METABOLISM RATE OF PEARL OYSTER SPAT *Pinctada maxima* IN DIFFERENT TEMPERATURES AND SALINITIES

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Abstract

Metabolic rate or energy budget is one of the most sensitive tools available for individual assessing environmental changes like temperature and salinity, and also prerequisite for individual growth and survival. The aim of this study is to obtain informations of energy budget for routine metabolism on different levels of temperature and salinity, and was to know the levels of optimum temperature and salinity. Randomized block design was applied with three replications. The result showed that optimal temperature and salinity on P. maxima spat is 28 ^{o}C and 32–34 ‰. Energy budget to routine metabolism increased was attributed temperature and salinity increased due to the optimal, than would be decreased were temperature and salinity increased. The highest level of oxygen consumption and energy budget for routine metabolism of spat occurred at 28 ^{o}C; salinity 32–34 ‰. The energy budget at 25 days old spat is 2.66 ± 0.07–2.77 ± 0.05 C/g wet weight^{-1} hour^{-1} (11.13 ± 0.32–11.60 ± 0.20 Joule/g wet weight^{-1} hour^{-1}). The energy budget at 35 days old spat is 1.90 ± 0.07 – 2.01 ± 0.20 C/g wet weight^{-1} hour^{-1} (7.96±0.28 – 8.43±0.82 Joule/g wet weight^{-1} hour^{-1}).

Keywords: Pinctada maxima; spat; temperature; salinity; metabolic rate

INTRODUCTION

The development of pearl culture in Indonesia turned out to be one of the triggers for the increasing demand for spat and oysters ready for operation (Winanto, 2004). Currently the production of spat from hatchery is still very limited, so most pearl culture efforts rely on the collection of spat from nature. Pearl culture requires knowledge and concern for the condition of resources, so it will have a positive impact on its management efforts (Arnaud-Hoand 2003).

Marine bivalves generally passively live, so that survival is greatly affected by environmental changes (Jeong and Cho 2007). Studies on bivalvia outekology (including pearl oysters) have been conducted and clearly show that some physical parameters of the waters influence the development, growth and survival (Alagarswami and Victor 1976; Kinne 1964; Marsden 2004; Yukihira *et al*. 2006).

Studies on the temperature and availability of feed on *Pinctada maxima* and *P. margaritifera* pearl oysters in Australia's Great Barrier Reef have been conducted by Yukihira *et al.* (1998ab, 1999, 2000, 2006). Slamet *et al*. (1998) in the waters of North Bali was recorded a temperature of 28−29 ^{o}C and salinity of 32−34 ‰ is a good range for the survival and growth of pearl oyster *P. maxima*. According to the BBL (2001) and Tun and Winanto (1987) seeds production of pearl oysters should be done in a location with water salinity 32–35 ‰. Larvae and spats show good development, growth and survival at a temperature of 26−28 ^{o}C.

The response of aquatic organisms to temperature and salinity can be determined through the level of energy spent on metabolism. Positive management of energy expenditure is a prerequisite for individual growth and survival and it can be an important criterion for evaluating environmental influences (Smaal and Widdows 1994). Metabolic rate can be measured by calories expended or oxygen consumption rate. Measurements can be made using a calorimeter or respirometer. Metabolic rate can also be measured at the basal and/or active level. Basal or standard metabolic rate (basal metabolism), which is a measurement made by fasting test animals for 1-2 times 24 hours. Routine metabolism is a measurement that is carried out by continuing to provide feed every day. Active metabolism is a measurement made in organisms that are actively swimming or fast swimmers. Maximum feeding (Msda) is metabolic energy for eating activities (Feeding Metabolism, Mt), such as digesting and absorbing food, or often called metabolic energy for standard dynamic action (Msda) (Affandi *et al.* 2009; Soria *et al*. 2007; Wirahadikusumah 1985).

Spat rearing in the laboratory requires optimum environmental conditions, because of the conditions are still very vulnerable and sensitive, especially to changes in temperature and salinity. Larvae and spat production in hatcheries, both in quality and quantity, require optimal maintenance environmental conditions, such as for growth, development and physiological processes that keep organisms in a balanced and controlled condition (O'Connor and Lawler, 2004; Gricourth *et al.*, 2006). During the process of large-scale spat production in hatcheries, information is needed on the effect of temperature, salinity and feed on growth and survival (Alfaro 2005; Asha and Muthiah 2005; Martinez-

Fernandez *et al*. 2004). The impact of environmental factors on organisms has long been known, even one factor can be modified by other factors, so it is necessary to conduct a comprehensive study to determine the negative effects caused (Kinne 1964; Yukihira *et al*. 2000; 2006).

The purpose of this study was to obtain information on energy expenditure for routine metabolism at different temperature and salinity levels, as well as to determine the optimum temperature and salinity levels in *Pinctada maxima* pearl oyster spats.

METHODS

The study was conducted in the laboratory for 2 months. The room is equipped with a cooling device (AC), to increase the water temperature a heater was used. Temperature measurement was carried out using a Hg thermometer, while salinity was measured using a refractometer (Atago, Japan).

To get the media water salinity (S) in accordance with the treatment (30‰ and 32‰), fresh water was added. Dilution of seawater is carried out by calculation, multiplying the volume of diluted seawater (liters) (V1) by the level of salinity (‰) to be diluted (St), divided by the product of the volume of fresh water added (V2) by the volume (liters) of seawater. diluted (V1). It can be mathematically stated as follows:

$$S = \frac{V_1 \ x \ St}{V_2 \ x \ V_1}$$

The experimental stages started with live feed culture, spawning, larval rearing and spat rearing.

Live Feed Culture

Live feed is prepared one month before the experiment begins. The types of live feed used are phytoplankton *Isochrysis galbana*, *Pavlova lutheri* and *Tetraselmis tetrathele*. The inokulum used comes from pure breeding on a lab scale, then propagated to a density of about 8-10 million cells / ml. Fertilizer media for live feed culture is the Walne's and Hirata formula (Alagarswami *et al*. 1987; CMFRI 1991).

Experimental Design

The experiment was used a factorial complete randomized design. The treatment used consists of 2 factors, namely (I) temperature, and (II) salinity. Factor I consists of three levels of factors, namely a temperature of 26 ^{o}C (A); 28 ^{o}C (B); and 30 ^{o}C (C). Factor II consists of three levels of factors, namely salinity 30 ‰ (D); 32 ‰ (E); 34 ‰ (F). Each treatment is three times repeated.

Larval Rearing

Larvae of *P. maxima* are kept in a fiberglass tank of 2 tons volume. Larvae are obtained from the spawning of the *P. maxima* broodstock by using a combination of temperature shock and exposure methods (CMFRI 1991; Winanto 2004). The stocking density of larvae is regulated according to the development stage (BBL 2001). The feeding schedule and water media used refers to the experiment of Winanto (2004).

Spat Rearing

P. maxima spat test animals aged 25 days, mean size 330 x 300 μm (AP x DV) were used in this experiment. The rearing tank uses a 20 liter plastic buckets. The schedule for feeding and water media and their management refers to the experiment of Winanto (2004).

The day before the experiment started, the spat was attached to the collectors measuring 20 x 30 cm. Spats are taken using a brush, then spread over the collectors that have been arranged horizontally. Spat density 1 ind/cm^2. After the spat sticks firmly (24 hours), then the collectors is put into the experimental containers.

Observed Parameters

1. Oxygen consumption

The measurement of the rate of oxygen consumption was carried out by placing the test animal in a dark colored plastic container measuring 20 L. The experimental design to determine the rate of oxygen consumption was in the form of one unit of equipment consisting of four containers. **Container A** for saturated water stock; **Container B** as a test animal container; **container C** for measuring the rate of oxygen consumption; and **container D** as a place to accommodate the remaining waste water (Figure 1). Dissolved oxygen was measured with a DO meter (YSI 550A, type

03J0820 AJ). To determine the weight of the test animals, the samples were weighed using the Dever Instruments analytical balance (d = 0.0001 gr).

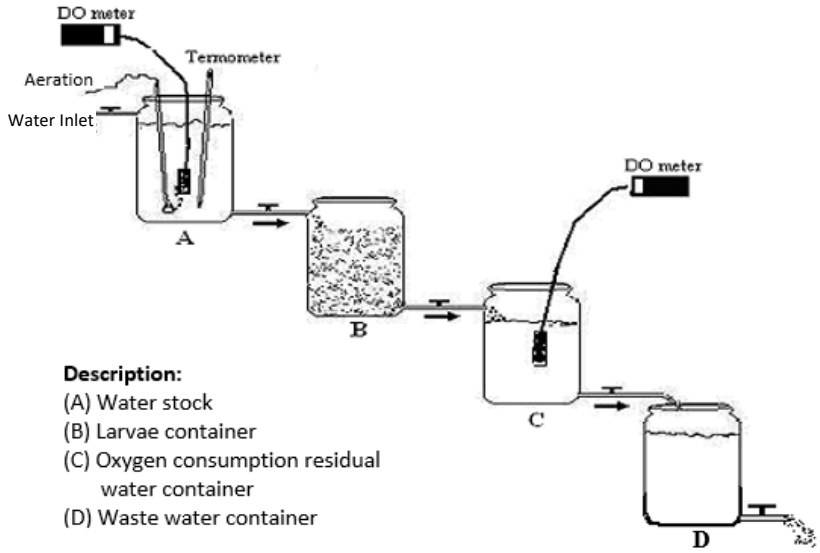

Figure 1. Experimental design for measuring the oxygen consumption rate of pearl oyster *P. maxima* spat.

The measured variable was the concentration of oxygen consumption by spat in the closed system, observations were made every hour for 24 hours. The measurement of the value of oxygen consumed is carried out by calculating the difference between the initial dissolved oxygen content in mg/liter $[O_2]o$ and the end of the observation in mg/liter $[O_2]t$, divided by the observation time/hour (T) and the number of test animals in units of weight (mg) (W) (Soria *et al*. 2007), or mathematically expressed as follows:

$$\textit{Konsumsi Oksigen} = \frac{[O_2]o - [O_2]t}{T \times W}$$

2. Routine

Rout
day during
amount of
mlO_2 = 19
2000).

Description:
(A) Water stock
(B) Larvae container
(C) Oxygen consumption residual water container
(D) Waste water container

in the condition that the test animals were fed twice a
rate of routine metabolism, it is done by converting the
follows; 1 mgO_2 = 0.7 mlO_2 (Jeong and Cho 2007); 1
1 calorie (calories) = 4.184 Joules (Somanath *et al*.,

D

3. Water quality

Water parameters measured during the experiment included temperature, salinity, pH, dissolved oxygen (DO), nitrate, nitrite and ammonia.

Data analysis

The data obtained during the study were analyzed using the F-test. If there is data that is not normally distributed, it will first be transformed used the natural logarithm (Ln). If the F-test showed a significant effect ($P < 0.05$) on each treatment, then the analysis was continued with the Tukey test (Neter *et al*. 1990). Data processing is done by using SPSS software version 17 for Windows.

RESULTS AND DISCUSSION

Oxygen Consumption

Observation of spat oxygen consumption level showed that the highest value occurred at 28 °C, salinity 34‰ (25 days: 0.863 mg O_2/g wet weight/hour; 35 days: 0.627 mg O_2/g wet weight/hour) and the lowest was at treatment temperature 26 °C, salinity 30‰ (25 days: 0.084 mg O_2/g wet weight/hour; 35 days: 0.069 mg O_2/g wet weight/hour) (Table 1).

Table 1. Oxygen consumption rate (mg O_2/g wet weight/hour) *P. maxima* spat (mean ± SD) at various temperatures and salinities.

Factor I / Factor II	Salinities (‰) (D) 30	(E) 32	(F) 34
Temperatures (°C) :			
• 25 days old			
(A) 26	0.084 ± 0.032a	0.286 ± 0.028b	0.303 ±0.026b
(B) 28	0.510 ± 0.025c	0.829 ± 0.024d	0.863 ± 0.015d
(C) 30	0.331 ± 0.022e	0.660 ± 0.026f	0.687 ± 0.014f
• 35 days old			
(A) 26	0.069 ± 0.032a	0.182 ± 0.031b	0.204 ± 0.018b
(B) 28	0.328 ± 0.029c	0.593 ± 0.021d	0.627 ± 0.061d
(C) 30	0.213 ± 0.019e	0.507 ± 0.034f	0.533 ± 0.026f

Note: Numbers followed by different letters in the same row and column indicate a significant difference between treatments at the 5% level.

The results of the variance analysis showed that temperature and salinity had a significant effect ($P \leq 0.05$) on the oxygen consumption level of spat and there was a significant interaction effect ($P \leq 0.05$) between temperature and salinity. The results of the Tukey mean test showed that the temperature treatment was significantly different ($P \leq 0.05$). but the salinity treatment of 32‰ (E) was not significantly smaller ($P \geq 0.05$) than the salinity of 34‰ (F) and the treatment D (salinity 30‰) was significantly smaller ($P \leq 0.05$) than E and F.

The study of the oxygen consumption rate of spat showed results that supported the study of the tolerance of spat to temperature and salinity. namely the highest level of oxygen consumption of spat occurred at a temperature treatment of 28 °C; salinity 32‰. 34‰ (BE. BF). The level of oxygen consumption in the spat is lower than the larvae. the decrease in oxygen consumption is about 59.71% (BE). 60.99% (BF). Allegedly. this decrease was caused by different behavior. Spat life is sedentary. while the larvae are planktonic which have an active behavior of swimming. thus requiring greater energy which is reflected by high oxygen consumption. Dharmaraj (1983) measured the oxygen consumption of *P. fucata* pearl oysters (50–60 mm) from cultured locations. the value was 1.34 ml O_2/hour. while *P. sugilata* (10–20 mm) originating from near the coast. showing a value of 0.62 ml O_2/hour.

Oxygen consumption of *Crassostrea gigas* oysters of various sizes at 13 °C; 33‰ salinity ranged from 0.23–1.91 mg O_2/hour/g dry meat weight and during the study there was no significant difference between 0.53 and 0.67 mg O_2/hour/g dry meat weight (Jeong and Cho 2007). In *Cerastoderma edule* bivalves. the oxygen consumption rate ranged from 0.014–0.087 l O_2/mg meat weight/hour (Kittiwatanawong 2007). Another study on abalone (*Haliotis discus hannai*) with a length of 92.6 mm; dry weight 12.1–14.39 g. the value of the oxygen consumption rate at a temperature of 25 °C. salinity 31.6 – 32.0‰ is 0.30–0.32 ml O_2/hour/g dry weight (Lee *et al*.. 2007) . Several research results compiled by Gosling (2004) noted that the oxygen consumption rate of *C. gigas* at a temperature of 27–28 °C was around 0.51ml O_2/hour/g dry weight. The oxygen consumption rate of *Mytilus edulis* was 0.38 ml O_2/hour/g dry weight at 15 °C; the oxygen consumption rate of *Ostrea edulis* was 0.962 ml O_2/hour/g dry weight (15 °C) and at 25 °C 2.655 ml O_2/hour/g dry weight; the rate of oxygen consumption of *C. virginica* at a temperature of 20 °C 0.372 ml O_2/hour/g dry weight and at a temperature of 30 °C 0.423 ml O_2/hour/g dry weight.

The results of this experiment are different from the results of several studies above. because apart from the different sample sizes and species. those studies are generally carried out in nature or by taking samples from nature. while in this experiment the samples come from the laboratory. Allegedly. spat that comes from nature must acclimatize to the treatment conditions. so it requires relatively large energy. On the other hand. spats that come from the lab or from larvae that have been reared in the lab. do not need to spend a lot of energy to acclimatize to the laboratory conditions. In addition. spats that come from the lab do not need to waste energy for filtration. because the water media in the laboratory is of good quality after going through several stages of the filtration process. On the other hand. spats that come from nature must spend more energy on filtration. because there are still dissolved particles that are trapped in the mantle and gills. According to Ropert and Goulletquer (2000) the filtration rate is influenced by the particle size and the stable particle size ranges from 7-8 µm. the larger the particles size. the more energy is needed for filtration.

Routine Metabolism

Energy expenditure for routine metabolism of spat is highest at a temperature of 28 °C and a salinity of 34‰ (BF). The lowest energy expenditure occurred at a temperature of 26 °C. salinity 30‰ (AD). The results of the analysis of variance showed that there were significant differences in metabolic rate ($P \leq 0.05$) in each treatment of temperature. salinity and the interaction of temperature and salinity. Furthermore. Tukey's test also showed that there was a significant difference in metabolic rate ($P \leq 0.05$) in each treatment of temperature. salinity and the interaction between temperature and salinity. While the salinity treatment of 32‰ (E) was not significantly smaller than the treatment of 34‰ (F). but E. F were significantly different ($P \leq 0.05$) than the salinity treatment of 30‰ (D) (Table 2)

Table 2. Energy expenditure for routine metabolism (C-J/g wet weight/hour) *P. maxima* spat at various temperatures and salinities.

Factor II	Salinities (‰)		
Factor I	(D) 30	(E) 32	(F) 34
Temperatures (°C)			
25 days old:			
(A) 26 (Calorie)	0.27 ± 0.10a	0.92 ± 0.09b	0.97 ± 0.08b
(B) 28 (Calorie)	1.64 ± 0.08c	2.66 ± 0.07d	2.77 ± 0.05d
(C) 30 (Calorie)	1.06 ± 0.06e	2.12 ± 0.09f	2.20 ± 0.05f
(A) 26 (Joule)	1.12 ± 0.42a	3.84 ± 0.38b	4.07 ± 0.35b
(B) 28 (Joule)	6.86 ± 0.34c	11.13 ± 0.32d	11.60 ± 0.20d
(C) 30 (Joule)	4.45 ± 0.29e	8.87 ± 0.36f	9.22 ± 0.20f
35 days old:			
(A) 26 (Calorie)	0.22 ± 0.10a	0.59 ± 0.10b	0.66 ± 0.06b
(B) 28 (Calorie)	1.05 ± 0.09c	1.90 ± 0.07d	2.01 ± 0.20d
(C) 30 (Calorie)	0.68 ± 0.06e	1.63 ± 0.11f	1.71 ± 0.08f
(A) 26 (Joule)	0.93±0.43a	2.45±0.41b	2.74±0.24b
(B) 28 (Joule)	4.41±0.38c	7.96±0.28d	8.43±0.82d
(C) 30 (Joule)	2.86±0.25e	6.81±0.45f	7.16±0.34f

Note: Numbers followed by different letters in the same row and column indicate a significant difference between treatments at the 5% level.

The experimental results show that the temperature is 28 °C; salinity 32‰; 34‰ (BE and BF) were the optimum conditions for spat survival and growth rate. Under the right conditions. spats can allocate maximum energy for development and growth. On the other hand. at a temperature of 26 °C salinity 30‰ (AD). the energy expenditure of spat is lowest. so the survival and growth rate of spat are also low. According to Goddard (1996) at optimum temperature and salinity conditions a maximum metabolic rate occurs. so that maximum survival and growth rate can be achieved.

The data obtained explain that energy expenditure for metabolism in spat is obtained from the filtered food (filter feeder). According to Crisp (1984); Dame (1996) energy balance can be estimated through a comparison between the energy obtained from food and the energy used for internal metabolism. If the result is positive. this energy balance can be defined as the scope for growth. or a representation of the energy used to grow (somatic tissue) (Resgalla *et al*. 2007).

P. maxima spat. throughout its life attach-settled to the substrate. Spat's main physical activity is only to filter food. so it requires relatively less energy. According to Bayne and Newell (1983) the energetic cost of feeding activity in *M. edulis* increases exponentially with filtration rate; decrease in energy requirements can reach two or three times than in the active state.

In this study. energy expenditure for routine metabolism at the age of 25 days is greater than the age of 35 days. Allegedly. the 25-day-old spat expends more energy. because it is still in the transitional period of life as benthic. so it must produce a lot of bisus to stabilize its position on the substrate. On the other hand. at the age of 35 days. the spat has settled. so the condition is relatively more stable. The production of bisus thread is only done to balance the growth of the shell. so that it consumes less energy. According to Morse (1990); Pawlik (1992); Zhao et al. (2003) metamorphosis and larval attachment is a critical period in controlling invertebrate population dynamics. Most of the bivalves have an bisus thread in the post larval stage. the bisus thread serves as a stabilizer during the metamorphosis process from larvae to spat.

Measurement of basal metabolic rate in juvenile abalone (*Haliotis fulgans*) with an average weight of 36.2 g was performed after fasting. Mentioned. abalone uses 108.10 calories derived from protein catabolism. equivalent to 2.99 C/g/day. His other research on *H. carrugata*. spent 40-50 C/g/day calories and the proportion used for basal metabolism was around 2.8-3.5 C/g/day. This value tends to increase and the energy obtained comes from carbohydrate metabolism (Viana *et al.* 2007). Species *Mytilus californianus* with a dry weight of 1 g. at a temperature of 13 ^{o}C consumes an average of 20.6 J/liter calories. the value is divided for the basal metabolic component 2.72 J/hour and the cost of digesting food is 8.15 J/hour.

This experiment limits the salinity treatment to only 34‰. because apart from preliminary studies and literature references. it also considers the natural habitat of pearl oysters. which generally live in oceanic-influenced waters. so it is assumed that the treatment at salinity over 34‰ is not significantly different. The results also showed that oxygen consumption and energy expenditure for routine metabolism at 32‰ salinity were not significantly different ($P \geq 0.05$) with 34‰ salinity. Supporting observations were put forward by Soria *et al*. (2007) on juvenile scallops (*Agropecten purpuratus*) oxygen consumption at 34‰ salinity was greater than 38‰ salinity. but oxygen consumption at 38‰ and 42‰ salinity treatments was not significantly different. Furthermore. there was no significant difference ($P \geq 0.05$) in the oxygen consumption rate at a salinity of 34‰. 38‰ or 42‰ with a temperature of 10 ^{o}C and 22 ^{o}C.

Changes in salinity can affect the temperature tolerance of poikilothermic aquatic organisms (Garside and Chin 1972). Tolerance to the maximum temperature shown by isoosmotic animals when in an environment. is an adaptation strategy that is generally owned by invertebrate animals. It should be noted. in a number of species showing no or only small extracellular osmoregulatory power. the intracellular isoosmotic regulation mechanism will lead to a number of regulatory cell volumes and it is the best adaptation strategy in the medium. The mussel *M. granosissimus* species. originating from the sea and then adapted to a salinity of 3‰. will experience stress and may die if not returned to the sea. In certain species. the water medium is more the cause of salinity stress conditions so that the variable depends on the adaptability of the species (Gilles and Jeuniaux 1979).

In this experiment. the highest rate of routine metabolism of spat occurred at 28 ^{o}C and salinity of 32‰; 34‰. or is the optimum temperature and salinity for *P. maxima* spat. The same opinion was conveyed by Syafiuddin (2005) and Thomas *et al*. (2000) that the optimal metabolic rate occurs at optimal temperature conditions. Changes in environmental temperature also change energy aimed at increasing the growth rate. respiration rate or oxygen consumption rate. For example. animals in the tropics have a higher metabolic rate than animals living in subtropics. this phenomenon can occur due to differences in water temperature. The metabolic rate of tropical animals increases by 10% for every 1% increase in water temperature. Biochemical reactions based on temperature compensation are believed to affect the functioning of the nervous system. as well as quantitative and qualitative changes in the rate of enzyme reactions (Gosling 2004). Temperature also influences biological phenomena in aquatic animals. which is not surprising because osmoregulation in aquatic animals may be responsive to thermal influences (Vernberg and Silverthorn 1979).

CONCLUSION

1. The highest level of oxygen consumption and energy expenditure for regular metabolism of spat occurs at 28 ^{o}C; salinity 32–34‰. The energy budget at 25 days old spat is 2.66 ± 0.07–2.77 ± 0.05 C/g wet weight^{-1} hour^{-1} (11.13 ± 0.32–11.60 ± 0.20 Joule/g wet weight^{-1} hour^{-1}). The energy budget at 35 days old spat is 1.90 ± 0.07 - 2.01 ± 0.20 C/g wet weight^{-1} hour^{-1} (7.96±0.28 - 8.43±0.82 Joule/g wet weight^{-1} hour^{-1}).
2. The optimum temperature and salinity for rearing spats is 28 ^{o}C. and 32–34‰.

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