J. Aquaculture
Vol. 20(2) : 81-84, 2007

한국양식학회지
Journal of Aquaculture

Batch-Specific Quality of the Reproductive Outputs and Nursery Acclimation in the Seed Production of *Patinopecten yessoensis* - case study on Korean coasts of the East Sea

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We studied two research items that can be undetermined issues in the artificial seed production of the Japanese scallops, *Patinopecten yessoensis*, batch-specific quality of the reproductive outputs and nursery acclimation, in the embayed waters of Yangyang, Gangwon, Korea. The first batch of the spawner showed better results in terms of survival, growth, and resistance against parasitic ciliate infection over the second batch that was obtained in 5 days after first batch from the same spawner. The early attached spats directly placed in the farming ground (the unacclimated) were resistant against the water current of the open environment, by showing survival of about 80% for a month, a normal survival compared with other results. However, the spat survival during the nursery acclimation was significantly lowered in comparison with that in the unacclimated condition ($P<0.01$). We discussed the research results of the two items, particularly focusing on their availability for mass seed production for aquaculture in the Korean coastal waters of the East Sea (Sea of Japan).

Keywords: Batch, Seed, Nursery acclimation, Patinopecten yessoensis

Introduction

Japanese scallop *Patinopecten yessoensis*, a cold tolerant species, distributes mostly in the subfrigid zones including the East Sea (Sea of Japan). Gangwon province is known as the southern limit of the natural distribution in Korea. During a last decade, decline of wild stocks was noticeable and thereby aquaculture business has been introduced in the province.

Gangwon has long coasts available for scallop aquaculture. However, the farmed scallops are often exposed to daily extreme temperatures that can be a cause of local mass mortalities (NFRDI Report, 2005). According to the report, the frequencies of the mortalities are dependent on the physiological status of the species as well as farmed strains. Chinese strains, acclimated to extreme temperatures of the Yellow Sea, appear to be resistant against temperature variation in Gangwon province. In the present study, we studied two research items that believably influence physiological status of the hatchery-based seeds of *Patinopecten yessoensis*: the batch selection and nursery acclimation in the embayed waters.

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Materials and Methods

Spawners and spawning

A total of 450 *Patinopecten yessoensis* (Chinese strain) weighing 73±13 g (mean ± SD) in total weight, were brought into the scallop hatchery of the East Sea Fisheries Research Institute (ESFRI), Gangwon, Korea. Upon arrival, males and females were separately contained in two raceways and the water temperature was maintained at 6±0.5°C without feeding for 3 days for acclimation. After the acclimation, healthy 120 females and 60 males were selected, air-exposed at 22°C for 1 hr, and placed into a spawning tank (20 ton) containing filtered seawater at 12±0.5°C to stimulate the spawning (1st batch). After the 1st spawning, the spawned scallops were re-contained in tanks as same way as the first acclimation for 5 days and a second spawning stimulation was applied to the spawner and termed 2nd batch.

Larval culture

Just after spawning, the spawners were withdrawn from the tanks. A gentle aeration was thoroughly given to facilitate aerobic condition for all parts of the hatching tank and dispersion of the zygote. Trochophore larvae were rinsed using a net (mesh size, 20 microns) and transferred into 4 larval culture tanks (water volume, 6 tons); 2 for larvae from the first batch and another 2 for larvae from the socond batch at concentration of 5,000 larvae L^{-1}. The larvae were initially fed with *Isochrysis galbana* (or *I.* aff. *galbana*) and *Chaetoceros gracilis* for 5 days. With the larval development, *Phaeodactylum tricornutum* and *Tetraselmis suecica* were additionally supplied for nutritional balance. The food concentrations were maintained $1–10\times10^4$ cells mL^{-1}, depending on larval growth and feeding activity. Water temperature was elevated to 16±0.5°C with a daily increase of 0.5°C and then decreased down to 12°C from day 18 to reduce cold shock after attachment. All cultural seawater was replaced by filtered and sterilized seawaters on an every 2-day basis. During the water replacement, samples were made for larval survival and growth measurements. The larvae failed to suspend or infected with parasites were considered to be mortal. All the larval culture methods unmentioned were followed by the protocol of Bourne et al. (1989) and others (MacDonald, 1988; Foighil et al., 1990; Shumway, 1991).

Post-larval culture and nursery acclimation

When the larvae metamorphosed to pediveliger, specially designed substrates (palm thread, ϕ5 mm×20 m) folded into a plastic net bag (30×20 cm; mesh size, 1 mm) were set in the culture tank for larval attachment (200 substrates for each tank). The substrates carrying spats were withdrawn from the tanks and distributed to raceways at density of 100 substrates per raceway (water volume, 8 ton). The post-larvae were fed with combination of 4 algal species used for algal culture at concentrations of $1\text{-}3\times10^5$ cells mL^{-1}, depending on algal species and spat feeding activity. Feeding schemes were 4 times a day with each feeding lasted for 1 hr without water flowing. Some of a month old attached spats were moved to a scallop farming ground as a reference, while others were moved to embayed waters for nursery acclimation. For a nursery acclimation, both ends of a rope (10 m) to which 10 substrates were evenly attached in a row were attached to a long rope of culture system to make a U-shaped suspension by a ballast (5 kg) attached to center of the rope.

Statistics

The data were statistically analyzed using student *t*-test in the Sigma Plot Software.

Results and Discussion

Is the first batch better than the second?

Physiological status of the bivalve is affected by their biological processes as well as alteration of environmental condition such as pollution and infection (Auffret and Oubella, 1997; Fisher et al., 1996; Choy et al., 2007). Among the biological processes, spawning behavior is one of the most influencing parameters (Cho and Jeong, 2005). Table 1 summarizes the spawning outputs from the first and second batches of *P. yessoensis*. There were no noticeable differences between early events of the reproductive outputs originated from the two batches. On the contrary, survival to trochophore of the second

Table 1. Comparison of Spawning outputs from first and second batches of *Patinopecten yessoensis*

Batch	No. spawner		No. egg spawned ($\times10^6$)	% fertilization	No. trochophore ($\times10^6$)*
	Female	Male			
first	120	60	143.8	100	123.9
second	106	43	83.6	100	79.9

*The number of larvae dead and failed to suspend was excluded.

Table 2. Percent occurrence of the dead and infected larvae from first and second spawning activities of *Patinopecten yessoensis*

Spawning	Day after first appearance of trochophore					
	0	4	8	12	16	18*/20**
first	4.7(0.73)†	11.1(1.39)	19.1(2.05)	37.1(3.24)	49.1(5.96)	54.3(4.28)
	0 (0)‡	0(0)	0.8(0.85)	4.2(1.56)	11.3(3.54)	15.7(5.8)
second	4.7(0.88)†	21.2(2.19)	26.4(3.06))	44.3(3.81)	47.2(6.25)	56.6(6.94)
	0(0)‡	0(0)	3.9(0.42)	13.7(2.26)	21.4(3.39)	29.4(2.69)

*Day for first batch
**Day for second batch
†Percent mean ± standard deviation for number of larvae dead and failed to suspend.
‡Percent mean ± standard deviation for number of larvae infected with ciliated parasite.

batch (95.6%) was better than that of the first batch (86.2%).

Reproductive outputs from the first and second batches of *P. yessoensis* were further studied in terms of larval viability and parasitic infection (Table 2). Unlike the early events, veliger larvae from the first batch showed better survival over those from the second batch. This was particularly significant in the parasitic infection ($P<0.01$). Since the parasitic infection was first found both in the experimental groups in day 8 after trochophore with a statistical significance between the two groups ($P<0.01$), the significance continued throughout the experiment.

Ciliate infections of veliger larvae of bivalve can cause significant morbidity and mortality (Elston et al., 1999). The cumulative ciliate infections measured in this study were around 15% and 30% for the larvae from the first and second batches, respectively. However, the actual cumulative infection was likely higher than the measurements in the present study since it did not take into account the infection potential, which was eliminated by removal of the infected larvae in our serial measurements.

Studies confirm that physiological status of bivalve changes seasonally, probably following the reproductive cycle of the animals (Park et al., 1999; Ringwood et al., 2002; Cho and Jeong, 2005). Bivalve eggs generally contain large amounts of lipids which, in comparison with proteins and carbohydrates, have high energy contents and are thus costly in energetic terms (Honkoop et al., 1999). The information on spawning cost of the scallop and its damage transfer to reproductive outputs are still lacking. In hatchery-based seed production, it is important to understand spawning cost of the scallop because the animal, like many other bivalve species, spawns many times in a given spawning cycle. Even though we did not determine energetic budgets of spawning activity, the first spawning appeared to be costly in the budget.

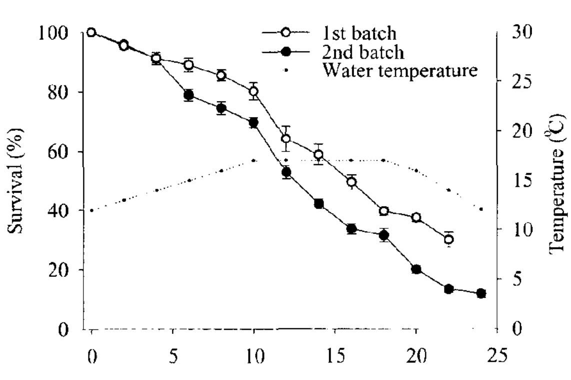

Fig. 1. Survival of larvae from the first and second batches of *Patinopecten yessoensis*. The cultures were continued until first appearance of ESL_{20}, a larval culture with 20% of eye-spotted larvae. Statistical significance: survivals of the larvae from the first batch were better over those from the second one from day 6 with significance of $P<0.01$. Error bar stands for mean ± standard error.

Table 3. Survival of post-larvae after attachment on the substrate during seed production of *Patinopecten yessoensis*

Larvae*	Week after attachment			
	0	1	2	3
first batch	-*	1,836(212)†	1,744(149)	951(340)
second batch	-	1,217(533)	632(387)	205(113)

*Uncountable.
†Mean survival. (number per substrate) (±standard deviation).

The larval survival of the first batch was superior to the second batch (Fig. 1). Furthermore, the first batch achieved better growth over the second one: the time to metamorphosis with a frequency of 20% of eye-spotted larvae (ESL_{20}) was 22 and 24 days for first and second batches, respectively. Survival of the post-larvae from the two batches showed similar trend to that of larval veligers (Table 3). These results, together with the parasitic infection data, imply that larvae of the first batch are more viable than that of the second batch, probably due to maternal energetic cost for the first spawning (Cho and Jeong, 2005).

Is the nursery acclimation necessary?

Both of juvenile and adult Japanese scallops respond to water current velocity and direction. The two physical parameters affect the feeding activity of the scallop (Sakurai et al., 2004). Sakurai and Seto (2000) found that scallops negatively respond to water velocities over 20 cm/sec and suggested water velocities not faster than 10 cm/sec for optimum growth. For this reason scallop aquaculturists dealing with artificial seed production routinely have their attached post-larvae acclimated to embayed nursery ground for a period of time prior to locating them in open farming ground. The nursery ground might keep the post-larvae from being detached from the substrate. However, at the same time, some negative aspects of nursery acclimation are expected. One of the negative aspects is attachment of fouling organisms on the nets, which in turn might have the nets less ventilated. Therefore, nursery acclimation has been a key issue in the artificial seed production.

Growth and survival of *P. yessoensis* seeds with and without nursery acclimation were studied (Fig. 2). The post-larval growth was not influenced by the nursery acclimation,

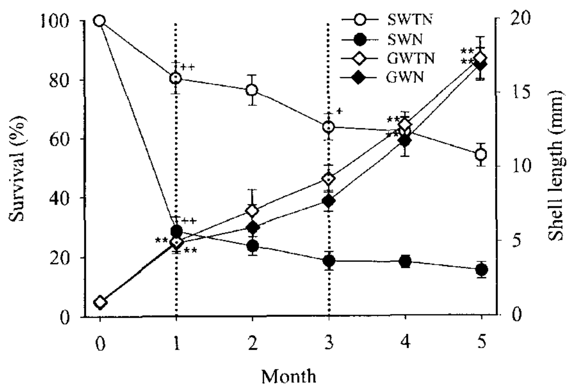

Fig. 2. Growth and survival of *Patinopecten yessoensis* seeds with and without nursery acclimations. A month old post-larvae were acclimated to open waters for a month in the embayed nursery ground (to the first vertical dotted line) and then placed in the open farming waters ground. The second vertical dotted line implies the time the spats were removed from the substrates into nursery lantern cages. Abbreviations: SWTN, spat survival without nursery acclimation; SWN, spat survival with nursery acclimation; GWTN, spat growth without nursery acclimation; GWN; spat growth without nursery acclimation. Statistical difference: $^{+}$significantly lowed with $P<0.05$; $^{++}$significantly lowed with $P<0.05$; **significantly elevated with $P<0.05$.

while a significant survival difference observed between with and without nursery acclimation ($P<0.01$). In our study the survival was about 80% for unacclimated spats, while it was around 30% for unacclimated ones.

The extremely low survival of the acclimated spats might not be due to mass mortality, but due to loss from the nursery nets. Settled spats tend to re-settle on another place if their ambient environment is unfavorable for their survival (FAO, 2004). In the weekly observation we found a number of fouling organisms and thick mud settled on the nursery nets, which made us detached the fouling organisms from the nursery nets. Although we don't have any concrete data, we believe a number of early spats were detached while being shaken and lost from the nets. The survival of unacclimated spats for a month, 80%, was still high, considering the study by Bourne (1988) who found frequent heavy mortalities at this size of *P. yessoensis*. Therefore, within the extent of present study concerned, it appeared that the nursery acclimation was not necessary during *P. yessoensis* seed production in the Korean coastal waters of the East Sea.

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Manuscript Received: October 9, 2006
Revision Accepted: May 1, 2007