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Settlement and metamorphosis of *Rapana venosa* (Gastropoda: Muricidae) with implications for artificial culture*

YU Zhenglin[1, 2, 3, 4], YANG Meijie[1, 2, 3, 4], SONG Hao[1, 2, 4], HU Zhi[1, 2, 3, 4], ZHOU Cong[1, 2, 3, 4], WANG Xiaolong[1, 2, 4], LI Haizhou[5], ZHANG Tao[1, 2, 4, **]

[1] *Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*

[2] *Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China*

[3] *University of Chinese Academy of Sciences, Beijing 100049, China*

[4] *Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China*

[5] *Shandong Fu Han Ocean Sci-Tech Co. Ltd., Haiyang 265114, China*

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Abstract The rapa whelk *Rapana venosa* transforms from a phytophagous larva to a carnivorous juvenile after settlement and metamorphosis. The high mortality rate and low metamorphosis rate (<1%) of larvae are among the key factors limiting the recovery of the resources of *R. venosa*. We studied these two processes and showed that the larva has the ability to settle and metamorphose in the middle and late 4 spiral whorl period, and the use of substrates (scallop shells with oysters) can significantly improve the settlement and metamorphosis rate of larvae. The presence of darkness, juvenile bivalve shellfish, and oyster polysaccharide could effectively increase the rates of larval settlement and metamorphosis. Our findings broaden the understanding of settlement and metamorphosis in gastropods and can be used to improve population control, resource recovery and commercial breeding strategies for *R. venosa*.

Keyword: larval development; substrate; oyster polysaccharide; commercial breeding

1 INTRODUCTION

The development of planktonic larvae into juveniles is a key stage in the life cycle of many benthic marine invertebrates (Song et al., 2016a), and settlement and metamorphosis are two closely related and crucial processes in the development of larvae (Yang et al., 2015). Rodríguez et al. (1993) defined settlement as a process that starts with the search for a suitable substratum and ends with metamorphosis. Leise and Cahoon (2012) indicated that Metamorphosis can transform a larva into a juvenile, via a process that involves physical, physiological, and behavioral changes, including changes in organs, external morphology, and lifestyle (Leise and Cahoon, 2012).. As a result of these changes and adaptation to the new microhabitat, planktonic larvae exhibit a high mortality rate during settlement and metamorphosis (Song et al., 2016b). Therefore, the settlement and metamorphosis of shellfish have a vital impact on the dynamics, distribution, and development of natural populations (Song et al., 2016c).

Settlement and metamorphosis of marine invertebrates usually occurs when highly developed competent larvae contact environmental inducers (Jackson et al., 2002). External inducers of metamorphosis of shellfish larvae can include biological, chemical, and physical factors (Burke,

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** Corresponding author: tzhang@qdio.ac.cn

1983; Rodríguez et al., 1993; Li et al., 2006; Laimek et al., 2008; Sánchez-Lazo and Martínez-Pita, 2012; Wang et al., 2012). Biological factors primarily include food, adult secretions, and natural competitors and predators. Chemical factors include microbial membranes and related biological secretions, exogenous neurotransmitters and their precursors, metal cations, and substances affecting signal transduction. Physical factors include temperature, salinity, and settlement surface roughness and color.

Rapana venosa (Valenciennes, 1846) is a widespread carnivorous snail and an economically important shellfish in China although it is an invader in aquatic ecosystems in other countries (Song et al., 2016b). Currently, the supply of *R. venosa* depends mostly on the harvesting of wild resources. In recent years, the intensity of harvesting is increasing, and the decline of wild resources is a serious concern (Pan et al., 2013a). Therefore, it is necessary to carry out artificial breeding, aquaculture, and resource recovery in China and other native countries, whereas population control is urgent in invaded countries. Artificial breeding of *R. venosa* has been attempted in China since the 1990s (Yuan, 1992; Wei et al., 1999), but the rate of larval metamorphosis is very low (<1%), and therefore artificial breeding is far from industrialized and seriously restricts the development of an aquaculture industry for *R. venosa*.

There are many in-depth studies on shellfish larval metamorphosis in species such as the phytophagous red abalone, *Haliotis rufescens* (Barlow and Truman, 1992; Searcy-Bernal et al., 1992; Biscocho et al., 2018), and the bivalve *Crassostrea gigas* (Coon et al., 1985; Fitt et al., 1990; Wang et al., 2015). Considerable progress has been made in research on external inducible factors, neuroendocrine regulation, receptors, signal transduction, and mechanism model construction. During metamorphosis, *R. venosa* changes from phytophagous to carnivorous (Yu et al., 2018), and the process is more complicated than that in phytophagous snails and bivalve shellfish. There are few reports of settlement and metamorphosis of *R. venosa*. Yang et al. (2015) indicated that chemical cues (e.g., epinephrine, L-3, 4-dihydroxyphenylalanine, and γ-aminobutyric acid) can induce the metamorphosis of *R. venosa*. Song et al. (2016a, b) studied the metamorphic mechanism of *R. venosa* at the molecular level. At present, understanding of the settlement and metamorphosis of *R. venosa* is limited, and further studies are needed, e.g., on the influence of external factors (light intensity and prey). Light is an important environmental factor affecting the metamorphosis of aquatic organisms (Gao et al., 2016) and prey is a key factor in the metamorphosis of marine invertebrate larvae (Li et al., 2006). In addition, it is known that polysaccharides can induce the settlement and metamorphosis of many species of coral larvae (Morse and Morse, 1991, 1996), and oysters, which are a common prey of *R. venosa*, are rich in polysaccharides (Shi et al., 2015). However, the inducing effect of oyster polysaccharide on gastropods has not been studied.

Therefore, we studied the following: (1) the time of larval settlement and metamorphosis; (2) the search for efficient methods to induce larval metamorphosis; (3) factors affecting larval settlement and metamorphosis (light, prey, and oyster polysaccharides). The aim is to improve the low rate of larval metamorphosis for cultured populations. Research on the settlement and metamorphosis of *R. venosa* is beneficial to artificial breeding, aquaculture, and resource recovery.

2 MATERIAL AND METHOD

2.1 Larval culture and developmental stages

Adult *R. venosa* broodstock were collected from Laizhou Bay (37°17′7″N, 119°35′10″E) in Shandong Province. Culturing of parental whelks, mating, spawning, hatching, and larval rearing were carried out based on Yang et al. (2007). Planktonic larvae were cultured in 3 m×6 m×1.2 m cement pools with a density of 0.1 ind./mL, temperature of 23–25°C, and salinity of 29.8−31.7. Larvae were fed *Isochrysis galbana*, *Platymonas subcordiformis*, and *Chlorella vulgaris* three times daily (at 5.0×10^4 cells/mL).

Larval development was monitored using a microscope. The planktonic larval development of *R. venosa* can be divided into six stages according to the spiral whorl, the shape of the velum, and the organs present: 1 spiral whorl (A, 320–340 μm shell length (SL)), 2 spiral whorls (B, 340–550 μm SL), early 3 spiral whorls (C, 550–780 μm SL), middle and late 3 spiral whorls (D, 780–1 000 μm SL), early 4 spiral whorls (E, 1 000–1 250 μm SL), and middle and late 4 spiral whorls (F, 1 250–1 500 μm SL) (Pan et al., 2013a).

2.2 Experimental design

2.2.1 Time of settlement and metamorphosis and substrates

The development of the planktonic larval foot begins at D stage and the development of the foot

indicates that the larvae begin to have the ability to settle (Pan et al., 2013a). Therefore, larvae at D, E, and F stages were selected to observe settlement and metamorphosis. During metamorphosis, larvae undergo a feeding transformation from phytophagous to carnivorous (Yu et al., 2018). Therefore, substrates (scallop shell) with an equal number and size of juvenile oysters (*Crassostrea gigas*, <10 mm SL) were selected. All experimental larvae (200 individuals in each experimental group) culture was as described in Section 2.1. Two groups of experiments: (1) substrates were placed into three groups of larvae at different stages, and no substrates were used as a control group; (2) in order to understand the effect of scallop shells on larval metamorphosis, different substrates (1: scallop shells with oysters; 2: scallop shells without oysters; 3: no substrate) were tested with F-stage larvae for three days. All the experimental groups provided single-celled algae. Larval development was observed using a microscope. The rates of settlement, metamorphosis, and mortality of larvae were recorded. The experiment was repeated three times for each group.

2.2.2 Illumination

F stage larvae were selected for the illumination experiment. Larval culture was as described in Section 2.1. A total of seven illumination conditions were tested: red (R), white (W), blue (B), green (G), yellow (Y), dark (D), and dark 12 h plus white 12 h (DW); at 1 000±100 lx illumination (by luxmeter testo 540, testo AG, Germany)for three days. To improve the metamorphosis rate of larvae, scallop shells with oysters were provided, and these were placed perpendicular to the light source to prevent shading. All the experimental groups provided single-celled algae. The rate of metamorphosis of larvae was recorded and the experiment was repeated three times for each group.

2.2.3 Prey

F stage larvae were selected for the prey experiment. Larval culture was as described in Section 2.1. Three species of juvenile shellfish (5 mm SL), *C. gigas* (Cg), *Mercenaria mercenaria* (Mm), and *Mactra chinensis* (Mc), were selected to induce larval settlement and metamorphosis. The experiment was divided into two induction modes. In the feeding group, the *R. venosa* larvae could directly feed on shellfish. In the isolation group, shellfish were separated from *R. venosa* larvae by silk sieve (mesh: 0.1 mm); therefore, shellfish secretions could enter the water environment of the *R. venosa* larvae, but the larvae could not feed on the shellfish. All the experimental groups provided single-celled algae (the control group were provided with single-cell algae, but not juvenile shellfish). The rate of settlement and metamorphosis of larvae was recorded and the experiment lasted ten days. The experiment was repeated three times for each group.

2.2.4 Oyster polysaccharide

Oyster polysaccharide, derived from *C. gigas* (Shi et al., 2015), is a homogeneous glucose polymer. F stage larvae were selected for this experiment. Larval culture was as described in Section 2.1. Different concentrations of oyster polysaccharides (0, 0.1, 1, and 10 mg/L) were provided daily. The rate of settlement and metamorphosis of larvae was recorded and the experiment lasted ten days. The experiment was repeated three times for each group.

2.3 Statistical analysis

The data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Data related to the time of settlement and metamorphosis and provision of substrates were analyzed using a two-way ANOVA, Tukey's post-hoc test was performed to identify significant differences between groups. Data related to illumination, prey, and oyster polysaccharide were analyzed using a one-way ANOVA; Tukey's post-hoc test was performed to identify significant differences between groups. All statistical computations were conducted using SPSS v. 16.0 software (SPSS Inc., Chicago, IL, USA) and α-values <0.05 were considered to be statistically significant.

3 RESULT

3.1 Metamorphic characteristics of larvae

The foot, shell, velum, and feeding habits of larvae at D, E, and F developmental stages are shown in Fig.1 and Table 1. These changes in morphology and feeding habits are closely related to larval settlement and metamorphosis. In D and E stages, the growth and development of foot, velum and shell indicated that the zooplankton larvae could not complete the settlement and metamorphosis behavior at these two stages. The foot development of F stage larvae was complete, and when without inducer, larvae would continue to float, only a small number of larvae would

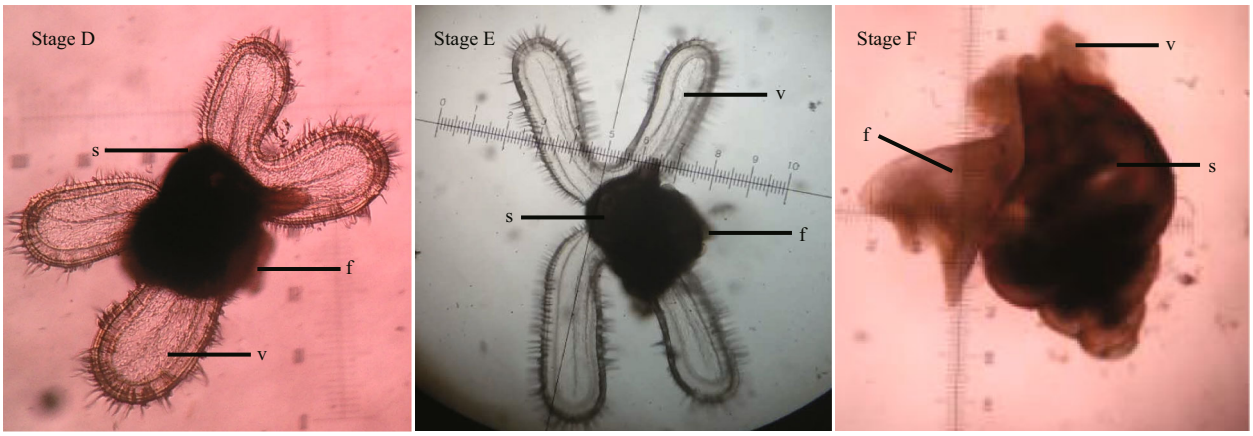

Fig.1 Pictures of veligers in stages D, E, and F

The foot (f), shell (s), and velum (v) are marked.

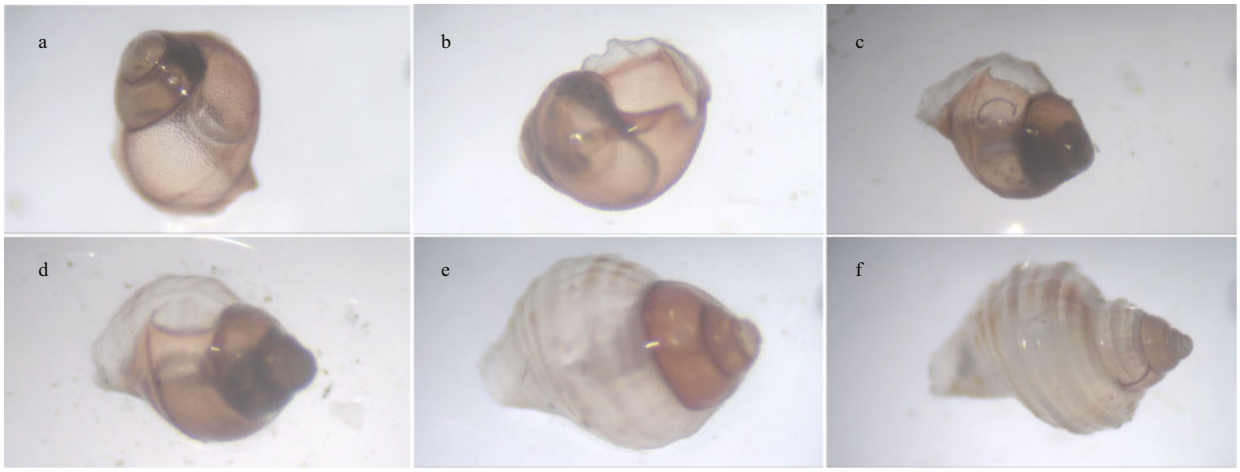

Fig.2 Morphological changes in the adult shell during the metamorphosis of *R. venosa*

The adult shell is white and the shell length are a: 1.38 mm, b: 1.53 mm, c: 1.77 mm, d: 1.85 mm, e: 2.01 mm, f: 2.35 mm.

Table 1 A comparison of the feet, shell, velum, and feeding habits of larvae at different developmental stages

Stage	Foot	Shell	Velum	Feeding habit
D	The foot primordium was thicker, but the foot could not expand freely and the larva could not rely on the foot to crawl	3 spiral whorls; 780–1 000 μm SL	The center of the velum is recessed to the base of the velum, resulting in a 4-leaf velum	Phytophagous. Feed on single-cell algae and remain planktonic
E	The foot is further elongated and thicker, and can be freely retracted. A few larvae try to crawl on their feet	Start growing 4 spiral whorls; 1 000–1 250 μm SL	The length of the single-leaf velum was further increased, and was noticeably longer than that of D stage	Phytophagous. Feed on single-cell algae and remain planktonic. Larvae sometimes alternate between plankton and settlement
F	The feet are well developed, can be freely retractable, and the larvae can crawl with their feet	4 spiral whorls; 1 250–1 500 μm SL. Edge of aperture thickening	In the absence of an inducer, the velum stretches in the form of a butterfly. In the presence of an inducer, the velum retracts into the shell and begins to degenerate until it disappears	Polyphagous. In the absence of inducers, the larvae feed on single-cell algae and remain planktonic. In the presence of inducers, the larvae feed on the juveniles of oysters and settlement begins

settle. When the larva floated too long and did not complete the metamorphosis, there would be a large number of deaths. When providing inducers, larvae would complete the settlement behavior within 24 hours and began the process of metamorphosis.

The morphological changes in the adult shell of *R. venosa* are shown in Fig.2. As shown in Fig.2a, the larvae is in F stage, and is mainly planktonic and feed on unicellular algae. The adult shell has not started to grow, and the shell is dark brown. As shown in Fig.2b, c, d, during metamorphosis, the larvae are mainly benthic and occasionally planktonic, feeding on unicellular algae and bivalves. The white adult shell begins to grow. As shown in Fig.2e, when

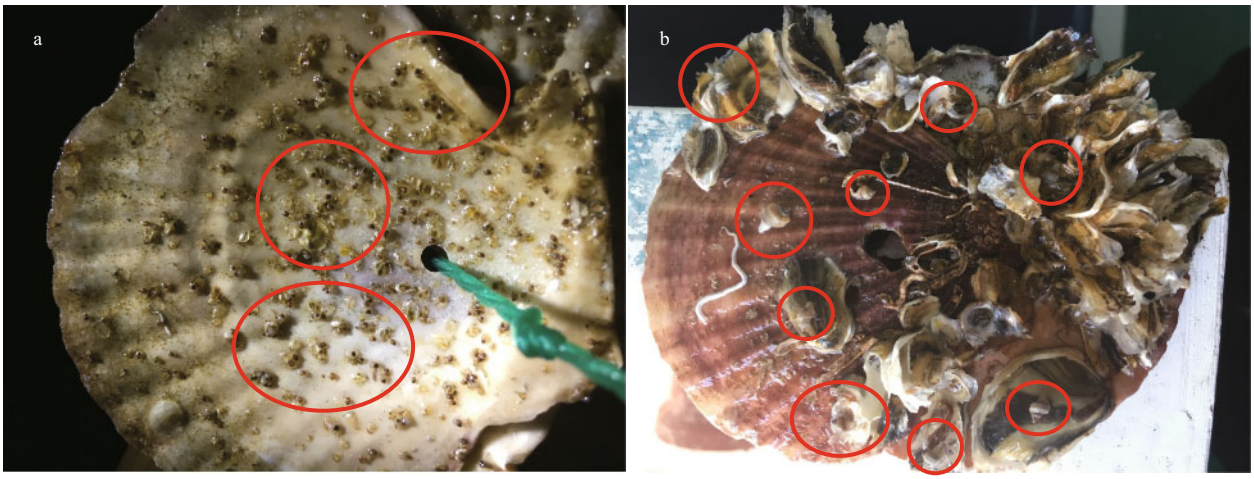

Fig.3 Scallop shell substrates, settlement (a) and metamorphosis (b) of larvae

Red circles indicate juveniles of *R. venosa*.

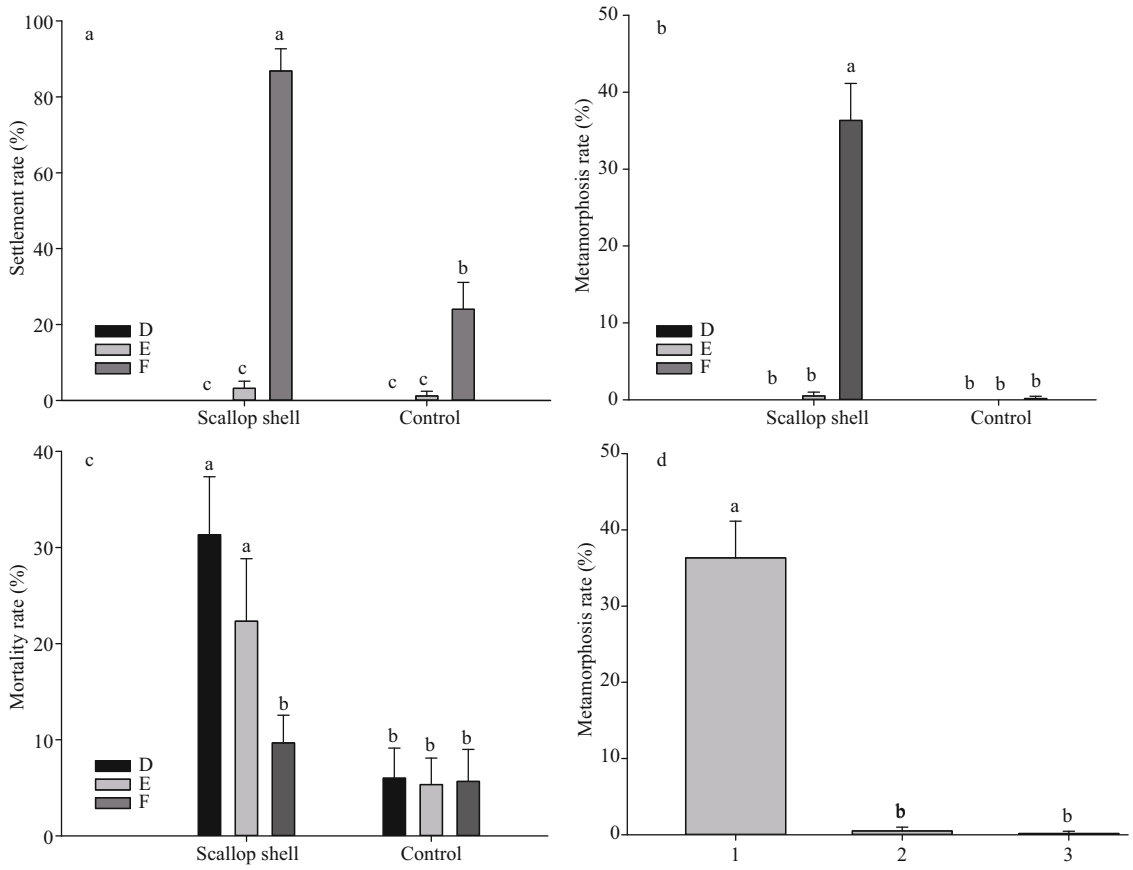

Fig.4 Settlement rate (a), metamorphosis rate (b), and mortality rate (c) of larvae in stages D, E, and F; metamorphosis rates of larvae when offering different substrates (d)

1: scallop shells with oysters; 2: scallop shells without oysters; 3: no substrate. Bars show standard errors. Means with different symbols (a, b, c) are significantly different (P<0.05).

metamorphosis is almost completed, larvae live mainly in the benthos, feeding on bivalves, and not in the plankton. The white adult shell occupies the whole spiral layer. As shown in Fig.2f, the larvae complete the metamorphosis into juvenile snails, benthic life, feeding on shellfish, and the adult shell is white.

3.2 Time of settlement and metamorphosis and provision of substrates

As shown in Fig.3a & b, scallop shells with oyster can effectively induce larval settlement and metamorphosis. As shown in Fig.4a, b, c, the

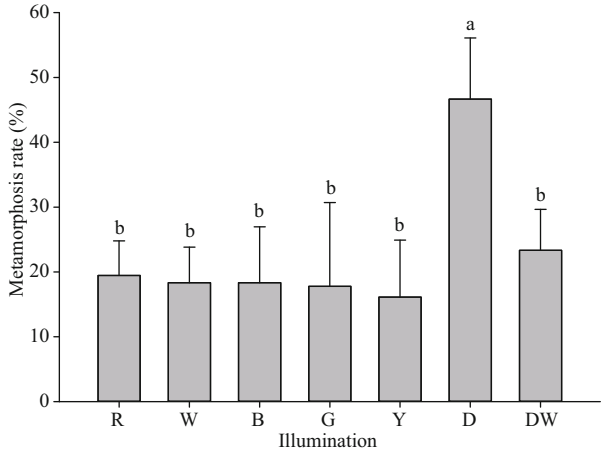

Fig.5 Metamorphosis rates of larvae under different illumination conditions

Bars show standard errors. Means with different symbols (a, b) are significantly different (P<0.05).

settlement rate, metamorphosis rate, and mortality rate of larvae differed significantly among the different stages (F=318.08, P<0.001; F=168.37, P<0.001; and F=9.48, P<0.001, respectively) and different substrates (F=113.41, P<0.001; F=172.24, P<0.001; and F=55.80, P<0.001, respectively). Different stages and substrates had significant interaction effects on the settlement rate, metamorphosis rate, and mortality rate (F=100.86, P<0.001; F=165.29, P<0.001; and F=9.01, P<0.01, respectively). The settlement rate, metamorphosis rate, and mortality rate of larvae with scallop shell substrates were higher than those of control groups (P<0.001). The settlement rate and metamorphosis rate of F stage larvae were higher than those of D and E stage larvae (P<0.001); however, the mortality rate of F stage larvae was lower than those of D and E stage larvae (P<0.05).

As shown in Fig.4a and b, when scallop shell with oysters was provided, the F stage larvae had high settlement (86.83%) and metamorphosis (36.33%) rates, whereas stage D and E larvae had very low rates of settlement (0, 3.17%, respectively) and metamorphosis (<1%). When scallop shells were not provided, the metamorphosis rate of F stage larvae was very low (<1%), while the settlement rate was as high as 24.17%. Settlement and metamorphosis rates were very low in D and E stage larvae (<1%). The results showed that the larvae began to settle and metamorphose significantly in the F stage, and the scallop shell with oysters had a good effect on the settlement and metamorphosis of larvae.

As shown in Fig.4c, when scallop shell with oysters was provided, the larvae of stages D and E had higher mortality rates (31.33%, 22.33%, respectively) than that of F stage larvae (9.67%). When scallop shells were not provided, there was no significant difference in mortality among D (6.00%), E (5.33%), and F (5.67%) stage larvae (P<0.05). There was no significant difference in mortality between scallop shell and control groups in F stage larvae (P<0.05). The results showed that premature provision of substrates increased larval mortality and only when the larvae reached the F stage can they begin to settle.

As shown in Fig.4d, at the F stage, the rate of metamorphosis was significantly difference among different substrates (F=166.05, P<0.05). The rate of metamorphosis in scallop shells with oysters groups was significantly higher than that in scallop shells without oysters groups and no substrate groups (P<0.05). There was no significant difference (P>0.05) in the rate of metamorphosis between scallop shells without oysters groups and no substrate groups.

3.3 Illumination

Different illumination conditions had a significant effect on the metamorphosis rate of planktonic larvae (F=9.547, P<0.001). The metamorphosis rate of larvae in dark (46.67%) conditions was significantly higher than that in other illumination (16.11%–23.33%) groups (P<0.05; Fig.5). The rate of metamorphosis of planktonic larvae decreased significantly when light was provided, which indicated that darkness was beneficial to the metamorphosis of larvae.

3.4 Prey

There were significant differences in the effects of different prey inducers on the settlement rate of planktonic larvae (F=30.13, P<0.001). Compared with the algae group, the settlement rate of larvae could be significantly increased by the presence of shellfish juveniles (P<0.05), including shellfish that could be eaten directly by larvae and those that could not be eaten by larvae (Fig.6a).

As shown in Fig.6b, the effects of different prey inducers on the larval metamorphosis rate were significantly different (F=34.56, P<0.001). The metamorphosis rates of Cg-F (35.67%), Mm-F (28.00%), and Mc-F (27.00%) were significantly high than those of Cg-I (14.33%), Mm-I (7.33%), and Mc-I (7.67%), respectively (P<0.05). Group Cg-F had the highest rate of metamorphosis, although there was no significant difference between group Mm-F and group Mc-F (P<0.05). The rate of metamorphosis of the algae group was very low (<1%). As shown in Fig.4c, the effects of different prey inducers on the time

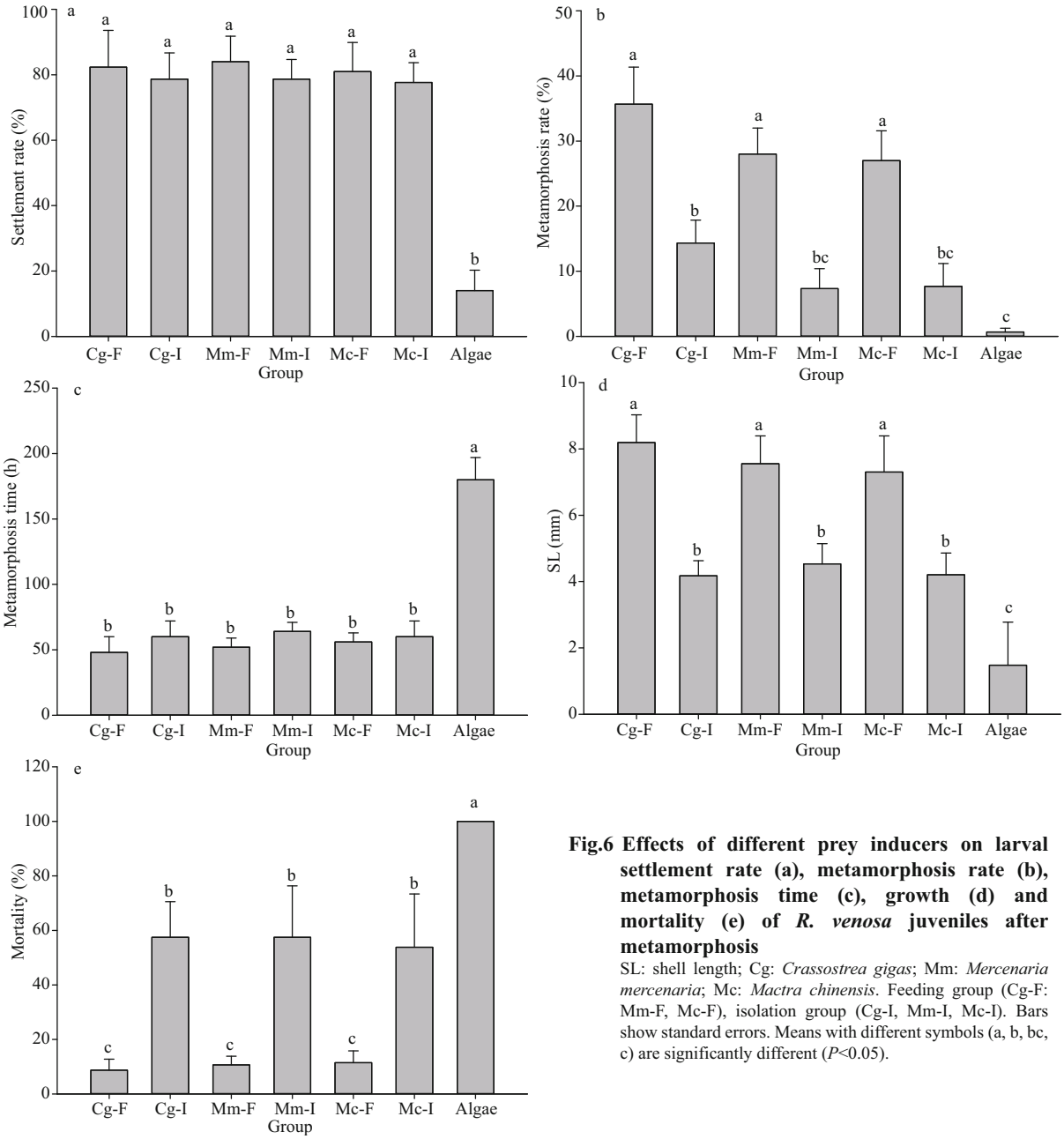

Fig.6 Effects of different prey inducers on larval settlement rate (a), metamorphosis rate (b), metamorphosis time (c), growth (d) and mortality (e) of *R. venosa* juveniles after metamorphosis

SL: shell length; Cg: *Crassostrea gigas*; Mm: *Mercenaria mercenaria*; Mc: *Mactra chinensis*. Feeding group (Cg-F: Mm-F, Mc-F), isolation group (Cg-I, Mm-I, Mc-I). Bars show standard errors. Means with different symbols (a, b, bc, c) are significantly different (P<0.05).

required for larval metamorphosis were significantly different (F=41.98, P<0.001). The larval metamorphosis time of algae group was the longest (180 h), but there was no significant difference between the larval metamorphosis times in the other groups (Cg-F, 48 h; Mm-F, 52 h; Mc-F, 56 h; Cg-I, 60 h; Mm-I, 64 h; Mc-I, 60 h). However, larvae in group Cg-F took the shortest time to complete metamorphosis.

The effects of different prey inducers on the growth of *R. venosa* juveniles after metamorphosis were significantly different (F=23.23, P<0.001; Fig.6d). The SLs of Cg-F (8.19 mm), Mm-F (7.55 mm), and Mc-F (7.30 mm) were significantly higher than those of Cg-I (4.17 mm), Mm-I (4.53 mm), and Mc-I (4.20 mm), respectively (P<0.05). Group Cg-F had the longest SL, and there was no significant difference in SL between group Mm-F and group Mc-F (P<0.05). Group algae had the shortest SL (2.22 mm) and almost no growth after metamorphosis. The effects of different prey inducers on the mortality of *R. venosa* juveniles after metamorphosis were significantly different (F=19.89, P<0.001; Fig.6e). The mortalities of Cg-F (8.73%), Mm-F (10.66%), and Mc-F (11.46%) were significantly lower than those of Cg-I (57.48%), Mm-I (57.50%), and Mc-I (53.79%), respectively (P<0.05). Group Cg-F had the lowest mortality, and there was no

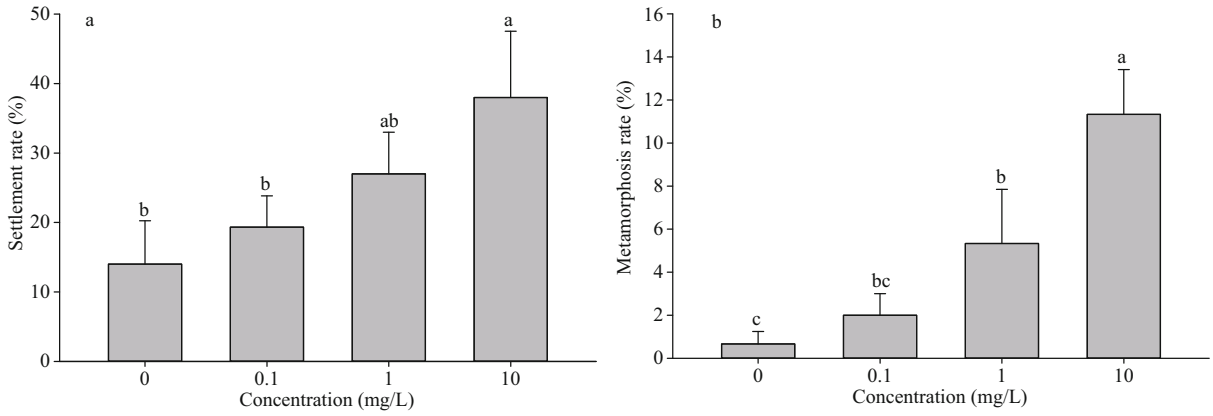

Fig.7 Effects of oyster polysaccharide concentration on larval settlement rate (a), and metamorphosis rate (b)

Bars show standard errors. Means with different symbols (a, b, ab, bc, c) are significantly different (P<0.05).

significant difference in mortality between group Mm-F and group Mc-F (P<0.05). All *R. venosa* juveniles in the algae group died.

3.5 Oyster polysaccharide

The settlement rate (F=6.99, P<0.05) and metamorphosis rate (F=22.63, P<0.01) of the larvae was significantly increased by the presence of oyster polysaccharide (Fig.7). Compared with the control group (0 mg/L), the rate of larval settlement and metamorphosis was significantly increased (P<0.05) when the concentration of polysaccharide was high (10 mg/L), but the effect of polysaccharide on inducing settlement and metamorphosis was not significant (P=0.78) when the concentration was low (0.1 mg/L).

4 DISCUSSION

4.1 Metamorphic characteristics of larvae, time of settlement and metamorphosis, and provision of substrates

For *R. venosa*, the development of the foot is the basis of its settlement, while its metamorphosis is characterized by feeding conversion, velum degeneration, and adult shell growth (Pan et al., 2013a). The initiation of settlement and metamorphosis of *R. venosa* has been reported to occur at 620 μm shell length (Wei et al., 1999), 850 μm shell length (Yang et al., 2007), 1 180–1 240 μm shell length (Harding, 2006) or 1 250–1 500 μm shell length (Pan et al., 2013 a). In the present study, we found that larvae could only reach a competent stage when the larval shell length reached 1 250–1 500 μm; when the larval foot was fully developed, it could freely stretch and crawl, and the velum began to degenerate gradually. When the larval shell length was 620 and 850 μm, the larva was in the C and D stage, respectively; the larva's foot was not able to expand freely, and the velum was still in the developmental stage and could not degenerate. Larvae at these stages are not yet capable of settlement. When the larval shell length was 1 180–1 240 μm, the larva was in the E stage. At this stage, the larva's foot had already been able to expand freely, and the length of the single leaf velum had reached the maximum length. The larvae were mainly planktonic, although a few larvae were observed alternately floating and settling. Therefore, only a few larvae were capable of settlement and metamorphosis in the E stage (Fig.4).

During metamorphosis, the larva of *R. venosa* changes from phytophagous to carnivorous (Yu et al., 2018). Yang et al. (2007) and Liu et al. (2015) found that diatom corrugated plate could induce *R. venosa* larvae to complete settlement and metamorphosis. However, Pan et al. (2013b) found that it is difficult for the larva to complete the process of settlement and metamorphosis by plant bait alone, and the fresh animal bait had a remarkable attraction to the larva. In this study, we found that although some larvae could settle, it was difficult to complete metamorphosis when only plant bait was provided. When scallop shells with oysters were provided, the settlement rate and metamorphosis rate (Fig.4a, b) of larvae increased significantly. Oysters attached to scallop shells are the main inducers of larval metamorphosis, since scallop shells themselves cannot induce larval metamorphosis. This kind of feeding transformation is similar to that of *Babylonia areolata*, whose juveniles feed on single-cell algae, and they prefer to feed on crabs after metamorphosis (Liang et al., 2005).

Premature input of substrates can cause a large number of deaths of *R. venosa* larvae. Liu et al. (2015) also observed this phenomenon, and they attributed the larval death to the contamination of the water by the substrates. In our experiments, we found two

different modes of larval death in D and E stages. The first mode is that the larva adheres to the substrates. Biofilms (Watnick and Kolter, 2000) or other impurities produced on scallop shells will stick the larva to the scallop shells. The larva's foot is not yet fully developed and cannot be used to roll over. Therefore, the larva can't move freely, causing it to die. F stage larvae can use their foot to roll over without being stuck by scallop shells, so the mortality rate is significantly lower than that in stages D and E. In the second mode of death, the larvae die near the substrates, and their empty shells gather beneath the substrates. This hinders the water circulation under the scallop shell. The larvae in this area are stimulated and the velum retracts into the shell resulting in a higher mortality rate of these larvae. F stage larvae can use foot movement, so there is no significant impact on the larvae at this stage. Therefore, the substrates should be released only when the larva develops to F stage. The scallop shell itself does not induce larval metamorphosis and its role is to collect juvenile oysters.

The production of the adult shell is an important feature in the metamorphosis of *R. venosa* larvae. The changes in the adult shell can serve as indicators of metamorphosis. This is of great significance for seed production as the changes in the adult shell can be used to guide changes in diet during seed production. Since the larva changes from phytophagous to carnivorous during metamorphosis, it is necessary to observe the growth of adult shell and adjust the feeding strategy in time when the larva grows to F stage (Fig.2a). When the adult shell begins to grow (Fig.2b), the larvae should be fed with scallop shell with oysters, otherwise, the larvae will begin to die in large numbers. Provision of unicellular algae should continue until the larvae complete metamorphosis and become juvenile snails (Fig.2f).

4.2 The effect of illumination on the settlement and metamorphosis of larvae

The effect of light on shellfish larvae differs for different species. Some larvae have positive phototaxis and some have negative phototaxis to light, and some larvae have no response to light (Bao and You, 2004). Gao et al. (2016) found that blue or green light could increase the rate of metamorphosis of Pacific abalone *Haliotis discus hannai* trochophore larvae and shorten metamorphosis time. However, our results for *R. venosa* are different; the larval metamorphosis rate is highest in the dark, but lower in different light colors. Similarly, metamorphosis of anadromous sea lampreys, *Petromyzon marinus*, does not require the influence of light (Cole and Youson, 1981). Therefore, in order to improve the rate of larval metamorphosis in *R. venosa*, the larval living environment should be kept dark.

4.3 The effect of prey and oyster polysaccharide on the settlement and metamorphosis of larvae

Prey is an important external factor that can induce larval settlement and metamorphosis of shellfish larvae. Until the required inducer is found, the larvae of shellfish continue to live in the plankton. In the absence of suitable inducers, many larvae delay settlement and metamorphosis (Pechenik, 1990; Rodríguez et al., 1993). Many foods contain active substances that induce larval settlement and metamorphosis of shellfish (Song et al., 2016a). The larvae of red abalone, *Haliotis rufescens*, prefer to feed on red algae, *Lithophyllum* sp. and *Lithothamnion* sp., and larvae can be induced to settle and metamorphose by some analogues of y-aminobutyric acid (Morse et al., 1979). Dibromomethane from red algae of the family Corallinaceae can effectively induce the metamorphosis of the larvae of the gastropod *Crepidula fornicata* (Taris et al., 2010).

Inducers are also needed for the settlement and metamorphosis of the larvae of *R. venosa*. Yang et al. (2016) found that the addition of new seawater in the sedimentation tank can induce metamorphosis of larvae of *R. venosa*, possibly because of the presence of chemical signs of animal food in the seawater. Unlike phytophagous shellfish, the settlement and metamorphosis of *R. venosa* is not induced by unicellular algae, but instead by bivalve shellfish. Our experiments indicated that bivalves could release chemical signals that induce the settlement of larvae, and when the larvae could not perceive these chemical signals, they would continue to float and delay settlement. Similarly, the chemical signals released by bivalves could effectively induce larval metamorphosis, however, the induction effect of bivalve shellfish secretion was significantly lower than that of the bivalve itself, indicating that the metamorphosis of larvae was affected by a variety of factors. Although the chemical signals released by bivalves could induce settlement and metamorphosis, the growth of juvenile snails after metamorphosis was still dependent on feeding on bivalve shellfish to obtain enough energy (Fig.6d, e). Because the settlement and metamorphosis of *R. venosa* larvae is strongly dependent on the juveniles of bivalves, their settlement and metamorphosis behavior can be effectively controlled

by controlling the number of shellfish, thus affecting the population dynamics of *R. venosa*.

The Pacific oyster *C. gigas* had the best induction effect on the settlement and metamorphosis of larvae of *R. venosa*. It is possible that substances specific to oyster larvae, which may include oyster polysaccharides, induce larval metamorphosis. Cell wall polysaccharides from coralline algae can induce settlement in a variety of coral larvae (Morse and Morse, 1991, 1996), and bacterial polysaccharides can induce metamorphosis in larvae of the polychaete *Neodexiospira brasiliensis* (Kirchman et al., 1981; Coon et al., 1985). In the present study, we found that a suitable concentration of oyster polysaccharide could significantly increase the settlement rate and metamorphism rate of *R. venosa* larvae. The use of oyster polysaccharide as an inducer would allow the supply of oyster larvae to be reduced, and this would greatly decrease the cost of raising seed.

5 CONCLUSION

In conclusion, the larvae of *R. venosa* were capable of settlement and metamorphosis only in the F stage. The substrates should be provided only at this time because premature delivery will lead to many larval deaths. The use of a dark environment, bivalve shellfish, and oyster polysaccharides could significantly increase the rates of settlement and metamorphosis of larvae. Further study is required to determine the induction mechanism of oyster polysaccharides. The identification of efficient inducers is of great significance in reducing the *R. venosa* seed production cost. However, aquaculture of *R. venosa* must be prohibited outside its natural range.

6 DADA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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8 AUTHOR CONTRIBUTION

ZHANG Tao conceived and designed the experiments. YU Zhenglin and YANG Meijie performed the experiments. YU Zhenglin analyzed the data. YANG Meijie, SONG Hao, HU Zhi, ZHOU Cong, WANG Xiaolong, and LI Haizhou contributed reagents, materials, and analytical tools. YU Zhenglin wrote the manuscript.

9 COMPLIANCE WITH ETHICAL STANDARD

Declarations of interest: None. Human and animal rights: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Conflict of Interest: Authors declare that they have no conflict of interest.

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