Relationship Between Stage of Gonad Maturity and Level of Osmotic Work of Sea Cucumber, *Paracaudina australis*

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Abstract

The holothuroid spawning can be succesful depend on varoius factors such as salinity, temperature, primary productivity and other physiological adaptation toward environment influences. The salinity fluctuation is one of the environment factors, which influence and can cause various osmotic pressure of coelomic fluid of Holothoroid and also gonad maturity stage. Holothuroid (sea cucumber), Paracaudina australis, is overexploited in Kenjeran waters, Surabaya, East Java. The study was aimed to reveal relationship between gonad maturity stage and level of osmotic work of sea cucumber P. australis. Fifty samples of P. australis were collected monthly. Coelomic fluid samples of approximately 200–1000 µl were obtained from all samples using disposable insulin syringes. There were 37 holothuroids classified in the late maturity (stage 5), 74 holothuroid in the earlier maturity stage (stage 1). Gonad maturity stage 5 (late maturity) could be reached on December 2016. Contrasly, the gonad maturity stage 1 (earlier maturity) was found on August 2016. The analysis of coelomic fluid showed that the average value of level of osmotic work ranged 70 to 91 mOsm/L H_2O in the earlier gonad maturity stage. Contrasly, in the late maturity, the average value of level of osmotic work ranged 118 to 156 mOsm/L H_2O. There is relationship between gonad maturation stage and level of osmotic work. From stage of gonad maturity 1 to 5, there were upward trend level of osmotic work in coelomic fluid of P. australis.

Keywords*: gonad maturity, osmotic, Paracaudina australis*

Introduction

Sea cucumber *Paracaudina australis* is belong to Phylum Echinodermata, Class Holothuroidea, Ordo Molpadida, Family Caudinidae, Genera Paracaudina (Pawson, 1970; Pawson, 2007; O'Loughlin *et al.*, 2011). *P. australis* lives in mud-sandy habitat and has wide distribution patern from temperate to tropical area (O'Loughlin *et al*., 2011). There are 7 sea cucumber species in the Eastern Part of Surabaya Coastal Water, namely *Phyllophorus* sp., *P. australis, Colochirus quadrangularis, Holothuria sp., H. sanctori, H. forskali*, *H. turriscelsa* (Winarni *et al*., 2015). There are two species (*Phylloporus* sp. and *P. australis*) that well known as the material of creaker snack.

Ecologically all sea cucumbers have important role due to their bioturbation activities and benthic ecosystem (Mangion *et al*., 2004). Over exploitation of sea cucumber affecting ecosystem, especially in benthic ecosytem. Decreasing stock of the sea cucumber population was caused by large demand for food and medicine purpose in Asian market (Toray *et al*., 2008; Purcell, 2010). The condition of population sea cucumber *P. australis* from Kenjeran waters of Surabaya has been over exploited due to the daily activity of fishermen.

Recent studies of *P. autralis* mainly covered on taxonomical and habitat, *e.g*. O'Loughlin *et al*. (2011) and Winarni *et al*. (2015). Research on gonad maturity stage and level of osmotic work is lacking. Osmotic pressure of coelomic fluid in holothurid is influenced by water quality such as salinity, freshwater from river, temperature, *etc*. Most of the recent studies showed that echinoderms become isosmotic with the ambient water by exchanging of water and ions in their coelomic fluid, and altering the concentrations of intracellular ions which play an important role in metabolic processes and affect enzymes of metabolism intermediary (Yancey *et al*., 1982; Diehl, 1986; Stickle and Diehl, 1987). The research reveals relationships between gonad maturity stage and level of osmotic work on sea cucumber *P. australis*.

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Material and Methods

Location of study and sampling

This study was conducted in Kenjeran waters, Surabaya, East Java - Indonesia. *P. australis* were collected at the same day of data collection. On each sampling, 50 individuals *P. australis* were randomly collected by mini trawl. Their coelomic fluid (200-1000 µl) were then taken carefully from those 50 samples. using a disposable insulin syringe.

Water samples were collected from the study area to determine phosphate, nitrate, nitrite and amonia concentrations. Water quality checker was used to measure salinity, temperature, pH and depth. All water quality data were measured monthly from August to October 2016. Coelomic fluid and water samples were stored in the iced cool box and immediately brought to laboratory and they were frozen to -20°C until analysis and measuring the osmolality. Osmolality was determined using Automatic Micro Osmometer Roebling (Anggoro and Nakamura, 1996)

Analyses

The level of osmotic work was calculated based on difference between osmotic pressure of coelomic fluid in *P. australis* and osmotic pressure of medium. The measurement of osmotic pressure used Automatic Micro Osmometer Roebling (Anggoro and Nakamura, 1996), as follows:

LOW = (P. Osm Coelomic fluid – P.osm medium)

Where:
LOW = Level of Osmotic Work (mOsm.L^{-1} H_2O)
P. osm Coelomic fluid = osmotic pressure of coelomic fluid (mOsm.L^{-1} H_2O)
P.osm media = osmotic pressure of media (mOsm.L^{-1} H_2O).

Maturity stages were determined according to physical characteristics following Conand (1981) and Shiell and Uthicke (2005) with modification, as follows: stage 1 (resting or indeterminate tubule); stage 2 (growing tubule), stage 3 (is immaturing tubule), stage of 4 (matured tubule) and stage 5 (late matured and partly spawned tubule).

Results and Discussion

Gonad maturity stage

The results showed that on August 2016 the dominant stage of the gonad in *P. australis* is stage 1, *i.e.* 15 females and 16 males (Figure 1). This condition indicated that gonad of most population of *P. australis* in Kenjeran waters was still growing in August to November 2016. While in December 2016, the gonad maturity stage 5 indicated the highest number of individuals (17 individuals) than with the others gonad maturity levels (1, 2, 3 and 4).

In September 2016, the number of female with stage 2 gonad maturity was 12 individuals, while stage 3 was 12 individuals. Figure 1 also showed that the number of male with the gonad maturity stage 1, 2 and 5 was 1, 3 and 3 individuals, respectively. The number of the male reached gonad marutity stage 1 has found 15 individuals. Whereas, the female reached gonad maturity stage 2 has found 11 individuals. Therefore, the gonad maturity stage 3, 4 and 5 had small number of individual (Figure 1.). The number of the female and male reached gonad maturity stage 2 had a number of individual 11 and 8. Contrasly, for stage of gonad maturity 1, 3, 4 and 5 had small a number of individual. In the Figure 1 showed that on August, September, October and November 2016 has the same pattern of gonad maturity stage except for the December. In December 2016, male reached gonad maturity stage 5 had 17 individuals. Whereas, for the stage of gonad maturity 1, 2, 3 and 4 had small number of individual.

The water quality measurements showed the average value of temperature was 29.82±0.201ºC, salinity was 29.74±0.352 ppt, DO value was 3.93± 0.368 ppm, value of pH was 7.22±0.097, value of phosphate was 0.09±0.054 ppm and value of nitrate was 0.64±0.172 ppm (Table 1.). The value of salinity, temperature and pH were fit with Andriyono *et al*. (2016). The salinity fluctuations may have significant effects on internal osmotic concentration in internal sea cucumber (Andriyono *et al*., 2016). Decreasing in ambient salinity medium could change the osmotic pressure of the coelomic fluid and could become stabilize again within 6 hours after the salinity changed (Meng *et al*., 2011).

The differences in water quality parameters such as temperature, nutrient, and salinity (Table 1.) can cause differences spawning time for *P. australis*. According to the sampling date, *P. australis* samples were collected at the new moon for August, September, October and November. Whereas, in the December 2016, *Paracaudina australis* samples were collected at the full moon. This does not fit with the researchs that showed most of the sea cucumber such as *Holuthuria spinifera, Holothuria scabra* and *Cucumaria frondosa* have spawing in the new moon (Conand, 1993; Hamel and Mercier, 1995; Asha and Muthiah, 2005). In the temperate region, most of sea cucumber have spawning time in spring and summer (Hamer *et al*., 1993), while in

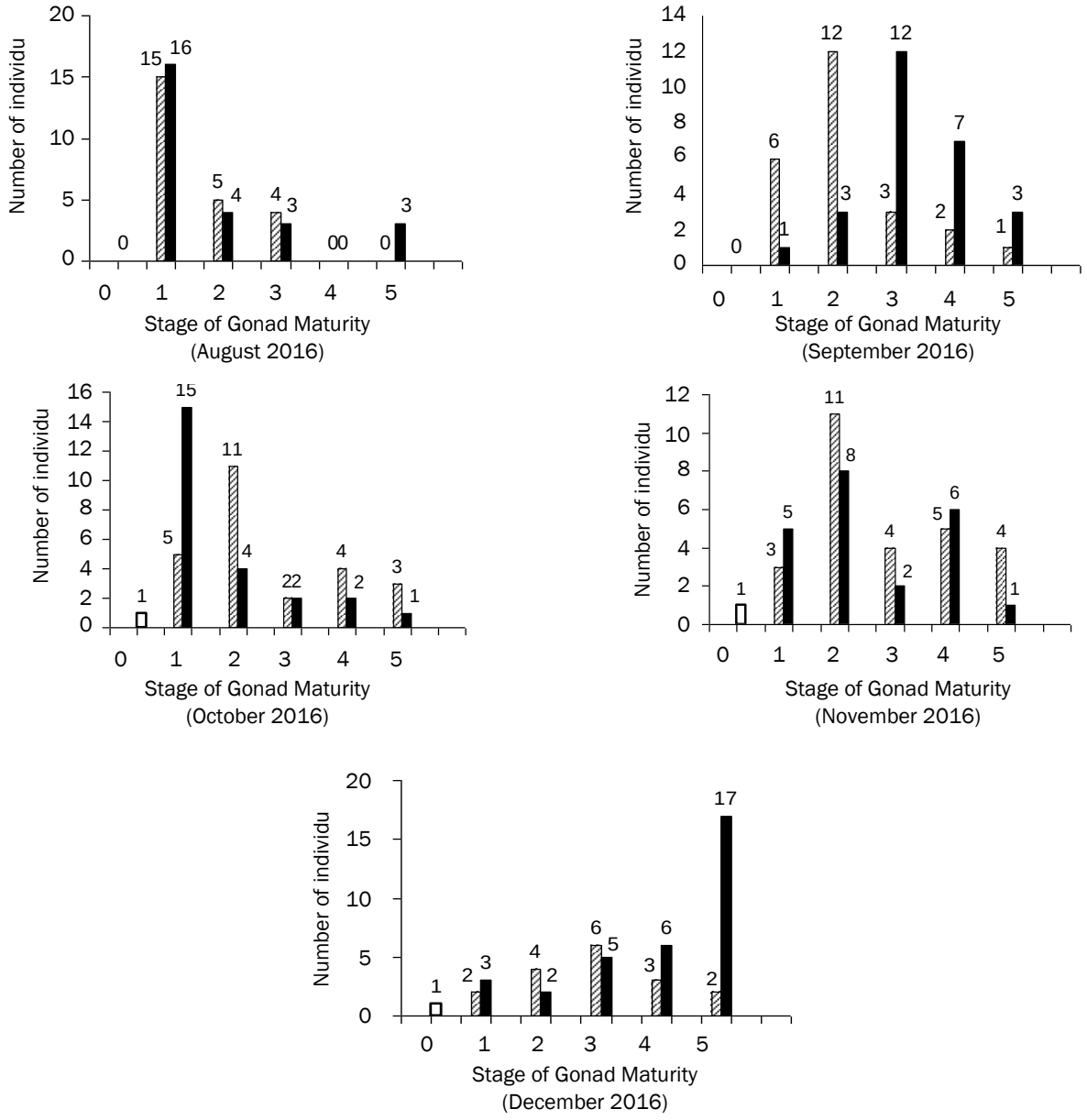

Figure 1. The number of individual *Paracaudina australis* on gonad maturity stage
Note : ■ = Male, ▨ = Female, □ = Undentified

the tropical region, most of sea cucumber have spawing time throughout the year (Conand, 1993).

Relationship between gonad maturity stage and level of osmotic work

Most of sea cucumber (Holothuroid) are unique marine invertebrate, being huge considered as osmoconformer and stenohaline, those animal has not tolerate wide variations in sea water salinity or they can not survive. The value of osmotic work *Holothuria grisea* were exposed to the air under sunshine was the biggest than exposed to the air under cloudy weather and under rain (Vidolin *et al*., 2002).

Osmolality pressure on the coelomic fluid of *P. australis* were various value and difference each other depend on gonad maturity stage. Osmotic pressure in the coelomic fluid of *P. australis* is higher than medium. Most of holothurids conduct water absoption mechanism if the concentration ion in the sea water is lower than in the body mass of holothuroid and thus, the body of holothuroid will become bigger for keeping isotonik in their body (Vidolin *et al*., 2002; 2007).

According the research result, in August till December 2016, there were the sama parttern of graph (Figure 2.). The value of level of osmotic work in female was higher than male. It is mean that female was more necessary energy than male for physiology adaptation process at every stage of gonad maturity. From gonad maturity stage 1 till 5, the level of osmotic work increase moderately from August to December 2016 (Figure 2.).

Coefficient regression value between stage of gonad maturity and level of osmotic work for all months of observation is close to 1 and is positive. this means the relationship between gonad maturity level and level of osmotic work was positive (Table 2). The greater the maturity level of the gonads at *P. australis* and the higher the osmotic work rate. This also means the higher energy required by sea cucumbers in physiological adaptations for gonadal development.

Based on the observation, the coefficient regression value between gonad maturity stage and osmotic work level were greater than 0.9. This indicates that there is a positive correlation between stage of gonad maturity and osmotic work level. (Table 2.).

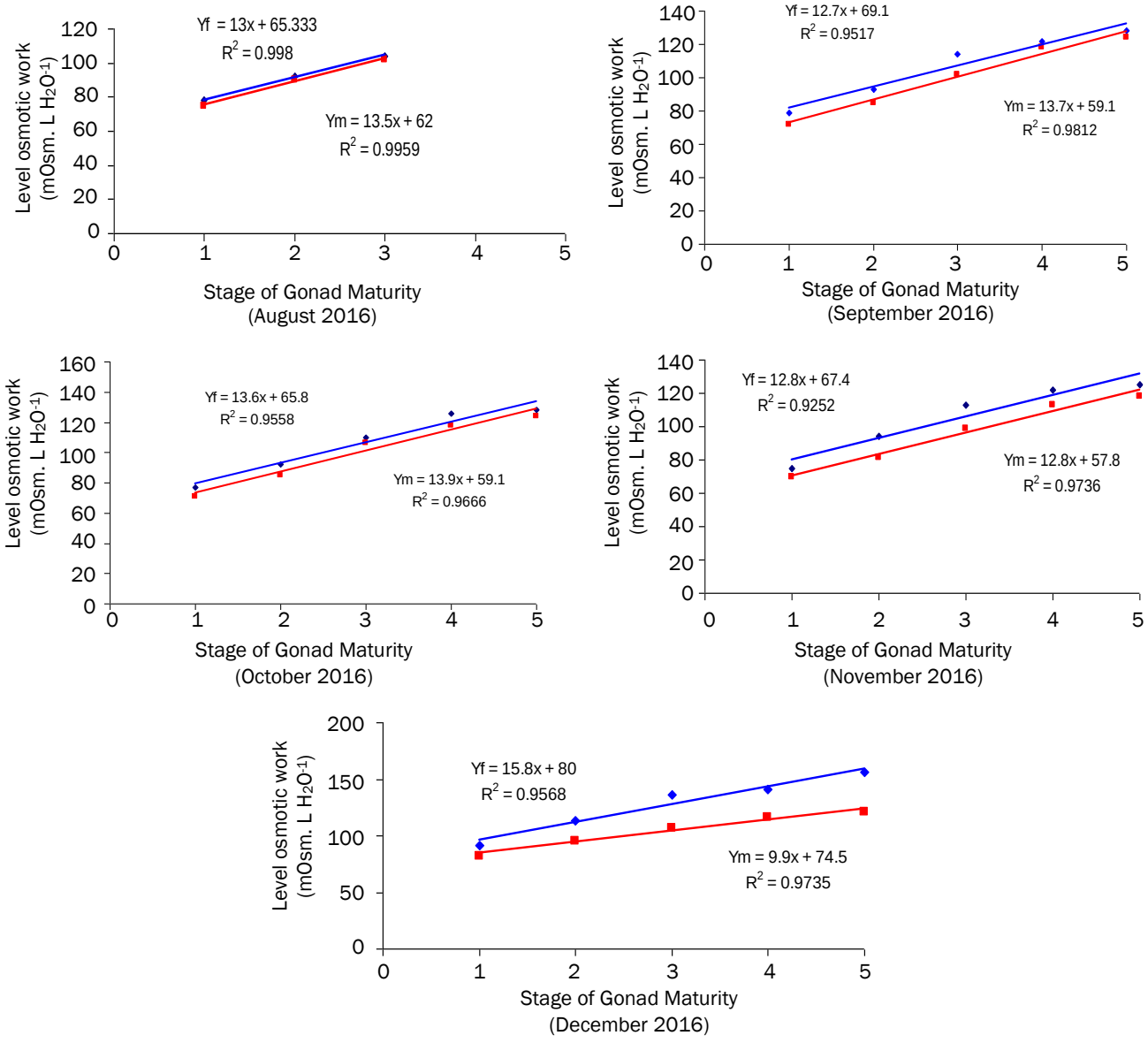

Figure 2. The level of osmotic work of coelomic fluid *P. australis* on gonad maturity stage
Note. ◆ = Female, ■ = male

Based on the observation, the coefficient regression value between gonad maturity stage and osmotic work level were greater than 0.9. This indicates that there is a positive correlation between stage of gonad maturity and osmotic work level. (Table 2). Research on coefficient of regression between stage of gonad maturity and osmotic work level on sea cucumber is still rare. However, studies of isosmotic media in other invertebrates have been conducted as the results of Anggoro and Muryati (2007) showed that salinity of the osmotic medium significantly affected Ca-chorionase enzyme activity, energy efficiency and efficiency of egg hatching *Metapenaus elegans.* Another research showed that salinity fluctuation greatly affected the development of male gonads crab *Eriocheir sinensis*, increasing osmolarity and ionic concentration in hemolymph (Long *et al*., 2017).

Table 1. The average value of water quality on Kenjeran Water, Surabaya from August to December 2016.

No.	Item	Value
1.	Temperature (℃)	29.82±0.201
2.	Salinity (ppt)	29.74±0.352
3.	DO (ppm)	3.93±0.368
4.	pH	7.22±0.097
5.	Light Penetration (cm)	45.97±11.751
6.	Depth (m)	3.15±1.359
7.	Phosphate (ppm)	0.09±0.054
8.	Nitrite (ppm)	0.03±0.016
9.	Nitrate (ppm)	0.64±0.172
10.	Amoniak (ppm)	0.04±0.027

Table 2. The value coefission of regression between stage of gonad maturity and level of osmotic work of coelomic fluid *P. australis*.

Month	Female	Male
August	0.998	0.996
September	0.952	0.981
October	0.956	0.967
November	0.925	0.974
December	0.957	0.974

Conclusion

There were high positive correlation and relationship between gonad maturity stage and level of osmotic work rate of coelomic fluid *Paracaudina australis*. The higher value of stage of gonad maturity, the higher value of level of osmotic work for *P. australis*. According to regression analysis, from August to December 2016, there were regression value between stage of gonad maturity and level of osmotic work rate had range 0.952–0.998.

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