



Concentration of Honey as a Coating in Dry Storage on Hatching Power and Survival of *Moina macrocopa Naupli*

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ABSTRACT

Moina macrocopa is a natural food found in tropical waters. Ehippia can be stored and hatched at any time just like *Artemia* cysts. Embryo stability can be maintained by regulating water content through coating technology. Honey can be used as a coating because it has osmotic properties to prevent dryness during the dormancy period. This study aims to determine the effect of honey coating on the hatchability of ehippia and survival of *M. macrocopa*. This research is an experimental research with a completely randomised design (CRD) consisting of 6 treatments and 4 replications. The results showed a significantly different effect ($p < 0.05$) on the hatchability of *M. macrocopa* ehippia using the optimal concentration of 10% honey. However, the coating did not have a significantly different effect on the survival rate of *M. macrocopa naupli*.

Keywords: Coating, Ehippia, Honey, *Moina macrocopa*.

INTRODUCTION

Shrimp hatcheries continue to increase, reaching 18% each year, followed by demand for natural feed (Sahabuddin, 2018; Radkhah and Eagderi, 2022; Purba, 2012). *Moina macrocopa* is a natural food that is often found in tropical waters (Villegas, 1990). Cultivation of *M. macrocopa* is considered less practical because it takes a long time. *M. macrocopa* can produce dormant eggs called ehippia, these eggs are the result of sexual reproduction of *M. macrocopa*. Ehippia production technology has been developed with a combination of environmental and feed manipulation (Mubarak et al, 2017; Oktaviani et al, 2020). Ehippia can be hatched at any time and stored dry just like *Artemia* cysts. Research by Pancella and Stross (1963), reported that ehippia in *Daphnia* could be

stored dry for 66 days with a low hatchability of around 0.3%. Hatchability is influenced by light and temperature induction factors. In addition, hatchability can be influenced by regulating water content so that there is no release of bound water which can disrupt the life of dormant embryos (Mubarak et al., 2017; Radkhah & Eagderi, 2022). Naturally, ehippia has protection against drying, namely the presence of a layer of trehalose on dormant eggs (Roger et al., 2019). The technique of adding coating to living creatures has been carried out on *Artemia* cysts using salt (Utomo, 2017). Salt has hygroscopic properties that can attract water and inhibit microorganisms, selectively. Apart from using salt, sugars can also be used as preservatives because they have high osmotic

pressure so they can cause plasmolysis (Ratnasari et al, 2014).

Honey is a sugar solution that is too saturated from fructose and glucose (Alvarez et al., 2013). According to Ahmadi and Estiasih (2009), fructose in honey is osmotic which can attract water to the material and reduce water activity. In dry conditions, the sugar content in honey can form an intracellular "glass" matrix that can prevent the release of bound water that can cause the death of dormant embryos. Based on these problems, the addition of coatings on ephippia using honey is needed to help regulate water content and maintain bound water in embryos.

The purpose of this study was to determine the effect of different concentrations of honey on the hatchability of ephippia and the survival rate of *M. macrocopa naupli*. It is hoped that this study can provide information on the hatchability of ephippia and the survival of *M. macrocopa naupli* through the development of ephippia dry storage technology using honey coating.

MATERIAL AND METHOD

This research was conducted from September to December 2023 at the Anatomy and Cultivation Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Airlangga. The equipment used during the study were gallons, plastic cups, 1800 lux lamps, aeration, hoses, beakers, filter cloth, petri dishes, microscopes, eppendorf tubes, storage boxes, blenders, and refrigerators. Materials used during the study were *M. macrocopa*, rice bran, lemuru oil, honey, aluminium foil, distilled water, and fresh water.

Research design

This study used a completely randomised design (CRD). The treatment with honey coating consisted of six treatments and four replications. Where the control treatment

(P0): 5% pure fructose, P1: 2.5% honey coating, P2: 5% honey coating, P3: 7.5 honey coating, P4: 10% honey coating, P5: 12.5% honey coating

M. macrocopa culture

The *M. macrocopa* used to produce ephippia was cultured from natural inoculants in 4 gallons with 10 litres of water per gallon. *M. macrocopa* was fed bran feed with a mixture of lemuru fish oil. The amount of feeding was 0.3 ml.L⁻¹ with the frequency of feeding twice a day (Mubarak et al., 2017). Furthermore, the resulting *M. macrocopa* pups were then transferred into 12 other gallons at a density of 20 ind.L⁻¹.

Harvesting Ephippia *M. macrocopa*

The ephippia used came from *M. macrocopa* chicks that had been cultured using bran feed. Harvesting of ephippia can be done after reaching population density, namely after day 14. Ephippia are harvested by siphoning the bottom of the gallon because ephippia are sinking. The siphon results are filtered using a plankton net with a mesh size of 50-60 and then rinsed using clean water. The sediment filtered on the plankton net was filtered again using a 0.01 mm sieve. Ephippia was taken using a pipette and stored in an eppendorf tube wrapped in aluminium foil. Ephippia were then stored in a refrigerator for two weeks. This aims to help the ephippia enter the dormant phase (Schwartz & Hcbert, 1987).

Preparation of Coating Solution

The preparation of coating solution for each treatment is 100 ml. The honey used was pure honey from Perhutani partners with a fructose content of about 38.5%, while the fructose solution used was pure fructose in powder form. The honey solution was made by mixing distilled water that had been brought to a temperature of $\pm 70^{\circ}\text{C}$ with honey

and fructose, then stirred until homogeneous using an iron spatula in a beaker glass.

Coating of Ehippia *M. macrocopa*

There were 25 ehippia used in this study for each coating treatment. The coating was applied by dripping the coating solution on the ehippia as much as 0.05 ml per treatment using a syringe needle and then the solution was shaken to all parts of the ehippia so that the ehippia was perfectly coated. The remaining solution after coating was withdrawn using the help of a syringe needle on a petri dish so as to leave only the ehippia that had been coated. Honey drying of the ehippia coating was carried out by storing the ehippia in a refrigerator for 2 days at 4 – 5 °C. This aims to accelerate the drying of the coating (Susanto, 2011).

Dry Storage of Ehippia *M. macrocopa*

Ehippia that has been coated, then removed from the refrigerator to continue to storage at room temperature. The room temperature used ranged from 30-32°C. Ehippia was placed in a petri dish with a closed position and then wrapped in aluminium foil. Petri dishes that have been completely closed are then stored in a closed cardboard box to ensure that the petri dishes are in dark conditions. Storage of ehippia at room temperature was carried out for 15 days and 30 days with different containers.

Hatching of *M. macrocopa* ehippia

Hatching of ehippia was carried out after the 15th and 30th days with calculations starting from the 24th, 36th, and 48th hours. While the survival rate was observed after the 24th hour. Ehippia to be hatched were rinsed using fresh water with the help of a pipette. The hatching container uses a plastic cup filled with water with additional aeration and under the irradiation of 1800 lux lights in accordance with the research of (Mubarak et

al 2017). The percentage of hatchability was calculated by comparing the number of *naupli* that hatched with the total number of ehippia according to the equation of Haghparast et al. (2012), and the survival rate refers to Effendy (1997) by comparing the number of *naupli* alive at the end of observation and the number of *naupli* at the beginning of observation, as the following equation.

$$\text{Hatching rate (\%)} = (N_i/N_e) \times 100\%$$

$$\text{Survival rate (\%)} = (N_t/N_0) \times 100\%$$

During treatment, all water parameters are controlled in optimal conditions and measured every day using a water quality checker.

Data analysis

The data obtained were processed using Analysis of Variance ($p < 0.05$) to determine the effect of the treatment given and then Duncan's Multiple Range test was carried out to determine the effect and significance of the differences in each treatment.

RESULTS AND DISCUSSION

Observation of the structure and morphology of the ehippia using an optical microscope (B-150) with 100x magnification (Figure 1). The ehippia in this study hatched after more than 24 hours. This is in accordance with research conducted by (Mubarak et al. 2017). Microscopic observation of the embryo was carried out after the 12th hour which showed the development of the embryo in the form of eyespots (Figure 2).

Eyespots are a form of embryo development at stage III after the embryo has rehydrated from the dormancy period. Stage III is based on the appearance of morphological development such as the formation of antennae or eyespots and the presence of an abdominal shape in *M. macrocopa* (Chen et al., 2018). Ehippia

forms with honey coating of 2.5% to 7.5% of the embryos appear larger than the embryos before coating. This also occurs in ephippia coated with 5% fructose. Meanwhile, ephippia with 10% and 12.5% honey coating

gave the embryo a more concave shape in the middle compared to other treatments. In each treatment, a thin layer was visible outside the ephippia shell.

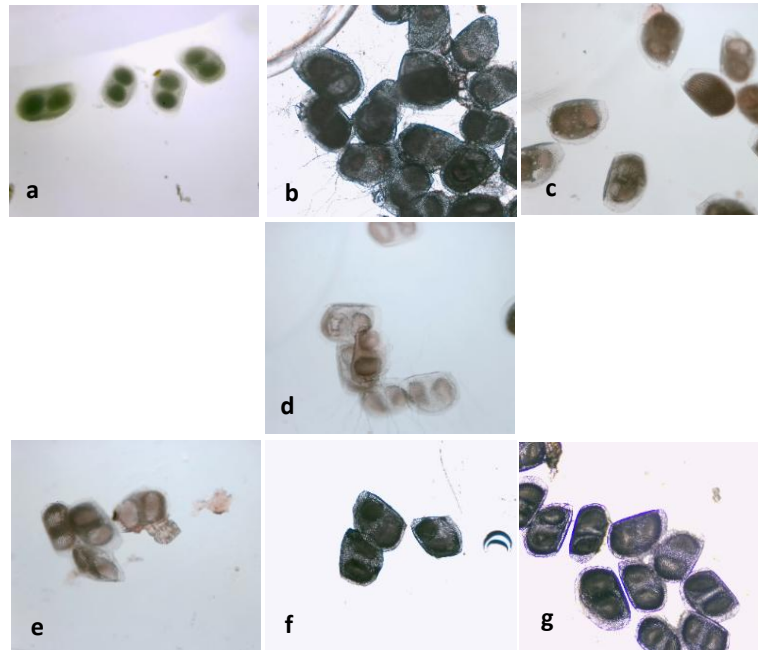


Figure 1. Structure and Morphology of Ephippia (a. ephippia without coating; b. ephippia with 5% fructose coating; c. ephippia with 2.5% honey coating; d. ephippia with 5% honey coating; e. ephippia with 7% honey coating; f. ephippia with 10% honey coating; g. ephippia with 12.5% honey coating).

Honey contains 17.1% water, 82.4% carbohydrates, most of which are glucose and fructose (Suranto, 2008). The honey solution in this study plays a role in the osmosis process in *M. macrocopa* ephippia, where this causes a change in shape due to the movement of water from low to high concentration. At low concentrations, namely 2.5% to 7.5%, the embryo appears larger because the water concentration in the embryo is higher, resulting in a process of water movement from the outside to the inside.

Meanwhile, with higher honey concentrations, namely 10% and 12.5%, the embryo appears concave in the middle because the concentration outside the ephippia is higher than the concentration inside the embryo.

Thus, it causes free water in the embryo to be drawn out and shrinkage occurs due to a decrease in cellular water activity. The presence of honey as a coating can form hydrogen bonds so that membrane integrity is better maintained and reduces excessive membrane damage. The use of a honey solution with a high level of viscosity can increase the thickness of the ephippia shell wall layer so that it can maintain the cellular structure and keep the embryo alive (Garcia and Barret, 2002).

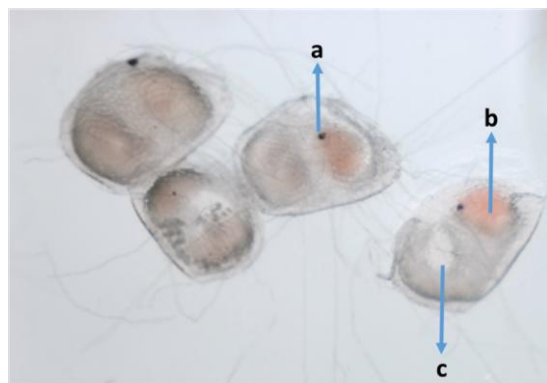


Figure 2. Embryos in ephippia develop at the 12th hour of 30 days of storage, (a. eyespots; b. embryos develop; c. embryos do not develop)

Hatchability of Ephippia

Ephippia hatchability testing was carried out after 15 and 30 days of storage by calculating the *nauplii* hatched after 24, 36, and 48 hours. The percentage of hatchability is presented in Table 1.

Table 1. The hatchability of *M. macrocopa*

Treatment	Average Hatchability (%) $\pm$ SD	
	Day - 15	Day - 30
P0	22,50 $\pm$ 5,74 ^c	31,50 $\pm$ 5,00 ^{ab}
P1	20,5 $\pm$ 2,51 ^c	26,50 $\pm$ 5,74 ^b
P2	22,00 $\pm$ 4,32 ^c	29,50 $\pm$ 4,12 ^b
P3	27,00 $\pm$ 4,76 ^{bc}	32,50 $\pm$ 8,54 ^{ab}
P4	38,00 $\pm$ 3,65 ^a	41,0 $\pm$ 5,29 ^a
P5	32,5 $\pm$ 7,37 ^{ab}	24,0 $\pm$ 8,16 ^b

Note: Different superscripts in the treatment column indicate differences in the percentage of hatchability over the length of storage time.

The honey treatment with a concentration of 10% gave a higher hatchability percentage compared to the control treatment using fructose. This is because honey has more complex content compared to fructose, such as the presence of antioxidant compounds, namely phenolic acids, flavonoids, β -carotene, vitamins (C and E) as well as antioxidant minerals such as manganese, zinc,

copper and selenium to support embryo development and accelerate egg incubation (Malgundkar et al., 2019). However, the 10% honey concentration treatment did not have a real effect on the length of storage time as shown in Figure 3.

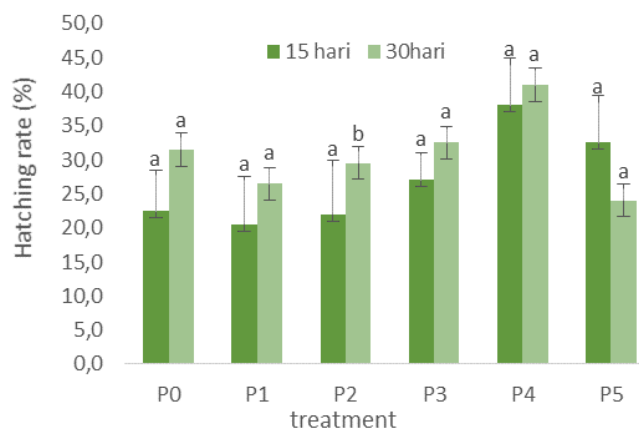


Figure 3. Graph of percentage of hatchability against storage time

Ephippia hatching is influenced by two factors, namely external and internal factors. Internal factors can be caused by the embryo lacking nutrition which will affect the cell division process and of course will have an impact on low hatchability values (Sinjal, 2014). Meanwhile, for external factors, ephippia hatching occurs due to several inductions, including temperature, exposure to light and photoperiod, as well as changes in wet-dry media (Wood & Banta, 1937). Ephippia that have entered the dormant phase will be sensitive to environmental changes such as light and temperature. Exposure to light will stimulate dormant ephippia by penetrating the protective shell to continue metabolic processes in the embryo (Crowe, 1987). Ephippia in a dormant state will experience a decrease in oxygen consumption and metabolic activity (Stross, 1971) so that if the dormant ephippia is exposed to light it will oxidize the formation of reactive oxygen (ROS), including the compound hydrogen peroxide (H_2O_2). The H_2O_2 compound is an

oxidizing agent that can damage the protective cell walls of the ephippia, causing the hatching process due to excessive light stimulation (Van der Linden, 1986).

Apart from induction from light exposure, temperature is one of the factors that stimulates ephippia hatching. According to Rojas et al. (2001) temperature plays a role in egg incubation, hatching success and hatching duration. Ephippia which have entered the dormant phase will be sensitive to changes in temperature (Pradana et al., 2009). The recommended temperature for hatching ephippia is between 24 – 30 °C (Davidson, 1969).

Survival rate of Naupli *M. macrocopa*

Observations of survival for 24 hours after the naupli hatched with the average survival can be seen in Table 2. Coating of ephippia with different concentrations did not have a significant effect ($p>0.05$) on the survival rate of *M. macrocopa naupli* on both ephippia and storage. 15 and 30 days.

Table 2. Moina macrocopa Naupli Survival Percentage

Treatment	Mean Survival rate (%) $\pm$ SD	
	Day - 15	Day -30
P0	79,25 $\pm$ 6,37 ^a	84,75 $\pm$ 3,85 ^a
P1	78,50 $\pm$ 8,05 ^a	85,00 $\pm$ 5,79 ^a
P2	83,75 $\pm$ 5,90 ^a	91,75 $\pm$ 5,95 ^a
P3	87,50 $\pm$ 1,87 ^a	79,75 $\pm$ 9,71 ^a
P4	77,50 $\pm$ 8,96 ^a	81,75 $\pm$ 9,91 ^a

Cladocera survival is said to be good if it is above 50%, while survival values below this value are said to be poor (Schlechtriema et al., 2006). The high survival rate is due to the fact that at the stage after embryogenesis, *M. macrocopa naupli* have egg yolk remaining. The egg yolk is used as a temporary food reserve for *M. macrocopa naupli* to survive.

When food reserves run out, *M. macrocopa naupli* need external food supplies. The feed given is rice bran mixed with lemuru fish oil. This feeding has been adapted to the parent so that *M. macrocopa naupli* can survive with the new food media through the inheritance of tolerance to environmental changes.

Feed is one of the factors that influences the growth of *M. macrocopa*. Bran contains protein (12 - 13%); fat (16 - 20%); high levels of B vitamins and minerals (6 - 9%), while lemuru oil contains EPA and DHA. This composition is a source of energy and helps *M. macrocopa naupli* to survive (Mubarak et al., 2017). The availability of feed and sufficient nutritional content can provide growth so that it has an impact on the survival of an organism (Simamora et al., 2021). Another factor that influences survival is the maintenance medium. During the observation period, water quality values were obtained which were in the optimum value range as presented in Table 3.

According to Jimenez et al. (2003) the optimum temperature for the growth of *M. macrocopa* is in the range of 24 - 31°C; while the solubility of oxygen according to Homer and Waller (1983) is in the range of 4 – 4.8 mg.L⁻¹. The dissolved oxygen concentration in the cultivation media influences the level of filtration and hemoglobin function; and a good pH value for *M. macrocopa* is in the range of 6.5 - 8.4 (Pennak, 1978).

Table 3. Water Quality Data during *M. macrocopa* observations

Treatment	Water quality parameters					
	Temp °C		pH		DO (mg.L ⁻¹)	
	Day 15	Day 30	Day 15	Day 30	Day 15	Day 30
P0	25,6 – 26,3	26,0 – 26,7	7,7 – 8,1	7,2 – 7,8	4,3 – 4,8	3,9 – 4,5
P1	26,2 – 27,1	25,4 – 25,9	7,5 – 8,0	7,3 – 8,1	3,9 – 4,8	4,0 – 4,7
P2	25,5 – 26,2	26,8 – 27,1	7,3 – 8,1	7,2 – 7,7	4,2 – 4,8	4,1 – 4,6
P3	25,7 – 26,3	25,7 – 26,5	7,1 – 7,4	7,3 – 7,8	4,2 – 4,8	4,1 – 4,7
P4	25, 2 – 26,0	25,9 – 26,7	7,3 – 7,9	7,6 – 8,1	4,1 – 4,6	4,2 – 4,9
P5	25,6 – 27, 0	26,1 – 26,7	7,3 – 8,0	7,5 – 8,1	4,1 – 4,5	4,2 – 4,9
Optimum	24 – 31 °C *		6,5 – 8,4**		4 – 4,8 mg.L ⁻¹ ***	

Note: *(Jimenez *et al.*, 2003), ** (Pennak, 1978); *** (Homer and Waller, 1983)

CONCLUSIONS

Based on the research results, it can be concluded that the honey solution as a coating material with different concentrations has a significantly different effect on the hatchability of Ehippia *M. macrocopa* with the optimal concentration using a honey concentration treatment of 10% at 15 and 30 days of storage, while the honey solution as a coating material does not provide significant influence on the survival of *M. macrocopa naupli*. However, it is necessary to carry out research regarding proximate analysis of *M. macrocopa* ehippia coated with honey.

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AUTHOR CONTRIBUTIONS

The contribution of each author is as follows, KST; collected the data, drafted the manuscript, and designed the table as well as the graph. ASM and NND; devised the main conceptual ideas and conducted a critical revision of the article. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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