Original article

Daya tetas dan kelangsungan hidup larva cumi *Uroteuthis chinensis* dalam wadah budidaya

Hatching rate and survival of larvae of mitre squid Uroteuthis chinensis *in aquaculture tank*

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Abstract

Development of squid culture is one effort to prevent the decline of squid population in nature due to continuous capture. Problem faced in the aspect of squid culture is the unavailable information regarding the culture technique of squid in controlled tank. This research was conducted to evaluate the hatching rate of mitre squid Uroteuthis chinensis, and survival of larvae in the controlled tank. The result showed that the hatching rate in culture tank was high, i.e 95.92%, while the survival rate of larvae after hatching was relatively low, only reached 4.25%. The low survival of squid paralarvae was expected because of the administration of single live food which resulted in the low predator response and imbalance of nutrient intake for the development and growth of paralarvae.

Keywords : squid, hatching, surviavl, Uroteuthis, tank, paralarvae

Introduction

Indonesia has a huge potential to develop the squid fishery sectorsince there are vast sea areaspossible to be intended for both capture fisheries and aquaculture development of squid. Today,the supply of squid in the market still relies on capture fishing, and yet the amount of basic information related to the progress direction to farming aspect is also considered as an obstacle to the development. Aquaculture development of squid is one effort to prevent the decline of squid population in the wild due to continuous capture. The problem encountered in squid cultivation aspect is that the aquaculture technique and maintenance of squid in controlled culture tank has yet been known. Therefore, in performing the hatching activities, the major component in the form of eggs still depends on wild-caught sources.

Squid eggs have the sticky surface that enables them to stick to the hard substrate in the bottom waters. Squid embryo eats nutrients available in the egg yolk until it is ready to hatch. This embryo breaks the egg shell by using a tiny branch-like brush structure on the tail. Research conducted by Tallo (2006) in the waters of the Mutiara Gulf of Alor Regency of East Nusa Tenggara Province showed that the squid eggs,which are taken directly from the attractor of FAD (Fish Aggregating Device) for squid then transferred to the floating cages,hatch at the age of 28-30 days. Deep-sea species and pelagic squid purely release their eggs one after another into the water or eggs remain floating as plankton. There is no larval stage in Cephalopod thus eggs hatch into adult-likeforms and immediately able to swim and feed (Roper *et al.* 1984).

In nature, squid eggsface such challenges in the hatching process either because of changes in the environmental quality orbeing killed by natural predators. The activity of collecting and maintaining the squid eggsis one effort to save and increase the hatching rate of eggs which further is expected to be a source of seeds that can be used in the grow-out activities. Many groups of Cephalopod have been able to be kept under laboratory conditions for the purposes of both research aspects of physiology and behavior and as model organisms. Difficulties in the cultivation in many groups of, Cephalopod, particularly squid species are closely related to theholonektonic life cycle, those are the planktonic phase of paralarvaeand adult phase with intense swimming activity (Goncalves et al. 2009). The high mortality rate during the experimental period of culture is generally caused by fishing techniques and inadequate tank design which lead to skin damage and bacterial infections.

Groups of Cephalopod will hatch into planktonic carnivorous paralarva. Results of the research showed that the hatched paralarvae require live food. In squid culture condition, failure to accept the first feeding can lead to starvation and become one of the leading causes of squid mortality, especially during the planktonic stage (Villanueva 1994). The domestication process of squid, specifically in larval rearing, has been done in some previous studies. Villaneuva (1994) performed the culture of *Loligo vulgaris* and *Octopus vulgaris*paralarvaein a controlled tank with a temperature of 19-23 °C. The tank wasin the form of ablack plastic cylinder witha diameter of 40 cm, acapacity of 50 liters and flow rate of 120 liters/hour. Meanwhile, the use of *Artemia* sp. as the single feed source for squid paralarvaewas reported to have limited success in several studies. Research carried out on natural populations of squid revealed that the small planktonic crustaceans and groups of decapoda zoeae were found in the stomachs of squid paralarvae (Villanueva 1994).

Syari (2014) was a success in creating an effective tool to collect the squid eggs by usingthe attractor of FAD for squid. However, information related to the hatching rate of eggs and survival of larvaein the controlled tank is still unknown. Therefore, the research was conducted to evaluate the hatching rate of squid eggs and survival of larvae in the controlled tank.

Materials and methods

Collecting of squid eggs

The process of squid egg collection was performed around Tuing Waters, Tuing Hamlet, Mapur Village, Bangka Regency, Bangka Belitung Island which was located in the northeastern part of the waters of Bangka Island. This area is the part of waters of the South China Sea which associate with the high seas. The process of egg collection wascompletedwiththe help of local fishermen who used polyethylenenets. A total of 1055 squid eggs which were obtained by fishermen were collected and transported using the open method of transportation. Visual appearancesof eggs were then observed.

Tank and culture system

Squid egg hatching was conducted on culture tank in the form of aquarium with dimension of 60x50x40 cm and volume of 80-90 liters of seawater. Tanks were equipped with a simple recirculation system, filtration system (top filter) and aeration as

well (Figure 1). Culture tanks were also equipped with water pump as artificial stream to maintain the circulation and movement of sea water in the aquarium hence resembled the natural condition. Hatching tank was placed in dark room conditions with temperature and salinity ranged between 26-28^{o}C and 26-27 ppt, respectively. Monitoring of squid egg hatching was carried out every day, while water quality were maintained stable through siphoning and water exchange.

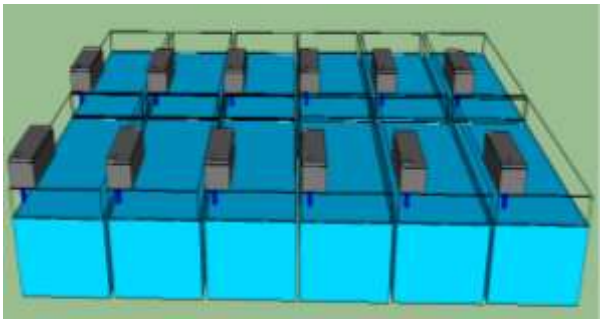


Figure1. Design and construction of hatching tank of squid egg.

Maintenance of after-hatch squid larvae was conducted in the hatching tank. The first feeding of squid larvaewas done by providing natural food of commercial *Artemia* naupliithree times a day and ad libitum based on the amount and population of nauplii given. The addition of feed was applied when the population of *Artemia* nauplii in the maintenance tank decreased.

Hatching rate, survival, and larval behavior

Hatching rate is the ratio percentage between a number of eggs hatch into larvae and the number of eggs successfully fertilized. The number of eggs hatched into larvae during the observation period were determined by the following equation:

Hatching rate = **Error! Reference source not found.**

Moreover, survival rate of after-hatch larvae was calculated using the equation below:

Survival rate
= **Error! Reference source not found.**

Mortality rate of paralarvae was determined by using this equation:

Mortality rate =**Error! Reference source not found.**

Observationof daily cumulative mortality rate of larvae was performed every day and based on the following equation:

Cumulative mortality rate
= **Error! Reference source not found.**

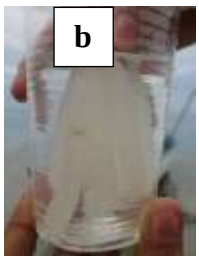
In addition to observation of the hatching rate and survival rate of larvae, the behavior of larvae during maintenance period was also observed and recorded.

Data Analysis

Data obtained during the research were including data of hatching rate and survival rate which further were tabulated by using MS. EXCEL 2013 and analyzed descriptively.

Result and Discussion

Squid eggs collected from local fishermen were incubated in hatching tank which was equipped with the top filter. The use of top filter aimed to maintain the quality of water through a filtration system by removing the suspended impurities in the water. Meanwhile, there were two types of eggs collected, namely young eggs (Figure 2a) and old eggs (Figure 2b). Squid eggs have the morphological appearance as a notched jelly capsule or pea pods with eachindentation represents the new individual candidate. In old eggs, one egg capsule may consist of more than two individual candidates withthe clearly visibleindentation or segments. The length of old egg capsules can range between 7-9 cm. While one egg capsule in young eggs can reach 3-6 cm yet the indentation is not visible.



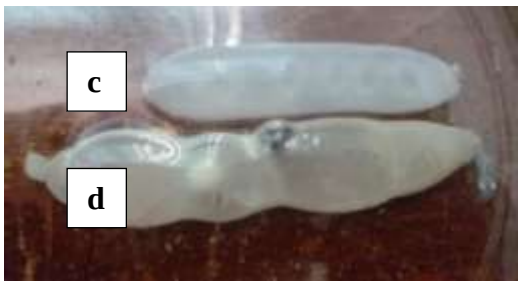


Figure2. Morphology of Bangka squid egg *U. chinensis*: old egg (2a and 2d) and young egg (2b and 2c)

Figure3. Morphology of squid paralarvae *U. Chinensis* at 5 days after-hatching

Based on observations, the eggs collected from nature were able to hatch after 1-2 days of incubation period. As these collected eggs had spent enough time for incubation and embryo development in nature, incubation period required to hatch eggs in the controlled tank in the laboratory was relatively short. Villanueva *et al*. (2011) mentioned that eggs of oceanic squid (*Illex coindetii*) obtained from in vitro fertilization were able to hatch with an incubation period of 11, 7 and 5 days at a temperature respectively of 13.1, 17.0 and 21.0 °C. The results showed that the hatching rate of squid eggs (Figure 4) was high at 95.92%. This finding indicated that squid eggs collected from the wild were able to hatch into planktonic paralarva (Figure 3) on rearing conditions in the controlled tank. The study results of Daniello *et al*. (1989) revealed that the water quality affected the hatching rate of *Loligo vulgaris* in a controlled tank. The eggs of *L. vulgaris*were able to hatch and develop normally in seawater with a salinity value of 34-42% and at a pH of 7.8-8.4.

The study on feeding spectrum conducted by Vovk (1985) showed that fish, crustaceans and cephalopods were major feed types found in the stomach of longfin squid (*Loligo pealei*). Meanwhile, on mantlelength of 0-5 mm, the dominant types of food found in the stomach of longfin squid were the group of Copepods and *Euphausiid*. Another study also mentioned that *Artemia* was not only able to be used in the larval rearing but also success in maintaining the survival of *O. Vulgaris* paralarvae (Iglesias *et al*., 2006; 2007).

In this study, squid paralarvaesuccessfully maintained for one week showed relatively low survival rate at only 4.25% with high mortality rate equal to 95.75% (Figure 4). Based on observations on the day-2 after-hatching (Figure 5), the cumulative mortality rate of paralarvae was known to be relatively low, reached 6.32%, howeveron day-3 after-hatching,no larval mortality was found (Table 1).

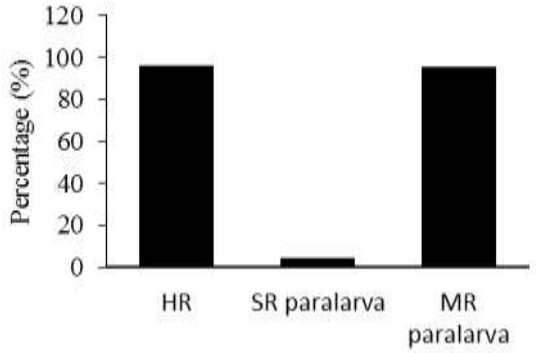


Figure4. Hatching rate, survival rate and mortality rate of Bangka squid paralarvae *U.chinensis*

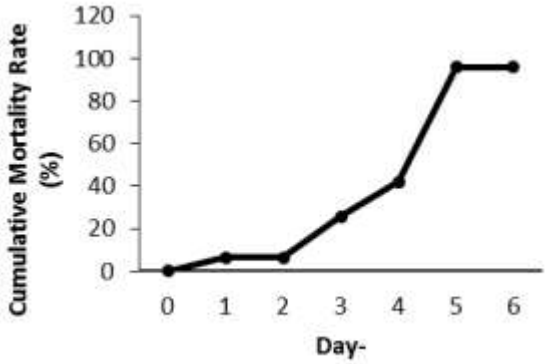


Figure5. Daily cumulative mortality rate of squid paralarvae *U. chinensis*cultured in controlled tank

Moreover, at the 4th and 5th days after hatching, the cumulative mortality rate increased amounted to 25.59% and 41.90%, respectively. The mortality of paralarvae also increased toward at the 6th days after hatching reached 95.75%. Paralarvae mortality during maintenance was suspected to be related to the paralarvae behavior which became less active in swimming and less responsive to the live food given (Table 1).

The low predation activity toward live food on the first day after-hatching was caused by physiologically unprepared paralarvaeto receive exogenous feed (Iglesias *et al*. 2006). Results of the subsequent study by Iglesias *et al.* (2006) found that the first five minutes after the addition of live food

was the most active period, indicated by paralarvaeattacked and caught prey. This process was stimulated by the first visual contact with the live food thus it is recommended to distribute the feed supply with greater frequency. However, several minutes after paralarvaecaught their prey, the number of attacks on prey showed a decrease. This was presumably because paralarvaeused their arms to hold their prey.

Table 1. Accumulation of the number of egg and paralarvae of *U. chinensis* during the culture period in controlled tank

Number of Egg Obtained	Culture Period day-	Number of live larvae on day-	Number of mortality of larvae on day-	Description
1055	1	0	0	No eggs were hatched.
	2	948	64	Paralarvaeactively moved and spread uniformly in the water column, yet were not responsive to feed. Paralarvae body were in blackish color. Paralarvae deaths occurred in small amounts.
	3	948	0	Paralarvaewere active and started to receivelive food of *Artemia*, characterized by paralarvaeactively pursuing the live food. Paralarvae body were in blackish color. Unhatched squid eggs changed color to brown.
	4	753	195	Paralarvae actively swam but were less active in feeding response. Paralarvae body colour started to changed to become translucent. Paralarvae mortality were high. Unhatched eggs color changed to brown. It was expected that eggs were dead or failed to hatch.
	5	588	165	Paralarvae suffered mass mortality in the morning. Paralarvae body started to be translucent. Most paralarvae gathered on one side of the aquarium. The movement and feeding response of paralarvae were less active.
	6	43	545	Paralarvae were less active and many of them gathered on the corner of the aquarium. A small portion of paralarvae showed an active swimming behavior. Paralarvae body generally turned into translucent white. Paralarvae suffered mass mortality in great number. Feeding responses were less active.
	7	43	0	Paralarvae remained quite active with translucent white body color and less active feeding response.

Predation activities on live food were also found to decrease on day 4, 5, and 6 after-hatching. This result was expected to be correlated to the lower survival rate of larvae after hatching (Figure 5). The low activity of predation was thought to be caused by the administration of relatively small size *Artemia* nauplii with the range of 400-500 µm (Lavens and Soorgelos 1996). Research conducted by Iglesias *et al.* (2006) reported that the size of *Artemia*given as paralarvae feedaffected the predation level of paralarva against live feed. Results of his study also showed that the predation rate of two days oldparalarvae wasfound to be higher in the group fed by large size *Artemia*(total length of 1.4 ± 0.4 mm) thanthat in the group fed by small size *Artemia*(total length of 0.8 ± 0.1 mm). Thus, it was able to explain that the lower the level of predation against feed, the lower the nutrient intake into the body of paralarvae. Inadequate intake of nutrients will affect the development and survival of paralarvae.

In addition to the feed type, nutrient content in live food given was expected to be responsible for the success of paralarvae maintenance. A study by Navarro and Villanueva (2003) showed that the type of polyunsaturated fatty acids (PUFA), phospholipids and cholesterol contained in live food greatly affected the success of Cephalopod paralarvae maintenance. The administration of single live food in the form of *Artemia* nauplii was thought to be responsible for the high mortality rate of larvae since it cause dan imbalance of nutrient intake for the development and growth of paralarvae. As reported by Okumura *et al*.

(2005), fatty acid composition of *Artemia* (small or large type) used as live food in *Octopus vulgaris* was found to contain DHA (Docosahexaenoic Acid) in a tiny amount, ranged from 0.05-0.10%. While the composition of DHA in *O. Vulgaris* paralarvae reached 26.01-28.50%. The research results also indicated the importance of omega-3 fatty acids (DHA) for the successful maintenance of paralarvae of Cephalopod group.

In addition to fatty acids, amino acids composition in live food were also affected the paralarvae culture. Villanueva *et al.* (2004) reported that the composition of the essential amino acids should be considered in the determination of feed for the cephalopod. The amino acids include lysine, leucine and arginine are known to form half of total essential amino acids in paralarvae. Arginine plays a role in the metabolic processes of adult Cephalopod group. During the anaerobic metabolism process, arginine phosphate is hydrolyzed to improve arginine's ability to condense with pyruvate and forms octopine as the final product when stress occurred. Deficiency of various types of important nutrients will hamper the growth of paralarva because fatty acids and amino acids are needed as raw materials to compose and form the structure of an organism.

Okumura *et al.* (2005) reported that the size of live food given significantly affected the survival rate of *O. Vulgaris* paralarvae. Paralarvae of *O. vulgaris* fed by large type *Artemia* significantly achieved higher survival rates and growth (45.9%) compared with those fed by small type *Artemia*(2.7%). This difference was thought to be caused by differences in the nutritional profile of both types of *Artemia*.

Conclusion

Based on the result, the low survival rate of larvae is determined by the size, type, and composition of nutrient contained in live food given to paralarvae of mitre squid *U. chinensis*

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