Acta Oceanol. Sin., 2023, Vol. 42, No. 9, P. 101–112
https://doi.org/10.1007/s13131-023-2158-x
http://www.aosocean.com
E-mail: ocean2@hyxb.org.cn

Two-stage reproduction derived from cells of thallus could directly contribute to seeds for green tidal algal *Enteromorpha* (*Ulva*) *prolifera*/*clathrata* bloom, with disclosure of their ephemeral trait

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Received 5 July 2022; accepted 2 November 2022

Abstract
Green tidal algal *Enteromorpha* species complete their life cycles by the isomorphic alternation of generations. The provenance of green tide caused by them in the western Yellow Sea has been disputed. The cell reproduction derived from adult thallus was observed on *E. clathrata* collected from Shantou City, Guangdong Province in this study. Subsequently, it further found that *E. proliferia* collected from Qingdao City, Shandong Province and Qinhuangdao City, Hebei Province, produced reproductive cells by somatic cells of its early infantile thallus or branch. The latter is functionally similar to that the seedlings of red alga *Porphyra yezoensis* produce the monospores, and could exquisitely explain the ephemeral or opportunistic trait and environmental adaptation ability of *Enteromorpha* species. Changes in growth conditions may induce the two types of cell reproduction. They contribute to the bloom, and can effectively reveal the seasonally occurring large-scale and on-year and off-year phenomenon. The latter may have played a decisive role in its formation. This paper analyses the legal status of the species name, the type of generation during bloom, ephemeral traits, the role of microscopic propagule, the area of origin, on-year and off-year phenomenon, early warning and prevention and control of the species, and so on. On this basis, further study on the influence of environmental factors on cell reproduction of early infantile thalli or branches will achieve a positive effect for early warning and prevention and control of the green tidal algal bloom.

Key words: green tidal algae, Yellow Sea, opportunistic trait, on-year and off-year, early infantile, alternation of generations, prediction on the occurrence process

Citation: Huang Bingxin, Ding Lanping, Zhang Yao, Guo Youxuan, Liang Junxia, Xie Yanqi, Chu Yue. 2023. Two-stage reproduction derived from cells of thallus could directly contribute to seeds for green tidal algal *Enteromorpha* (*Ulva*) *prolifera*/*clathrata* bloom, with disclosure of their ephemeral trait. Acta Oceanologica Sinica, 42(9): 101–112, doi: 10.1007/s13131-023-2158-x

1 Introduction

Enteromorpha (*Ulva*) *proliferia* and *E. clathrata* are common green tidal algae in warm temperate waters, with a life cycle completed by the isomorphic alternation of generations by cell reproduction of adult thallus (Hiraoka et al., 2003; Shen, 2022). Since the eruption of *E. prolifera* in the Yellow Sea in the summer of 2007, many scholars have focused on its various aspects scientifically. The reason for the large-scale outbreak has always been one of the main issues. Some scholars considered that microscopic propagules were the main source of the green tidal algae during its blooming period (Zhang et al., 2011; Liu et al., 2012, 2013; Wang et al., 2020; Han et al., 2022). However, there are three viewpoints on the definition of microscopic propagule (Shen, 2022). Based on the reproduction mode, there are four kinds of the life cycle of *E. prolifera*, which are summarized as asexual reproduction (spores and discrete single cell of parent thallus), sexual reproduction (fusion between male and female gametes), parthenogenesis (germination direct by single female/male gamete), and vegetative reproduction (somatic cell mass and branch segments of parent thallus and so on). Moreover, based on the thallus maturity, there are two kinds of cell reproduction: mature thallus reproduction and vegetative reproduction.

The discovery of these reproductive pathways improved and supplemented the life history of *E. prolifera*. However, there were still some gaps in the comprehensive interpretation of the large-scale status during the blooming period and the phenomenon of on-year and off-year. Some scholars attributed the original of its seedlings to the reproduction of vegetative or animal-derived callus-induced cells or germination of sink reproductive cells. Because the life cycle of normal annual thallus also occurs once every year. However, these results did not address the prevention and control of its bloom. It still appears regularly every year. Whether there are other supply or supplement pathways of seed for the blooming green tidal alga in the Yellow Sea has been actively explored by scholars.

We observed *E. clathrata*, another green tidal alga collected from Shantou City, Guangdong Province, in 2013, which is closely similar to *E. prolifera*. Obtained evidence that adult thalli

Foundation item: The National Natural Science Foundation of China under contract Nos 31970216 and 31670199.
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were the main provenance for the reproduction of large-scale blooming green tidal algae (Xie, 2013; Ding et al., 2014), presented in International Symposium of Advanced Research on Green Tides[1]. It has been mentioned successively on *E. prolifera* after our work. Subsequently, the release of reproductive cells from seedlings of the latter was unintentionally observed when we examined the growth state of marine algae microscopically in the lab culture tank at the end of 2018, previously collected from Qingdao City, Shandong Province, in August 2017. But we did not publish these data due to the lack of a complete evidence chain. In mid-winter 2021, we once again observed the seedling reproduction of *E. prolifera*. It was collected as an epiphytic macroalga through the thermostatic incubation culture of living red alga *Gelidium amansii* and green alga *Codium fragile* from Qinhuangdao City, Hebei Province, in September 2021 (Zhang et al., 2023). They can be called cell reproduction by early infantile thallus/filament, with a function similar to the monospore produced by the seedling of the red alga *Porphyra yezoensis*. But it is fundamentally different from that of *P. yezoensis*. The former is the reproductive cell born by somatic cell division, while the latter is the discrete form of a single somatic cell.

The habits and characteristics between *E. clathrata* and *E. prolifera* are very similar (Ding et al., 2014), and both are common green tide algae. They have the same life cycles, especially since the cell reproduction process of the adult thallus is mainly the same. The cell reproduction of early infantile thallus/filament could exquisitely explain the “ephemeral” or “opportunistic” trait and environmental adaptation ability of *Enteromorpha* species. Based on cell reproduction of both adult and early infantile thalli/filaments in our findings, it can effectively be analyzed to the above-mentioned large-scale outbreak and on-year and off-year phenomenon (Ding et al., 2009) of green tidal algae in the Yellow Sea, and the latter may have played a decisive role in its formation. On this basis, further study on the influence of environmental factors on cell reproduction of infantile thallus/filament will be able to predict its bloom effectively. Here we are willing to publish our data in the hope of providing reference strategies for the forecasting and prevention of green tidal bloom in China.

2 Materials and methods

2.1 *Materials*

The fresh sample of *E. clathrata* collected in March 2012 from the salt pan of San’ao Village, Nan’ao County, Guangdong Province (23°20′N, 116°55′E). After being rinsed with seawater, the healthy thalli were selected and temporarily cultivated at room temperature (18–22℃) for three days before the experiment. The culture conditions for the experiment included the temperature 20–23℃, photoperiod 12L:12D, light intensity 35–40 μmol/(m^2·s), salinity 30, medium f/2, and culture cycle of 12 d.

The living seedlings of *E. prolifera* were isolated from the descendants of marine macro-algae in the indoor culture tank at the end of 2018. They were the epiphytic ones on the fresh samples of *Cladophora* species cultivated and previously collected from Qingdao City, in August 2017. The culture conditions included the culture tank 56 L, room temperature 20℃, photoperiod 12L:12D, fluorescent lamp 20 W, brine adjusted salinity to 30, ventilation by an air pump, and water circulation by a jet water pump.

Living seedlings of *E. prolifera*, as one of the epiphytic algae by thermostatic incubation, were isolated from the fresh samples of both *Codium fragile* and *G. amansii* (Zhang et al., 2023). The fresh samples were collected in Qinhuangdao City, in September 2021. The latter two were temporarily cultivated indoors at room temperature (about 20℃) for the following experiment. The culture conditions for *C. fragile* included the temperature of 22℃, photoperiod 12L:12D, light intensity 60 μmol/(m^2·s), salinity 30 and culture cycle of 14 d, for *G. amansii* under temperature of 25℃, photoperiod: 12L:12D, light intensity 75 μmol/(m^2·s), salinity 30 and culture cycle 14 d.

2.2 *Experimental equipment*

Intelligent illumination incubator (GXZ-300D/380D, Ningbo Jiangnan Instrument Factory, Ningbo, China), culture tank 30 L, electronic scale, illuminometer, salinometer, pipette, and UV-Vis spectrophotometer (Shimadzu UV-2550, Kyoto, Japan).

3 Result

3.1 *Cell reproduction of adult E. clathrata under constant temperature conditions*

3.1.1 *Cell development process of adult thalli submerged in seawater*

Under the condition that the thalli were wholly submerged in seawater, their color varied with the growth process from bright green to yellow-brown. The starch nucleus in the cell first became larger and finally disappeared, and the pigment also changed from filling the entire cell to only part concentrated at one end of the cell (Fig. 1).

3.1.2 *The release and germination types of reproductive cells in adult thalli under semi-drying conditions*

Under the semi-drying condition that the thalli were partially exposed to air and others submerged in the seawater, there are two ways of releasing reproductive cells and attaching them to germination, respectively in the seawater and on the parent thalli (Figs 2a, b).

The reproductive cells were released into the seawater when the adult thalli of *E. clathrata* were submerged. Figure 2a shows the development of a single reproductive cell into a seedling after it was released. It can be observed from Figs 2a4–a6 that polar differentiation occurs when the reproductive cell divides into three cells. Its upper part continued to divide and grow, and the lower part finally developed into the basal part of the seedling. When more than 30 cells are produced, the basal part can be seen, and the polarity is already obvious.

The reproductive cells were also released when the adult parent thalli were exposed or partly submerged. However, they could not enter the seawater and retain or settle in the body of the latter or epiphytic to the outside surface of their remnant. Figure 2b shows the germination of reproductive cells in the adult parent thalli or their remnants into seedlings.

3.2 *Cell reproduction of infantile E. prolifera under room temperature—reproductive cells born from the middle and lower cells of infantile thalli*

In August 2017, fresh samples of marine *Cladophora* species were collected from Qingdao City and cultured in the tank at room temperature shown in Fig. 3a. Before long, this species, with its epiphytic macroalgae and their offspring, began to flourish in the tank. Until December 2018, the release of reproductive cells from seedlings of *E. prolifera* was observed unwittingly when these algae were examined microscopically (Fig. 3b). The

[1]Ding Lanping, International Symposium of Advanced Research on Green Tides, May 09–11, 2014, Shanghai, China.

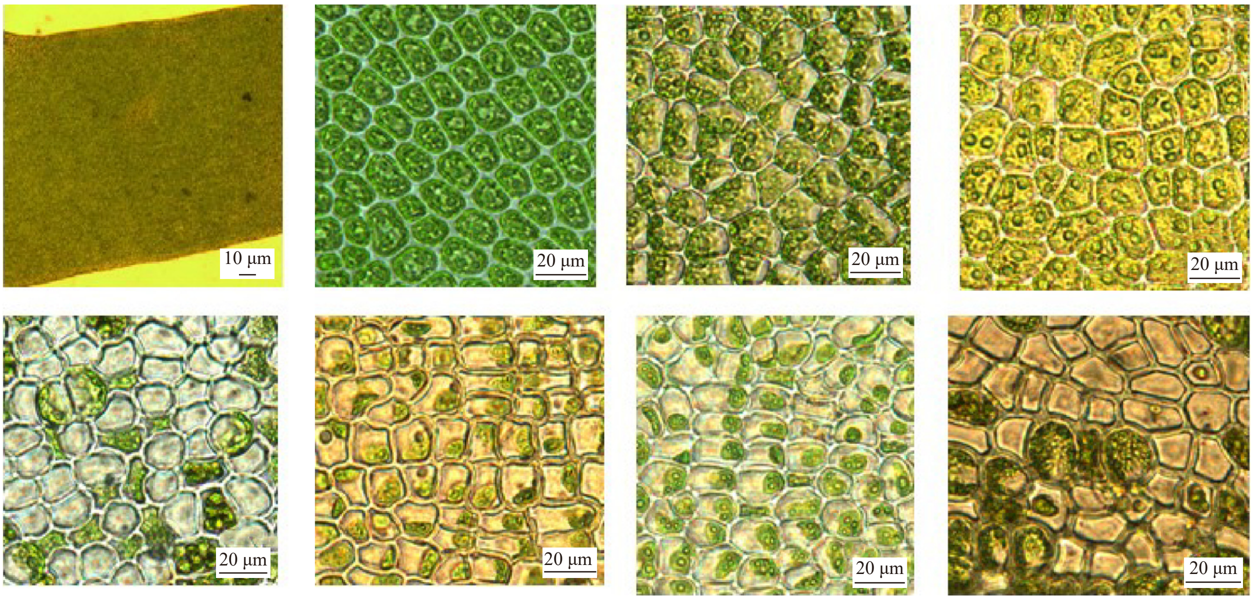

Fig. 1. The somatic cells development process of *Enteromorpha clathrata* shows the juvenile to maturity in turn.

seedling in Fig. 3b is about 2.4 mm in length with a basal rhizoid. The reproductive cells were produced and completely released about 0.8 mm in length upwardly from the elongated cell near the base of the seedling. At the upper part of it, some reproductive cells are being born continuously. The pigments are dark green at the elongated cell near the base and upper vegetative cells. Except for the base, about 42% of the seedling's length produced reproductive cells. Figure 3c shows an apparent remnant of it after its reproductive cells had released. The average size of the parent cells is about 17.8 μm × 15.5 μm based on the apparent cell wall of the seedling's remnant when the reproductive cells are released. As a result, there are about 44 to 50 layers of cells in this 0.8 mm long section of seedlings mentioned above. At the upper part of the seedling, where reproductive cells are formed, it is obvious that the seedling is composed of 2 rows of cells with eight reproductive cells formed by a single parent cell (4 × 2 sides). Theoretically, a seedling of 1 mm in length can produce at least 1 760 reproductive cells because the thalli of *Enteromorpha* species are composed of double-layered cells before they don't form the tubular structure.

3.3 *Cell reproduction of adolescent filamentous thalli of E. prolifera under constant temperature—reproductive cells born from the branches near the base of adolescent thalli*

3.3.1 *Adolescent E. prolifera with reproductive cells formed and released at 22℃*

After being cultured for 14 d at 22℃, the formation and release of reproductive cells in the adolescent filamentous thalli were observed (Fig. 4). The thallus in Fig. 4a is about 7 mm in length with basal holdfast about 0.5 mm long (Fig. 4b), in which three branches are produced from its upper part. The lower parts of the three branches all developed into light-colored elongated cells (Figs 4b, c). Two strong branches continued to grow or branch, and developed into dark green vegetative branches without any reproductive structure. And the third branch (see the short arrow) successively produced seven secondary ones near its base (Fig. 4a). They together developed into a reproductive branch group. Their common features are slender branches, pale color, and slightly creeping growth with 2–3 columns of cells (Figs 4d1, d2 and f6). The formation and release of reproductive cells in different parts of these branches shown in Figs 4e1–e7 and f1–f5. The cell sizes of the reproductive branches were measured and averaged to 17.30 μm × 14 μm based on Figs 4d2, f1, and f6. The average number of reproductive cells was about 12 per parent cell based on Figs 4e2, e3, e6, e7, and f6. According to the biological reproduction rule, a theoretical number of reproductive cells should be 16 or 32 per parent cell. Therefore, an adolescent branch of *E. prolifera* 1 mm in length can theoretically produce at least 1 840 reproductive cells.

3.3.2 *Adolescent E. prolifera with reproductive cells formed and released at 25℃*

After being cultured for 14 d at 25℃, the branches produced abundantly near the base of adolescent *E. prolifera* and all were not more than 1 cm in length. It was observed that many branches of the adolescent thallus started to form and release reproductive cells from about 400–890 μm near the base (Fig. 5a). The branches of released reproductive cells are lighter in color or colorless (Fig. 5a). Near the base of the thallus and the middle and upper parts of the branches, some didn't form reproductive cells and dark green. The other darker green ones were releasing the reproductive cells (Fig. 5b).

4 Analysis and discussion

4.1 *Disputes of species name*

For more than a decade, there has been a serious dispute on the scientific species name of the green tidal alga that occurred in the Yellow Sea. It involves the legitimacy of the generic names *Enteromorpha* and *Ulva*. In 2003, a proposal made to combine the two genera into the genus *Ulva* (Hayden et al., 2003). And many subsequent scholars have accepted this viewpoint. However, *Enteromorpha* has been adopted as an independent legal genus name in all twelve versions of the "International Code of Botanical Nomenclature" since 1956, especially the latest three versions (2006 for Vienna Code, 2012 for Melbourne Code, and 2018 for Shenzhen Code, the last two versions are also known as "International Code of Nomenclature for Algae, Fungi, and Plants"). Their scientific name changes and chronicle have re-

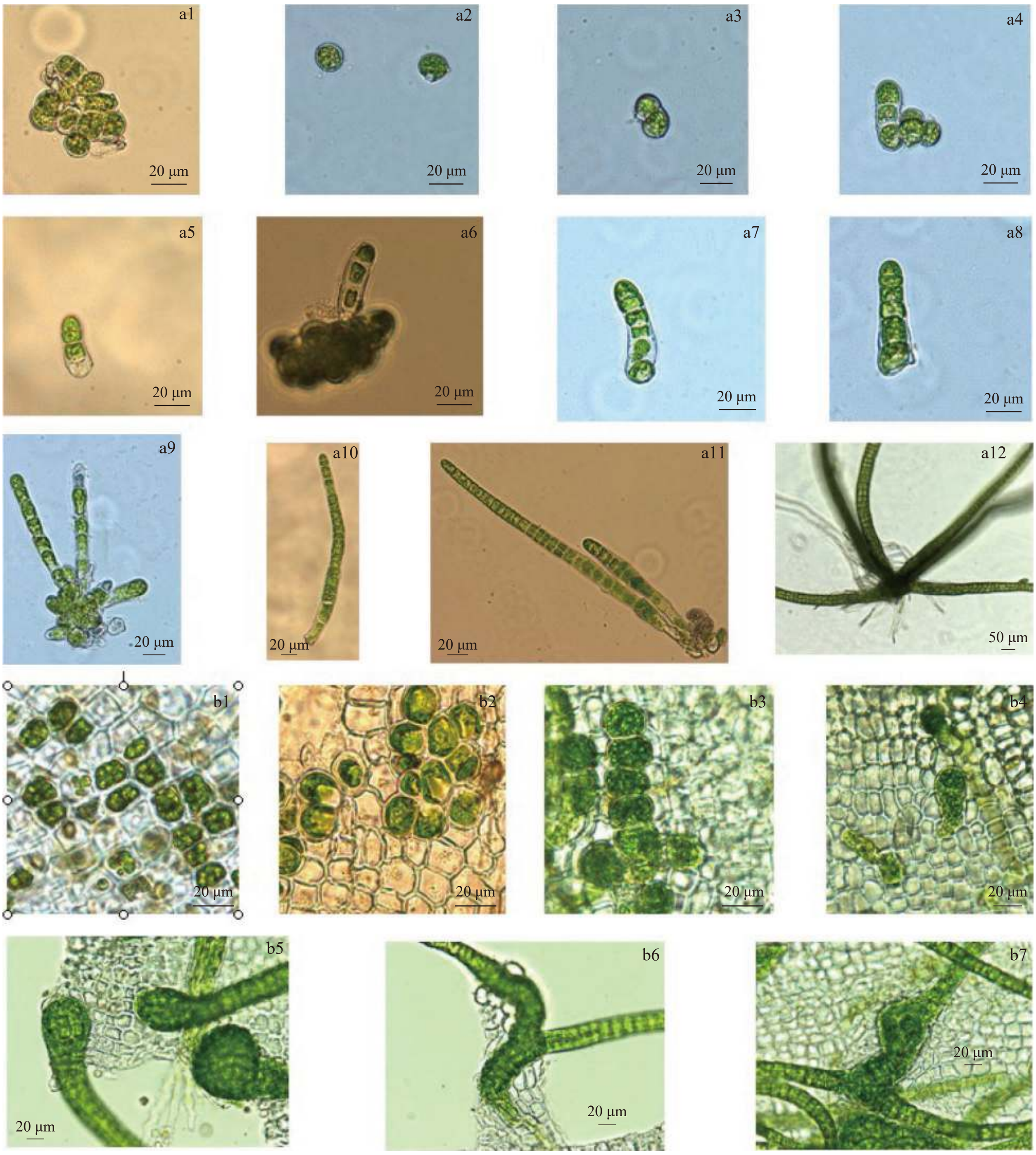

Fig. 2. Reproductive cells born and germinate into the seawater under the semi-drying condition (a); the reproductive cells germinated in the adult parent thalli or their remnant (b).

cently been analyzed (Ding, 2022). Therefore, *Enteromorpha prolifera/clathrata* is still adopted as the legal species name (Ding and Luan, 2009; Ding et al., 2009, 2014; Ding, 2013).

4.2 *"Ephemeral" or "opportunistic" trait of E. prolifera explained by the cell reproduction of early infantile thalli*

For a long time, *Enteromorpha* species have been called "ephemeral" or "opportunistic" marine algae because of their amazing survivability. However, what is this ability mainly reflected? From the observation of cell reproduction in early infantile thalli, the essence of this ability should be instant maturation and rapid reproduction. As a normal multicellular thallus, its cell reproduction is theoretically impossible to achieve "ephemeral", because it often takes dozens of days or even months to grow before it can reproduce. Conversely, this property of *E. prolifera* can very appropriately explain these abilities. These abilities are passed down from generation to generation to achieve the immediate regeneration of offspring.

According to our observations, the early infantile thalli at 250–300 μm above the base or their branches begin to produce reproductive cells. The few parent cells above the elongated cells of the basal rhizoid were just beginning to produce them in Fig. 3. Under suitable environmental conditions, the infantile thalli or their branches in this length take only a few days to reach the re-

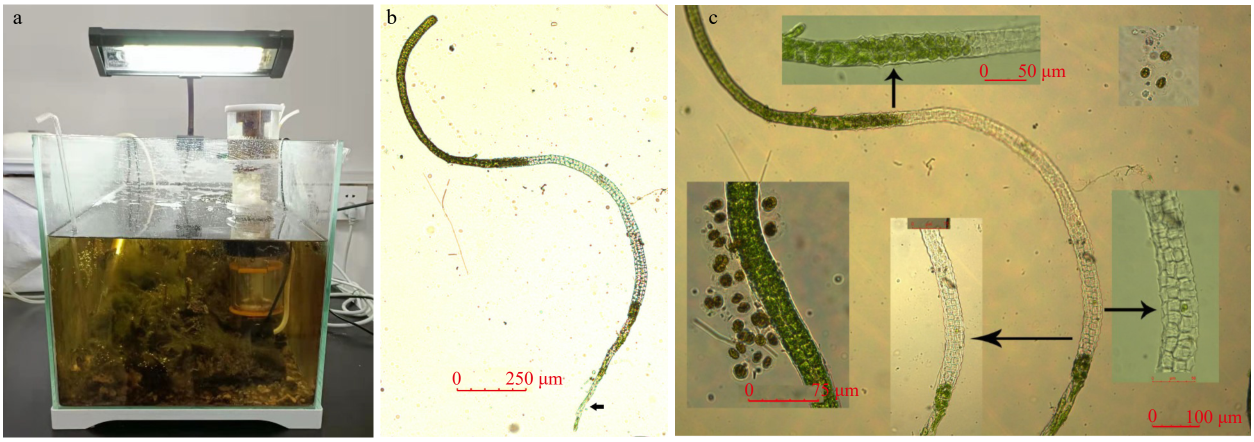

Fig. 3. Cell reproduction of infantile *Enteromorpha prolifera* under room temperature. a. The indoor culture tank of infantile *E. prolifera*. b. The seedlings of *E. prolifera* (the thallus, short arrow shows the basal rhizoid). c. The part of the seedling unformed reproductive cells, and the reproductive cells formed near the base of the seedlings and remnants of cell walls after released.

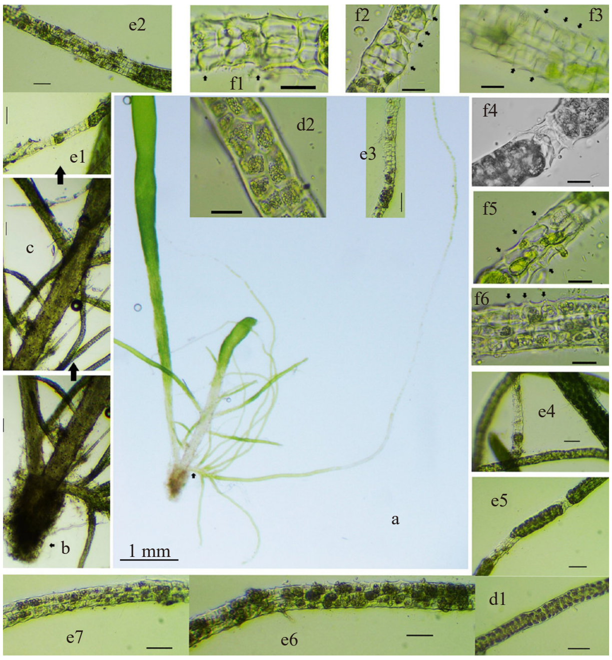

Fig. 4. Adolescent *Enteromorpha prolifera* with reproductive cells formed and released at 22℃. a. Adolescent thalli (short arrow show the formation and release of reproductive cells at first-order branches); b. Base of the adolescent thalli (short arrows show the holdfast);c. Near the base of the adolescent thalli; d1–2. Branch cells of the adolescent thalli (d2 shows magnification image); e1–7. Formation of branches and their type and morphology released reproductive cells; f1–6. Reproductive cells released by the branched of adolescent thalli (magnification image, short arrows showed the ostiole releasing the reproductive cells) (scale bar: a. 1 mm; b. 100 μm; c. 100 μm; d1. 50 μm; d2. 20 μm; e1–7. 50 μm; f1–6. 20 μm) .

productive stage (but it may take longer under unfavorable environmental conditions) and complete their cycle of life and death.

Thus, there is no doubt that the reproduction of early infantile thalli is the optimal revelation of the "ephemeral" or "opportunistic" trait of *E. prolifera*. The trait may be closely related to environmental stress, such as light and temperature. It is speculated in Fig. 4 that low or excessive light intensity may be one of

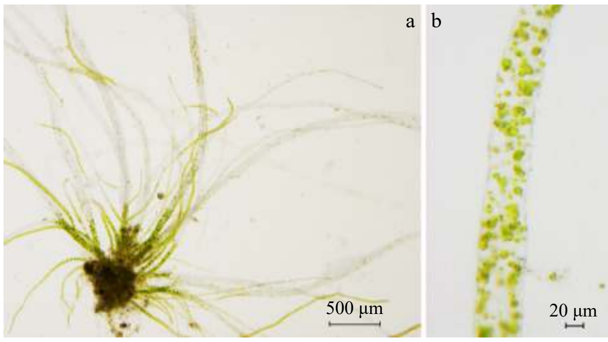

Fig. 5. Reproductive cells formed and released by the filamentous thallus of adolescent *Enteromorpha prolifera* at 25℃. a. Reproductive cells formed from the upper cells of the filamentous thallus and their remnants after released. b. Reproductive cells are releasing from some branches.

the major factors that induce this process under a suitable temperature (22℃). Such light condition often causes chlorophyll deficiency.

4.3 *What does the reproduction of early infantile thalli contribute to E. prolifera blooming? Provide a huge amount of initial seeds*

According to the rules of modern biological science, a large number of seeds, whether of asexual or sexual types, is a prerequisite for a mass blooming of the species. There have been many reports about the initial seeds of *Enteromorpha* species blooming in the Yellow Sea (Gao et al., 2010; Zhang et al., 2011; Liu et al., 2012; Song et al., 2015; Huo et al., 2016; Miao et al., 2018; Wang et al., 2020). It can be summarized as sexual, asexual and vegetative reproductions. Of course, the seed derived from the division of parent cells has an unparalleled advantage theoretically in quantity because they are increased geometrically. In response to the blooming *E. prolifera* in the Yellow Sea, its reproduction of individual adult thallus is repeated annually under a similar hydrological environment. The number of its initial seeds produced will not theoretically change subversively. However, the main cause of large-scale (huge biomass) or on-year and off-year bloom lies in the quantitative change of macroscopic adult *E. prolifera*. Adult individuals are most likely derived from the initial seeds and offspring of their early infantile thalli. This phenomenon has been fully reflected in the cultivated red alga *P. yezoensis*. The initial seeds and monospores contribute to its yield, in which the seedlings derived from the monospores play a vital role. Under the conditions of indoor temperature (about 20℃) (Fig. 3), 22℃ (Fig. 4), and 25℃ (Fig. 5), the formation and release of reproductive cells on the branches of *E. prolifera* infantile thalli were observed in this study. The reproductive cells released at 20℃ and 22℃ theoretically exceeded 1 760 per 1 mm long branch and 1 840 per 1 mm long branch, respectively. Compared with its vegetative branches, the number of seeds produced by this infantile reproductive branch with a length of 1 cm exceeds 8.8×10^3 (zygote) or 1.76×10^4 (spores) and 9.2×10^3 (zygote) and 1.8×10^4 (spores). Considering that the male and female gametes of *E. prolifera* can produce new male and female gametophytes through parthenogenesis (Hiraoka et al., 2003; Wang et al., 2020; Shen, 2022), the total number of seeds is closer to that of spores. As a result, there is no doubt that they are the main contributors to the rapid increase in the number of adult *E. prolifera*.

4.4 *Is so-called vegetative reproduction really important?*

It has been reported that micro-propagule is the main initial seeds of blooming *E. prolifera* (Gao et al., 2010; Liu et al., 2010, 2012; Zhang et al., 2010; Fang et al., 2012; Song et al., 2015; Huo et al., 2016; Miao et al., 2018, 2020; Han et al., 2019; Wang et al., 2020). However, there are three different views among scholars on the exact boundary of its concept (Hoffmann, 1987; Clayton, 1992; Santelices et al., 1995; Worm et al., 2001; Gao et al., 2010; Zou and Xia, 2004; Zhang et al., 2010; Liu et al., 2012; Song et al., 2015; Han et al., 2019; Wang et al., 2020). These views overlap and differ from each other and do not form a unified opinion. Until recently, a scholar has reviewed and analyzed this concept pertinently in detail and proposed his suggestion (Shen, 2022). In algal biology, "propagule" is a scientific terminology in a strict sense. Its original meaning refers to an independent structure of asexual propagation produced by the thalli of some algal species, such as *Sphacelaria* species (brown algae), during their normal life cycle. It can germinate into a seedling after it falls off its parent thallus. It is obviously that the life history of *Enteromorpha* species is not the same process. The thallus of *Enteromorpha* species itself does not produce an independent asexual reproduction structure. Still, like other plants, it can produce complete plants from a single cell, callus, or fragment due to totipotency. It reflected in two aspects: (1) the branches of thalli normally form fragments broken from one or more parts by external environmental pressure; (2) the branch easily falls off due to constriction of its base and forms asexual proliferation. The Latin species name of *E. proliferia* was derived from its asexual habit. The first may be induced primarily by artificial farming activities, grazing by marine herbivores, or extreme environmental conditions (e.g., brittle fracture caused by high temperature), while the second may be related to natural environmental conditions such as tides and waves. Comparatively, the "micro-propagule" mentioned above is even an ecological concept. It has been cited to uncover the generating process of blooming *E. prolifera*. However, it essentially obscures the bioscientific subdivisions implicated. In order to achieve the purpose of prevention and control of blooming *E. prolifera*, we need to comprehensively recognize, locate and divide the different stages of its life history and their roles in the generating process. The process of scientific life history should not be simplified. It is more practical to use "proliferation" to describe the stage derived from callus mass or fragment in the floating life history of *E. prolifera*. In our observed materials of infantile *E. prolifera,* only the upper part of the seedlings in Fig. 3 forms an independent fragment because its lower cells produce reproductive cells. And the early infantile branches of Figs 4 and 5 can also form similar separate fragments. However, compared with the cell reproduction mentioned above, the number of broken or detached fragments is almost negligible.

4.5 *Enlightenment from a picture*

In July 2021, a friend in Jiangsu Province passed us a few pictures of naturally juvenile *E. prolifera* on the shoals in Xiangshan County, Ningbo City, Zhejiang Province, and the South Yellow Sea. There are very similar growth conditions. We were struck by the scenes that the juvenile thalli of *E. prolifera* prefer to settle on the flat areas of the shoal highlands rather than be covered by the floating thalli after the tide out. But unfortunately, only this picture at Xiangshan County, is left, and the other at the South Yellow Sea cannot be found again. It is inconsistent with the viewpoint of propagule derived from the asexual fragment as the initial seed. If the initial seeds are mainly asexual propagules, they can only drift with water movement because of lacking attached components such as holdfasts or rhizoids. The floating thalli of *E. prolifera* should cover more low-lying shoal areas after the tide out. And they float again in the water when the tide rises. During the few hours between the tide rise and fall, the attached components can't form instantly and have an effect. By contrast, this picture is consistent with the settlement of reproductive cells released from the infantile thalli that we have observed. Large numbers of reproductive cells are released into seawater due to water and possible autologous movements. They are settled in the flat areas of the shoal highlands when the tide falls. The tide also may have a driving effect on the release of reproductive cells of *E. prolifera* because it has been reported that there is a certain rule between the reproduction of green algae and lunar motion (tide) (Togashi and Cox, 2001; Gordon and Brawley, 2004). The reproductive cells are mainly attached by holdfast on the sandy (larger particles) surface (as shown in Figs 4a, b), and mainly by rhizoids on the muddy (smaller particles) surface (as shown in Fig. 3). When the growth of infantile *E. prolifera* reaches a certain size, the former floats due to excessive buoyancy, and the latter is still mainly benthic. This is probably the main reason for the difference in the initial floating biomass of *E. prolifera* in the East China Sea and the Yellow Sea.

4.6 *On-year and off-year phenomenon on the biomass of blooming E. prolifera*

In the past, the phenomenon of on-year and off-year mainly involved fruit yield. We pointed out for the first time in 2009 that the bloom of *E. prolifera* in the Yellow Sea might also show this phenomenon (Ding et al., 2009). In the following ten years, its biomass had changed many times. Nutrition is often blamed for the phenomenon (Huang et al., 2021). However, based on the knowledge of plant reproductive physiology, forming more reproductive structures (flowers and fruits in fruit trees) is the essential prerequisite to the phenomenon (Taiz et al., 2015). Therefore, we believe that this phenomenon of floating *E. prolifera* is mainly determined by the following three factors.

Firstly, the early infantile thalli and oppressive environmental factors enhanced the production of floating ones. Secondly, the adult thalli with their seeds and ecological factors further increase the floating population and the number of infantile thalli. Thirdly, adequate nutrition is essential as well.

The infantile thalli of *E. prolifera* can supply seeds for the supplement of the seedlings in the sea (Figs 3–5). This ability is similar to that of *P. yezoensis*, which produces monospore. This process is completed in the early growth stage of the infantile thalli. Therefore, the number of early infantile thalli largely determines the changes in the number of blooming *E. prolifera* thalli in the year or the following year. The supplement of the seedlings from the adult floating population (Fig. 2) promoted this phenomenon to a certain extent. Once the mature adult thalli emerge from the water, they continue to release seeds (Fig. 5) under this environmental stress, which further exacerbates this phenomenon. The influence of nutrition on these three aspects may belong relatively to the smallest ones based on the characteristics of plant reproductive physiology because the current seawater does not seem to be very deficient in nutrients (Xia et al., 2009).

In recent years, some scholars have studied the nutrition of *E. prolifera* in the South Yellow Sea (Li et al., 2013). At the surface seawater of the accumulation area of blooming *E. prolifera*, the average values of the DIN and PO_4^{3-} are 14.89 μmol/L and 0.27 μmol/L, respectively (Xia et al., 2009). The maximum absorption rates of DIN and phosphate were 0.022 mg/(g·h) (in terms of N) and 0.006 mg/(g·h) (in terms of P) , respectively, and the absorption rates of inorganic nitrogen were faster in nutrient deficiency (Wang and Wu, 2015). The N/P ratio of each water layer from the surface to the bottom in some areas of the Yellow Sea is much higher than the Redfield value (Xia et al., 2009). And inorganic nitrogen content is sufficient and does not limit the growth of marine algae (Ma and Li, 2022).

4.7 *Major environmental factors inducing this process*

Light, photoperiod, and temperature changes (vernalization) are the main environmental conditions induce the reproduction of plants (Taiz et al., 2015). On the west coast of the Yellow Sea, they are significantly affected by natural conditions, shelter by other organisms, and disturbance by human activities. Especially for the population of "ephemeral" *E. prolifera*, the dynamic change of the above environmental conditions in a short period is the most likely to induce the number of early seedlings. It has also been reported that tides influence the formation and release of green algal reproductive cells (Togashi and Cox, 2001; Gordon and Brawley, 2004). Although the environmental conditions and nutrition on a larger scale fluctuated to a certain extent every year during the blooming period of floating *E. prolifera* in the Yellow Sea, they were all within its normal growth and development requirements.

In our observed materials, the seedlings or early infantile branches of *E. prolifera* under 1 cm in length can produce reproductive cells, as shown in Fig. 3, from a few cells above the basal rhizoid. The lighting and temperature changes mentioned above may have induced this process. The seedlings or early infantile branches that can produce reproductive cells are gray-green and lack chlorophyll. However, they lose their reproductive ability when their cells are filled with abundant chlorophyll and dark green color (Figs 4 and 5). It has been reported that the color changes of *E. prolifera* thalli are related to photosynthetic activity (Lin et al., 2009). The cells in the dark green positions of the thalli are in the vegetative stage, and those in the medium and light green parts are in the formation stage of sporangia/gametangia (Wang et al., 2020). Our observation results are consistent with those results.

Therefore, based on the color changes of the reproductive seedlings and early infantile branches, we speculate that light coercion is one of the environmental conditions for the induction of the "ephemeral" or "opportunistic" trait under suitable temperatures. The light induces the development of protoplasts into chloroplasts, chromoplasts, and amyloplasts in the plant (Taiz et al., 2015). Plant etiolation occurs when the light intensity is insufficient. When the light intensity is too high, the chlorophyll is degraded, especially the darker chlorophyll *a* destroyed, and even the production of photoprotective carotenoids is induced. And the plant will be yellow-green or lighter in color. When the seedlings or early infantile branches of *E. prolifera* are subjected to insufficient or strong light intensity in the early stage of their growth, their cells are pale in color and lack photosynthetic products. And they may be forced to induce self-producing offspring to achieve "ephemeral".

The environments along the coast of the western Yellow Sea that may lead to the heavy change of light intensity for the seedlings or early infantile branches of *E. prolifera*, such as water turbidity, shading due to the flourishing growth of other biota or inorganic mantles, and exposure to intense light in clear water areas and so on, may induce its cell reproduction theoretically. At 20℃, 22℃, and 25℃, cell reproduction was observed in the seedlings or early infantile branches of *E. prolifera* in this study. Relatively, this range may be a more suitable or higher temperature condition. It may also be combined with light to promote this process.

It is reported that light intensity on the sea surface on rainy days in the South Yellow Sea is less than 30 μmol/(m²·s), and the maximum light intensity on sunny days can reach 2 000 μmol/(m²·s) (Cui et al., 2015). The experimental light intensity in this study is only about twice that on rainy days and is relatively low. In the lower temperature range (5–15℃) and higher light intensity range (72–216 μmol/(m²·s)), the Fv/Fm of *E. linza* decreased with the increase of light intensity and photoinhibition occurred (Ma and Li, 2022). It may be beneficial to cell reproduction.

In the natural water body, lower temperature and changes may be more conducive to the cell reproduction of seedlings or early infantile branches of *E. prolifera*. It conforms to the rule of plant physiology and the "ephemeral" trait of *E. prolifera*. In our samples, this capability appeared only when the length of the seedlings or infantile thalli was less than 1 cm. Their growth was relatively slow when the temperature was lower. There may be another possibility. With the continuous growth of seedlings or infantile thalli, they break through the shelter and result in weakening of light resistance, and losing their "ephemeral" trait. Hence, we concluded that changes in light and temperature jointly acted on the induction of the cell reproductive process in the seedlings or early infantile branches of *E. prolifera*.

In a word, as far as the current data are concerned, how light and temperature regulate the reproduction of early infantile thallus and the regularity of their occurrence may be a significant issue worthy of in-depth study in the future. Interestingly, some scholars found that nitrogen nutrition, especially nitrate nitrogen, promotes the formation of *E. prolifera* reproductive cells (Wang et al., 2020). However, the primary function of nitrogen is to promote nutrient accumulation for plant growth and development based on plant physiological characteristics (Taiz et al., 2015).

4.8 *Analysis of the genesis of blooming E. prolifera in the Yellow Sea based on the observation results*

In the western part of the South Yellow Sea, the water temperature at the surface and in 10 m layers follows a similar pattern, with clear seasonal variations (Fig. 6). Affected by the warm current of the Yellow Sea, the water temperature showed an upward trend from April to July with a maximum of surface layer 25.2℃

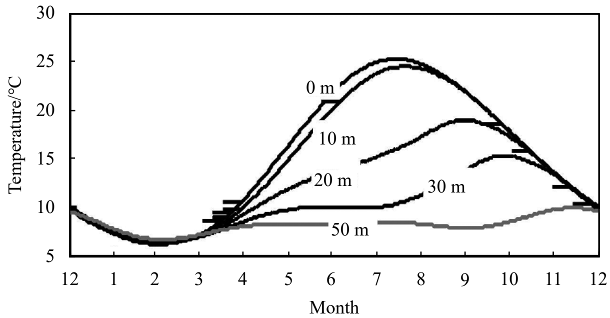

Fig. 6. Seasonal variation of water temperature in the western part of the South Yellow Sea (Hu, 2013).

in mid-July, and a downward trend from August to February with a minimum of 6.2℃ in early March followed (Hu, 2013). The blooming *E. prolifera* in this water area was closely related to surface water and water-air interface environmental conditions. Its floating population highly occurred in May (water temperature about 16–22℃), continued increasingly from June to July (about 22–25.2℃), and gradually disappeared in August (25–22.5℃) (Hu, 2013; Yang et al., 2017).

It reported that the temperature range was between 10℃ and 30℃ for the germination of green algal reproductive cells deposited on the sea floors. And the germination number of such cells of *E. prolifera* increased first and then decreased with the increase in temperature, and reached the maximum value at 15℃ (Liu et al., 2015). The suitable ranges of light and temperature for the germination of reproductive cells of *E. prolifera* were between 40 μmol/(m²·s) and 160 μmol/(m²·s), and 15℃ and 25℃, respectively (Han et al., 2015). The growth conditions of *E. prolifera* were as follows: salinity 16–40 (optimal 24–28, maximum growth 24), temperature 10–30℃ (optimal 20–25℃, maximum growth 25℃), light intensity >9 μmol/(m²·s) (optimal >18 μmol/(m²·s), maximum growth 72 μmol/(m²·s)) and pH 6–10 (optimal 8–9, maximum growth 8); the release conditions of reproductive cells were as follows: salinity 12–40 (optimal 28–40, maximum release 32), temperature 15–35℃ (optimal 20–30(~35)℃, maximum release 35℃), light intensity >9 μmol/(m²·s) (optimal >36(180–300) μmol/(m²·s), maximum release rate 144 μmol/(m²·s)), pH 6–10 (optimal 8–9, maximum release rate 9) (Wang et al., 2007b; Han et al., 2015). Some green algae, such as *Enteromorpha* species have cold tolerance (Kamermans et al., 1998), and the lethal low temperature is lower than –18℃. The cryogenic extermination treatments of *Enteromorpha* species and other green algae that are epiphytic to the nets and ropes, isolated from the culture seedlings of *P. yezoensis*, exploited this biological property (Ma et al., 1998). The lowest water temperatures in the South Yellow Sea in winter and spring are much higher than their lethal lows. Therefore, the growth and reproduction of epiphytic green algae such as *Enteromorpha* species show regularity with the annual water temperature change. They should theoretically be alternating between the autumn-winter and the spring-summer populations.

The experimental temperature in this study was between 20℃ and 25℃. It was included in the range of surface water temperature from mid-May to early July and from August to mid-September in the South Yellow Sea. Two periods corresponded to the cultivation activities of *P. yezoensis*, the end of the harvest or the beginning of rafting in this area. It was representative to explain the occurrence and bloom of *E. prolifera*.

4.8.1 *Prediction on the occurrence process of E. prolifera population from autumn to winter in the South Yellow Sea—reproduces mainly by infantile filamentous thalli and forms filamentous thalli*

Based on the change in water temperatures in the South Yellow Sea, the autumn-winter population of *E. prolifera* should germinate and grow in the cooling period from mid-August to early March. In the high-temperature season in the South Yellow Sea, the green algae including *E. prolifera* decline. The autumn-winter population mainly comes from the local natural ones, including the cell reproductive seedlings that had passed high-temperature seasons in the summer and autumn (their reproductive cells crossed the summer through dormancy) and those derived from their infantile branches.

In the cultivated region of *P. yezoensis* along the coast of the South Yellow Sea, from September to October during the autumn, millimeter-level green algal seedlings, including *E. prolifera* can be seen by the human eye on the substrates such as rafts and net ropes for its artificial cultivation. At this time, the water temperature is about 23–20℃. It is consistent with the reproduction and growth of *E. prolifera* seedlings (Figs 3 and 4).

Subsequently, the water temperature drops until 15–10℃ can still release the reproductive cells. But the sunshine's short duration and low intensity shorten the suitable growing season. The growth rate of seedlings is slow until the water temperature drops to its limited growth range of 10℃ or below (around the beginning of December) (Ma and Li, 2022; Hu, 2013). The thalli will remain in a relatively microscopic filament.

At this stage, *E. prolifera* generally shows the appearance of a microscopic filament. It is easily ignored by the shelter of some larger thalli, such as *E. linza* and *Ulva* sp., that thrive at winter temperatures when investigated.

4.8.2 *Prediction on the occurrence process of E. prolifera population in spring and summer in the South Yellow Sea—reproduces in microscopic filamentous thalli and common tubular adults to form tubular or filamentous thalli*

In early March, affected by warm currents and temperature rise, water temperature and light intensity in the South Yellow Sea began to rise, gradually producing environmental conditions suitable for the growth of *E. prolifera*. After the autumn-winter population of green algae including *E. prolifera* spent the winter, their microscopic filamentous thalli continued to grow, or some cells transformed to reproduce cells (Figs 3 and 4). Each parent cell produces 4 or 8 or 16, or 32 reproductive cells (Wang et al., 2007a; Ye et al., 2008; He et al., 2019; this study). Simultaneously, the reproductive cells deposited on the sea floors also germinate into seedlings. They together start the period of spring-summer population, roughly from early March to mid-late July.

The spring-summer population of *E. prolifera* can be divided into three stages: the attaching stage (characterized by adhering and the reproduction of microscopic filamentous thalli), the floating stage (characterized by floating and the reproduction of microscopic filamentous and macroscopic tubular thalli), and the declining stage (characterized by floating and macroscopic filamentous thalli).

(1) Attaching stage of spring-summer population

The microscopic filamentous thalli of *E. prolifera*, after overwintering (overwintering autumn-winter population), produce reproductive cells. These reproductive cells either adhere to the substrates or move in ups and downs by the movement of seawater. Those free ones are settled on the high platform of the shoal when the tide is out. They grow into green seedlings, as shown in Fig. 7, under suitable environmental conditions.

Fig. 7. Juvenile *Enteromorpha prolifera* at the intertidal mudflat of Xiangshan County, Ningbo City, Zhejiang Province.

Based on our observations and speculation, these seedlings and microscopic filamentous thalli composed of those from the substrates and platform of the shoal are theoretically the primary triggers of subsequent blooming *E. prolifera* in the South Yellow Sea. But farming operations such as nori cultivation ended in April. The effective artificial attaching substrates in the sea are reduced or disappear. By early May, the water temperature in the shoal began to rise to 16℃ or above. The microscopic filamentous thalli settled on the shoal grew rapidly and became the main initiator of the blooming *E. prolifera*.

(2) Floating stage of spring-summer population

As mentioned above, the seeds of *E. prolifera* on shoal germinate downward to form holdfasts or rhizoids due to the differences in the attaching substrates and upward to erect branches. With their increasing volume or the formation of a tubular structure, the seedlings or early infantile thalli of *E. prolifera* on a shoal in the South Yellow Sea begin to separate from the sandy substrate under the influence of seawater buoyancy. Their drifting population is formed under the influence of tides and currents.

In the following drifting time, the population continued to grow and reproduce under suitable water temperatures, sufficient light, and abundant nutrients. The population continued to increase their biomass, expanded the number, and some formed floating ones.

The average summer water temperature in the Yellow Sea is suitable for the growth of *E. prolifera* (Ma and Li, 2022). The suitable temperature range is 20–30℃ with the optimum 25℃ for the photosynthetic physiological activity of floating *E. prolifera* (Wang et al., 2007b; Jiang et al., 2015). Under 15–25℃ and 200–600 μmol/(m^2·s), the specific growth rate of *E. prolifera* was 1.5 to 3.5 times that of *E. linza*, *E. flexuosa* and *E. compressa* in this water area (Ma and Li, 2022). However, due to the change in environmental conditions caused by the sinking and floating, the submerged thalli or seedlings attaching to parent thalli continue to grow into tubular macroscopic ones.

As mentioned above, nutrient such as nitrogen and phosphorus are abundant in the western waters of the South Yellow Sea and do not limit the vegetative growth of floating *E. prolifera* (Xia et al., 2009). Therefore, in this period, the floating population as a whole showed a mixed appearance of microscopic filamentous and macroscopic tubular thalli, especially dominated by the biomass of the latter. But in general, rich nitrogen not only encourages their excess growth but also delays their reproduction.

The photosynthetic carbon fixation rate of *E. prolifera* floating on the water surface was 4.23 times that of submerged water (Feng et al., 2012; Wang et al., 2020). The instantaneous net photosynthetic performance of *E. prolifera* significantly improved under high light intensity (280 μmol/(m^2·s)) (Wu et al., 2018). The thalli floating above the water can grow exceedingly in a short time. They or their attaching seedlings develop into a filamentous shape. But this process is not sustainable under overall environmental stress. It has been reported that sporangium formation in *E. prolifera* is also related to photosynthesis, facilitated by cyclic electron transport in photosystem II (Wang et al., 2016; Wang, 2017). It suggests that a lack of effective photosynthetic activity can promote the reproductive process. Photosynthesis accumulates organic matter and energy for reproduction, which is the basis for the continuation of life. But there is an upstream and downstream relationship between photosynthesis and the reproductive process in plant physiology (Taiz et al., 2015). When it comes time to reproduce, strong photosynthesis activity hinders the reproductive process. The main reason for photosynthesis and reproductive competition is phosphorus restriction.

Seawater conditions in the summer are also suitable for *E. prolifera* to release reproductive cells. This process also strongly adapts to environmental changes (Ma and Li, 2022). Light intensity significantly affected the releasing reproductive cells of *E. prolifera*. When light intensity was increased (18–144 μmol/(m^2·s)), the release of reproductive cells was promoted (Wang et al., 2007b). The optimal release conditions were salinity 32, temperature 35℃, light intensity 144 μmol/(m^2·s), and pH 9 (Ma and Li, 2022). Such environmental conditions cannot be satisfied in the surface water in the clear water area of the South Yellow Sea in summer. It only partly explains the reproduction of populations floating on the water surfaces to a certain extent, but it is also unsustainable.

Therefore, in the middle and late periods of the floating spring-summer population, the main source of population expansion may be the reproduction of macroscopic tubular thalli, namely the reproduction of macroscopic mature thalli (Figs 1 and 2). Because their cells are larger, the number of reproductive cells formed is greater. At this stage, the macroscopic mature thalli, which act as a primary force of marine replenishment seedlings, are mainly submerged in seawater.

(3) Declining stage of the spring-summer population (characterized by floating, macroscopic filamentous thalli)

After the floating population of *E. prolifera* in the South Yellow Sea had drifted from a coastal region of turbid water to a nearshore region of clear water, the water temperature and light intensity were suitable for its growth and adult reproduction. The biomass continues increasing, and the floating remains in the waters for a long time, subject to unique local circulation. Huge biomass results in the clusters of the populations.

However, the normal growth and development of the populations inhibited because of the continuous increases in water temperature and light intensity in the South Yellow Sea. Photosynthetic pigments of the thallus cell were destroyed, the color gradually changed from strong dark green to yellowish-green, and the individual growth gradually developed to a filamentous appearance. The reproductive cells derived from macroscopic mature or subsequent microscopic filamentous thalli born under suitable conditions adhered to their parent thalli or residues, and continued germinating under tolerable conditions. However, due to the obvious environmental stresses such as light, temperature, salt, air, nutrition, and tide, thallus can only survive as a short filamentous shape. The filamentous thallus holds a larger specific surface area and lighter weight. It floats more easily on the water surface than the macroscopic larger thallus. Under such harsh environmental stress of the water-air interface, insufficient accumulation of organic matter inhibited the formation of the reproductive structure of filamentous thallus floating on the water surface. In particular, high temperatures and intense light intensity may cause them to lose the further reproductive ability or reproductive cell activity.

4.8.3 *Generation alternation of E. prolifera population in the South Yellow Sea*

Based on the "ephemeral" or "opportunistic" trait of *E. prolifera*, it can complete the generation alternation and/or overlapping life history process at each stage of its occurrence in the South Yellow Sea. The number of cycles of its life history mainly depended on the reproductive frequency of infantile branches induced by environmental conditions. Therefore, the blooming *E. prolifera* in the coastal area of the South Yellow Sea may have multiple annual life cycle processes, i.e., the multiple alternations and/or overlapping or the repetition within generations between sporophyte and gametophyte generations. As marine algae with alternation of isomorphic generations, the blooming *E. prolifera* in the Yellow Sea were previously reported to be mainly sporophyte generations (Zhao et al., 2019) and dominant gametophyte generations (Wang et al., 2020). However, our previous study on alternating isomorphic generations in *Chaetomorpha valida* suggests that sporophyte and gametophyte generations may coexist at the same growth stage based on temperature characteristics (Deng et al., 2011). As the blooming species under a similar coastal environment, their life history process is similar, although *E. prolifera* has a more variable one due to its ephemeral trait.

At the birthplace of the South Yellow Sea in the season of suitable growth and reproduction, the population of *E. prolifera* alternates its generations in ten days, or a month, or a season, or half a year and carries out its breeding. However, when they drift away from their birthplace as a blooming population, the reproductive process is forced to be interrupted at the last moment due to intense environmental stress (high temperature, high light intensity, high oxygen, nutrition deficiency, away from coastal attaching substrates, etc.), which leads to the tragedy of a life without descendants.

4.9 ***Updated direction on the forecast, prevention and control of blooming E. prolifera***

The bloom of *E. prolifera* in the South Yellow Sea has occurred for more than ten years. Organizations at all levels are also actively exploring approaches and strategies for its forecast, prevention and control. However, it was not done well. The on-year and off-year of blooming *E. prolifera* in the Yellow Sea have been confirmed. It makes it more difficult for effective forecast, prevention and control. In fact, we have all realized that the optimal time for its prevention and control is mainly in the early stage of blooming *E. prolifera*. However, due to the lack of understanding about the early reproduction of infantile *E. prolifera* in the past, some seemingly reasonable treatment measures and procedures have not achieved the desired effect. The discovery of early infantile thalli reproduction in this study provides new insights into its effective forecast, prevention and control. A similar study has been reported abroad (Lotze et al., 1999).

The investigation should carry out in the early stage of blooming *E. prolifera* to grasp the reproductive rule of early infantile thalli with its main environmental regulatory factors. Once the rules are fully understood, such as which environmental factors and human activities induce the process, targeted measures can be taken to effectively prevent and control the blooming population. Of course, the cell reproduction derived from adults and their offspring's microscopic filamentous thalli will be reduced when the floating biomass can be effectively declined in the early stage of the floating population occurrence. The massive bloom may also alleviated to a certain extent.

Although we have observed the reproduction of early infantile *E. prolifera*, many details remain to be explained, such as how they survive in the hot summer. Some scholars believe that it passes the summer through the microscopic propagules in the sediments (Liu et al., 2015). However, the concept of microscopic propagules is too general to be accurately defined, as mentioned above. We also initially wanted to disclose our observations after we had thoroughly explored these issues. But considering the urgent prevention status of blooming *E. prolifera* in the Yellow Sea, it has to be disclosed as soon as possible. The purpose is to facilitate other scholars and departments with more accumulated research foundations to implement the layout as soon as possible, reveal its occurrence rules, and achieve goals for its forecast, prevention and control.

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