Harvey) Okamura (Sargassaceae, Phaeophyta), being endemic along the northwest coast of Pacific Ocean, has traditionally been used as food-stuff in China, Japan and Korea. Market demand for this alga is increasing owing to its high economic value in nutrition, pharmacy and industrial utilization. In China, *H. fusiformis* is one of the most common species along the coastline from the Liaodong Peninsula of Northern China to Leizhou Peninsula of Southern China, covering both the sub-tropical and sub-temperate coastal waters (Tseng et al., 2000). However, the natural resource of *H. fusiformis* was severely damaged due to overexploitation in the last decade. Large-scale aquaculture of this alga is the ultimate way for the conservation and sustainable utilization of the resource. However, the expected aquaculture has not yet been fully achieved because of the very low technical level of artificial breeding. The main cause is a lack of biological knowledge (especially of reproductive biology) about this alga, although a few studies have been carried out on the physiology, ecology, morphology, reproduction and commercial utilization of *H. fusiformis* (Sohn,

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1993; Hwang et al., 1994a,b; Park et al., 1995; Sun et al., 1996; Hwang et al., 1997; Lee & Kamura, 1997; Li, 2001; Ruan & Xu, 2001; Luo & Li, 2002; Zou et al., 2003; Zou & Gao, 2004; 2005a,b).

The reproductive biology of this species is not well documented, although both sexual and asexual reproduction is known to occur seasonally (Sun et al., 1996; Li, 2001; Ruan & Xu, 2001). The population of *H. fusiformis* is pseudo-perennial in that the rhizoidal holdfasts are perennial, whereas the sporophytes are annual. The holdfasts can produce or regenerate new seedlings (sporophytes), contributing greatly to the population maintenance of *Hizikia*. Sporophytes begin to produce receptacles during the maturation season. Generally, *H. fusiformis* displays a trend in growth and reproductive phenology along the latitudinal gradient of its distribution; *Hizikia* populations growing in the temperate and subtropical zone tend to mature earlier compared to those growing in higher latitudes, and this is assumed to be associated with the latitudinal temperature gradient (Tseng et al., 2000). However, there is little quantitative information about the timing and the process of sexual reproduction.

On the coast of Yunao Bay, Nanao Island, which is located at the border between the East China Sea and South China Sea, the sporophytes of *H. fusiformis* appear in autumn (December), are most abundant in spring and disappear completely in summer (August) when temperature of surface seawater reaches 28–31 °C. During the mature season (from April to June), the reproductive tissues (receptacles) develop mainly on the lateral branches of *Hizikia* sporophytes. Gametes are discharged from the numerous spherical conceptacles distributed subepidermally in the mature receptacles. In our previous work, we investigated the impacts of key environmental factors on the egg release during the peak period of sexual reproduction (Zou & Gao, 2005a). In the present work, we collected *H. fusiformis* from Yunao Bay, Nanao Island, to examine the seasonal pattern of sexual reproduction, and concurrently to examine whether there existed a reproductive size dependence in the natural population of *H. fusiformis*.

Material and methods

Sampling was performed at Yunao Bay, Nanao Island, Shantou, China (23°20'N, 116°55'E). Yunao Bay is a small bay sheltered by reefs with a mixture of hard

substrata of stones and boulders that are dispersed on the sandy sea bottom. The patchily distributed *H. fusiformis* plants grow on hard substrata from the low intertidal zone to a depth of 1.5 m. The site is exposed to relatively strong wave action throughout the year.

Samples were collected semi-monthly from April to July 2003 during the sexual reproductive period of *H. fusiformis*. Seawater temperature was recorded at the time of algal sample collection. At each sampling time during low spring tides, several patches were chosen randomly among the *H. fusiformis* population and all the plants in the patches, were harvested intact including as much of holdfasts as possible from the substrata by gentle scraping or pulling. Approximately 500–800 plants were collected at each sampling time. The plants were placed in plastic bucket and brought back to the laboratory of the Nanao Experiment Station of Marine Biology, adjacent to the collection site.

The length of all plants sampled was measured from the attachment to the longest frond, but holdfasts were excluded as they were very variable. The percentage occurrence of different sized plants (divided into 8 length classes: ≤ 5.0 , 5.1–10.0, 10.1–15.0, 15.1–20.0, 20.1–25.0, 25.1–30.0, 30.1–35.0, ≥ 35.1 cm) was calculated from the total number of the plants. The average length of the 30 largest plants among the samples at each collection date was used for growth analysis of the *H. fusiformis* population. At the same time, all the plants sampled were inspected for the presence of receptacles, and the maturation rate (i.e. the percentage of plants with detectable receptacles) in each length class and in the total number of the plants were calculated. At the peak of the sexual reproductive period, the number of receptacles was counted for a single plant. Reproductive allocation (RA) was estimated as the proportion of reproductive biomass per plant according to Thompson and Stewart (1981) and McCourt (1985). RA for an individual plant was calculated as: $RA (\%) = [R/(R+V)] \times 100$, where R is the biomass of the reproductive structures (receptacles including their supporting branches), and V is the biomass of vegetative tissues (excluding holdfasts).

Results

Figure 1 shows the changes in surface seawater temperature at Yunao Bay during the growth and maturation season of *Hizikia fusiformis* in 2003. Water temperature

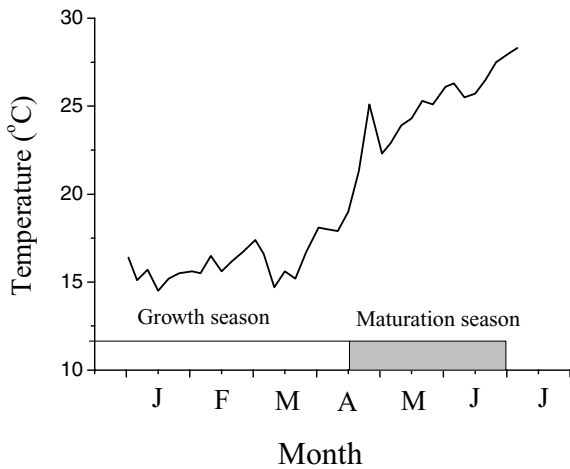


Fig. 1 The surface seawater temperatures at Yunao Bay during the growth and maturation season of *Hizikia fusiformis* in 2003

increased from ca. 16 °C in early January to ca. 30 °C in early July.

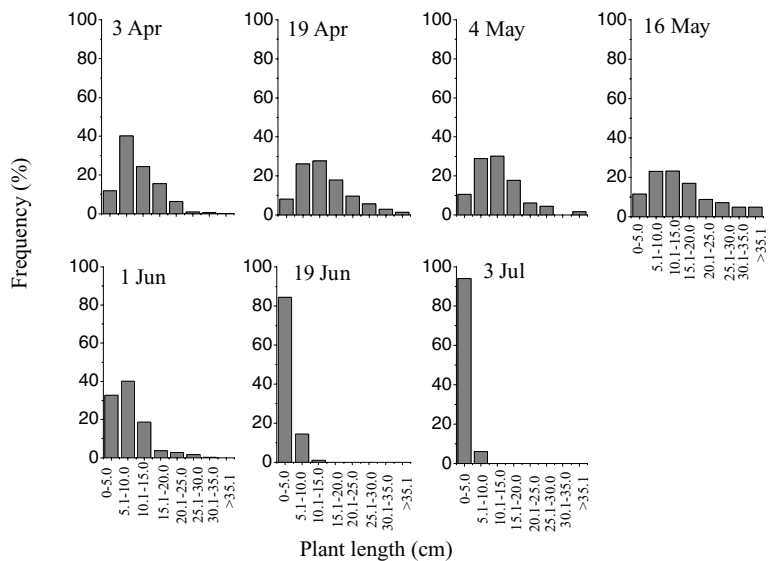
The size distribution of the plants in the *Hizikia* population during the maturation season is presented in Figure 2. On 3 April, more than 50% of the plants were less than 10.1 cm in length. From 19 April and onwards, until 16 May, the plants belonged mainly to a bigger length class (> 10.0 cm). However, on 1 June, the frequency of longer plants was strongly reduced. On 19 June almost all the plants had declined to less than 10.0 cm, because the larger plants began to senesce

and to rupture. On 3 July, almost all the plants were less than 5.1 cm in length. Fig. 3 shows that the *Hizikia* plants continued to gain length from the beginning of the maturation season to reach a maximum mean length of 34.8 cm in mid-May, after which the mean length decreased rapidly due to the senescence and rupture of the larger plants.

Figure 4 shows the changes in the proportion of plants bearing receptacles. The mature plants (i.e. plants bearing receptacles) were first collected on 19 April, when 30% of the plants bore receptacles. The percentage of mature plants increased gradually until 16 May, when a maximum percentage of 70% was observed. From this date onwards, there was a sharp decline in the percentage of fertile plants and on 3 July, no fertile plants were recorded.

On 19 April, 80% of plants longer than 25.0 cm, and 100% of the plants longer than 35.0 cm were observed to bear receptacles (Fig. 5). However, the receptacles were small (>2 mm in length) and immature, and no egg release was observed on this date. On 4 May more than 80% of plants longer than 15.0 cm bore receptacles. The receptacles had a length of 2–6 mm, and egg release could be observed in some of them. On 16 May most (more than 90%) of the plants longer than 15.0 cm were reproductive, and even small numbers of plants shorter than 5.1 cm bore a few receptacles. At this time, the receptacles reached their maximum size of 5–10 mm and a copious release of eggs could be

Fig. 2 Changes in frequency distribution of plant length of *Hizikia fusiformis* population during the reproductive season in 2003



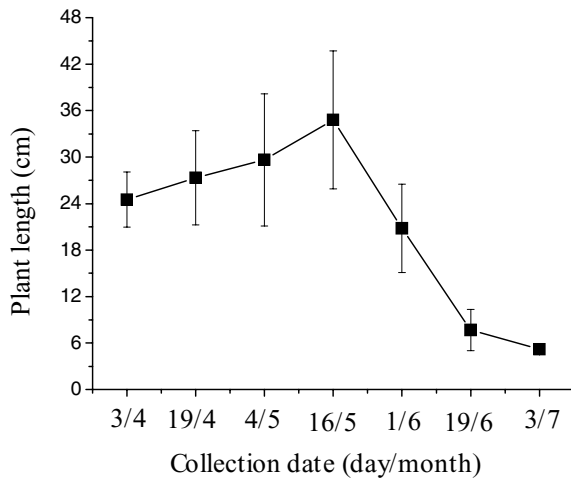


Fig. 3 Changes of plant length of *Hizikia fusiformis* population during the reproductive season in 2003. Data are the averages with standard deviations for the 30 largest plants at each collection time

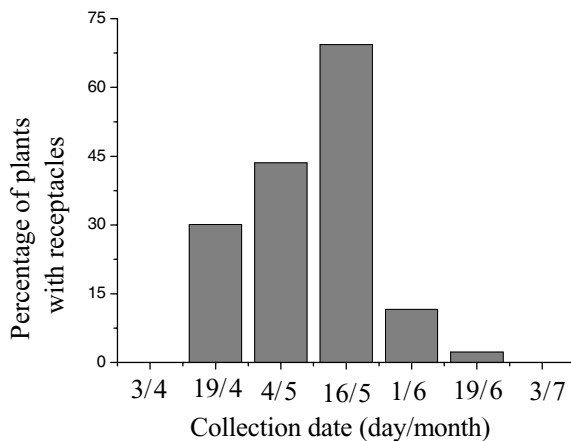


Fig. 4 Changes in the percentage of plants bearing receptacles in *Hizikia fusiformis* population during the reproductive season in 2003

discerned. After 16 May, some of the larger plants in size started senescence and ruptured. The proportion of plants with length of 25.1–30.0 cm class possessing receptacles was 80% for the samples collected on 1 June, while the maturation rate in plants shorter than 25.1 cm was lower on 1 June than that on 16 May. On 19 June, all the plants sampled were shorter than 15.1 cm, and the maturation rate was 75% within the plants in the 10.1–15.0 cm class.

The size distribution of reproductive plants is presented in Figure 6. The major proportion of the reproductive plants was in the larger plants during April and

May, but in the smaller ones in June. Between April (the beginning of mature season) and early May, most of the mature plants belonged to the 10.1–30.0 cm length class. No small plants (< 10.1 cm length) on 19 April, and only a few small ones on 4 May, had reached maturity. However, by the 16 May, 16% of reproductive plants were less than 10.1 cm in length. On 19 June, there was a pronounced change in the length distribution of the reproductive plants, with all reproductive plants in the length class of 5.1–15.0 cm.

There was a positive relationship between the number of receptacles (NR), as well as the reproductive allocation (RA) and plant length (L) at the peak of reproductive maturation (Fig. 7). Regression analysis gave the following: $\log(\text{NR}) = 0.4079 + 1.4722 \times \log(L)$ ($R^2 = 0.4176$, $P < 0.001$, $n = 85$); $\log(\text{RA}) = 0.1494 + 0.9363 \times \log(L)$ ($R^2 = 0.1871$, $P < 0.001$, $n = 101$). The maximum NR and RA measured in a single plant in the *Hizikia* population were 1220 and 64.3%, respectively.

Discussion

This study showed that the population of *Hizikia fusiformis* along the coast of Yunao Bay, Nanao Island, Shantou had a spring-summer mature period from April to June, which was earlier than the fertile period in the higher latitude *H. fusiformis* population of Dongtou Island, Zhejiang (Sun et al., 1996; Li, 2001; Ruan & Xu, 2001). The higher seawater temperature at Nanao Island than at Dongtou Island might be responsible for the earlier fertility period in the *Hizikia* population of Nanao Island, possibly because *H. fusiformis* requires a specific amount of energy for reproductive maturation, as suggested by other authors (De Wreede, 1976; Deysher, 1984; Arenas - Fernández, 1998) in *Sargassum* spp. Lüning and tom Dieck (1989) have stated that some environmental factors, such as photoperiod and/or seawater temperature, trigger the algal maturation processes. It has been reported that short days and/or decreasing seawater temperature result in the onset of maturation in some *Sargassum* species (De Wreede, 1976; Prince & O'Neal, 1979; Glenn et al., 1990; Gillespie & Critchley, 1999; Yoshida et al., 2001). However, the present study shows that *H. fusiformis* began to mature in spring-summer, coincident with increasing day length and increasing seawater temperature. Uchida (1993) also reported

Fig. 5 Changes in the percentage of mature plants within varied plant length classes in *Hizikia fusiformis* population during the reproductive season in 2003

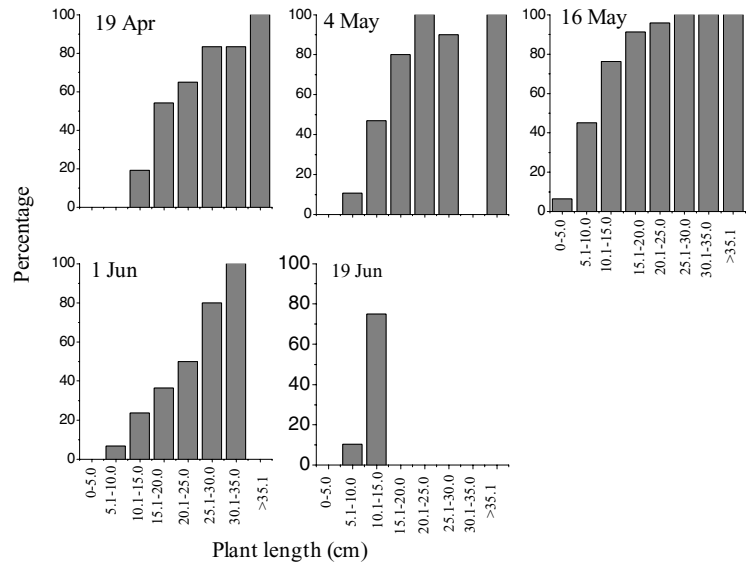
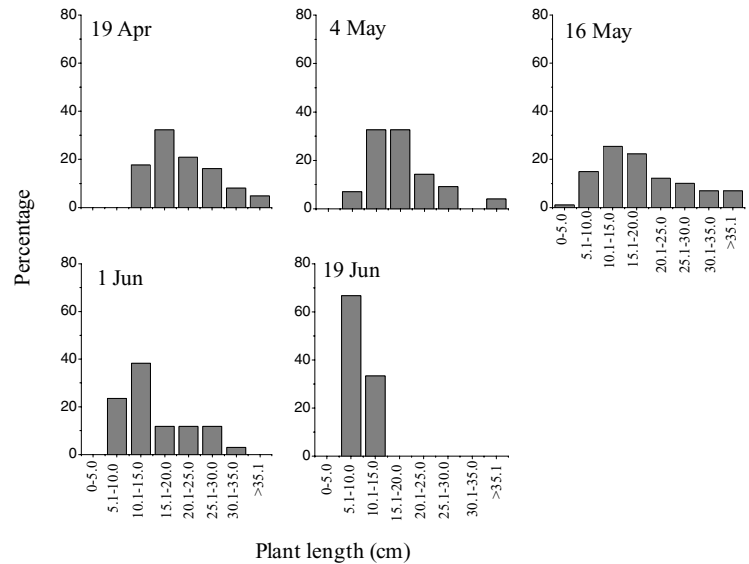


Fig. 6 Changes in the length distribution of mature plants in *Hizikia fusiformis* population during the reproductive season in 2003

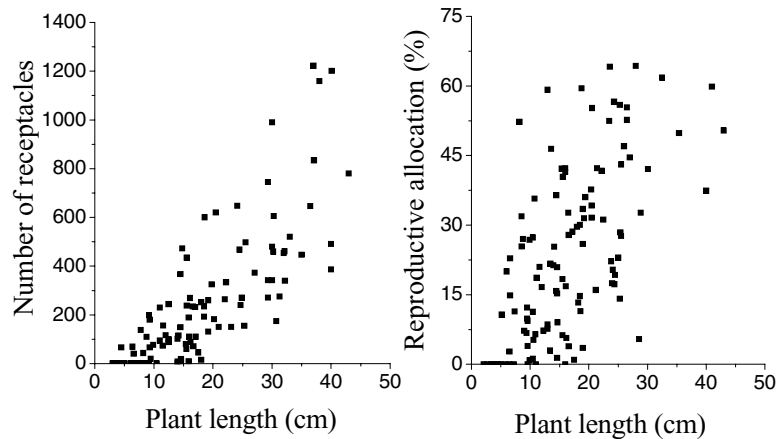


that long-day conditions induced receptacles formation of *S. horneri* in laboratory culture. Therefore, in *H. fusiformis*, elevated temperature and photoperiod, or combined temperature/daylength, should be considered as important for reproductive maturation.

The present results showed that the onset of reproductive maturation did not occur in all the plants in the *H. fusiformis* population. Changes in the percentage of fertile plants show a gradual maturation in this population, implying a development constraint. In such

a complex structured seaweed such as *H. fusiformis*, thalli must achieve a critical size before reproductive maturation can occur as reported by McCourt (1984) and Arenas and Fernández (1998) in *Sargassum* spp. The present study shows that the percentage of mature plants of *Hizikia* was greater in larger plants than in smaller ones. At the beginning of mature period only plants longer than 35.0 cm were sufficiently developed to bear receptacles on the leaf axils, and 10.1 to 35.0 cm length range plants were partly

Fig. 7 Relationship between the number of receptacles, as well as the reproductive allocation, and plant length in *Hizikia fusiformis* population at the peak of reproductive season in 2003



mature. The smallest plants (≤ 5.1 cm in length) could never become reproductively mature, except for a very small proportion (6%) of small plants at the peak of the reproductive period which produced some small receptacles.

At the beginning of reproductive season, *H. fusiformis* still exhibited a positive growth rate. After *H. fusiformis* had reached their maximum length at the peak of fertility, they showed a pronounced negative growth rate due to senescence and rupture. It appeared that the senescence and mortality of *Hizikia* plants were size dependent, since they occurred mainly in larger plants. De Wreede and Kllinger (1988) pointed that reproduction of seaweeds might be cost-free, or more probably cost-reduced, because reproductive structures might be self-supporting in terms of carbon budgets. In fact, our previous results have shown that the receptacle tissue of *H. fusiformis* had a much higher photosynthetic capacity than the vegetative tissues (Zou & Gao, 2005b). This might indicate a reduced dependence of these reproductive structures on the vegetative parts for much of their nutrition. Therefore, the negative relationship between growth and reproduction in *H. fusiformis* does not involve the traditional view of reproductive allocation, which involves a trade-off between these two processes and between the alternate strategies of sexual reproduction by means of receptacle formation and asexual reproduction by means of investment in the holdfast where new shoots are initiated, as suggested by McCourt (1984). The coincidence of fertility, termination of growth and/or senescence has also been reported in other brown algae such as *S. muticum* (Norton, 1977; Arenas et al., 1995) and *Fucus distichus* (Ang, 1992). Rico and Fernández (1997) also

showed that the senescence of *S. muticum* along the north coast of Spain coincided with the onset of a period of N-limited growth. Further investigation is required to exactly establish the factors (such as elevated temperature, light intensity and nutrient limitation, as well as some physiological factors) controlling senescence of *H. fusiformis*, once the plants reach maximum size and the peak of reproductive maturation.

The results in the present study show that there is a positive plant size dependence for reproductive allocation and the number of receptacles in *Hizikia*. This agrees with the results reported for many other brown algae such as *F. spiralis* (Roberston, 1987), *Alaria nana* (Pfister, 1992), *Ascophyllum nodosum* (Mathieson & Guo, 1992; Åberg, 1996) and *S. muticum* (Arenas and Fernández, 1998). The interpretation for the reproductive size-dependence in *Hizikia* could be that larger plants may generate a greater number of reproductive meristems per unit of vegetative biomass as in the case of *S. muticum* (Arenas & Fernández, 1998). However, Ang (1992) did not find a correlation between reproductive allocation and plant size in *F. distichus* which exhibits continuous reproduction throughout the year (Ang, 1992), whereas *H. fusiformis* in our study only has a very shorter sexual reproductive period (from April to June). Therefore, the discrete reproductive event in such alga as *H. fusiformis* might give a clearer picture of the size dependence of reproductive allocation.

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