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Original Article

Variability in larval period, post-setting growth and survival of the oyster *Crassostrea belcheri* produced by gamete stripping method

Suwat Tanyaros,* Woraporn Tarangkoon

Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang Campus, Trang 92150, Thailand

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ABSTRACT

Gamete stripping is the most practical method in oyster production because the time of fertilization can be precisely controlled. However, it was found that there was much greater variability in the speed of egg development compared with natural spawning. In this study, three groups of larvae (aged 21, 27 and 29 d) were successively separated from fertilization to the pediveliger stage among all the larvae produced by the stripping method. No differences in the spat growth rate were found among the larval periods at 15 d of the experiment ($p \geq 0.05$), but spat growth and survival rates of juvenile oysters in the larval periods of 21 and 27 d were significantly higher than those for 29 d at the end of the experiment (30 d). The positive correlation between larval growth rates and the spat daily yield at 30 d was significant. These results indicated that the gamete stripping method produced high variations in the larval period and post-setting growth and survival in the oyster, *Crassostrea belcheri*.

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Introduction

From the first reported *in vitro* oyster fertilization in 1879 to the appearance of modern production hatcheries, hatchery practices have seen more than one hundred years of development (Helm et al., 2004). Today, knowledge about oyster reproduction and rearing techniques has improved greatly. Hatcheries successfully achieve controlled development of spat from fertilization to post-larvae for many oyster species. Induced spawning and gamete striping or sacrification are commonly used for obtaining gametes in oyster hatcheries as using stripped gametes is the most practical method for producing triploid oysters because the time of fertilization can be easily controlled (Allen Jr. and Bushek, 1992). Although “strip” spawning is the recommended method for obtaining gametes, it was found that there was much greater variability in the speed of egg development in “stripped” eggs than in those obtained from natural spawning (Allen Jr. and Bushek, 1992). This technique is an alternative practice in fully mature oysters in cases where induced spawning is ineffective.

The white-scar oyster, *Crassostrea belcheri* (Sowerby, 1871), is one of the most common commercial bivalves in Thailand, and many studies have been done on its biology and culture over many years (Department of Fisheries, 1994). Oyster seed production from hatcheries has been continuously developed and is a subject of great interest in Thailand (Tanyaros et al., 2000, 2008, 2012). Larvae production under hatchery conditions for this oyster species has been obtained by induced spawning (Sahavacharin et al., 1984; Nugranard et al., 1987) and gamete stripping (Tanyaros and Kitt, 2011, 2012; Tanyaros et al., 2012). However, larval rearing in hatcheries using both techniques results in variability in size within the same batch. The variability in larval period, post-setting growth and survival of the oyster *C. belcheri* produced using the gamete stripping method has not been previously investigated. Smaller grades must take into account the time and cost for nursing them to reach the pediveliger stage at which they are ready to set. The question arises as to whether small grade larvae produced using the stripping method will grow at the same rate as large grade larvae after setting. The objective of the current study was to determine the variability of the larval period and the growth performance of juvenile *C. belcheri* oysters produced by the stripping method.

Materials and methods

Experimental larvae

Mature *C. belcheri* broodstocks (mean shell length 14 ± 1.5 cm) were collected from the wild during the spawning season from

* Corresponding author.
E-mail address: stanyaros@gmail.com (S. Tanyaros).
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Takuatung district, Phangnga province, Thailand. The seasonal spawning of natural *C. belcheri* normally occurs from June to September each year (Department of Fisheries, 1994). The collected oysters were cleaned and acclimated in sand-filtered seawater (30 psu) in 1000 L fiberglass tanks with a closed-recirculation system. An algal mixture containing 6% *Chaetoceros calcitrans* and *Tetraselmis suecica* per milligram oyster (dry algal weight/dry meat weight) was provided throughout the period. The brooders were checked daily under a binocular compound microscope (CH_2; Olympus, Tokyo, Japan) for their ripeness. Two days later, 10 and 4 ripe females and males, respectively, were observed prior to being sacrificed, gametes were obtained, and the eggs fertilized using a ratio of 100 spermatozoa per oocyte (Collet et al., 1999). The mean percentage of fertilized eggs was 85.0%. The embryos were stocked for further development in 500 L culture tanks at a density of 15 embryos/mL in filtered (1 μm) and UV-treated seawater. The embryos developed to D larvae within 24 h, and then the density was reduced to 5 larvae/mL. Seawater renewal and tank cleaning were conducted every 2 d. At each draining, larvae were sieved and graded for size to prevent growth retardation. D larvae were fed daily with *Isochrysis galbana* at a density of 20,000 cells/mL. When the size of the larvae was greater than 100 μm, a mixed diet of *I. galbana* and *C. calcitrans* was fed to them daily and the algal density was increased with increasing age (Tanyaros, 2013).

Sieving groups

When the pediveliger larvae were first observed (competent larvae and the presence of eyespots), they were collected by sieving with a 250 μm mesh. Larvae retained on the 250 μm nitex sieve were then transferred to a 10 L plastic bucket. A perforated plunger agitator was used for evenly suspending the larvae in a bucket and then three samples were taken for the estimation of larval number. Each sample was counted using a Sedgewick-Rafter counting chamber under a binocular compound microscope. The time of larval development from fertilization to the pediveliger stage from each grading was recorded and 20 samples of the larvae were measured for shell width (dorso-ventral measurement) using a micrometer under a binocular compound microscope. Pediveligers were then transferred to setting units for settlement and the smaller individuals were returned to the larval rearing tanks. Thus, sieving group G1 corresponded to the fastest-growing larvae and sieving group G3 to the slowest-growing larvae.

Settlement procedure

A closed recirculation system was chosen to determine post-setting growth and survival. The details of the experimental system were described in Tanyaros and Chuseingjaw (2016). In brief, three sets were used, each consisting of a submersible pump, a rectangular 105 L (50 × 70 × 30 cm) fiberglass tank for placement of the nursing units, a cylindrical plastic 30 L container (60 cm diameter and 25 cm depth) for food storage, and three sets of cylindrical polyvinyl chloride (PVC) pipe (15.2 cm diameter × 12 cm length) were used as nursing units. Each cylindrical PVC pipe was drilled at 10 cm from the bottom for a 1.86 cm diameter overflow pipe. A screen using 600 μm mesh was fixed by a PVC clamp to the bottom of each cylindrical PVC section. The volume of water in each nursing unit was 1.82 L during system operation. The oyster chips used as a substrate for oyster setting were made from clean oyster shell, which had been sun dried and broken into particles using a stone mortar and pestle. The particles were graded so that only those that passed through a 500 μm screen but were retained on a 250 μm screen were used for oyster setting in the experiment. The particulate material was spread over the screen in the setting unit. Substrates were conditioned by immersion in clean seawater for 24 h before use. Into each setting unit, 2000 pediveligers were released. During pre-setting, the water from the water storage tank was pumped into the setting unit. The water was injected so that it down-welled into each setting unit and then drained through the overflow pipe in the fiberglass tank before being returned to the storage tank. The rate of water flow into each setting unit was adjusted by the valve on the inflow pipe to 0.5 L/min (Tanyaros et al., 2012). Water salinity was maintained at 30 psu over the study period.

Growth and survival measurement

After all larvae had been set on substrate, the direction of the water flow was changed to up-well through the setting units, and the water flow rate in each setting unit was increased to 4 L/min using the adjustable valve (Tanyaros et al., 2012). The water was totally renewed every 2 d and food was added twice a day (morning and evening) at a rate of 25,000 cells/mL of *C. calcitrans* and 25,000 cells/mL of *T. suecica*. The spat was allowed to grow in the system and then 20 samples of oyster spats from each replicate were taken bi-weekly to measure the shell width and length using a binocular stereomicroscope. The ocular scale on the stereomicroscope was calibrated using a micrometer prior to measurement. At the end of the experiment, the juvenile oysters were sieved separately from substrates using a 900 μm screen and measured for wet weight. The daily yield was then calculated using the method of Dégremont et al. (2007). All juvenile oysters from each experimental unit were counted and then the survival rates were calculated and expressed as percentages.

Statistical analysis

Growth performance and survival rate data from the experiments were analyzed to test for differences among sieving groups applying one-way ANOVA using SPSS 17.0 for Windows (SPSS Inc; Chicago, IL, USA). When significant effects were found, a further analysis using Tukey's test was used to compare among the means. The regression analysis was used to test the relationship between the larval growth rate of each sieving group, spat daily yield and survival rate.

Results

Variability of larval period

In this experiment, gamete stripping produced a high variation in the larval period. The three groupings of larval periods (from fertilization to the pediveliger stage) were 21 d (G1), 27 d (G2) and 29 d (G3) with the pediveliger component accounting for 20.3%, 49.2% and 30.5%, respectively. However, no significant differences ($p > 0.05$) were found in the mean shell widths of pediveliger among the different larval periods (Fig. 1).

Growth and survival of juvenile oysters

After setting, juvenile oysters were nursed in a closed recirculation system for one month. At 15 d, no significant differences ($p > 0.05$) were found in the mean absolute shell width and length among the different larval periods. The mean absolute shell width was 52.5 ± 13.4 μm/d, 59.2 ± 17.1 μm/d and 69.8 ± 13.9 μm/d and the absolute shell length was 52 ± 11.3 μm/d, 67.8 ± 14.6 μm/d and 67.7 ± 15.8 μm/d for the larval periods of 21, 27 and 29 d, respectively.

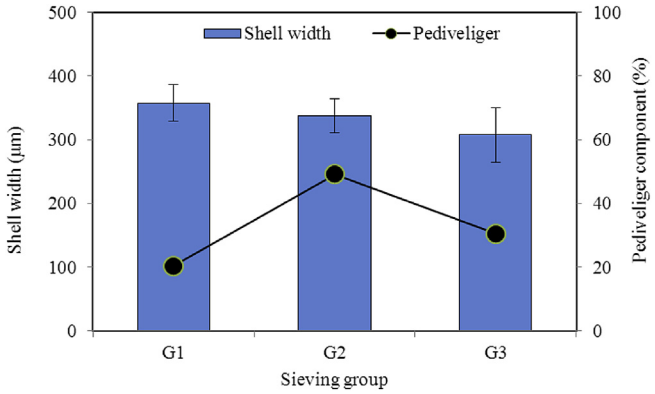

Fig. 1. Mean (±SD) shell width and component of pediveliger larvae obtained from different larval periods. (G1 = 21 d, G2 = 27 d and G3 = 29 d).

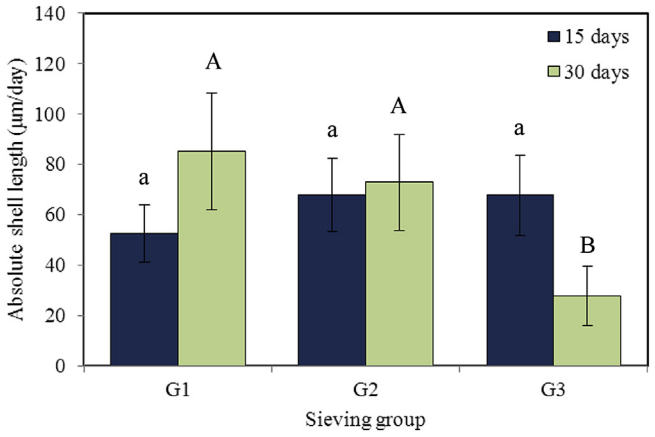

Fig. 3. Mean (±SD) absolute shell length of juvenile oysters from different larval periods after nursing for 15 and 30 d. Columns labeled with different letters represent means that were significantly different ($p < 0.05$). (G1 = 21 d, G2 = 27 d and G3 = 29 d).

However, the mean absolute shell width and length of juvenile oysters in the larval periods of 21 and 27 d were significantly ($p < 0.05$) higher than those for 29 d at day 30 of the experiment. The mean absolute shell width was 80.7 ± 19.4 µm/d, 92.2 ± 20.2 µm/d and 19.8 ± 7.7 µm/d and the absolute shell length was 85.2 ± 23.3 µm/d, 72.8 ± 19.0 µm/d and 21.7 ± 11.7 µm/d in the larval periods of 21 d, 27 d and 29 d, respectively (Figs. 2 and 3). The correlation between the larval growth rate of each sieving group and the spat daily yield at 30 d was significant (correlation coefficient, $r^2 = 0.9687$). A positive correlation between the larval growth rate of each sieving group and survival rate was found ($r^2 = 0.7879$). The lowest survival rate for juvenile oysters was found in the third batch of sieving (larval period of 29 d) compared with the first and second batches of sieving (21 and 27 d, respectively).

Discussion

Obtaining gametes is a vital step in bivalve hatchery operations. Stripping gametes is the most practical method in oyster hatcheries because the time of fertilization can be easily controlled (Allen Jr. and Bushek, 1992). Fully mature gamete stripping has been done in several oviparous oyster species, such as the Sydney rock oyster, *Saccostrea commercialis* (Nell et al., 1996) and in the Pacific oyster, *Crassostrea gigas*, the American oyster, *Crassostrea virginica*, and the mangrove oyster, *C. rhizophorae* (Helm et al., 2004). This is a common practice and a convenient way for spawning these species, following a suitable period of conditioning. Gamete quality can be characterized by fertilization rates and by embryo development yields and these are related to the multiple cellular and subcellular parameters of the gametes (Song et al., 2009). In the current study, the mean percentage of embryos developed was high (85.0%) and similar to the results found by Collet et al. (1999) in *C. gigas* (87.6%) using the same method. However, the yield for larvae produced by gamete stripping has been shown to be lower than that obtained from natural spawning (Allen Jr. and Bushek, 1992). Fully mature eggs produced from natural spawning may have a higher nutritional accumulation than those produced by stripping, with effects on subsequent larval development. Prolonging the swimming period of the larvae may also have detrimental effects once energy reserves fall below a critical level; Pechenik et al. (1993) indicated that larval age and lipid content jointly affected attachment and metamorphosis in *Balanus amphitrite*. The importance of lipids as a major energy reserve for larval development in oysters (*Ostrea edulis*) has been shown in some studies (for example, Millar and Scott, 1967; Holland and Spencer, 1973). Oyster larvae do not synthesize docosahexaenoic acid or show very low biosynthesis activity for it, and cannot satisfy their own growth requirements (Waldock and Holland, 1984). Therefore, depletion of energy reserves may reduce their chances for success in the later stages of development. Delay in larval development was found in the present study as the first sieving group (G1) reached the pediveliger stage at 21 d, longer than the results obtained from natural spawning (18 d) in experiments conducted by Sahavacharin et al. (1984) and Nugranard et al. (1987) using the same species and under similar environmental conditions, while the last sieving group (G3) reached the pediveliger stage at 29 d. It has been admitted that the reproductive success of *C. gigas* is highly variable and that this variability is partially due to inconsistent gamete quality, sperm–egg interaction and differential viability among genotypes (Boudry et al., 2002).

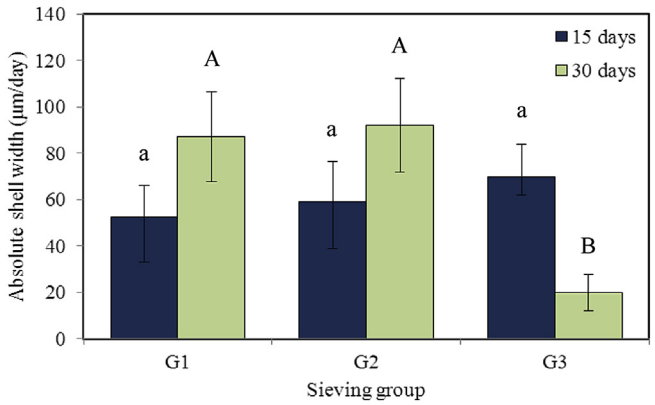

Fig. 2. Mean (±SD) absolute shell width of juvenile oysters from different larval periods after nursing for 15 and 30 d. Columns labeled with different letters represent means that were significantly different ($p < 0.05$). (G1 = 21 d, G2 = 27 d and G3 = 29 d).

In the preset experiment, feeding, environmental conditions and handling techniques were similarly controlled in each sieving group. However, the larval period affected the spat growth rate significantly. A low growth rate and a low survival rate for the third sieving group (G3) were found at day 30 of the experiment. These results support the general assumption of commercial hatchery operators that the final grading of a hatchery batch of larvae is slower growing and therefore of lower value than the top grading. Systematic culling of the smallest larvae is suggested in order to reduce the time of larval rearing and to reduce size variability. The correlation between sieving groups and spat daily weight gain was

positive. A similar relationship was found in *C. virginica* (Newkirk et al., 1977; Losee, 1979) and *C. gigas* (Collet et al., 1999). These results indicate that the larval period in oyster hatcheries has great importance on growth in the juvenile stage. Similarly, Losee (1979) found that hatchery-reared *C. virginica* that reach metamorphosis earlier than sibling larvae, achieved greater subsequent growth rates. The speed of growth is high at an early stage and decreases at later stages as shown in the present study. Experiments have been carried out on some invertebrate species in which the length of the larval period or the nutritional conditions of the larvae were altered and the effects on subsequent juvenile growth studied with *B. amphitrite* (Pechenik et al., 1993; Harder et al., 2001) and *Crepidula plana* and *Crepidula fornicata* (Pechenik and Eyster, 1989). In the present experiment, the effects of the larval period (rate of larval growth) on early spat growth and survival rates were evident in *C. belcheri* when the environmental conditions were maintained as constant as possible during all larval and post-setting stages. From these findings, artificially bred *C. belcheri* produced high variations in the larval period, post-setting growth and survival. The last sieving group should be discarded in order to reduce the operating cost due to this group's low growth and survival rate.

Conflict of interest

There is no conflict of interest.

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