Hatching and harvesting techniques for *Artemia* cysts with different effects of salinity in the district of Situbondo, East Java

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Abstract. *Artemia* sp. is a natural food that is very important in the hatchery of marine fish, crustaceans, freshwater fish consumption and ornamental fish. This happens because *Artemia* has a high nutritional value and the size that matches the mouth opening of almost all types of fish larvae. *Artemia* can be applied in a variety of fish and shrimp hatcheries. Good seawater, brackish and fresh. The advantage of *Artemia* compared to other natural foods in aquaculture is that they are traded in the form of cysts, so they can be used at any time if needed. The specialty of *Artemia* sp. as plankton is to have tolerance (the ability to adapt and defend themselves) in a very wide range of salt levels. At very high salt levels where no organism can survive, it turns out that *Artemia* sp. can tolerate it. The method used is an experimental method with the experimental design used is a Completely Randomized Design (CRD) with 3 replications and 5 treatments namely 15 ppt, 45 ppt, 60 ppt, 75 ppt 90 ppt and 30 ppt as a control. While the supporting parameters are water quality in culture media which includes temperature, pH, and oxygen.

1.Introduction
The development of the fish farming business is increasingly felt. This is indeed in line with the times and technology. Fish cultivation currently tends to utilize the available land as much as possible so that production per unit area also increases. With the increasingly intensive business of fish farming, the more is also felt the importance of the role of fish food to accelerate the growth rate of fish. At the level of seed and seed, cultivation must be able to cultivate natural feed in large quantities. To get it, farmers must know the types of natural foods that have advantages and can be cultivated also have high protein content and nutrients, one of which is *Artemia* [1].

*Artemia* sp. is a natural food that is very important in the hatchery of marine fish, crustaceans, freshwater fish consumption and ornamental fish. This happens because Artemia has a high nutritional value and the size that matches the mouth opening of almost all types of fish larvae. *Artemia* can be applied in a variety of fish and shrimp hatcheries both sea, brackish and freshwater [2].

The advantage of *Artemia* compared to other natural foods in aquaculture is that it is traded in the form of cysts so that it can be used at any time if needed. *Artemia* can adapt to various environmental conditions.
conditions, is a filter feeder and can grow in high density, and Nauplius size according to what is needed by most fish and shrimp larvae [3].

*Artemia* is zooplankton from the Arthropod phylum and the Crustacean class. *Artemia* is needed as natural food for various fish larvae. The need for *Artemia* as larval feed is highly dependent on the opening of the mouth and the rate of digestion of fish larvae. Fish larvae have a faster digestive rate and complete nutritional needs, all these needs (until now) can only be met by natural food, especially *Artemia*. *Artemia* sp. is often used as larval feed because of its tolerance to very wide salinity, although there are alternatives such as Rotifer (for smaller mouth openings) and *Daphnia* (wider mouth openings). *Artemia* needs in the production of fish and shrimp seeds on an intensive scale must be met within a few hours because the rate of digestion in larvae is so fast. Whereas in normal time incubation of the *Artemia* system in seawater is 24-36 hours at a temperature of 25°C [4].

One thing to consider in hatching *Artemia* is the media. The basic ingredient of the media is natural seawater, but as an alternative, artificial seawater can also be used by adding non-iodized salt or common salt (95% NaCl) to freshwater to produce salinity [5].

*Artemia* cysts can be hatched on media that have 5-35 ppt salinity, although in the original habitat they can live at high salinity [6]. The optimum salinity to hatch *Artemia* cysts is 30 ppt [7]. Where the salinity of 30 ppt *Artemia* sp. lives and develops well so that *Artemia* sp. does not require much energy to adapt to the environment or media where it lives.

Salinity is one of the most important physical factors in marine life. There is often a complex relationship between two factors, where temperature can modify the effect of salinity, thus changing the salinity tolerance limit of an organism [8]. The factors that influence *Artemia*’s life are salinity, dissolved oxygen, temperature, pH, aeration. One feature of *Artemia* is its ability to adapt to a wide range of salinity. One of the advantages of this microorganism is its ability to adapt to various environmental conditions, such as salinity.

### 2. Material and Methods

#### 2.1. Place and time of research
This research has been carried out at the Salafiyah Syaфиyah Islamic Boarding School in the Sukorejo Village, Banyuputh District, Situbondo Regency. This research was conducted from May 11 to June 11 in 2019.

#### 2.2. Tools and materials
The tools used in this study include Aerators, Aerated stones, Measuring tubes, Thermometers, pH pens, Ohaus scales, Digital scales, Stirring rods, Spatulas, Watch glass, Refractometers, Oxygen meters, Microscopes, Sedgewick rafter 1 mm, Micropipette. Materials used include seawater, aquades, freshwater, *Artemia*, and common salt.

#### 2.3. Procedure of research preparation
Preparation of containers and media, make an *Artemia* hatching container by using a used bottle such that the container does not leak when used by using an aeration hose, silica glue, scissors/cutter, making a bottle holder using wood/bamboo, so that the bottle can stand properly with an upside-down position, Arranging the container and aeration before use, make sure the container and aeration function properly, make the hatching media with water of different salinity by using fresh water and salt as much as 1 liter each (bottles A, B, C, D, and E), insert the prepared media in the hatching container.

The hatching of *Artemia* cysts without decapsulation starts from weighing *Artemia* cysts which will be hatched as much as 3 grams/liter, making artificial seaweed media by adding common salt (NaCl 95%) to freshwater with each salinity as *Artemia* culture media, performing hydration/soaking cysts of *Artemia* with fresh water in a beaker glass for 1-2 hours, filter *Artemia* with plankton net then put it into a container and hatching media (artificial seawater) that has been prepared with strong aeration,
close the hatching container using black plastic, observe and note the progression of the *Artemia* cysts, counting and recording how much the hatching power of *Artemia* is at different salinity.

2.4. *Data analysis*

Data obtained were analyzed using one-way ANOVA. If $F$ arithmetic $\geq F$ table 5%, then there is a significant influence and continued with the LSD test (Least Significant Difference) to determine the difference between treatments.

3. Result and Discussion

3.1. *Artemia sp. Hatchability*

From the results of the study, the treatment of different levels of salt in the hatching media of *Artemia* sp. cyst and analyzed using a Completely Randomized Design (CRD) yielded the percentage of hatching power of *Artemia* sp.

**Table 1.** Percentage hatchability of *Artemia* sp. with differences in salinity (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt).

| Treatment (t) | Replication (n) | Total | Average |
|---------------|-----------------|-------|---------|
|               | 1 | 2 | 3 |       |       |
| 15 ppt            | 11.00 | 22.01 | 27.51 | 60.52 | 20.17 |
| 45 ppt            | 22.01 | 49.53 | 38.52 | 110.06 | 36.68 |
| 60 ppt            | 38.52 | 66.04 | 82.55 | 187.11 | 62.37 |
| 75 ppt            | 5.50  | 55.03 | 77.05 | 137.58 | 45.86 |
| 90 ppt            | 0    | 33.02 | 49.53 | 82.55  | 27.51 |

Source: (Processed data, 2019)

Information: A (treatment with salinity 15 ppt)
B (treatment with salinity 45 ppt)
C (treatment with salinity 60 ppt)
D (treatment with salinity 75 ppt)
E (treatment with salinity 90 ppt)

From Table 1, above, it can be seen that the best hatching capacity of *Artemia* sp. is in treatment C with a value of 187.58%. Furthermore, successively followed by treatment D with a value of 137.58%, treatment B with a value of 110.06%, treatment E with a value of 82.55%, and the lowest is treatment A that is 60.52%. The percentage of hatchability percentage of *Artemia* sp. can be seen in Figure 1.
Figure 1. Graph of percentage of hatchability of Artemia sp. with different levels of salinity

In the graph above, it can be seen that the effect of differences in salinity (15 ppt, 45 ppt, 60 ppt, and 90 ppt) does not have a very significant effect on the hatchability of Artemia sp. because the value of $R^2 = 0.052$. Furthermore, analysis of variance is performed using a Completely Randomized Design analysis (CRD) to determine the effect of differences in salinity on the hatchability of Artemia sp. from the results of research on different salinity treatments in the hatching medium. The results of the calculation of variance are presented in Table 2.

Table 2. Analysis of variance in the effect of differences in salinity levels on hatchability of Artemia sp.

| SK | Db       | JK       | KT     | F count | F table 5% | F table 1% |
|----|----------|----------|--------|---------|------------|------------|
| Treatment | 4       | 3251,1   | 812,8  | 1.48 ns | 3.48       | 5.99       |
| Error     | 10      | 5472.46  | 547.246|         |            |            |
| Total     | 14      | 8723.66  |        |         |            |            |

Information: ns: no significant effect

Based on the results of the analysis of variance analysis found that the calculated $F$ value = 1.48 < $F$ table 5% = 3.48 means that the results of the analysis show that there is no effect of differences in salinity (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt) so that among treatments did not significantly affect the hatchability of Artemia sp., the decision making is to accept H0 and reject H1 because in this study the percentage of hatchability of Artemia sp. has a range of values that are not much different and also Artemia sp. can adapt and defend themselves in the range extensive salt content. Artemia sp. lives planktonically in waters whose salinity is very high (15-300 ppt) where no organism can survive, it turns out that Artemia sp. can tolerate it [9].

The fourth research that produced Artemia sp. nauplii gave no significant difference, this happened because in each test and treatment produced a population that had a range of values not too far away so that the ANOVA test showed results that were not significantly different [10]. The effect of differences in salinity on Artemia sp. is the potential to increase the number of nauplii Artemia sp. different nauplii populations in each treatment due to environmental supporting factors such as temperature, pH, oxygen, and the amount of food contained in different aquatic environments [11].
In the results of the analysis of variance analysis showed that differences in salinity did not significantly affect the hatchability of Artemia sp., that is because the hatching media used in the Artemia sp. culture were artificial seawater, so the hatching media were spared from fungi and parasites which resulted in the hatching of cysts Artemia sp. Media on hatching cysts Artemia sp. can use seawater that has been filtered with sand filters [12]. This was shown to prevent cysts from fungi and parasites that inhibit the hatching rate of Artemia sp., as the best alternative to avoid fungal and parasitic contamination that inhibits the hatching rate of Artemia sp. cysts and also to get high hatchability using artificial seawater, artificial seawater this is made by adding non-iodized salt to freshwater in a ratio of 1:1 (1 gram of salt, producing 1 ppt).

Freshwater and common salt as the hatching media of Artemia sp. and states that common salt contains ions and minerals such as sodium (Na⁺), Potassium (K⁺), Magnesium (Mg ²⁺), Calcium (Ca ²⁺), Carbonate - Bicarbonate (CO₂⁻ and HCO₃⁻) and Chloride (Cl⁻) which are very important in maintaining the balance of Artemia sp. [13].

In this study, the decision resulted in the absence of a very significant effect on differences in salinity levels (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt) on the hatchability of Artemia sp., but the best percentage produced was 187.11% with salinity treatment 60 ppt. This is consistent with previous research which states that the highest peak population of Artemia is with a salinity of 60 ppt [10].

### 3.2. Water quality

The results of measurements of water quality in each treatment during the study can be seen in Table 3.

**Table 3.** Water measurements at the time of the study

| Parameters (average) | Treatment | Water Quality Standards |
|----------------------|-----------|-------------------------|
|                      | A         | B                       | C         | D         | E         |
| Temperature (°C)     | 30,08     | 30                      | 29,88     | 30        | 30        | 6 – 35    |
| DO (mg/L)            | 1,3       | 1,2                     | 1,8       | 1,9       | 1,4       | 1,2 – 2,8 |
| pH                   | 6,5       | 6,43                    | 6,4       | 6,3       | 6,3       | 6 – 8,5   |

a. **Temperature**

Temperature is an important supporting parameter of water quality during maintenance. Every change in temperature affects biological processes, especially towards structural and functional responses. Increased water temperature can affect directly or indirectly on development, growth, biological processes including, metabolism, osmoregulation, and respiration. But Artemia sp. also has a fairly wide tolerance for temperature, namely 6 – 35°C [14]. The optimal temperature needed ranges from 25-30°C [15].

In studies that have been carried out temperature measurements at the beginning of the spread of Artemia sp. cysts at the Science Laboratory of Salafiyah Syafi’iyah Islamic Boarding School averaged around 29°C then at the time the temperature measurement at the end of the study changed to 30.5°C. This temperature change did not affect because it was still in optimal temperature.

From the results of this study, the Artemia sp. culture temperature was obtained and calculated by a Completely Randomized Design (CRD) analysis, then the variance obtained was an F -2.5 <F table of 5%. This shows that the water temperature during the study did not significantly affect each treatment, namely with different levels of salinity (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt). The ANOVA temperature table can be seen in Table 4.

**Table 4.** Range of average temperatures during the study

| Sources of Diversity | Db | JK   | KT   | F count | F Table |
|----------------------|----|------|------|---------|---------|
| Treatment            | 4  | 265225,02 | 66306,255 | -2,5²⁺ | 3,48 | 5,99 |
The concentration of oxygen in water is affected by temperature, partial pressure, gases in the air and water, salinity, and easily oxidized compounds contained in water. The higher the temperature, salinity, and partial pressure, the solubility of oxygen in the water will decrease [16].

Seeing the information above and comparing the range of dissolved oxygen (DO) in the Artemia sp. culture in the research that has been done that is the range of 1.2 to 2.8 mg/l so the dissolved oxygen content in the Artemia sp. culture is still at its optimal point, this is due to the culture Artemia sp. is in a closed room. The level of dissolved oxygen in the maintenance of Artemia sp. is in the range of 0.6-5.61 mg/l [17]. The content of dissolved oxygen is good for supporting the culture of Artemia sp. which is above 5 mg/l [18].

From the results of these measurements the amount of dissolved oxygen produced in the Artemia sp. culture and analyzed using a Completely Randomized Design (CRD), then the variance obtained was an F count of 0.712 < F table of 5%. This shows the meaning that oxygen in this study did not significantly influence each treatment, namely treatment with different salinity (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt). Table ANOVA of dissolved oxygen can be seen in Table 5.

### Table 5. Range of dissolved oxygen

| Sources of Diversity | Db   | JK  | KT  | F count | F Table 5% | F Table 1% |
|----------------------|------|-----|-----|---------|------------|------------|
| Treatment            | 4    | 1,06| 0,265| 0,712ns | 3,48       | 5,99       |
| Error                | 10   | 3,72| 0,372|          |            |            |
| Total                | 14   | 4,78|      |         |            |            |

Information: ns: No significant effect

Acidity or pH is one of the environmental factors that cannot be tolerated by Artemia sp., which is in the range of 6 - 8.5. A decrease in pH below 6 meals will die [14]. The acidity content of the Artemia culture range is 8. pH conditions can be tolerated in Artemia is between 7.3 - 8.4 [19].

The pH measurements made at the beginning of the spread of Artemia cysts in the Science Laboratory were 6.5 on each treatment with different salinity (15 ppt, 45 ppt, 60 ppt, 75 ppt, and 90 ppt). So, the pH in the Artemia sp. culture process can still be said to be optimal. From the research that has been done, it is obtained the calculation of variance which can be seen in Table 6.

### Table 6. Table of variance of pH

| Sources of Diversity | Db   | JK  | KT  | F count | F Table 5% | F Table 1% |
|----------------------|------|-----|-----|---------|------------|------------|
| Treatment            | 4    | 0,08| 0,02| 6,66**  | 3,48       | 5,99       |
| Error                | 10   | 0,03| 0,003|         |            |            |
| Total                | 14   | 0,11|      |         |            |            |

Information: **: Very real influence

Based on the results of the calculation of variance, it is found that the calculated F value = 6.66> F table 5% = 5.99, meaning that the analysis shows that the difference in salinity has a very significant effect on the hatchability of Artemia sp. This is because Artemia sp. cannot live at pH below 6. In studies that have been carried out, the acidity level of Artemia culture is still within the optimal level.

### 4. Conclusion

Based on research on the effect of differences in salinity on the hatchability of Artemia sp. as follows, the difference in the administration of salinity does not provide a very significant effect on the
hatchability of *Artemia* sp., this is because *Artemia* sp. has a very high tolerance to salinity levels. From the 5 treatments and 3 replications, the best percentage was obtained with 187.11% in 60 ppt salinity, then continued with the second highest with 137.58% in 75 ppt salinity, then with 110.06% with 45 ppt salinity, then with the amount of 82.55% with a salinity of 90 ppt and the smallest percentage is a salinity of 15 ppt, with a total of 60.52%. While the variance calculation values show F count 1.48 < from F table 5% with a value of 3.48.

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