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The effect of different salinity towards energy utilization levels and the effectiveness of bigfin reef squid egg hatching (*Sepioteuthis lessoniana*)

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Abstract
Sepioteuthis lessoniana is a member of *Cephalopoda* which has important economic value. Continuous use of *Cephalopoda* resources can lead to extinction. Efforts are needed to conserve *Cephalopoda* resources. Factors that influence the success of the conservation of these resources are through breeding cultivation activities. The purpose of this study was to assess the hatching of *S. lessoniana* eggs in various osmotic and energetic conditions. The method used was an experimental laboratory, using a completely randomized design. The treatment in this study was the difference in media salinity, namely 27 ppt, 30 ppt, 33 ppt, and 36 ppt salinity. Data was collected for media osmolarity and hemolymph, egg energetics, number of hatched and damaged eggs. The calculated parameters were the level of osmotic work, the efficiency of energy utilization, hatching efficiency, and survival. The results showed that the best *S. lessoniana* hatching occurred at 33 ppt salinity treatment with an osmotic work level approaching the iso-osmotic value of 2.03 ± 0.05 mOsm/lH2O so that the most efficient energy utilization was 90.72% and a high hatch rate was 91%.

Keywords: *Cephalopoda*, osmoregulation, salinity, *Sepioteuthis lessoniana*

Introduction
Sepioteuthis lessoniana is one of the important economical *Cephalopoda* members that is caught in many water areas throughout Indonesia. Most of the *Cephalopoda* production in Indonesia comes from catches in nature. Catching these activities if carried out continuously can lead to extinction. The use of *Cephalopoda* fisheries resources through fishing activities is the time to be accompanied by efforts to regulate fishing and aquaculture activities which include breeding (hatchery) and release to nature. These efforts can repair the damage to squid resources because stock enrichment can be done to improve and maintain the squid resource. To overcome the availability of squid stock in nature, it can be done by cultivating squid commodities even though it still relies on spawning in its habitat [1].

Seed breeding business can develop with the support of several studies on seeding. The success of the hatchery itself requires information and knowledge about the ecological and biological aspects of reproduction. The factor that can support the growth and hatching of *Cephalopoda* eggs is salinity. Salinity is closely related to the osmotic pressure of ions and water. The osmolarity of maintenance media is one of the factors that influence the hatching rate and the efficiency of energy utilization for aquatic organisms. It is important to know the optimal level of salinity and the efficiency of energy utilization for hatching *S. lessoniana* eggs, as a support for the success of breeding (hatchery) and release of seeds to nature (restocking). Data relating to the reproductive aspects of *S. lessoniana* squid that lives in Indonesia are still lacking in detail [2]. The lack of information is the background of the need for research on the aspects of hatching eggs and the effect of salinity on the success of hatching and to find out the optimal salinity for hatching squid eggs *S. lessoniana*. Salinity is a masking factor for the life of aquatic organisms, this is because salinity can modify the physical and chemical variables of water into a unified influence that will have an osmotic effect on osmoregulation and bioenergetics of aquatic organism [3]. Salinity and water temperature during hatching will affect the development of embryos in the egg so that it will affect the success of hatching [4].

Materials and Methods
Materials
The material used in this study was *S. lessoniana* squid egg capsules obtained in the coastal waters of Teluk Awur Jepara. The tools used in this research are sample maintenance container, water reserve container, water blower, water heater, pH pen, DO meter, Thermometer, Refractometer, Automatic Osmometer Roebling, Bomb Calorimeter.

Methods
The method used in this study was an experimental method with a laboratory scale. The design used was a completely randomized design with a 3x4 factorial pattern.

The experimental research was the act of the researcher conducting activities and observing directly on research objects [5]. The treatments given were different media salinity, namely hypo-osmotic (27 ppt) and (30 ppt), iso-osmotic (33 ppt), and hyperosmotic (36 ppt). Determination of iso-osmotic values based on preliminary tests of iso-osmotic media which at 33 ppt salinity. Determination of different salinity follows the reference [6], which says that squid eggs can live in the salinity range of 21.8-36 ppt. The water used in this study was seawater and diluted according to the experimental salinity treatment. The dilution made refers to the formula [7]:

$M_1 \times V_1 = M_2 \times V_2$

Information:

M_1: Salinity of diluted seawater (ppt)

V_1: Volume of diluted seawater (L)

M_2: Desired salinity (ppt)

V_2: Volume of water with a salinity desired (L).

Research Sample Preparation
Before entering into the test container, the test animals were sorted to separate the damaged and good eggs and the egg capsules were grouped based on the number of eggs contained in them so the test animals that will be put into the test container have uniformity.

Research Container Preparation
The test container consisted of 12 plastic jars with a diameter of 30cm which contained ± 23 litres of water, cone-shaped, and at the bottom was installed with water net so the test animals did not come out during the recirculation process. Each experimental container was given a test animal of 57 capsules, each capsule consisting of 3-5 eggs in it, with a total number of eggs in one container was 210 eggs.

Water Exchange System
The water exchange system was carried out using the water recirculation method using a control tub containing ± 80 litres of water and connected to the test containers using a water hose. There were 4 control tanks with 1 salinity treatment each. The tank serves to supply water and control the salinity and temperature conditions in the experimental container.

Measurement of Water Quality Parameters.
During the study, periodic measurements of water quality were carried out and then the data was recorded. Salinity and temperature were monitored every day and maintained its stability. The temperature used in this experiment was 300 C which is the best temperature to hatch squid eggs [8]. Observation of the growth of test animals was carried out for 15 days during the study. If salinity increases, water dilution was carried out by adding a quantity of freshwater gradually, and if it experiences a decrease, saltwater was added to increase gradually. To increase and decrease the salinity of the treatment to maintain the stability of the salinity of the experiment, gradual acclimatization was carried out [8].

Research Parameters
The measured variable was the Osmotic Work Level which was calculated based on the difference between the media osmolarity and blood osmolarity values. Osmolarity measurement using the Automatic Osmometer Roebling tool, while the Osmotic Work Level (OWL) was calculated using the formula [9]:

OWL = (P Osm Blood – P Osm Media)

Information:

OWL: Osmotic Work Level, mOsm / 1 H2O

P Osm Blood: Blood osmotic pressure, mOsm / 1 H20

P Osm Media: Media Osmotic pressure, mOsm / 1 H20

Measurement of heat value was done using the Bomb Calorimeter (cal/gr), and then the efficiency level of *S. lessoniana* larvae energy was calculated, determined by calculating the amount of egg yolk energy for embryo development and *larval* hatching process using the formula [10]:

$$EK\ \% = \frac{Ze}{Ne} x100\%$$

Information:

E: Energy efficiency from hatching larvae

Ne: The caloric value after the larvae hatch

Ze: Initial caloric value in a certain phase

The survival of *S. lessoniana* larvae was obtained from observations and calculation of the number of developing embryos and was able to come out of the egg capsule, then the results of these observations were calculated using the formula [11]:

$$SR\ \% = \frac{Nt}{No} x100\%$$

Information:

SR: Survival Rate (%)

Nt: Number of squid larvae that live at the end research

No: Number of squid embryos at the start research

Results and Discussion
Osmotic Work Level (OWL)
The measurement result of *S. lessoniana* hemolymph osmolarity showed that *S. lessoniana* squid was an osmoregulator organism, an organism that has a failure mechanism to maintain the stability of its *milieu interieur* by regulating osmolarity (salt and water content) in its internal fluid [12]. The results of the measurement of media osmolarity and hemolymph of *S. lessoniana* squid embryos can be seen in Table 1. The results of the measurement of media osmolarity and hemolymph of S. *lessoniana* embryo indicated that the value of media osmolarity was directly proportional to the increase in salinity, meaning that the higher the level of salinity, the higher value of media osmolarity.

Table 1: The measurement results of average media osmolarity and hemolymph osmolarity of *S. lessoniana* embryo

Treatment	Media (mOsmo/ l H2O)	*Hemolymph* (mOsmo/ l H2O)		
		Day-5	Day 10	Day 15
27 ppt	792.01 ± 0.04[a]	801.91 ± 1.10[a]	800.43 ± 0.22[a]	797.46 ± 0.28[a]
30 ppt	879.99 ± 0.04[b]	887.85 ± 0.59[b]	885.64 ± 0.24[b]	884.71 ± 0.26[b]
33 ppt	968.00 ± 0.03[c]	963.07 ± 0.55[c]	965.17 ± 0.09[c]	965.97 ± 0.05[c]
36 ppt	1057.33 ± 1.13[d]	1045.19 ± 0.45[d]	1046.76 ± 0.79[d]	1051.05 ± 1.31[d]

Note: Different letters in the same column show significant differences $p<0.05$.

The results were different from what happened to the hemolymph osmolarity values on measurements every day which decreases with increasing maintenance time. Salinity will affect osmotic pressure in the water, osmotic pressure will increase with increasing salinity [13]. The higher the salinity of the treatment there will be a tendency to increase the osmolarity of *hemolymph* [14]. Media osmolarity was greater with an increase in salinity, this was due to an increase in the concentration of dissolved ions [15].

Based on the results of measurements of media osmolarity and hemolymph, the osmotic work level was calculated. The difference of 3 ppt in the range of salinity of 27 ppt to 36 ppt turned out to give a picture of different osmoregulatory response patterns that differ from each salinity treatment. The response tended to look not so wide. This can be caused by *S. lessoniana* embryos being stenohaline or the organisms that can only adapt to slight changes in salinity. Each species has a wide range of temperature tolerances that was specific, but tolerance to salinity was very limited because *cephalopods* were stenohaline, except *Loliguncula spp*. [16].

The average value of the osmotic work level of *S. lessoniana* can be seen in Figure 1. The highest value occurs in the 36 ppt salinity treatment that was 6.27 ± 0.43 mOsm / lH2O, and the lowest was the iso-osmotic media 33 ppt salinity treatment which was 2.03 ± 0.05 mOsm / lH2O. The low level of osmotic work was allegedly to be an iso-osmotic medium for *S. lessoniana* embryos. Iso-osmotic was the concentration of body fluids equal to the media concentration [17].

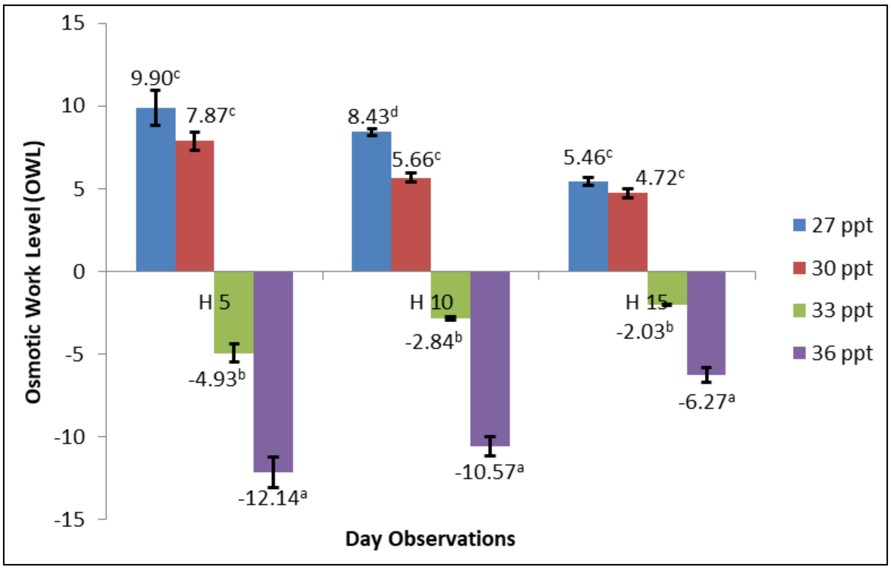

Fig 1: *S. lessoniana* Osmotic Work Level at Various Salinity Levels

The smallest value of osmotic work levels can also be referred to as iso-osmotic media because there was a low difference between media osmolarity and hemolymph in *S. lessoniana* embryos. The value of the low osmotic work level was allegedly to be the optimal salinity that the energy used in iso-osmotic media was smaller than hypo-osmotic and hyperosmotic media. Hyperosmotic and hypo-osmotic regulations were aimed at maintaining a balanced system between body fluids and their media fluids [18].

If the osmotic work level was low, then only a small amount of energy was needed for osmoregulation activities, causing the energy used for growth to be large [19]. The osmotic work level = 0 (or close to zero) means the regulation or regulation of iso-osmotic (media approaches iso-osmotic), the osmotic work level > 0 means the regulation of hyperosmotic (media is hypo-osmotic), and the osmotic work level < 0 means hypo-osmotic regulation (media is hyperosmotic) [20].

The results of measurements of osmotic work level also showed a decrease during the maintenance period, the decline was allegedly due to the increased use of osmoefector (organic and inorganic nutrients) in the hemolymph which was used as an ingredient for somatic tissue formation [2].

The Efficiency of Energy Utilization

Energy utilization will increase as a form of metabolic activity caused by differences in salinity levels. The use of energy-related to osmoregulation was needed by *S. lessoniana* embryos, it was a form of an effort to respond to the osmotic pressure differences in the maintenance media of *S. lessoniana* embryos were located. The results of the average measurement of the efficiency of egg yolk energy utilization from *S. lessoniana* embryos can be seen in Figure 2.

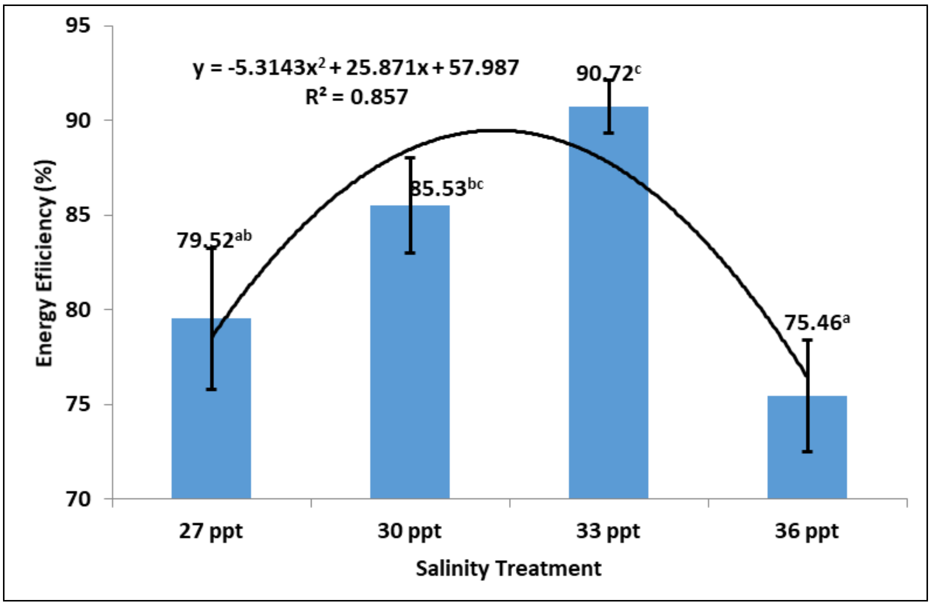

Fig 2: Efficiency Levels of *S. lessoniana* Energy Utilization at Various Salinity Levels

Based on Figure 2, the highest average efficiency values occurred in the 33 ppt salinity media at 90.72%, 30 ppt salinity at 85.53%, 27ppt salinity at 79.52%, and 36 ppt salinity at 75.46%. The highest efficiency value contained in the 33 ppt iso-osmotic media because the treatment has the lowest osmotic work level compared to other salinity treatments so that the use of energy for the osmoregulation process was small. The high efficiency of energy use was due to *S. lessoniana* embryos that were maintained in 33 ppt salinity media more to use that energy for growth. Media salinity was one of the physiological factors that influence feed utilization and growth [20]. In the hypo-osmotic and hyperosmotic conditions shown in the 27 ppt and 36 ppt salinity treatments where the osmotic work level was large, the energy expenditure used for the osmoregulation process will also be large and get lower energy efficiency values than iso- osmotic media. If the energy for osmoregulation activity increases, the energy that will be used for growth will decrease, resulting in decreased growth rate [21].

Survival Rate

The salinity of the maintenance media affects the egg quality and survival of *S. lessoniana* larvae. Based on the calculation of the survival rate of *S. lessoniana* larvae during the maintenance period, obtained the survival value of *S. lessoniana* larvae presented in Figure 3

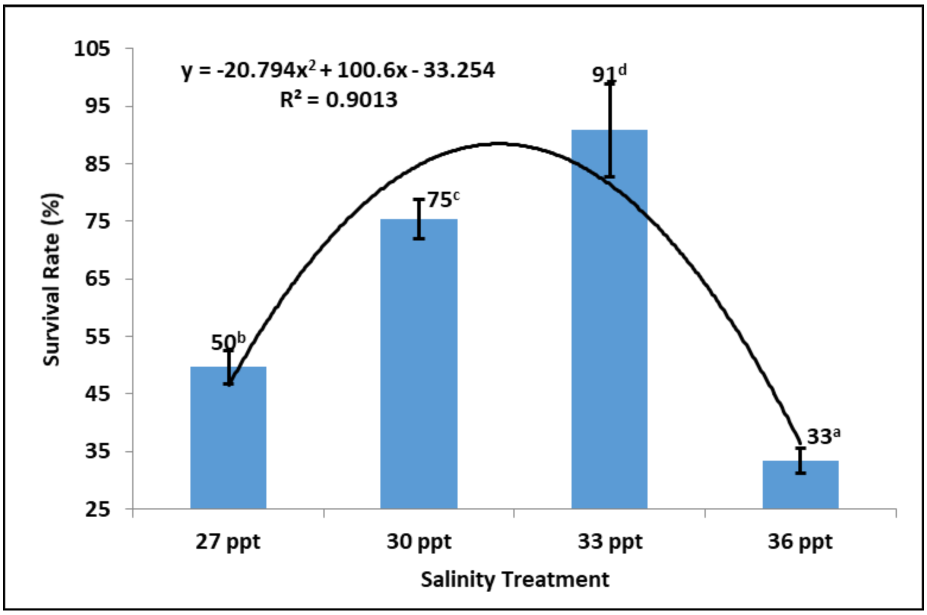

Fig 3: Histogram of Larva *S. lessoniana* survival rates at various levels of salinity.

Treatment of different levels of salinity turned out to influence the survival of *S. lessoniana* embryos. Based on Figure 3, the highest average survival rate was found in iso-osmotic media at 33 ppt salinity with hatching percentage of 91 ± 8%, and lowest in hyperosmotic salinity was 36 ppt with hatching percentage of 33 ± 2%, for hypo-osmotic media at 27 ppt salinity obtained hatch percentage of 50 ± 3% and at 30 ppt salinity of 75 ± 3%. In the maintenance of hyperosmotic media, 36 ppt salinity and 27 ppt, it was found that many eggs were damaged (dead) before they can eventually hatch from egg capsules. Iso-osmotic maintenance media at 33 ppt salinity can give an idea of the condition of eggs that are developing well even though there were also eggs that have been damaged. Eggs that fail to hatch were caused by embryos that died.

Due to changes in salinity in the osmoregulatory activity. In the process of egg hatching, osmoregulation was very closely related to salinity [22]. The osmoregulation process in *cephalopods* can take place very quickly and effectively, besides, the process was very sensitive and can cause sudden death [5]. The picture of the condition of the egg experiencing development and damage can be seen in Figure 4.

Fig 4: Egg Condition a). Good Egg Condition b). Broken Egg Condition

The low percentage of hatching on hyperosmotic media at 36 ppt salinity was suspected to be too high levels of salt in the maintenance media or media that was not iso-osmotic with eggs, so eggs use most of their energy to maintain their body fluid balance and energy cannot be used optimally for increasing the enzyme Ca-chorionase in the hatching process.

Embryonic death was a result of internal symptoms, namely disruption of the osmolarity balance between the media and egg fluid (cytoplasm) and perivitelline fluid so that only embryos that were resistant to the environment were successfully hatched [23].

Salinity was closely related to osmoregulation of aquatic animals, if there was a sudden decrease in salinity and a large enough range, it will be difficult for animals in the osmoregulation of their bodies that it can cause death [24].

Salinity that gives too high or too low osmotic work level will weaken the activity of the enzyme Ca-chorionase [10]. The enzyme activity of Ca-chorionase plays a role in the process of embrittlement and softening of the eggshell layer to help the embryo escape when it will hatch [25].

Conclusion

Based on these results it can be concluded that the media salinity has an influence on the osmotic work level, the efficiency of energy utilization, and the survival rate of *S. lessoniana* larvae. The results of this study indicated that optimal salinity in the process of hatching and energy utilization was in the media with the condition of 30-33 ppt salinity.

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