Evaluation of pearl oyster (*Pinctada maxima*) spawning using natural and artificial induction methods to increase the seed production

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Abstract. The increasing pearl jewelries production leads to an increasing demand for pearl oyster (*Pinctada maxima*) seeds of certain sizes. The induction spawning method is an important factor in producing pearl oyster seeds. This study aimed to evaluate natural and artificial induction methods for pearl oyster spawning using wild and farmed broodstocks to determine efficient seed production. Treatments applied were natural spawning induction of wild and farmed broodstocks (A), natural spawning induction of farmed broodstocks (B), and artificial induction of farmed broodstocks (C). After spawning completed, reproductive performances of the pearls for each treatment were observed with parameters of total number of eggs, number of hatching eggs, and the hatching rate. Pearl oyster seeds were reared for 43 days and fed twice a day with a mixture of phytoplankton. At the rearing stage, we observed the morphology, number, size, and survival of larvae. Natural spawning induction with wild male and farmed female broodstocks showed the highest total number of eggs (32,000,000 eggs) and the highest hatching rate (25%) compared to natural induction and artificial induction with all farmed broodstocks. However, natural spawning induction of wild male and farmed female broodstocks showed higher survival and normal seed growth to the spat stage compared to other spawning induction methods.

1 Introduction

The world's demand for pearl jewerly products is currently on a continuous rise, which is supported by increased pearl production by producer countries [1]. The pearl oyster (*Pinctada maxima*), known as the producer of "South Sea pearls" [2, 3], naturally distributed in the central Indo-Pacific region from Myanmar to the Solomon Islands, including Southeast Asia, the Philippines, South China Sea, and Australia [4, 5]. This biota has considerable economic value, as nearly all parts of the oyster's body have benefits and selling value, including the pearl, the shells, the flesh, and the organism itself (seed and broodstock) [6]. Indonesia is a pearl-producing country along with Australia, Philippines, and Myanmar [7].

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The export value of this commodity in 2016 reached USD 45,293, with importers including Hong Kong, Australia, Japan, and China [8].

Pearl farming is a very challenging and labor-intensive business [9]. The increased pearl production and achievement targets of farmers have caused the demand for pearl oyster seeds of certain sizes to escalate. Therefore, the hatchery production of pearl oyster seed and broodstock has become a critical aspect of pearl oyster culture [10]. The main methods of seed production include hatchery, semi-artificial seed breeding in the controlled environment, wild seed collection, and intermediate nursery in the controlled environment (collectors or pocket nets) or shallow seas [11]. The common problem encountered in pearl oyster farming is low seed production, which is presumed to be caused by low larval survival at certain rearing ages (< 10%) [3, 12, 13]. The low survival of the seeds depends strongly on the broodstock used and hatchery management, particularly during the early stages of larval development [14]. Pearl oyster hatcheries usually collect wild broodstocks from several Indonesian waters, but several broodstocks do not go through controlled spawning, and there has been mixing between one broodstock and another broodstock from another population (mixed population). As a result, the quality of the seeds produced by each population is not known. Therefore, an improved method of hatchery management is needed [15]. Many experiments related to the genetic performance of pearl oysters have been conducted, but they have not been optimally applied by farmers [16]. Artificial breeding is one of the steps to reduce the dependence on wild pearl oyster capture and improve the broodstock quality and seed production [17]. Several environmental stimuli can initiate the release of sperm and eggs [18]. Spawning induction is a major factor in bivalve seed production.

The success of a spawning process is highly reliant on a combination of endogenous and exogenous factors [19]. Some exogenous parameters that can trigger a spawning process include salinity, temperature, light, lunar phase, dissolved oxygen, mechanical shock, pH, and chemical stimulation [20]. Methods commonly used to initiate spawning or so called as induction spawning methods include removal from the water and air-drying, increased of temperature, light density or pH, addition of gamete suspensions to the spawning medium, dense microalgae concentration or hydrogen peroxide solutions, and combinations of these methods [19, 21]. According to [22], serotonin intragonadal injection is a common method used for spawning induction in bivalves. Many pearl oyster hatchery units in Indonesia applied the thermal shock method to initiate spawning in the pearl oyster [16].

Previous studies reported the performance of pearl oyster seed production using controlled spawning and the application of cross-breeding from the wild broodstocks to produce pearl oyster [14, 15]. However, those studies applied a particular induction spawning method and used wild broodstocks. In this study, we evaluate natural and artificial induction methods for pearl oyster spawning using wild and farmed broodstocks to determine the efficient seed production.

2 Methods

2.1 Broodstock collection

The broodstocks used in this study consisted of wild and farmed pearl oyster broodstocks. The wild broodstocks were collected from the Jembrana waters, while the farmed broodstocks were collected from the rearing of farmed pearl oysters obtained from Atlas South Sea Pearl Ltd, PT. Timur Otsuki Mutiara, and PT. Cendana Indopearls (Table 1). The broodstocks were adapted to the location of the study in North Bali by placing them along a long line with pocket nets hanging at a depth of 4–6 m. The broodstocks were selected based

on a number of criteria, such as: gonad maturity stages III or IV, at least 3 years old, undamaged shells by biofouling organisms, and being free from fungi and other pests. In the selection stage, a broodstock observation was performed involving broodstock age and size measurement.

Table 1. The pearl oyster broodstocks for the treatments of spawning induction.

Codes	Sources	Total number
Melaya	Wild (Jembrana Waters)	4
D 253	Farmed (PT. ATLAS-K13)	7
D2 215	Farmed (PT. TOM)	6
Cip 10	Farmed (PT. CIP and PT. ATLAS)	13
D 259	Farmed (Melaya + K-190 + Cip Gold)	7
K-222	Farmed (PT. ATLAS D)	6

The pearl oyster broodstocks used in this study had certain characteristics, including being aged 3-6 years old and having a shell length of 16-20 cm (Table 2). Wild broodstock with the code Melaya is male and 16 cm in shell length. The male farmed broodstocks identified with code D 253, Cip 10, and K-222 aged 4-5 years old and had a shell length of 16-20 cm, while the female farmed broodstocks (D2 215, Cip 10, and D 259) aged 3-6 years old and had a shell length of 17-20 cm.

Table 2. The age and size of the pearl oyster broodstocks used during the study.

Codes	Sex	Age (years)	Size (cm)
Melaya	Male	-	16
D 253	Male	4	17
D2 215	Female	6	19
Cip 10	Male and female	-	18–20
D 259	Female	3	17–18
K-222	Male	5	16–17

2.2 Spawning

The spawning process was carried out on pearl oyster broodstocks that had matured gonads (males and females). The spawning induction methods used in this study were natural induction and artificial induction methods (sperm donor and thermal shock) (Table 3). This study used three treatments namely natural spawning induction of wild and farmed broodstocks (A), natural spawning induction of farmed broodstocks (B), and artificial spawning induction of farmed broodstocks (C). The experiment was carried out through one spawning cycle for each treatment. The natural induction used two male broodstocks and three female broodstocks, while the artificial induction used six male broodstocks and three female broodstocks. The broodstocks were prepared by cleaning the outer shells of broodstocks from attaching dirt and then rinsed them with clean seawater or clean freshwater. The cleaned broodstocks were stored in a cool room at a temperature of 17°C for 30 minutes with the dorsal part facing upwards and with labels specifying the codes and sexes according to the gonad condition.

Male and female broodstocks were placed in a fiber container with a size of 200 x 80 x 100 cm^3. Male broodstocks were placed near the inlet, while female broodstocks were placed near the outlet. Such an arrangement was based on the fact that male broodstocks are typically stimulated faster than their female counterparts. Male broodstock's state of being stimulated was marked by their release of sperm. The sperm passing through female broodstocks when the outlet tap was opened would stimulate the female broodstock and release eggs.

The natural induction was performed by water replacement at 100% of the total water volume every 15–30 minutes until male broodstocks released sperm and female broodstocks released eggs. The artificial induction could be carried out in one or two steps. The first artificial induction method used was sperm donor. The sperm donor was performed by sacrificing male broodstocks and extracting their sperms, then solving those sperm in a water-filled container. The water was slowly poured into the spawning container to stimulate other male broodstocks and female broodstocks. The induction could be continued into the second step (thermal shock), if the broodstocks were not induced to spawn. The induction with thermal shock was carried out by increasing the water temperature from 28°C to 34°C by pouring hot water slowly into the spawning container and stirring it gently. Afterward, sperm and eggs were incubated for 60–90 minutes in the spawning container without aeration for fertilization and the splitting process. After they were fertilized, the eggs were transferred into a rearing container.

Table 3. Spawning induction and successfully spawn broodstocks of pearl oyster during the study.

Spawning inductions	Male broodstock codes	Successfully spawn broodstocks (ind)	Female broodstock codes	Successfully spawn broodstocks (ind)
Natural	Melaya	1	D2 215	1
	D 253	1	Cip 10	2
Artificial	K-222	3	D 259	3
	Cip 10	3		

2.3 Observation of reproductive performances

The reproductive performances of pearl oysters were observed through egg observation using a random sampling method. We observed the total number of eggs, total number of hatching eggs, and the hatching rate. We used a Sedgewick rafter during the observation of pearl oyster eggs, aided by a binocular microscope with a 40x magnifcation.

2.4 Rearing and observation for larval development, total seed number, and survival

Larval rearing was conducted for 43 days, from the D-shape stage to the spat stage. Pearl oyster larvae were reared in a rearing container containing 5,000 L of water. To maintain the quality of the water and to prevent diseases during larval rearing, a total water replacement was performed every two days along with larval sampling to record the growth of larvae and the number of survived larvae. The water quality during the study was maintained at a temperature of 29.3–30°C, salinity of 32–36 ppt, and pH of 7.5–8.6. Larvae in the plantigrade stage were then moved to a collector created from the polyethylene rope. A single collector unit contained 20 ropes with a length of 30 cm. The collectors, were tied together using raffia yarn.

The pearl oyster larvae were fed with the mixture of live feed species with different composition in each stage. At the stages of D-shape, umbo, and pediveliger, we fed larvae with a mixture of *Pavlova lutheri*, *Chaetoceros amami*, *Chaetoceros calcitrans*, and *Chaetoceros simplex*, while at the plantigrade and spat, we fed larvae with a mixture of *Pavlova lutheri*, *Chaetoceros amami*, *Chaetoceros calcitrans*, *Chaetoceros simplex*, and *Nannochloropsis* sp. (Table 4). The daily feeding requirements were changed according to the pearl oyster larvae stages. The daily feeding amounts of the pearl oyster larvae ranged from 1,000 to 5,000 cells mL^{-1} (Table 5). The live feed was administered by pouring the live

feed mixture at 3–4 spots in the larval rearing container. Feeding was performed twice a day, in the morning and late afternoon.

Table 4. The composition of the live feed at different stages of the pearl oyster larvae during the study

Stages	**Live feed percentage (%)**				
	Pavlova lutheri	***Chaetoceros amami***	***Chaetoceros calcitrans***	***Chaetoceros simplex***	***Nannochloropsis* sp.**
D-shape	76	8	9	8	-
Umbo	46	19	16	19	-
Pediveliger	40	20	20	20	-
Plantigrade	20	20	20	20	20
Spat	39	15	15	15	15

Table 5. The daily mixed live feed requirements of pearl oysters during the study

Stages	**Daily feed requirements (cells mL^{-1})**
D-shape	1,000–3,000
Umbo	3,000–4,000
Pediveliger	2,000–5,000
Plantigrade	2,000–5,000
Spat	2,000–5,000

During the rearing stage, observations of seed performances were conducted. The observed parameters were larval development, larval size, total larval number, and survival at each stage of larval development

2.5 Data analysis

The data preparation was conducted in Microsoft Excel 2019. We analyzed the data and provided descriptive statistics with tables and figures.

3 Results

3.1 Reproductive performances

The natural spawning induction with male wild broodstock and female farmed broodstock (treatment A) produced a higher total number of eggs (32,000,000 eggs) and a higher hatching rate (25%) than the natural spawning induction with male bred broodstock and female bred broodstock (treatment B) (8,000,000 eggs and 12.5%, respectively) (Table 6). The artificial spawning induction in male and female bred broodstocks (treatment C) produced a high number of eggs, with 40,000,000 eggs, however, the hatching rate is low (10%) (Table 6).

Table 6. Total numbers of eggs, total numbers of hatched eggs, and hatching rates of pearl oysters produced by different spawning induction methods.

Spawning inductions	**Broodstocks (male x female)**	**Total number of eggs (eggs)**	**Total number of hatched eggs (eggs)**	**Hatching rate (%)**
Natural	Melaya x D2 215 (A)	32,000,000	8,000,000	25
	D 253 x Cip 10 (B)	8,000,000	1,000,000	12.5
Artificial	K-222 + Cip 10 x D 259 (C)	40,000,000	4,000,000	10

3.2 The morphology and performance of pearl oyster seeds

The first pearl oyster larval stage is the D-shape stage. This stage occurred when larvae entered 1–6 days of rearing (Figure 1). At the D-shape stage, larvae appearance is clear, with a size of 75–110 microns. The larvae then entered the umbo stage after 7–15 days of rearing. The larvae in the umbo stage were characterized by thickening the edge of the larval body, which later developed into a bump at the dorsal part of the larval body. In this stage, the larvae have a size of 90–120 microns. After 15 days of rearing, the larvae entered the pediveliger stage with a size of 120–225 microns. The larvae that entered this stage were characterized by a primordial foot protruding from the larval body. Furthermore, at 19–22 days of rearing, the larvae started the plantigrade stage and grew 150–450 microns. The plantigrade stage was marked by the clear shape of the larval body, which resembled the mature pearl oyster with shells, allowing the visibility of the internal larvae body from the outside. The larvae entered the spat stage after 23 days of rearing, with a size of 410–2400 microns (Table 7).

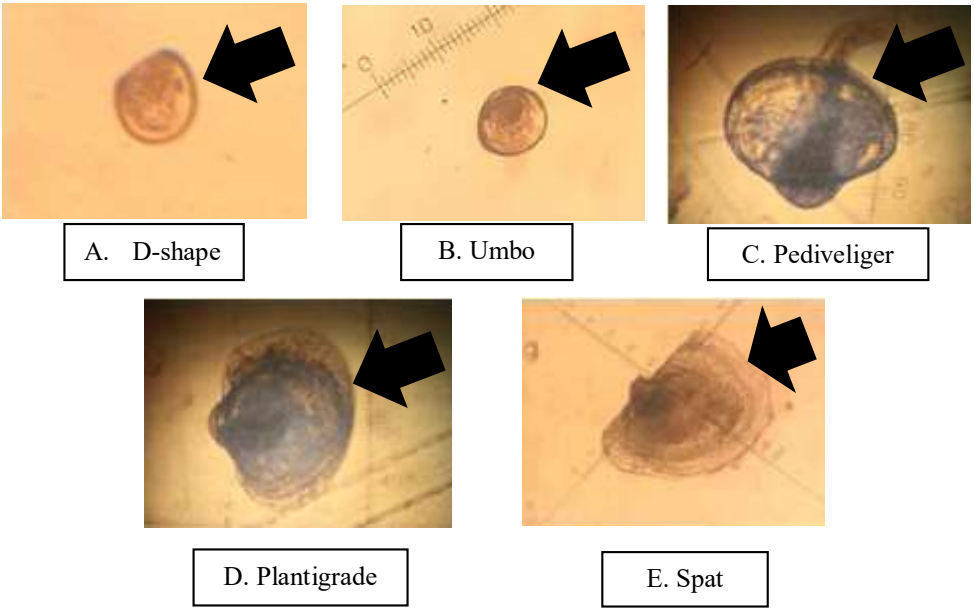

Fig. 1. The morphology of pearl oyster seeds. Larval developmental stages of the pearl oyster consist of A. D-shape, B. Umbo, C. Pediveliger, D. Plantigrade, and E. Spat.

Table 7. Age and size of the pearl oyster seed during the study.

Stages	Age (days)	Size (microns)
D-shape	1–6	75–110
Umbo	7–15	90–120
Pediveliger	16–18	120–225
Plantigrade	19–22	150–450
Spat	23–43	410–2,400

The number and survival of pearl oyster seeds dropped as they grew in rearing duration (Table 8). The natural spawning induction with males from wild broodstock and females from farmed broodstock (treatment A) showed higher survival of the umbo stage than the natural spawning induction with males from farmed broodstock and females from farmed

broodstock (treatment B) and the artificial induction of farmed broodstocks (treatment C) (Table 9). The natural spawning induction in male wild broodstock and female bred broodstock (treatment A) also produced pearl oyster seeds that grew normally into the spat stage based on the larvae size and development, while the larvae obtained from treatments B and C did not show the development to the next stages. The seeds yielded from the artificial induction demonstrated a slow growth rate and a high mortality rate at six days of rearing.

Table 8. Numbers of pearl oyster seeds yielded from different spawning induction methods.

Stages	**Number of seeds (ind)**		
	Natural (Melaya x D2 215) (A)	**Natural (D 253 x Cip 10) (B)**	**Artificial (K-222 + Cip 10 x D 259) (C)**
D-shape	8,000,000	1,000,000	4,000,000
Umbo	6,999,000	700,000	-
Pediveliger	5,200,000	-	-
Plantigrade	2,700,000	-	-
Spat	1,500,000	-	-

Table 9. Survival of pearl oyster seeds produced by different spawning induction methods.

Stages	**Survival (%)**		
	Natural (Melaya x D2 215) (A)	**Natural (D 253 x Cip 10) (B)**	**Artificial (K-222 + Cip 10 x D 259) (C)**
D-shape	-	-	-
Umbo	87.49	70	-
Pediveliger	65	-	-
Plantigrade	33.75	-	-
Spat	18.75	-	-

4 Discussion

The success of the pearl oyster hatchery activity is highly dependent on the broodstock and hatchery management, particularly in the early stages of larval development. Pearl oyster broodstock is typically captured from the wild, but there are also some pearl oyster broodstock obtained from breeding. Genetic and artificial breeding techniques have been conducted in breeding programs to improve growth and pearl quality through mass selection and hybridization [23]. Wild broodstock with a shell size of 15–16 cm and 2–2.5 years of age is ready for spawning, while farmed broodstock is ready for spawning when it reaches age of 3 years [24-25]. In this study, we used the same broodstock criteria as the previous study. However, the broodstocks in this study were larger than the broodstocks from the study by [26], with a standard shell length of 5.57 ± 0.32 cm.

Spawning in pearl oysters is commonly triggered by changes in the environment or the presence of water-borne gametes. A number of techniques are often used to induce spawning in bivalves after reaching a matured gonad stage, which consists of chemical stimulation, biological stimulation, and physical shock. Biological stimulation in bivalves can be performed through sexual stimulation and the use of microalgae as feed. Sexual stimulation is performed by exposing newly stripped sperm to the spawning medium of broodstocks or directly into the egg cells with the aid of a spawning container [19]. The presence of gametes in the water will provide a stimulus that triggers a spawning response from broodstocks [27]. According to Heslinga et al. [28], mature gonads contain pheromones that can induce bivalves to spawn. The sperm or eggs released by a broodstock will cause other broodstocks to spawn. Pouring gonads that are extracted from sacrificed bivalves into the spawning

medium can induce the spawning of other broodstocks. This sexual stimulation is the basic concept of natural spawning induction and artificial spawning induction by sperm donors in pearl oysters [19]. The sperm donor is only applied for inducing pearl oyster spawning if the pearl oyster broodstocks are not stimulated after going through a natural spawning induction in which male and female broodstocks with mature gonads are placed in the same spawning container. To increase the success rate of spawning in pearl oysters, then a combined spawning induction through thermal stimulation and gamete extraction is conducted [20]. The drawback of this method is that it sacrifices some bivalves at every spawning induction [19]. The thermal shock method is one of the spawning induction methods that apply physical shock to bivalves. The disadvantage of this method is that the slow increase in temperature may decrease in dissolved oxygen, which may cause death to broodstocks after spawning [29].

Fertilization occurs after a male broodstock releases sperm and a female broodstock releases egg. The pearl oyster egg will hatch in 18 hours after fertilization. Fertilized eggs were circular in shape and afloat on the surface of water or adrift in the water, while unfertilized eggs showed pink to red color and settled at the bottom of the container [25]. The embryogenesis phase commences with the polar body I and II bump, which marks the outset of cell division. The next stages are the morula, blastula, gastrula, and trochophore stages [30]. The numbers of eggs yielded in this study, both from the natural spawning induction and the artificial spawning induction, were fairly high, but the hatching rates were low. The hatching rate of eggs from the combination of wild male and female broodstock with natural spawning induction (treatment A) was higher than the hatching rate in the study reported by [31] with a hatching rate of 20%. According to [14], good spawning performance is shown by a high number of eggs and a hatching rate ≥ 40%. Poor egg and sperm quality were assumed to be as the cause for the low hatching rate. Therefore, during the fertilization process, many egg cells were failed to split perfectly. The natural spawning induction in male wild broodstock and female bred broodstock (treatment A) produced a higher number of eggs and a higher hatching rate than the natural spawning induction of farmed broodstocks (treatment B) and the artificial spawning induction in male and female bred broodstock (treatment C). According to Dhoe et al. [25], wild broodstock have higher quality than farmed broodstock with better egg and larval quality as well as survival compared to farmed broodstock.

The study by Wardana et al. [14] reported that pearl oyster larvae in the veliger (D-shape) stage are typically red, but larvae from cross-breeding tend to be white, off-white, and cream in color. In this stage, larvae demonstrated a fair amount of activity with a spinning motion on the surface. The D-shape stage occurs in larvae at the age of 1–10 days. Next, the umbo stage occurred in larvae at the age of 7–17 days, followed by the pediveliger stage. In this stage, larval motion was slowing down, and a tongue-like attachment organ developed from the inside of the larval body. This organ was used to find the attachment substrate. Furthermore, a collector also appeared at this stage. The pediveliger stage is a transitional period for larvae from the planktonic phase to the benthic phase [32]. This stage occured when larvae reach the age of 17–22 days. These larvae then came into the plantigrade stage when they reached the age of 20–26 days. The spat stage will normally occur at the age of 28 days. The pearl oyster larval development from the D-shape stage to the pediveliger stage in this study is consistent with the results of the study by Wardana et al. [15]. However, at the plantigrade stage and the spat stage, larvae developed faster than the results from the study by Wardana et al. [15]. This condition was influenced by the origin of the broodstock population. Seeds from cross-breeding showed faster development during the larval phase than seeds from intra-population spawning.

The survival of seeds in the pediveliger stage in this study was higher than the survival of seeds in the pediveliger stage in the study by Wardana et al. [14], at 45–65%. The final

survival in this study was in the range of survival of pearl oysters reported by Hui et al. [33] at 4.18-21.20%. The main obstacle to pearl oyster seed production is the very low survival of spats, as is also the case in this study. The low survival of pearl oyster seed from the pediveliger stage to spat was caused by the metamorphosis process from the planktonic phase to the benthic phase that required a lot of energy, the adaptation capacity that differed between individuals and fluctuating environmental factors. The factors influencing the survival of pearl oyster seeds include water biophysical conditions (temperature, pH, dissolved oxygen, and salinity), the presence of contaminants, depth, base substrate, brightness, freshwater supply, current pattern, and water fertility level.

5 Conclusion

Natural spawning induction in wild male and bred female broodstock resulted in higher reproductive performances compared to natural spawning induction in bred broodstocks and artificial spawning induction in farmed broodstocks. Natural spawning induction in wild male and bred female broodstock showed the best seed performance compared to other spawning inductions.

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