Review: Spawning Induction in Bivalve

Ludi Parwadani Aji
Technical Implementation Unit For Marine Biology Conservation, Research Centre For Oceanography, Indonesian Institute Of Science (LIPI), Biak - Papua Barat

Abstract: Spawning induction is eminent to bivalve hatchery production as it involves control of the timing of reproduction to make maximum fertilization. There is several ways to induce spawning in sexually mature bivalve which the major methods are chemical stimulation, biological stimulation and physical shock. Chemical induction can be obtained by using hydrogen peroxide, serotonin and sex steroids injection into the gonad or mantel of the broodstook. Biological stimulation is conducted by using microalgae as a diet for conditioning of adult bivalve and by introducing gonad extract into the medium or gonads of the broodstock. While, physical shock can be done by rapidly decreasing or increasing of salinity or temperature to mature bivalve. Physical shock was less efficient compare to chemical injections but still commonly used as it is less invasive and has been found to be quite effective in certain bivalves. In addition, using microalgae as a diet is mostly used for broodstook conditioning rather than to induce spawning. All and all, the different species of bivalve react differently depend on what spawning method is used.

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E-mail: ludi_bio@yahoo.co.id

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1 INTRODUCTION

Bivalve is one of the mollusk groups with a shell consisting of two valves joined along a hinge and enclose the body. Oysters, mussel, scallops, and clam are animals that can be classified as Bivalves. Bivalves are good aquaculture candidates as they are the greatest source of mollusk production and can be reared using simple technology [1]. They reproduce by spawning or the release of eggs and sperm into water which occurs when the reproductive organs are ripe. Male bivalve usually secrete several jets of white pasty milt as sperm, while, female which also spawned in a same manner producing jets of orange colored eggs [2].

Spawning induction is important to the production of bivalve seed. In bivalve aquaculture, availability of broodstocks may be a limiting factor, so it is often prominent to obtain as many gametes as possible from selected individuals [1]. Spawning is easier to induce in mature individuals than in non-mature, suggesting that internal physiological conditions have to be met before external stimuli effectively induce spawning. To know the maturity of the bivalve can be done by biopsies. It is monitoring the reproduction condition of bivalve by removing a small number of eggs from the gonad by inserting a hypodermic needle through the mantle into the gonad [3]. Broodstock also need to be washed with filtered seawater before applying any stimulus to induce spawning to minimize contamination [4].

Spawning of gametes appears to rely on a combination of endogenous and exogenous factors. Once reproductive maturity is reached (through neurosecretory control and environmental), a variety of environmental stimuli can initiate spawning. Parameters potentially triggering spawning including salinity, temperature, light, lunar phase, dissolve oxygen, mechanical shock, pH, and chemical stimuli have been examined through correlation with field observation and laboratory experiments [5]. Therefore, this paper will examine the major various techniques such as chemical stimulation, biological stimulation, and physical shock to induce spawning in sexually mature bivalve.

2 CHEMICAL STIMULATION

One of the most common techniques to induce spawning in mature bivalve is by chemical stimulation (serotonin injection, sex steroid injection, water bourne chemical).

Serotonin injection

The biogenic monoamine serotonin is already recognized as a neurotransmitter present in the nervous systems of bivalve that stimulates gills ciliary beating and heart beating [6]. Moreover, serotonin (5-hydroxytryptamine creatinine sulphate com-

plex) is also known on reproductive processes in bivalves including gamete release from gonad fragments and spawning due to salient neurohormonal effects [4]. Serotonin is supposed to bypass all other hormonal and central nervous system mechanisms associated with spawning and take action on the gonad muscles. Therefore, causing them to contract vigorously and drive out gametes [1].

Chemical induction by serotonin injection mostly used to giant clam and scallop. Serotonin has success to be an inducer of spawning in six bivalve species [2]. Spawning in the surf clam *Spisula solidissima*, the bay scallop *Argopecten irradians* and the American oyster *Crassostrea virginica* occur after injection of serotonin solution of 0.4ml of 2mM into the gonad. Similarly, the same dosage of serotonin injection into the anterior adductor muscle of the ribbed mussel *Geukensia demissa*, the ocean quahoq *Arctica islandica*, and the hard clam *Mercenaria mercenaria* also induced spawning. In addition, both male and female bivalve of *Mactra chinensis* that have injected by serotonin in their foot also spawn [4].

In giant clam, serotonin is inserted through the mantle below the excurrent siphon in order to avoid the heart. The needle must be inserted 1-2 mm into the gonad tissue prior to injection [7]. Another method is by inserting the needle into the gonad through the muscle covering the byssal opening. Usually clams responded by producing sperm a few minutes after injected by around 1-2 ml of 1-2mM serotonin in filtered seawater. The injected bivalve reacted to serotonin by foot probing, gaping, or increased pumping and initiated spawning around 15 minute after injected [8].

Spawning induced by serotonin has the advantages of easy to apply, lack of need to cycle temperature, speed, and synchronization induction. However, injection of serotonin by inexperienced technician may cause the death of bivalve due to puncture the viscera or heart of bivalve. Moreover, serotonin (neurotransmitter) does not induce spawning in all bivalve species tested and usually just induces male animals to spawn [9]. Quality of eggs is also a main concern since serotonin cause releasing of eggs and sperms whether they are mature or not [7].

Sex steroids injection

According to Wang and Croll [10], sex steroid can be alternative spawning inducers when injected to bivalve alone or combined with serotonin. Aliquots of 200 μl of one of the steroid (testosterone, estradiol, progesterone) solution were injected between the opened valves into the gonads of ripe sea scallops (*Placopecten magellanicus*). As a result, the injections of testosterone into scallops induced spawning in males, estradiol injections induced spawning in males and females, while, progesterone blocked spawning in both sexes. Furthermore, injections of sex steroids affected subsequent 5-HT- induced spawning as injection of estradiol promoted 5-HT- induced spawning in both sexes, whereas, injection of testosterone just potentiated spawning in males.

Hidrogen peroxide

Hidrogen peroxide has been reported to induce spawning in sexually mature bivalve. Beckvar [11] was successfully obtained the gametes of the giant clams (Tridacnidae) by induction of spawning with hydrogen peroxide. Hydrogen peroxide as a stimulating agent was tested by squirting 20-30 ml of a 3% solution into the incurrent siphon of specimens (*Tridacna gigas*, *T. squamosa, T. Derasa*) ranging in length from 30-40 cm. The giant clams reacted by spawning sperm within 15 minute and the other within 90 minute.

3 BIOLOGICAL STIMULATION

Other techniques to induce spawning in broodstock bivalve are biological stimulation (sexual stimulation and using microalgae as a diet).

Sexual stimulation

Sexual stimulation is accomplished by introducing freshly emitted spawn stripped or extract of ripe gonad from the gonads of mature bivalve either into the medium where the broodstock were immersed or directly into the gonads. The present of gamete in the water obviously provides a stimulus that triggers a spawning response in broodstock [12]. According to Heslinga [13], ripe gonads contain pheromones that induce other clams to spawn. When clams release sperms or eggs, others are likely to follow to spawn which is called epidermic spawning. A simple squirt of gonads extracted from a sacrificial clam into the incurrent siphon of sexually mature clam is adequate to induce spawn.

In the oyster and scallop, the gamete is obtained by striping the gonad from sacrificed individuals by scalpel and directly washed by filtered sea water. In giant clams, gonad material is removed and macerated in a blender using filtered seawater. The gonad extract is then pouring through a sieve to remove large particles or strands of membrane. After that, this suspension is added to the tank water or is squirted into the incurrent siphon using a syringe [3].

Sexual stimulation with addition of sperm or egg suspensions into the water on the Asian Moon Scallop, *Amusium pleuronectes*, caused the release of sperm but no eggs [12]. Similarly, introduction of macerated gonad into the inhalant siphon of adult clams mostly

just resulted in sperm release. In addition, the combination of thermal stimulation with extract of gamete is more effective than mechanical stimulation of spawning in *C. varia*[5]. The drawback of these method is that some bivalves has to be sacrificed each time spawning is induced. However, the remaining gonad extract or water containing gamete can be frozen and used later to stimulate complete spawning without sacrificing the bivalve[7].

Using microalgae as a diet

Bivalve tends to spawn in the favorable area with abounded of plankton. According to Breese and Robinson[14], marine algae such as *Pseudoisochrysis paradoxa* at a concentration of between 2 to 2.5 million cells/ml can induce spawning in the razor clam *Siliqua patula*. Furthermore, the gonadal recovery and the conditioning of the mature *Argopecten purpuratus* which was fed by microalgae mixed with lipid was higher than those were who fed by mixture of pure microalgae and microalgae-carbohydrate[15].

It seems that reproductive performance, gonad maturation and the quality of broodstock is influenced by food availability. Mature adult of Ostrea edulis produce more broods of larvae when fed with a mixture of microalgae[16]. Moreover, lipids and carbohydrate play a major role in gamete development of bivalve. Carbohydrates provide the main energy in particular for gametogenesis and lipid are used as substrates for gametogenesis and lost during spawning by female bivalve[15]. The availability of microalgae for bivalve diet usually is used for conditioning mature bivalve than to induce spawning.

4 PHYSICAL SHOCK

Shellfish including bivalve can be induced to spawn by subjecting them to high pH using NH_4^+ or K^+ ions, a combination of high pH and a temperature jump, UV-irradiated sea water[17], temperature shock[18], mimics changes encountered naturally (e.g. rising/faling tide) and salinity shock[19].

Salinity shock

Salinity is one of the environmental factors influencing reproduction of estuarine and marine invertebrate. Long-range seasonal fluctuations in salinity are important to the synchronization of the gametogenic cycle, reproductive pattern and stimulate spawning[20].

According to Stephen[21], the bivalve (*Saccostrea cuccullata, S. commercialis, Perna viridis* and *P. indica*) which was tested by subjecting to rapid salinity changes shown spawning activities. Bivalve was maintaining at 34ppt for 2 month before decreasing the salinity at 26ppt. As a consequence, sudden salinity change provides the stimulus of mature specimens to the prespawning condition. All 4 species responded by spawning which female oysters took less time to spawn completely than male, while, individuals who were maintained in stable salinity did not spawn. It can be notice that rapid decline in salinity from stable condition provides an effective signal for individuals in a population to synchronise spawning.

However, other studied by Sivalingam[19] shown that both salinity variations and pH were not very effective to induced spawning of *Mytilus viridis*. The mature bivalve was placed directly to several different salinity and pH. The experiment indicated that *M. viridis* have 50% salinity tolerance from 24 to 80ppt and 50% pH tolerance between 3.5 and 9. It seems that suddenly increase or decrease the salinity shock will be effective to induce spawning of bivalve.

Temperature shock

This occurs by rapidly changing the internal temperature of bivalve. To induce spawning of the saucer scallop *Amusium balloti*, water temperature was increased gradually up to around 24°C over a period of 2 hours. If spawning did not occur the heater was switched off and allowed water to cool. If necessary, Ice can be used to decrease the water temperature. Another way to induce spawning of scallop is by placing them in transparent plastic bowl, immersed in clean saltwater and positioned in direct sunlight. Solar heating have resulted in increasing of water temperature that may induce spawning in scallop. The drawback of this method is the slowly rise of water temperature and decrease in dissolve oxygen can cause mortality for adult individuals after spawning[18].

Furthermore, temperature cycling involves placing broodstock bivalve in water at a certain temperature then slowly increasing the temperature of the water over a period of time. For instance, the pearl oyster *Pinctada margaritifera* and *P. maxima* have successfully to spawn after increasing the ambient sea water temperature by 6°C for 30 minutes before removing and placing the oyster into a container conditioned at seawater temperature[23]. On the other hand, induction by temperature shock have a little success in *Amusium pleuronectes* when subjected to 10°C higher than ambient temperature of 27°C[12]. The likely explanation is because the scallop should be conditioned by lowering temperature rather than raising temperature as in the ca of the tropical mussel *Perna viridis*.

It seems that the tropical bivalve will spawn if they are induced by declining the temperature, whereas, the temperate bivalve will spawn effectively by increasing the temperature. According to Barber and Blake [5], the initiation of spawning occurs with both declin-

ing and increasing temperature. Spawning in scallop *Pecten alba* were associated with increasing water temperature, while, scallop *Argopecten gibbus* spawned by declining of water temperature.

5 CONCLUSION

The chemical stimulation, biological stimulation and physical shock have been used in bivalve hatchery production effectively. Different species response efficiently depends on what spawning induction method used. For example, serotonin or sex steroids injection mostly used for the giant clam and scallop because it is easy to insert needle in their gonad. While, using gonad extract to induce spawning is commonly used in low valuable species (oyster and muscle) and not for expensive one such as pearl oyster and the giant clam. Furthermore, salinity and temperature shock will give different result in spawning for tropical and temperate bivalve. Therefore, aquaculturist or hatchery manager can obtain the viable gametes for fertilization at any time and timing of spawning is not a main problem again for commercial aquaculture industries.

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