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Protease enzyme reactability effect of pearl oyster (*Pinctada maxima*) flesh shell growth at different water temperature and salinity

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Abstract

Shell growth of pearl oyster is affected by the availability of nacreous liquid containing calcium carbonate ($CaCO_3$). It is nearly similar to mucus from enzymatic metabolism product in protein digestion. This study was aimed at observing the protease enzyme reactability and its effect on the survival and growth rate of the pearl oyster seeds at different water temperature and salinity conditions. It was carried out on March 6th – May 7th, 2015, at Laboratory of Marine Bio-industry Technical Implementation Unit, Research Centre for Oseanography, Indonesian Institute of Sciences in Lombok. $CaCO_3$ concentration was analyzed using an Atomic Absorption Spectrophotometer (AAS) to know the reactability of protease enzyme and the relationship between $CaCO_3$ content and shell growth. ANOVA indicated that larval survival at spat phase cultured in the media of different temperature-salinity interaction showed different effect ($P<0.05$). Based on Honest Significant Difference test, spat survival at the treatment of tempersture-salinity interaction II (28±0.5°C and 32±1ppt) was the best. High spat survival and growth in treatment II were supported by the reactability of protease enzyme and higher $CaCO_3$, 0.0518 µmol/mL-min^{-1}(unit) and 98.873 ±0.04% than those of other treatments. In addition, water quality of the culture media was also discussed in this paper.

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Introduction

Pearl oyster (*Pinctada maxima*) culture business in Indo-Pacific waters, including Indonesia, is developing with increase in domestic or international market demand, for pearl oyster seeds**.** Research outcomes of several tropical and subtropical countries can be taken as references in raising the continuity of pearl oyster production. Shell growth and pearl seed formation are a product of biomineralization of nacreous layer containing calcium carbonate ($CaCO_3$) (Caiping *et al*., 2005; Miyamoto *et al*., 2005; Hamester *et al*., 2012; Naganuma *et al*., 2014). Pearl oyster shell holds calcium carbonate of 95%, while the rest is other organic matter (Feng *et al*., 2009; Hamester *et al*., 2012)

One of the important factors in formation process of pearl oyster shell at the larval stage is highly determined bythe availability of cacium carbonate produced by enzyme in protein digestion. Failures in shell formation of mabe oyster (*Pteria penguin*) at the *D-veliger* stage is largely determined by the availability of protein used for metabolism peocess of the enzyme in nacreous liquid formation that produces crystal element of $CaCO_3$ (Naganuma *et al*. (2014). Also, lack of nacreous liquid could inhibit the shell growth rate (Zaremba *et al*., 1996; Weiner and Addadi, 1997; Fritz and Morse, 1998 *in* Blank *et al*. (2003). Mollusc shell and pearl seed are made by minerals composed of $CaCO_3$ crystals and organic polymers (Caiping *et al*., 2005). The presence of protein content of the flesh highly influences the $CaCO_3$ precipitation in giving shell growth through nacreous liquid (Feng *et al*., 2009).

Environmental condition stress, such as temperature and salinity, can also affect the mollusc life, particularly oysters. According to Zeng *et al*. (2009), the enzymeatic activity level in the flesh of pearl oyster (*Pinctada martensii*) after hydrolized with different temperature treatmentsis proved to have reaction. High response in protease enzyme component is alcalase, 4.84±0.9mL^{-1}at 50^{o}C and pH 8.0 andfollowed with pancreatin, 4.79±0.89 µmol/ml/min.at 37^{o}C and pH 8.0. Observations on the reactability of protease enzymeand its effect ongrowth and survival of the pearl oyster seedlings at different temperature-salinity interaction conditions are still rare.This study was aimed to look at the reaction of protease enzyme of each temperature-salinity interaction treatment in relation with nacreous liquid production as organic material in the formation of crystal elements containing containing ($CaCO_3$for shell growth. This information is expected to be additional reference in further research development, particularly larval post-culture up to attachment to the collector in the laboratory.

Materials and methods

Breeding of pear oyster, P. maxima

Forty pearl oyster (*P. maxima*) spawners were selected from F1 oysters, 21 males and 19 females, with 12.0-14.7cm shell width and body weight of 550-730gr and 12.6-14.6cm shell width and body weight of 550-660gr, respectively, and spawning was done in the laboratory of PT. Autore Pearl Culture (Fig. 1). The spawning used temperature shock, and only 19 individuals, 8 males and 11 females, showed spawning response. Fertilized eggs were grown in the tank for 24 hours until the larvae reached D-veliger stage. The larvae were filtered through screen nets of 75µm, 63 µm and 57 µm mesh size,the biggest size on the top and the finest one on the bottom, and obtained 77.46 millions of larvae, then 900,000 larvae were taken for laboratory observations in the laboratory of Marine Bio-industry Technical Unit, Research Centre Of Oseanography. The filtering method after reaching *D-veliger*s tage is presented in Fig. 1.

Materials and Equipment

This experiment used VC-18 Wagon 82L-marked container (687 mm long x 478 wide x 390mm deep) with holding capacity of 82 liters.There were 18 units of containers with 3 treatments of temperature factor and 3 treatments of salinity factor so that there were 9 temperature-salinity interaction factors with 2 replications. The treatments of 9 interaction factors were 26±0.5^{o}C and 29±1ppt (I), 28±0.5^{o}C and 32±1ppt (II), 30±0.5^{o}C and 35±1ppt (III), 28±0.5^{o}C and 29±1ppt (IV), 30±0.5^{o}C and 32±1ppt (V),

26±0.5°C and 35±1ppt (VI), 30±0,5°C and 29±1ppt (VII), 26±0.5°C and 32±1ppt (VIII), and 28±0.5°C and 35±1ppt (IX), respectively.The experimental tankswere facilitated with heater that could be automatically set to the desired treatment temperature. Meanwhile, to obtain low temperature, a 2-inch thin paralon pipe of 43cm height was installed with lower end tightly closed, then frozen bottle ice was inserted reaching the bottom of the experimental tank.The frozen bottle ice inside the pipe helped to make the test medium be cool and the temperature was controlled by automatic heater in the range of the desired treatment temperature. To obtain the desired salinity treatment, pure salt solution was added to raise the salinity level and mineral water to reduce the salinity. Water aeration was also created to supply dissolved oxygen in each experimental tank until all larvae reach the benthic phase (juvenile).

Each experimental unit was randomized and wrapped with black plastic sheet to reduce white light intensity into the experimental tank (Fig. 2). Previous finding (Hamzah, 2013) revealed that pearl oyster larvae prefer dark areas and possessed higher survival percentage than that in the medium with light intensity. Also, larval attachment to the collector, both pearl oyster (*P.maxima*) and pinguin's wing oyster (*Pteria penguin*),shows higher attachment on the dark-colored collector than that in blue, green, yellow, and white colored one (Hamzah, 2003; 2007).

Stocking and Larval rearing

This experiment used 18 units of *VC-18 Wagon 82L* tank (L 687 x W 478 x H 390mm) with 82 liters of holding capacity. The experiment apllied 3 temperature and 3 salinity treaments, so that there were 9 temperature-salinity interaction factors with 2 replications. The position of experimental units had been randomized before the larvae were stocked (Fig. 2). The experimental tanks were wrapped with black plastic to reduce light intensity in the tank. Hamzah (2013) found that pearl oyster larvae preferred to live in the dark area, and the percent survival tended to be higher than that in the light one.

The larval attachment to the collector, either pearl oyster (*P.maxima*) or mabe oyster (*Pteria penguin*), was found higher on the dark collector than blue, green, yellow and white ones (Hamzah, 2003; 2007).

The test tanks were equipped with an automatic heater to set the water temperature as preferred for the experiment. To obtain low water temperature, a 2 inches-paralon pipe of 43cm long with tightly closed lower part was inserted frozen bottle ice and dipped into the tank bottom. The frozen bottle ice in the pipe was diffused and affected the temperature of the test media controlled by the heater, so that the media temperature remained around the treatment value. To raise the salinity level as to the treatment level, pure salt solution was added, and inversely the "aqua" water was given. Aeration was also set in each experimental unit and used after all larvae had reached the benthic phase (juvenile).

Culture media for larvae were sterilized from main tank (tower) to the experimental tanks through *UV,*andthe pipe edge was put a filter bag, so that the water used for the test animal observation was surely clean and free of wastes or particles that could press the larval life. *D-veliger* larvae were then stocked with 50,000 individuals/experimental unit, and therefore, number of larvae needed for 18 experimental tanks were 900,000 larvae. The calculation of *D-veliger* larvae stocking in each experimental unit used the following formula:

$$\mathbf{V = X/Y}$$

where : V = Volume of larvae stocked (mL)
X = Number of larvae desired (individual)
Y = Mean number of larvae sampled (larvae/mL)

Water was replaced every 2 days as much as 50% and totally replaced at day-4. After total water replacement (100%), water quality measurements, such as dissolved oxygen (DO) and acidity (pH), were doneusing DO-meterand pH-meter *HANNA H19124N*.

Feeding was provided 2 times a day, morning and afternoon, with *Pavlova luteri*, *Chaetoceros* spp., and *Isochrysis galbana*.The collector placement in the experimental tank of 20cm x 30cm was done after 75% of number of larvae had reached the eye spot phase, 2 colecttors/tank. This observation was conducted until all larvae attached to the collector and then grown up to the 60th day in the tank. Ninety large-sized seed were selected, 10 individuals per experimental tank, for enzyme and calcium carbonate analyses. Shell width was measured with a digital calliper, and body weight with *BSA323S-CW,d=1mg* digital balance.

Size determination and larval survival at the planktonic phase of *Umbo-veliger* and *Pedi-veliger* were known from the screen size,180µm, 100 µm and 60 µm, respectively. Number of larvae retained on each screen was separated and then counted under a microscope of 100x enlargement. A "Sedgwik Rafter" was set under the ocular lense of the micrioscope to count the larvae and repeated 3 times, so that number of larvae on each screen size could be gained. Meanwhile, early spats attached on the collector and tank wall were visually counted using a magnifier and hand counter. The development of oyster and juveniles up to small adult is presented in Table 1.

Protease enzyme reactability and calcium carbonate ($CaCO_3$) concentration

The analysis of protease enzyme reactability of the pearl oyster (*P. maxima*) tissue and calcium carbonate content of the spat shell was done in Chemistry Laboratory of Brawijaya University. Number of spats analyzed were 90 large-sized individuals, 10 ind./experimental unit. $CaCO_3$ content of the spat shell concentration was analyzed using an Atomic Absorption Spectrophotometer (AAS).

The protease enzyme reactability test employed a spectrophotometer as follows: One mL of coarse enzyme extract was inserted into the flask and added 2.5 mL of 1% casein solution in pH 6.5 buffered phosphate, incubated in a waterbath at 37°C for 10 minutes, then added 2.5 mL of 5% TCA solution, left for 30 minutes at room temperature, and then centrifuged at 3000 rpm for 5 minutes and filtrate collected. The filtrate absorbance was measured using a *UV-Vis* spectrophotometer at the wavelength (λ) of 275.2 nm. As blank, enzyme solution of the same treatment was used, but TCA was added before substrate addition**.** The reactability of protease enzyme was calculated through tyrosin content, i.e. conversion of protease enzyme value at the standard tyrosin using regression equation of Tyrosin standard curve developed by Simonian (2002) as follows:

$Y = 0.007X$

where : Y = Absorbance

x = Concentration

Determination of protease enzyme activity is expressed in unit, in which 1 unit is the amount of µmol of tyrosin produced in each 1 mL of enzyme/min. The calculation follows the equation below:

$$AE = \frac{[\text{Tyrosin}]}{\text{MW of Tyrosin}} \times \frac{V}{pq}$$

where: AE = Enzyme activity (Unit)

V = sample volume of each flask (1 mL)

MW = Molecular weight of Tyrosin (181 µg/µmol)

q = reaction time (10 min.)

p = Volume of crude protease extract (1 mL)

The survivorship of pearl oyster spat at the juvenile phase (attach to substrate) was analyzed using ANOVA (Sudjana, 1991).

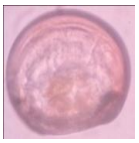
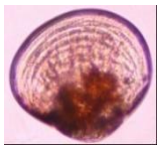
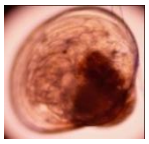
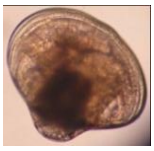
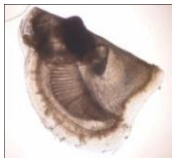
Results and discussin

Protease enzyme reaction andcalcium carbonate ($CaCO_3$) content.

Calcium carbonate ($CaCO_3$) content of the pearl oyster shell in each treatment unit and tyrosin level were calculated through protease absorbance value conversion in regression equation of tyrosin standard curve (Y = 0.007x).

Table 2 shows that the highest $CaCO_3$ content of the pearl oyster is found in temperature-salinity interaction II treatment (28±0.5°C and 32±1ppt),averagely 98.41±0.02%with protease enzyme reactivity of 0.0610µmol/mL.min^{-1}(Unit).

Table 1. Development of pearl oyster larvae up to spats in the laboratory for 60 days. Stage determination classification follows Haws & Ellis (2000).

No	Image	Phase	Remarks
1		Embryogenesis (Planktonic)) *D-veliger*	Shell starts being *D-shape*, hinge line is seen. (Observation at day -2)
2		*Umbo-veliger*	Formation of second shell occurs after bulge at dorsal part appears. (Observation at day-14)
		*Eye – spot*	Small black spots appear on both sides of the shell at 16-17 days old. These black spots are an indicator for immediate collector placement. (Observation at day -17)
4		*Pedi-veliger (final umbo)*	Bysus starts appearing at dorsal part used to attach. (Observation at day-20)
5		*METAMORPHOSIS (BENTHIC)* *Plantigrade*	Final transition of planktonic phase. Shell formation is complete with anterior, posterior and bysus, and attachment process to the collector starts. (Observation at day-22)
7		*Early spat*	Develop and grow to early spatin attachment to substrate. (Observation at day-34)
8		*Spat*	Morphological shape is complete, looks like small pearl oyster. (Observation at day-60)

Protein characterization of test animal flesh

Characterization data of pearl oyster seed flesh protein using *SDS-PAGE* method are presented in Fig.3. Band Marker possessing contrast color segment indicated the presence of protein characterization concentration of the oyster flesh based on temperature-salinity interaction treatment.

The test animals stressed under temperature-salinity interaction treatments demonstrates nearly similar protein characterization appearance, the lowest molecular weight of 40 kDa and the highest of 100 kDa, except in temperature-salinity interaction treatment 9S/I (26±0.5°C and 29±1ppt), the lowest was 45 kDa and the highest was 100 kDa.

Molecular weight concentration range of the oyster (*P. maxima*) flesh protein characterization in this study was not significantly different from other previous findings, in which the lowest molecular weight of nacreous liquid protein of *P. maxima* was 29 kDa and the highest was 94 kDa (Bedouet *et al*., 2001), while Mouries *et al*. (2002) found the lowest of 14 kDa and the highest of 94 kDa. Other findings on *P. fucata*were 14.4 kDa and 97.4 kDa (Caiping *et al*., 2005), 12 kDa and 97 kDa (Gong *at al*., 2008), and14 kDa and 100 kDa (Feng *et al*., 2009) for the lowest and the highest values, respectively.In Pinguin's wing oyster (*Pteria penguin*), the lowest value was 20 kDa and the highest was 116 kDa (Naganuma *et al*., 2014).

Table 2. Protease enzyme reactability analysis and calcium carbonate ($CaCO_3$) of pearl oyster shell.

No.	Temperature-salinity interaction treatment	Absorbance	Tyrosin concentration (µg/ml)	Reactability of protease enzyme µmol/mL.min^{-1} (Unit)	Mean content of calcium carbonate of the shell ($CaCO_3$) (%)
1	V (A3B2)	0.687	98.14	0.054	96.66±0.02
2	IX (A2B3)	0.724	103.43	0.057	97.71±0.04
3	VII (A3B1)	0.763	109.0	0.060	97.83±0.03
4	III (A3B3)	0.736	105.14	0.058	97.54±0.04
5	II (A2B2)	0.772	110.29	0.061	98.41±0.02
6	VI (A1B3)	0.502	71.71	0.040	96.23±0.03
7	IV (A2B1)	0.704	100.57	0.056	97.20±0.04
8	VIII (A1B2)	0.494	70.57	0.039	95.61±0.02
9	I (A1B1)	0.529	75.57	0.042	95.43±0.03

Note : I – IX = temperature-salinity interaction treatment.

Table 3. ANOVA of pearl oyster seed survival based on different temperature-salinity interaction treatment.

Sumber Variasi	df	SS	MS	F.$_{calc.}$	F. $_{tab.}$ 0.05	0.01
Factor : A	2	2,147,036.107	1,073,518.054	3.995 *	3.86	6.99
Factor : B	2	1,462,856.773	731,428.387	2.722 ^{ns}	3.86	6.99
Interaction:AB	4	4,796,008.56	1,199,002.14	4.462*	3.63	6.42
Error	9	2,418,595.50	268,732.833	-		
Total	17	10,824,496.94	-	-		

Note : * = significant effect at 95% confidence level; ns = non-significant.

Survival and growth

The survival of 34-day old early spats attaching to the collector and tank wall is shown in Fig. 4. It shows that percent early spats attaching to the collector was partially found the highest in temperature-salinity interaction II (28±0.5°C and 32±1ppt) treatment, 4.1% (K1), 4.62% (K2), and 3.88% (tank wall-D), respectively, and followed by treatment VI (26±0.5°C and 35±1ppt), 3.86%, 3.39%, and 1.82%, respectively, and then treatment III (30±0.5°C and 35±1ppt), 2.82%, 2.38%, and 2.76%, respectively. Percent survival of juvenile phase is cummulatively recorded the highest in temperature-salinity interaction II treatment, 12.6%, followed by treatment VI, 9.07%, and III, 7.96%, respectively (Fig. 5).

ANOVA indicated that temperature-salinity interaction treatment gave significant effect (P<0.05) on the survival of the early spats**.** Single factor of temperature significantly affected their survival, but single factor of salinity did not give significant effect (Table 3). Honest Significant Difference (HSD) test (Table 4) showed that temperature-salinity interaction II (28±0.5°Cand32±1ppt) treatment gave highly significant different survival rate from that of other temperature-salinity interactions. Single temperature treatment resulted in very significant difference between the temperature range of 27.5-28.5°C (28±0.5°C) and that of 25.5-26.5°C (26±0.5°C),

but no difference from that at the temperature range of 29.5-30.5°C (30±0.5°C). High survival of the early spats reared in the media of temperature-salinity interaction II (28±0.5°C and 32±1ppt) treatment could result from environmental condition suitability. As mentioned before, the highest percent larval attachability was recorded at salinity range of 29-32ppt and temperature of 18-26°C, 80% in 4 hours period, while high mortality was found at salinity level of 23ppt (O'Connor*at al.*, 2004). Similar finding was indicated by Tailor *et al.* (2004) *in* Kvingedal *et al.* (2008) that pearl oyster spatsgrew faster at the salinity level of 30ppt in 20 days than those at 25ppt, 34ppt, 40ppt, and 45ppt. This range condition is similar to that for growth and egg hatchability of the bigfin reef squid (*Sepioteuthis lessoniana* Lesson) in which temperature-salinity interaction of 28±0.5°C and 32±1ppt results in higher mantle length growth, 15.15mm, at day-16, with percent survival of 93.14% (Hamzah, 1997). Also,gold-mouth turban (*Turbo chrysostomus*, L.), other member of molluscs, has sufficiently wide temperature variations, 25.5-28.5°C,with mean survivorship of 93.3% (Hamzah, 2015).

Table 4. Honest Significant Difference Test of pearl oysterseed survival based on different temperature-salinity effect.

Single effect of A (Temperature factor)	Single effectof B (Salinity factor)			Main effect of A
	B1	B2	B3	
A1	929 a	1,631.5 ab	1,954 ab	1,504.83 a
A2	1,071.5 a	3,601.5 d	1,162.67 a	2,349 b
A3	2,267 bc	1,675 ab	1,695 ab	1,879 ab
Main Effect of B	1,632.5 a	2,302.67 a	1,797.67 a	
HSD AxB $_{0.01}$ = 1,029.88; HSD A & B $_{0.01}$ = 729.61				

Note: Values with same alphabet in the same column indicate non-significance.

Strong correlation between the reactability of protease enzyme of pearl oyster flesh and shell calcium carbonate ($CaCO_3$) content is presented in Fig. 6, indicating that the higher the protease enzyme reactability of the oyster flesh is, the higher the shell calcium carbonate content is produced.

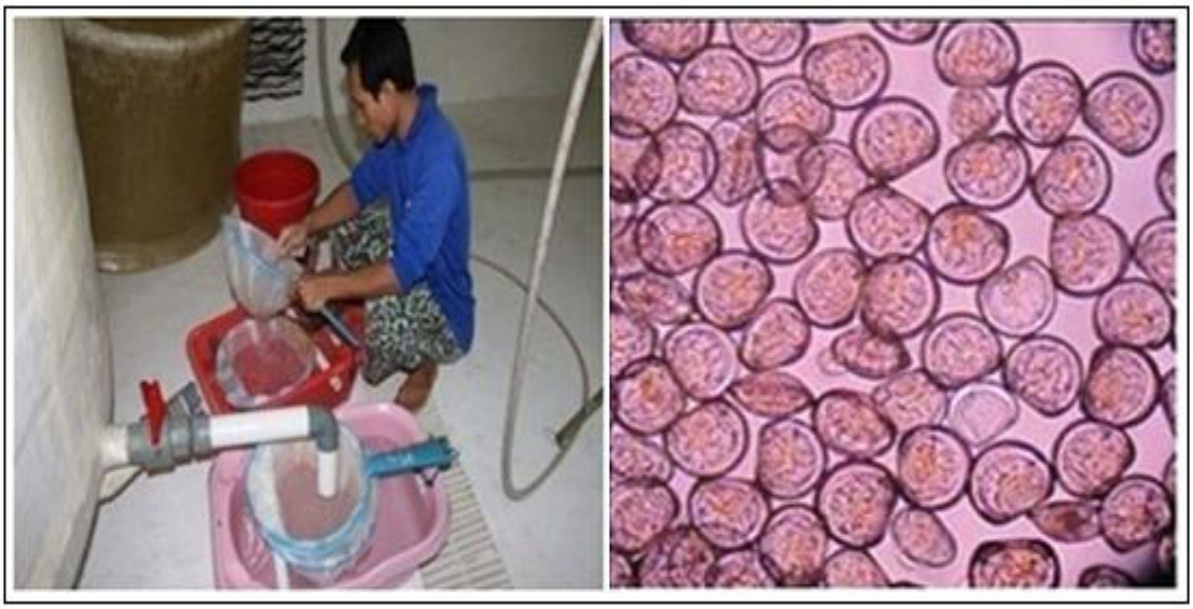


Fig. 1. Filtering method of larvae and morphology of larvae at the *D-veliger* stage after 24 hours of culture.

For instance, the reactability of protease enzyme in temperature-salinity interaction II (28±0.5°C and 32±1ppt) treatment tended to be higher, 0.061µmol/mL-min^{-1}(Unit) and followed by interaction III (30±0.5°C and 35±1ppt) treatment, 0.06 µmol/mL-min^{-1}(Unit) and balanced with high $CaCO_3$ content, 98.41% and 97.83%, respectively (Fig. 7). Determination value (R^2) of 0.713 indicates that percent $CaCO_3$ content was affected by temperature-salinity interaction as much as 71.3%, while the rest 28.7% was other external factor effects unexplain able in the model.

Fig. 2. Experimental tanks wrapped with black plastic shee.

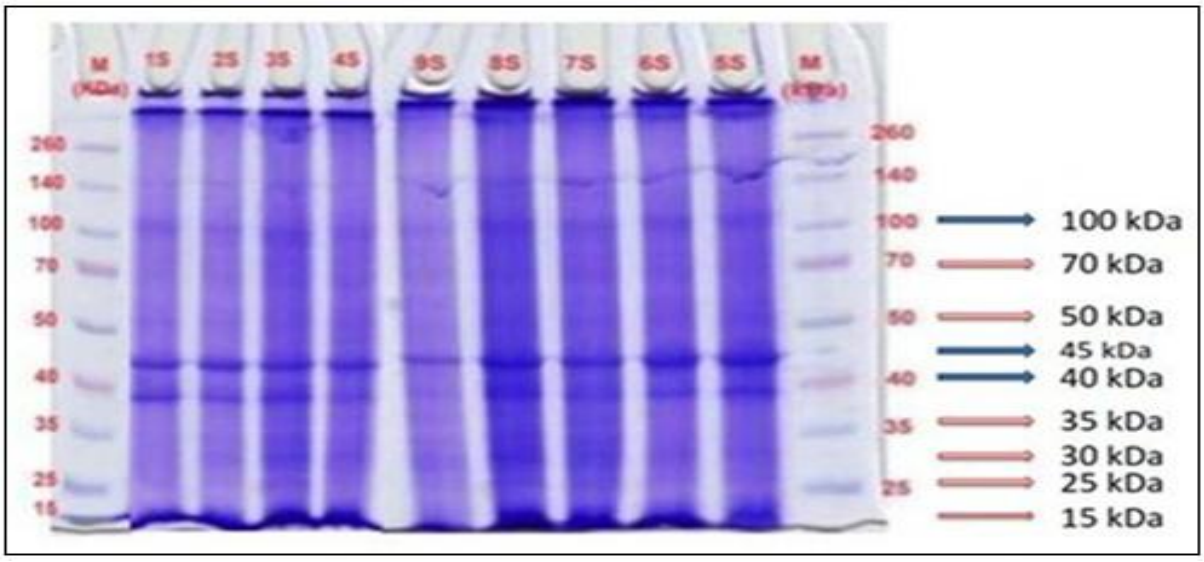


Fig. 3. Protein characterization of pearl oyster (*P. maxima*) seed flesh with *SDS-PAGE* based on temperature-salinity interaction treatment.

Thus, the presence of enough $CaCO_3$ content is highly needed in oyster shell formation. Several previous findings revealed that failure in shell formation of pinguin's wing oyster (*Pteria penguin*) at *D-veliger*phase was largely determined by the availability of protein component used for enzyme metabolism process in nacreous liquid production to produce crystal $CaCO_3$ (Naganuma *et al.*, 2014). Similar argument was also given by Zaremba *et al.* (1996), Weiner and Addadi (1997), Fritz and Morse (1998) *in* Blank *et al.* (2003), Caiping *et al.*(2005), and Feng *et al.* (2009).

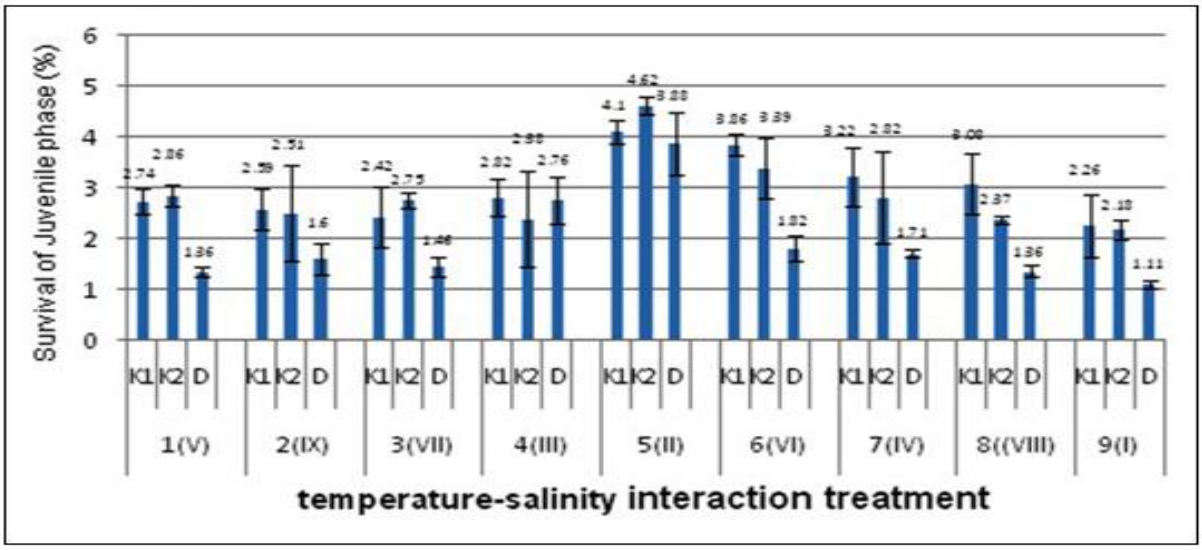


Fig. 4. Survival of 34-day old early spats attaching to the collector (K) and wall (D) based on different temperature-salinity interactions.

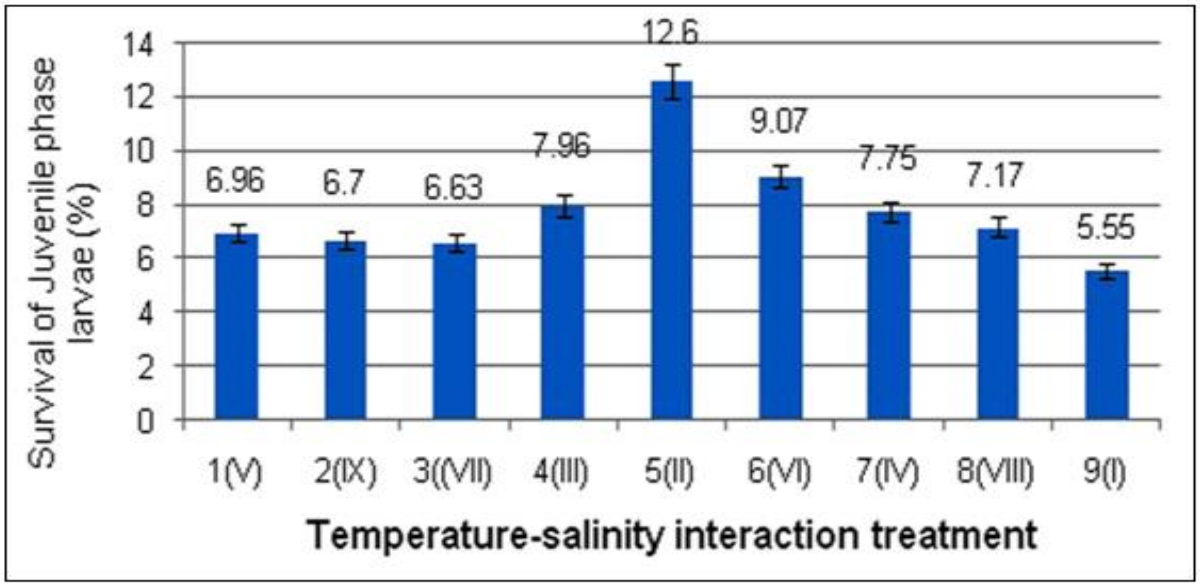


Fig. 5. Percent survival of 34-day old juvenile phase larvae based on different temperature-salinity interaction treatment.

Environmental condition is important in enzyme activity process in protein digestion. As mentioned by Zeng *et al.* (2009) that from several enzymes tested, high reaction was recorded in alcalase enzyme, 4.84±0.98mL^{-1}(Unit/10,000gr) at 50°C and pH of 8.0 and followed by pancreatin enzyme, 4.79±0.89 Unit/10,000gr at 37°C and pH of 8.0. Seed rearing (spat)in the ocean for approximately 4 months based on temperature-saliniity interaction treatment in the laboratory is presented in Fig.8. Spat growth of temperature-salinity interaction II (28±0.5°C and 32±1ppt) treatment in the ocean tended to be faster than other rearing treatments. Percent survival of the pearl oyster seed during the culture reflects that spats of the interaction II treatment has the highest production, 4.82% (2,410 individuals) and followed by the interaction VII (30±0.5°C and 29±1ppt), 4.11% (2,055 individuals) (Fig.9). Hence, it can be inferred that growth and percent survival of treatment II-spat rearing in the sea is enhanced by high reactability of protease enzyme and calcium carbonate content, 0.061 Unit and 98.41±0.02 %compared with that of other treatment outcomes.

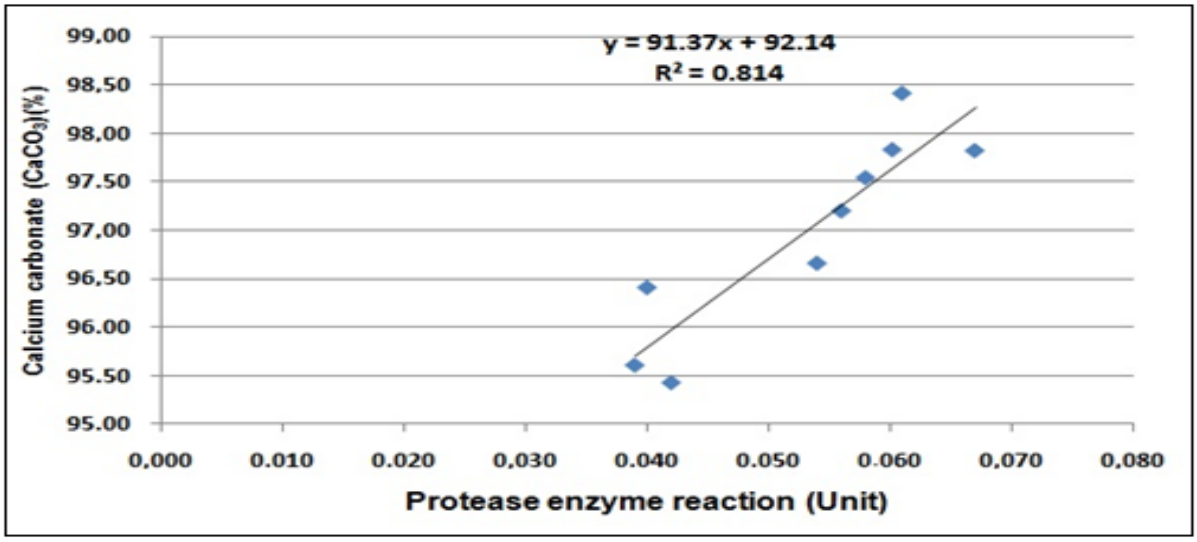


Fig. 6. Relationship between protease enzyme reactability and pearl oyster shell calcium carbonate ($CaCO_3$) content.

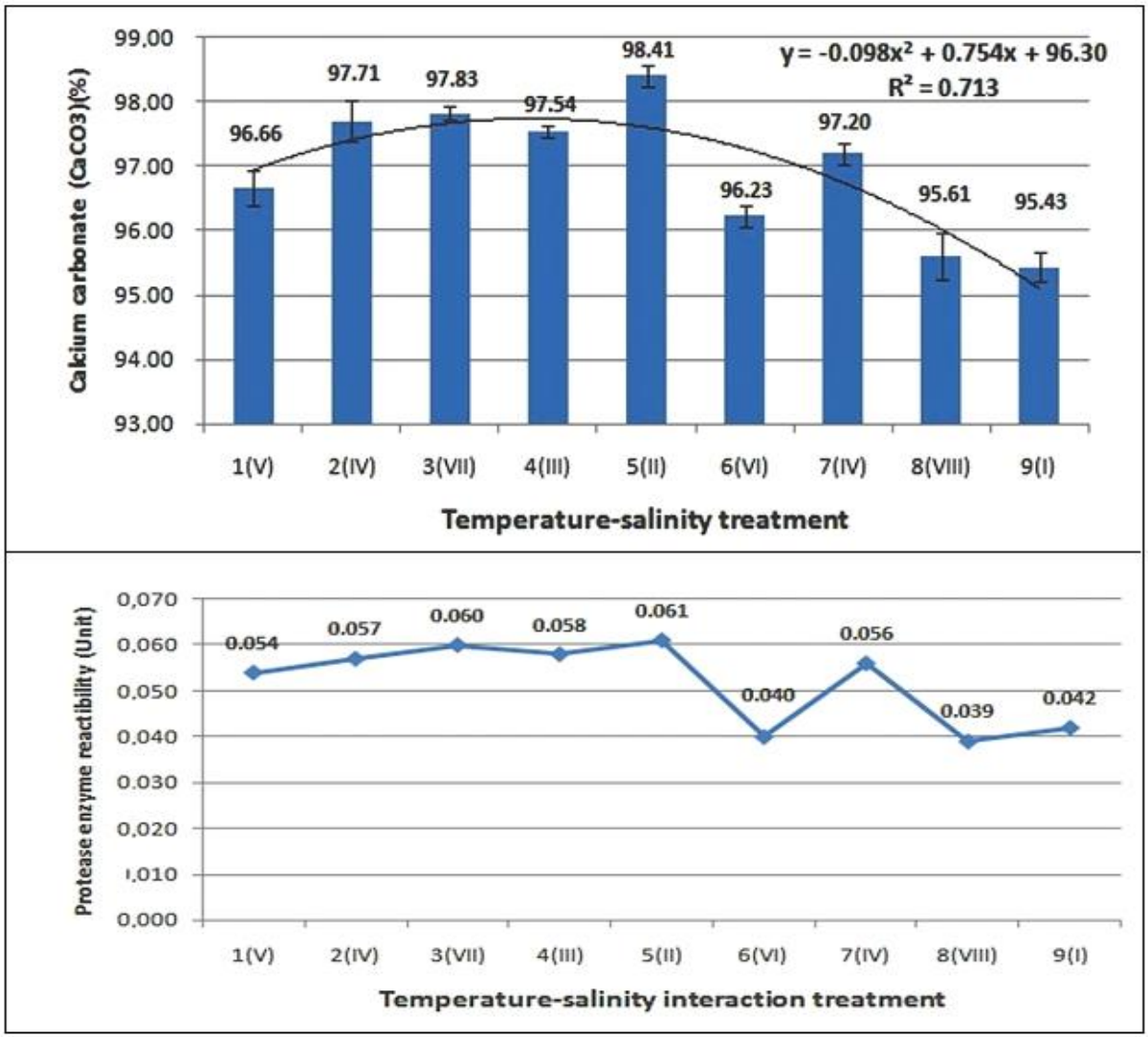


Fig. 7. Calcium carbonate ($CaCO_3$) content of pearl oyster shell (upper) and protease enzyme reactivity (Unit)(lower).

Water quality

Water quality conditions in the tank with planktonic phase (umbo-veliger and pedi-veliger) and spats (attach) during the observation period are shown in Fig. 10. It shows that dissolved oxygen fluctuation in the experimental tank with planktonic phase measured at the water replacement time ranged from 5.5 to 5.7ppm with daily mean concentration of 5.6ppm and at benthic phase, due to aerated, it varied from 5.9 to 6.8ppm with daily mean concentration of 6.52ppm. Dissolved oxygen concentrations during the observation period were still in the tolerable range of the pearl oyster larvae.

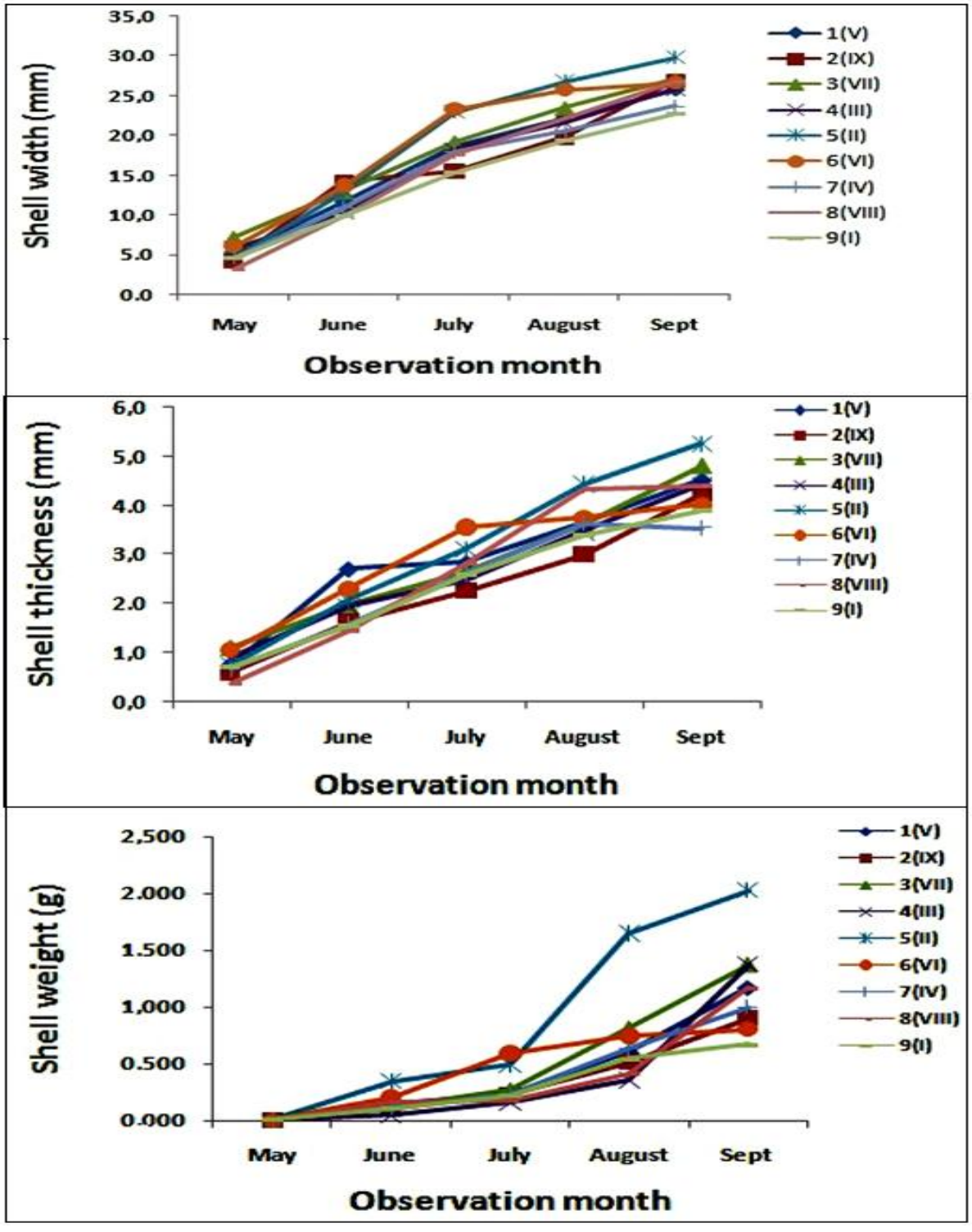


Fig. 8. Growth of laboratory-originated pearl oyster seed at day-120 in the sea.

It is different from that suggested by Effendi (2003) *in* Litaay (2011) that pH and DO for the oysters ranged from 7-8 and 7,5-7,8mg/L, respectively. Meanwhile, Sapii (2007) found that laboratory culture of pearl oyster larvae was still good enough under dissolved oxygen condition between 4.5-5.0ppm. Hamzah (2013) found that water quality range was still suitable for laboratory culture of pearl oyster larvae, 7.3 – 7.8, 26.5-28.0°C and 32-33ppt, for pH, temperature, and salinity, respectively. Pearl oyster seed culture in water temperature of 27-78°C and pH of 7.6 – 8.3 resulted in the highest survival rate, 83.3% (Hamzah and Setiyono, 2009). Water acidity (pH) value during the study period was still in normal range according to seawater quality standard, between 6.5-8.5 (KMNLH, 2004).

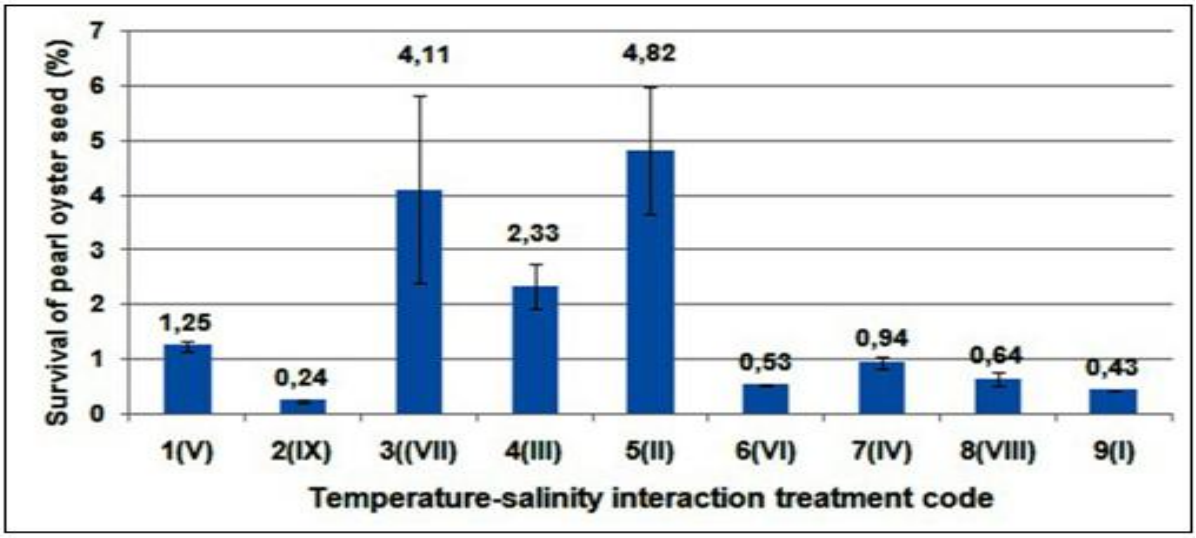


Fig. 9. Percent survival of pearl oyster seed rearing at day-120 in the sea based on temperature-salinity interaction treatment**.**

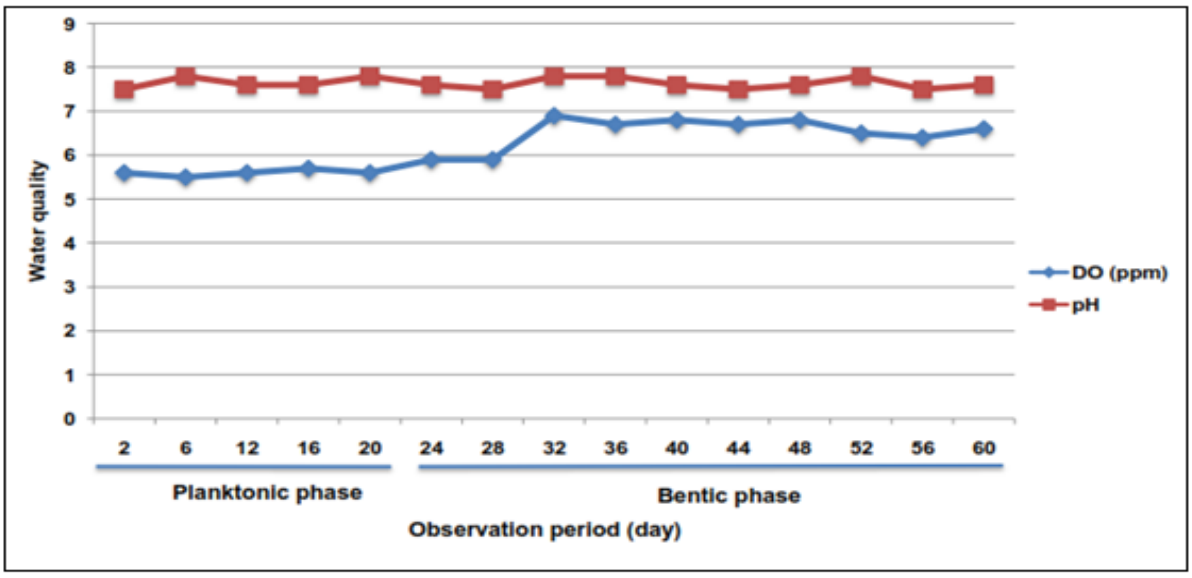


Fig. 10. Water quality for planktonic and benthic phase larvae rearing.

Conclusion

Percent survival of larvae and spats cultured in the media of temperature-salinity interaction II (28±0.5°Cand32±1ppt) treatment was found the highest, 12.6% and 5.84%, respectively, with mean growth rate of 6.29 mm shell width/mo., 1.13 mm thick/mo., and body weight of 0.52 g/mo. The growth rate of spats cultured at temperature-salinity

interaction II treatment media was enhanced by higher reactabilty of protease enzyme and $CaCO_3$ content, 0.061µmol/mL-min^{-1}(Unit) and 98.41±0.02%, than those of other interaction treatments.Protease enzyme reaction and calcium carbonate ($CaCO_3$) content of the oyster shell had strong correlation (R^2 =0,814).Thus, to obtain maximum quality and quantity of pearl oyster production, the pearl oyster (*P. maxima*) farming developers who possess hatchery laboratory producing spat collectors should do further marine rearing at the range of 34-40 days old.

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