The Effect of Different Salinity on The Growth of Phronima sp. In Mass Culture as Natural Feed

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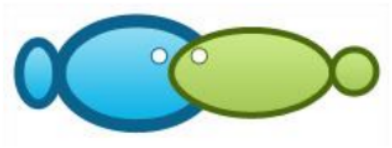

The effect of different salinity on the growth of *Phronima* sp. in mass culture as natural feed

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Abstract. When developed as a natural feed, *Phronima* sp. is one of the main microcrustaceans which can increase the production and growth of aquaculture organisms. *Phronima* sp. specimens can survive in a wide range of salinity values, but an optimal salinity can increase their growth and reproduction. The purpose of this study was to determine the growth rate of *Phronima* sp. at different levels of salinity. Salinity levels used in this study were 20, 25, 30, and 35 ppt. The experimental design used in this study was a completely randomized design (CRD). Data resulted from observations of the population density, growth rate (r), as well as water quality. The results showed that the salinity level of 25 ppt produced the best results, during the 18 day maintenance period, with a growth rate of 0.2739±0.02, for the following measured water quality parameters: dissolved oxygen (DO) from 4.40 to 6.97 mg L^{-1}, pH of 8.01-8.84 and temperature of 28.5-32.1°C.

Key Words: production, growth rate, water quality, *Chlorella* sp., *Chaetoceros* sp.

Introduction. The availability of natural food is a very important factor in aquaculture, therefore it is necessary to stimulate its production in order to stimulate the biomass growth and secure the success of cultivations. The natural food selection is based on a variety of criteria, such as: the larval mouth opening, digestibility, toxicity, nutrients concentration, availability and an accessible price (Agustina et al 2015). *Phronima* sp. development lacks of information about the optimal environmental conditions of their habitat, which is an obstacle in the development of aquaculture. *Phronima* sp. is a natural food with high nutritional content, and can be an alternative natural feed for *Artemia* (Herawati et al 2019; Fattah et al 2014 2015). Nutritional content based on proximate analysis conducted by Herawati et al (2019) showed that the highest value of proximate protein analysis was 58.90%, the proportion of fatty acids consisting of eicosapentaenoic acid was 7.53%, and the amino acid lysine was found at 44.16 ppm. The nutritional content of *Phronima* sp. depends on the culture media used. Lack of information about the environmental conditions following the habitat for life becomes one of the obstacles in aquaculture development, thus it is necessary to research the appropriate media life for optimal growth.

According to Padang et al (2012), the level of salinity and type of feed in *Phronima* sp. media are insufficiently discussed in literature. Nutrition of *Phronima* sp. larvae or in the early stages consists in a combination of *Chlorella* sp. and *Chaetoceros* sp. phytoplankton. A research conducted by Fattah et al (2014) explained that such a feeding scheme can maintain population stability and nutritional quality of *Phronima* sp. Natural feed, mainly microalgae, are source of protein, carbohydrates, and fat (Fattah et al 2014) which can accelerate the growth by providing all the necessary nutrients (Maryam et al 2015).

The environment is an important factor to be considered for the development of aquatic organisms. Eurihaline organisms capable of living in the sea, beaches, and estuaries can be grown within a wide range of salinity (15-34 ppt), the optimal values for *Phronima* sp. growth ranging between 20 and 30 ppt. Culture media salinity relates to

the ability of an organism to maintain osmotic pressure between the protoplasm and its environment (Supriyantini 2013). Zooplankton life is influenced by the water chemistry conditions and by its physical parameters. The environmental changes that occur in a water body will affect the presence of zooplankton, either directly or indirectly. A medium with an ideal salinity and nutrients availability are sufficient conditions leading to a rapid population growth (Fembri et al 2017). Diversity and abundance of zooplankton in the water can be used as a biological indicator in determining changes in water conditions (Raza'i 2017). Changes in salinity in the media life can result in metabolism and body activity modifications: salinity variations can influence several biochemical and physiological mechanisms whose function is vital to the survival of marine organisms. High salinity is an obstacle to the processes of growth and reproduction, as a consequence of the microalgae adaptation (Imron et al 2016). Therefore, the aim of this study was to assess the salinity influence on the growth and reproduction of *Phronima* sp., in order to be able to develop massive cultures of this species, as a natural food.

Material and Method

Culture of Phronima sp. The study method refers to the research methodology that has been used by Fattah et al (2014): *Phronima* sp. stocking density in a culture container was 3 ind L^{-1} and feeding consisted in doses of 1x105 cells ind^{-1} of a *Chlorella* sp. and *Chaetocerros* sp. combination. *Phronima* sp. used in this study came from the Live Feed Laboratory BBPBAP Jepara.
This media salinity experiment used diluted seawater originating from Jepara waters. Desired salinity was obtained by mixing seawater and freshwater, using the following formula:

$$V1xN1=V2xN2$$

Where:
V1 - diluted volume of seawater (L);
N1 - the salinity of the diluted seawater (g L^{-1});
V2 - water volume with required salinity (L);
N2 - salinity required (g L^{-1}).
Chlorine was added 60 ppm for media sterilizing and 30 ppm sodium thiosulfate was used to neutralize the chlorine.

This study used an experimental research method based on a completely randomized design (CRD), with 4 treatments and 3 replications. The treatments used in this study were:
A - salinity 20 ppt;
B - salinity 25 ppt;
C - salinity 30 ppt;
D - salinity 35 ppt.
The treatments were prepared according to a research of Fattah et al (2014), where the salinity ranged between 21-35 ppt.

Data collection. Measured variables included population density, growth rate (r), and water quality.

Phronima sp. population density. The population density of *Phronima* sp. was calculated daily by observing the culture medium before calculating the amount of *Phronima* sp. at each observation. Three repetitions were performed in order to generate reliable data and derive a daily growth pattern during the maintenance period.

Growth rate (r) The specific growth rate (ind/day) was calculated by the Krebs formula (1985) used by Cheng et al (2011) as follows:

$$r = (Ln\ N_T - Ln\ N_0) / T$$

Where:
r - rate of growth (ind day^{-1});
T - days needed to achieve maximum growth;
N_T - density *Phronima* sp. on day T;
N_0 - initial density *Phronima* sp.

Water quality parameters. The measured water quality parameters included: air temperature (°C), pH, salinity (ppt), and dissolved oxygen (mg L^{-1}) and were controlled every day. The necessary instrumentation consisted of a dissolved oxygen (DO) meter, a thermometer and a pH tester.

Statistical analysis. The growth rate (r) was statistically tested, showing anormal data distribution, homogeneity of variance and additivity of observations, followed by the "one way Anova" analysis, in order to determine the influence on the observed variables. If the influence was significant (P<0.05) or highly significant (P<0.01), a Duncan Multiple Range Test was applied in order to determine the true differences in median values of the treatments and thus to choose the optimal treatment (Srigandono 1981). This study used 4 complete random designs treatment with 3 replications. Population density was analyzed descriptively to determine daily growth. Specific growth rates were analyzed using analysis of variance (ANOVA) to determine differences between treatments. The parameters analyzed were population density, growth rate, and water quality parameters. Data analysis used Microsoft Excel 2013. The water quality data were analyzed descriptively and compared with the reference.

Results

The population density. Based on population density data for the study can be graphed growth given in Figure 1.

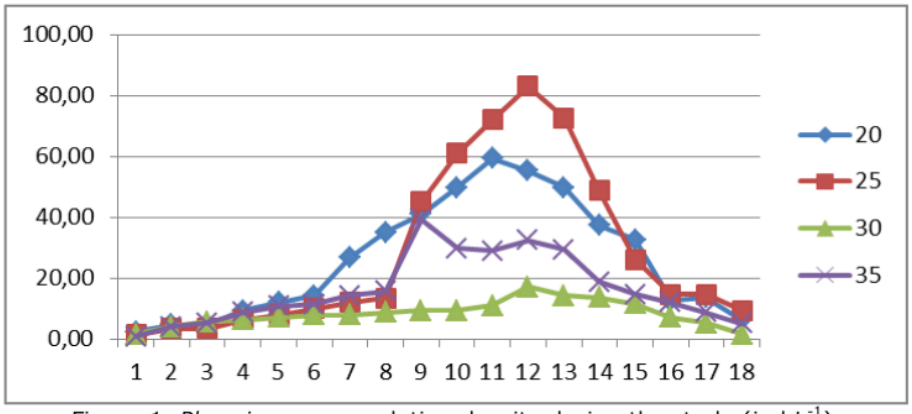

Figure 1. *Phronima* sp. population density during the study (ind L^{-1}).

Phronima sp. population density peak was the highest for the 25 ppt treatment, with a value of 83.00 ind L^{-1}, which occured on day 12. When the salinity was of 30 ppt, on day 12, *Phronima* sp. population density peak reached its lowest point of 17.00 ind L^{-1}.

Growth rate (r). Based on data for the *Phronima* sp. growth rate (r) a histogram was created and presented in Figure 2.

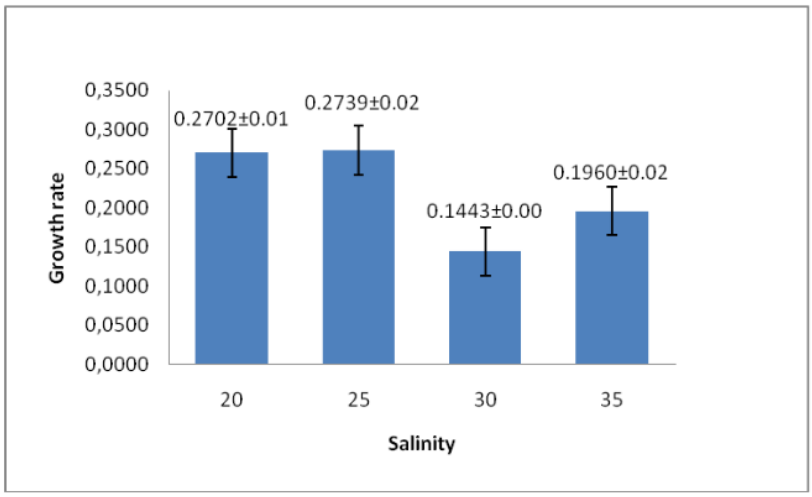

Figure 2. Histogram growth rate (r) *Phronima* sp. during the study (ind day^{-1}).

The results of the water quality parameters measurements in the *Phronima* sp. culture medium during the study are presented in Table 1.

Table 1

Measurement water quality parameters in culture media *Phronima* sp. during the study

Treatment	*Water quality parameter value range*		
	Temperature (^{0}C)	*pH*	*DO (mg L^{-1})*
A 20 ppt	29.2-30.8	8.2	4.69-5.38
B 25 ppt	28.7-30.5	8.09	4.53-4.98
C 30 ppt	27.2-29.1	8.1	4.78-5.05
D 35 ppt	28.9-29.7	8.13	4.91-5.23
Literature*	27-31	8.0 to 9.0	4.7-5.6

* Fattah et al (2014).

Discussion

Phronima sp. population density. The population density is the ratio between the number of individuals and the occupied unit area or space volume at a given time (Maryam et al 2015). From the calculation of the population density of *Phronima* sp. during the culture period of 18 days, differences can be observed in the population density peak phase at each treatment. Differences are presumably due to differences in the adapting ability of *Phronima* sp. to its environment. The maximum density achieved was on day 9 by *Phronima* sp. cultured at a salinity of 35 ppt. The addition of feed in the medium also determined the amount of *Phronima* sp. density in culture media. In order to support its growth, *Phronima* sp. absorbs nutrients from its living media. The salinity of seawater affects the absorption of nutrients, which occurs through a diffusion process consisting in the movement of molecules from the culture medium high concentration region to the the *Phronima* sp. cell lower concentration region (Padang et al 2012). Zainuddin et al (2017) stated that the difference in salinity influences the pattern of growth of the organism by increasing the osmotic pressure. The difference between the culture medium and the body liquid concentrations will have an effect on the growth and the diffusion of nutrients in cells.

Phronima sp. cultured in different salinity experiences different growth phasest. In the treatment with a higher salinity, the growth will be slower (Imron et al 2016). Peck et al (2015) stated that marine organisms can tolerate extreme salinity ranges, but along with the growth slowdown, it can cause a decrease of the available energy for

reproduction, which has a negative feedback on the number of individuals. Therefore, the population density of *Phronima* sp. can rapidly decrease when salinity conditions are no longer appropriate to the growth (Fembri et al 2017). An optimal salinity determines a maximum growth and reproduction rates.

The range of tolerance is often associated with the development period (Milione & Zeng 2008). The difference in salinity at each treatment affects the growth of *Phronima* sp. during maintenance. The results obtained in this study showed that there are differences in population density and the time of achievement of the exponential phase in each treatment. Calliari et al (2008) explained that the organism expresses a direct physiological response to high salinity, involving changes in the stages of life. Water salinity change may pose barriers to the organisms' development. The excess of Na^+ and Cl^- ions, due to high concentrations of salt in the media, can disrupt the osmotic balance between the interior of the cell and its media, causing loss of the intracellular fluids and eventually the death of the species (Zainuddin et al 2017). Consequently, adjustments to a higher salinity can cause *Phronima* sp. population decline or, in case of survival, if adaptative responses are still possible, the individuals' physiology will be influenced negatively.

Adaptation of *Phronima* sp. to the culture medium is best demonstrated by the large number of individuals produced at the time of maintenance. *Phronima* sp. growth showed the same pattern in the maintenance media. Despite the differences in the population density of *Phronima* sp. between mediums, the phase of increase and decrease in population density produced relatively the same results (Herawati et al 2019). Treatment B, with the highest population density, showed that *Phronima* sp. is able to adapt to the media, unlike treatments C and D, with a salinity of 30 and 35, respectively. It can be presumed that the salinity in the treatment B is more appropriate for *Phronima* sp. growth and survival, compared with treatments C and D. The growth and reproduction of individuals and populations during maintenance may vary depending on the environmental changes. Dam (2013) stated that living organisms adaptive mechanisms depend on the environmental conditions fluctuations, including the salinity. Anggoro et al (2013) explained that salinity has a very strong influence on the degree of reproduction. Also, the salinity effects on growth depend on two factors: the ability of the organism to regulate the internal osmotic and ionic abnormal concentrations and sudden fluctuations, and its ability to restore a normal osmotic pressure. Water quality in *Phronima* sp. culture media will also affect the population density. An increase or a decrease in the population density is another form of adaptability to the new environment as well as a form of energy transfer between growth and reproductive metabolisms (Maryam et al 2015).

Growth rate. The rate of growth is the increase in the number of individuals during a certain period (Maryam et al 2015). The species has an optimum salinity range, outside which organisms must spend more energy for the osmoregulation. The endurance of the living organism is affected by the balance between the osmotic pressure of the body fluids and the aquatic environment. Non-optimal salinity will lower the growth rate (Zainuddin et al 2017). In isoosmotic condition, the ion content in the blood of the organism is equivalent to the medium of life. Energy utilization for osmoregulation activity becomes smaller due to the salinity balancing between the body fluid and the environment of living media, so that the remaining portion of a larger energy can be used for growth. Spending energy on osmoregulation can be reduced if it is maintained in isosmotic media, which improves the feed efficiency, increasing the growth rate (Anggoro et al 2013).

Growth is a process of change in the weight, length, and volume. The rate of growth is influenced by internal and external factors. Internal factors include sex, heredity, resistance to parasites and diseases, such factors being usually difficult to control. External factors include the ability to use feed, the physical-chemical properties of water, such as water temperature, dissolved oxygen, ammonia, salinity, and photoperiod. External factors are usually associated with a living media environment and are easily controllable (Anggoro et al 2013). The difference in growth rate of *Phronima*

sp. is caused by several factors, such as the ability to adjust the isoosmotic condition of *Phronima* sp., wide range of salinity tolerance in *Phronima* sp., decreased phytoplankton cells that serve as a source of nutrients. According to Zainuddin et al (2017), the limiting factors that affect the growth rate are the nutrients in the culture media, caused by the decline of phytoplankton cells that serve as *Phronima* sp. feed. Darmawan (2014) explained in his research that the rate of growth increases with the abundance of phytoplankton and organic material contained in the media. Availability of feed at the beginning of the culture was still high, and it was utilized by *Phronima* sp. for the growth and reproduction process. Herawati et al (2019) explained that the difference in growth rate is also influenced by the ability of cells to metabolize the nutrients. Phytoplankton cells affected by the nutrients contained in the culture media. The nutrient availability, causing differences in the growth rates, depends on the: range of movement, availability of feed and water quality maintenance (Maryam et al 2015).

Conclusions. Based on the results of this study, *Phronima* culture sp. in a treatment with 25 ppt salinity provided a population density and the highest growth rate during the maintenance period and had a significant effect ($P<0.05$). Therefore, we can conclude that *Phronima* sp. can be cultured in a 25 ppt salinity medium to gain a population density and optimal growth rate.

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