The effect of individual selection on sperm motility in tropical abalone *Haliotis squamata*

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Abstract. High-quality broodstock is required to support successful cultivation activities of the tropical abalone. This study aimed to find out the structure of the sperm under the influence of individual selection. A total of 50 eggs per batch were measured in egg diameter, calculated by the degree of fertilization and hatching rate. Sperm collection is carried out from the natural spawning of male abalone from the wild (F0) and the selected offspring from individual Fillial-1, F2, and F3 selections. Samples of sperm were collected by artificial spawning and analyzed using the Sperm Class Analyzer® CASA System. The results showed that the wild abalone (F0) had the highest hatching rate but was not significantly different from other derivatives (P > 0.05). The average spermatozoa was 2.39 million cells/m, with the highest spermatozoa (F0) of 7.110 million cells/ml. The most increased sperm motility and rapid velocity were in the wild (89.78%; 34.00%) and the lowest of F2 (22.00; 4.00%). A significant difference in sperm motility and rapid velocity were found between F0 and other treatment. The consequences of individual selection will aid in studying reproductive factors and sperm motility, which are most likely crucial in tropical abalone fertilization.

1 Introduction

One kind of seashell that has both great commercial and exotic values worldwide is the abalone (*Haliotis* spp.). This species, which has a shell on its posterior and a snail-like structure, is a member of the gastropod family Haliotidae [8]. In Indonesia, *H. asinina* and *H. squamata* are common abalone species living in coral seas. Worldwide abalone production increased from 19.208 MT in 1989 to 44.187 MT in

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1996, with Taiwan and China being the top two producers. Other countries that produce abalone include Australia, Korea, the United States, Mexico, and Southeast Asia. The largest abalone catch results worldwide come primarily from Australia, Japan, Mexico, and New Zealand. Abalone prices in export are extremely high. *Haliotis discus* and *H. discus hannai* prices may reach US $66,000 per ton when exchanged for US red abalone and African species *H. midae*. Due to the high demand for abalone and the high price, abalone exploitation in the ocean has increased significantly and is no longer sustainable. As a result, an effort is required to ensure the long-term sustainability of natural abalone resources, notably by breeding.

Research on breeding selection based on phenotypes from natural offspring (F0) and generations of their offspring fillial F1, F2, and F3 has been successfully carried out at the Institute Mariculture Research and Fisheries Extension Gondol Bali since 2011. The availability of high-quality and large-amount seeds is essential for the success of abalone farming. The quality of broodstock significantly impacts the quality of the generated seeds. The male parent's involvement in abalone spawning is crucial for developing high-quality seeds. The egg quality depends on the spermatozoa produced by the male parent. The low quality of spermatozoa may contribute to the decreased hatchability of eggs. There hasn't been a lot of investigation into the role of selection in spermatozoa on abalone. However, studies on the spermatozoa of tiger shrimp (*Penaeus monodon*) have been conducted, among others, by [17] and [18]. The shell size of subtropical abalone species is larger than that of tropical species [23]. Therefore, it is crucial to integrate both tropical and subtropical abalone species regarding gonadal maturation, larval rearing, hybrids, and cross-breeding of various broodstock origins to increase seed quality and build effective abalone farming. In Indonesia, techniques like hybridization and sustainable cultivation do not yet constitute standards in the abalone aquaculture and breeding industries. Most of the marketed products from this species are taken from the wild. The country would likely develop the technique because Indonesia has natural coastal waters where abalone may be found. This experiment aimed to determine the structure of male offspring's sperm and its progeny under the impact of individual selection.

## 2 Materials and methods

Samples of wild F0 broodstocks were taken from Pekutatan Beach, Jembrana Regency, Bali (approximately 114°50'14" E longitude and 8°26'12" S latitude). For spawning, 25 males and 25 females from natural F0 broodstock that had reached TKG III gonadal maturity and had shell lengths between 6.5-7 cm. 12 concrete containers totaling 4.2 x 2.3 x 1.2 m³ each are used for maintaining larvae. Additionally, a substrate for larval attachment consisting of corrugated plastic material that measures 58 x 60 cm and has been overrun with benthic diatoms is added to the plate as the larvae feed. Veliger stocked as many as 150,000 ind./tank, or 50–75 ind./L [20]. To produce F2 and F3, followed by the F1 progeny from the hatchery.

### 2.1 Selection
The individual selection method refers to the Standard Operating Procedures for Abalone Breeding concerning individual selection by the Research and Development Center for Gondol Marine Cultivation in 2010. A 50% cut is performed after four months of age. If the size per individual is tiny (average size), then up to 50% of the population is promptly eliminated. If there is a significant range in size per individual, 100 individuals (5-10% of the population) will be measured (length and weight). Populations of varying sizes are segregated and maintained in the same container/environment. At 16–18 months, the F1 population will become broodstock, ready for spawning. The same individual selection technique is used to create F2 and F3.

2.2 Sperm collection

Male abalones with mature gonads were subjected to a temperature shock and stress with dry-up and pure oxygen. The sperm released after spawning is mixed with a Physiological Sodium solution and added to the sperm in the cuvette. Sperm collection in microtubes of 5 μl in the leja chamber of sperm motility and viability. The fertilization of sperm and eggs was then studied until hatched to calculate the hatching rate. A pipette is used to put the activated sperm into the counting chamber. It is then placed under a microscope Nikon Ci-L to examine and analyze sperm motility. The visualization of sperm motility was placed on the video. Rapid progressive motility, slow progressive motility, progressive motility, and immotile could all be calculated using computer software, and the system was connected to a database through the SCA® Production CASA System.

2.2 Eggs collection

To examine and measure egg diameter, degree of fertilization, and degree of hatching, about 50 eggs were randomly chosen from each spawning. A Nikon DSLR camera was used to capture the observations using Nikon SMZ1000 and Nikon Eclipse E600 microscopes.

2.3 Data Analysis

The study observed the performance of male abalone sperm in terms of sperm motility, rapid velocity, and sperm count. The motility level test will be conducted using a microscope integrated with the SCA (System Class Analyzer). Other reproduction parameters were measured, such as egg diameter, degree of fertilization rate, and hatching rate. The data obtained were analyzed using ANOVA and correlation analysis with the MS Excel program.

3 Results and discussion

3.1 Sperm performance

The average amount of spermatozoa abalone was 2.397 million cells/ml, with the highest spermatozoa (F0) of 7.110 million cells/ml. The concentration of sperm
discharged during the spawning process will influence the number of larvae produced. [7] explained that the sperm concentration of abalone *Haliotis asinina* 5 x 10^3 - 1 x 10^5 cells/ml will provide maximal fertilization average and normal trophophore (100%). Generally, the optimal sperm concentration for fertilization in other abalone species is 105-106 cells/ml.

The spermatozoa quality of the selected abalone showed a decrease with the length of reduced with each following generation. This is congruent with [13] stating that in *P. setiferus*, not only did the quality of spermatozoa decrease with time of rearing, but there was also a rapid decline in the quality of spermatophores after two weeks of rearing in the laboratory. Furthermore, according to previously reported by [1] and [2], differences in penaeid population are dependent on geographic factors.

![Fig. 1. Pattern of rapid progressive motility, slow progressive motility, progressive motility, and immotile.](image)

Live sperm are sperm that move fast, slow, or in the head or tail, while dead sperm are sperm that show no movement at all, either in the head or tail. The movements and motility patterns of F0, F1, F2, and F3 sperm are presented in Figure 2. The duration of motility and sperm fertilization for each type of fish is different. According to [21], spermatozoa will move (motile) towards microfil (micro-sized holes in the egg).

![Fig. 2. Progression and velocity sperm of abalone F0-F3 through individual selection.](image)
The highest progressive motile were F0 (89.8%), F1 (80.7%), F3 (50.4%), and F2 (22.0%). F0 and F1 were significantly different from F2 and F3 (P<0.05). F0 had the greatest sperm velocity (34%), while F2 had the highest static and non-progressive motile velocity (18%). This suggests that the quality of spermatozoa from F2 selection is the lowest compared to the others. Fast-moving sperm is necessary in breeding operations to create high-quality offspring [12]. According to [21], active spermatozoa will velocity (motile) toward microfil (micro-sized the openings in the egg). [5] revealed that sperm motility considerably impacts the success of sperm-egg fertilization. Even though there are inherent variances in penaeid shrimp populations based on geographical location, as reported by [1] and [2].

### 3.2 Egg diameter, hatching rate, and degree of fertilization

The diameters of natural F0 abalone eggs and results of F1, F2, and F3 selection were 181.25 m, 183.66 m, 185.5 m, and 186.00 m, respectively. The size of the F3 abalone eggs was greater than the others but not significantly different (P<0.05). Egg diameter indicates egg quality [4], although this statement contradicts the data obtained where the diameter of F3 eggs is not directly linked to hatchability and degree of fertilization. According to [14]. This occurs due to an increase in nutrient accumulation throughout the gonad maturation process and an increase in oocyte size, causing the size of the eggs to grow, particularly before spawning [14]. Egg size, primarily egg diameter, is a good indication of egg quality. The same trend may be seen in natural *H. asinina*, which has a yolk diameter of up to 180 π [4; 22].

![Fig. 3. Polyspermy penetration of *H. squamata* during fertilization.](image)
The percentage of fertilization rate of natural F0 abalone was 79.34 ± 9.65, whereas the percentages of F1, F2, and F3 as a consequence of selection were 84.66 ± 15.62, 64.57 ± 24.36, and 60.85 ± 18.00%, respectively. F2 had the highest fertilization rate (84.66%) compared to the others. However, it was not statistically significant (P<0.05). The low incidence of fertilization in progeny can be attributed to selection influences; nevertheless, poor sperm quality or high sperm density during fertilization might result in polyspermy with a significant proportion of active embryos (Fig. 3). 5-10 sperm per egg is the ideal density. Sperm density is frequently greater than 186.20/ml [8].

The hatching rate of F0, F1, F2, F3 and F4 s was 88.76 ± 8.39; 61.42 ± 7.85; 72.25; and 67.49% respectively. F2 and F3 fertility rates were 91.07% and 92.07%, respectively, with hatchability of 72.66% and 66.67% for F2 and F3 broodstock (Fig.4). The wild broodstock had the highest hatching rate. Still, it was not significantly different from the other derivatives (P > 0.05).

4 Conclusion

A significant difference in sperm motility and rapid velocity was found between F0 and F1, F2 and F3 in this experiment. These conclusive findings will help gain insights into the effects of individual selection. The consequences of individual selection will aid in studying reproductive factors and sperm motility, which are most likely crucial in tropical abalone fertilization.

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