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The Effect of Different Diet of Phytoplankton Cells on Growth Performance of Copepod, *Oithona* sp. in Semi-mass Culture

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Abstract

The effect of different diet of phytoplankton Cells on the growth performance of copepod, *Oithona* sp.in semi mass culture was examined in this research. This research used *Chaetoceros calcitrans*., *Chlorella vulgaris*., *Nannochloropsis oculata* and *Isochrysis galbana* as diet to known the *Oithona* sp. growth performance. This research was designed by using Completely Randomized Design (CRD) with four treatments and three replicates. Those treatments were **A.** the culture was added with *Chaetoceros calcitrans* cells diet, **B.** with *Chlorella vulgaris*.cells diet, **C.** with *Nannochloropsis oculata*. cells diet, and **D.** with *Isochrysis galbana*. cells diet, respectively. The results showed that the different diet of phytoplankton cells were highly significantly difference ($P < 0,01$) on *Oithona* sp. growth performance. The diet of *Chaetoceros calcitrans* cell gave the best performance of *Oithona* sp. growth, where reached (6.963 ± 0.38) ind · mL^{-1} of total density, (0.121 ± 0.003) ind · d^{-1} of specific growth rate, and eggs production of (16.50 ± 2.74) eggs · ind^{-1}.

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Keywords: Different diet; growth; *Oithona* sp.; phytoplankton cells; semi-mass culture

1. Introduction

As live food organism, *Oithona* sp. can be used as feed intermediate between rotifer and *Artemia*, or as a substitution of *Artemia*, but until recently it's existence has not been utilized optimally. Whereas Calcium content of *Oithona* sp higher than *Artemia* (Kusmiyati et al., 2000). The content of EPA and DHA is also higher than *Artemia*

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and rotifer. The high content of EPA/DHA will be helpful for growth improvement, the survival rate and reduce the occurrence of abnormality on shrimp larvae. *Oithona* sp. contain substances imunostimulant, attractant and some important digestive enzyme. Given the importance of *Oithona* sp. as a substitute for *Artemia* in the shrimp hatchery, hence the availability of *Oithona* sp. were sustainable was important.

Many research have done to show the effect of *Oithona* sp. compared with *Artemia* dan rotifer (*Brachionus* sp.) for marine fish fry. Many of them showed the increasing of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) composition on *Cromileptes altivelis* (Valenciennes, 1828) (Aliah et al., 2010), survival rate on *Hippocampus kuda* (Bleeker, 1852) (Redjeki, 2007), growth on *Chanos chanos* (Forsskål, 1775) [Raj et al., 2003] and growth and survival rate of *Lates calcalifer* (Santhanam and Perumal, 2012). There were still little information to increase *Oithona* sp. culture for aquaculture activity. According to Santhanam and Perumal (2012) and Vasudevan et al. (2013) there were still no ideal phytoplankton diets to culture *Oithona* sp. in semi-mass condition.

The phytoplankton *Chaetoceros calcitrans* (Ehrenberg, 1844) , *Chlorella vulgaris* Beyerinck (Beijerinck, 1890), *Nannochloropsis oculata* (Droop) (Hibberd, 1981) and *Isochrysis galbana* (Parker, 1949) were choosed for the feed to *Oithona* sp. culture in semi-mass condition. Many of them already well developed on mass culture, generally available on marine hatchery, and already used in *Oithona* sp. culture as mixed phytoplankton (Molejo'n and Alvarez, 2003; Santhanam and Perumal, 2012a; Zamora et al., 2014). The different phytoplankton was expected to give an effect for *Oithona* sp. growth performance. The most suitable single cell phytoplankton will show the best *Oithona* sp. growth performance (Kleppel, 1993). The purpose of this research was to know the effect of different diet of phytoplankton on growth performance of *Oithona* sp. and the kind of phytoplankton that give the best growth performance of *Oithona* sp.

2. Material and methods

This research conducted at BBPBAP - Laboratory of Brackish Water Aquaculture Development Research Center (BWADRC), Jepara, Central Java, Indonesia in 2015 by using Completely Random Design (CRD) with four treatments by using Completely Random Design (CRD) with four treatments. That treatment were : A. culture *Oithona* sp. by using phytoplankton, *C. calcitrans* ; B. culture *Oithona* sp. by using phytoplankton, *C. vulgaris*; C. culture *Oithona* sp. by using phytoplankton, *N. oculata* ; and D. culture *Oithona* sp. by using phytoplankton, *I. galbana*. Dry weight was used as the base in the calculation of the number of cells used in each treatment. The dry weight of each individual algae was 11.3 pg · $cell^{-1}$ to *C. calcitrans* [Lavens and Sorgeloos, 1996], 12 pg · $cell^{-1}$ to C. *vulgaris* [Lee *et al*., 2006], 6.1 pg · $cell^{-1}$ to *N. oculata* and 25 pg · $cell^{-1}$ to *I. galbana* [Lee et al., 2006].

2.1. Phytoplankton culture

C. calcitrans, C. vulgaris, *N. oculata* and *I. galbana* were got from Life Food Laboratory, BBPBAP Jepara. These phytoplankton were cultivated in that laboratory on Modificated Walne Medium, using filtered sea water sterilized by boiled. The cultures was incubated at 25 ºC to 28 ºC temperature, 24 ‰ to 34 ‰ salinity, pH 8 to pH 9, with a 24 h light photoperiod and at 1 500 lx to 1 800 lx .

Phytoplankton cultured in sterile erlenmeyer flask volume 6 L. Seawater sterilized through a membrane filter then accommodated in drum-sized 50 L. Sea water was then added with a solution of Natrium Hypochlorite (NaClO) 60 mg · L^{-1} for 10 min to 30 min. The declorination process then performed through the addition of NatriumTiosulfate solutions (NaS_2O_3) 80 mg · L^{-1} accompanied by aeration for 24 h. Declorination process results then filtered by planktonet 5 μm and autoclaved (1 atm, 121 ºF [1 atm = 101 325 Pa]) before it was ready to use. Phytoplankton culture used Modification Walne Medium with a dose of 0.5 mL for every 1 L sea water.

There was a difference between main chemical concentration Modification Walne Medium for culture of *C. vulgaris* and *N. oculata*, and Modification Walne Medium for culture *I. galbana* and *C. calcitrans*. The main concentration of chemical Modification Walne Medium for culture of *C. vulgaris* and *N. ouculata* used NH_4NO_3. Instead, culture of *I. galbana* and *C. calcitrans* used NaNO3. Vitamin B_{12} with dosage of 0.5 mL to 1 L sea water was added in all cultures microalgae. Silicate (Na_2SIO_3) with the same dosage also added to the culture of *I. galbana* and *C. calcitrans*.

Volume of inoculan phytoplankton was 10 % of the volume of media culture Lee et al. (1996). Harvesting algae

to feed *Oithona* sp. was conducted at the time of the exponential phase because they contain high nutrients (Aliah et al., 2010; Creswell, 2010). Stock density of microalgae (sel · mL^{-1}) was calculated each day by taking a sample of the microalgae and then counted under a microscope (Olympus CH20) 10 × magnification with a haemocytometer (Improved Neaubouer volume 0.0025 mm^3).

Observation of Bacterial Contaminant Population in Phytoplankton culture was done to know the relationship between the population bacterial contaminant and phytoplankton cells. Sample was taken from medium cultured of Phytoplankton and then inoculated on the agarplate bacterial medium (Zobbel's 2116E). Those agarplate were incubated at 25 °C during 5 d.

2.2. *Oithona* sp. *semi-mass culture*

Oithona sp were got from BBPBL [Life Food Laboratory, Marine Water Aquaculture Development Research Center (MWADRC)] , Lampung, Indonesia. A semi-mass culture of *Oithona* sp. was done as long as 15 d in 2 L plastic jars filled with sterilized sea water (30 ‰), under a 24 h light photoperiod and with gentle aeration. Initial density of *Oithona* sp. was 1 ind · mL^{-1} and consisting of many stages of *Oithona* sp. The 50 % of culture media was changed daily by fresh medium contain phyitoplankton for each treatment. The quantity of phytoplankton was (2 to 4) × 10^6 ind · mL^{-1} for *C. vulgaris* and *N. oculata* (1 to 2) × 10^6 ind · mL^{-1} for *I. galbana*. and *C. calcitrans* respectively.

The feeding method used *ad libitum*. The giving of phytoplankton was according to the dry weight of the phytoplankton and the number of *Oithona* sp. Every day was kept in dry biomass microalgae as much as 0.01 mg for each individual copepods (Lee et al., 2006). The formula of calculation of microalgae cells number was as follows:

$$\text{The number of Microalgae (cells)} = \frac{\text{the weight of feed (mg)}}{\text{dry weight microalgae (mg} \cdot \text{cell}^{-1})} \quad (1)$$

The density of *Oithona* sp have been counted once every 4 d during the 16-d observations. 5 mL to 10 mL sub-sample was taken from the culture medium to calculated the number of total *Oithona* sp, population growth rate (r) and the number of eggs.

Population growth rate (r) using the latest data of sampling activity. Population growth rate was counted using Krebs (1985) formulation that used by Cheng et al. (2011) :

$$r = \frac{\ln Nt - \ln N0}{t} \quad (2)$$

Where, t is the long of culture day to reach Nt (d), N0 and Nt was initial and the last density of *Oithona* sp. (ind · mL^{-1}).

Egg production was calculated by using modificated egg production rate formula from Zamora-Terol et al. (2014) below:

$$\text{Egg production} = \frac{\sum s \times e}{\sum n} \quad (3)$$

Where, s is egg sac; e is average number of egg in one sac; and n is number of *ovigerous* female (ind).

2.1 Statiscal analysis

Data was analized by one way Analysis of Variance (ANOVA) to determine the effect of different phytoplankton on growth performance of *Oithona* sp. culture in semi-mass condition. Least Significant Different (LSD) test (α = 0.01) by using SPSS 16 was conducted when the treatment had significantly effect. All statistical analysis were

conducted using SPSS program Ver. 16.

3. Result and discussions

3.1. Results

The different diet of phytoplankton cells gave a different effect on growth performance of *Oithona* sp. *C. calcitrans*. and *I. galbana*. Eventhough it were not high significantly different ($p > 0.01$) Only on, *Chaetoceros calcitrans* showed the best result on growth performance of *Oithona* sp. (Table 1). The small result of *Oithona* sp. growth performance was showed not only by *N. oculata*, but also by *C. vulgaris*. *Oithona* sp. fed by *Chlorella vulgaris,* it had relatively higher number of total density and egg production, however, it was not high significantly different ($p > 0.01$) with *N. oculata*. Total density was the total number of Nauplies, Copepodits and adult copepods, including adult with eggs (Fig. 1). Maximum total density was found in *C. calcitrans*, who high significantly different ($p < 0.01$) with another treatmens that showed the low population growth rate (Fig. 2) and zero egg production on the last day of the culture. The low growth rate was produced by phytoplankton that had a low egg production and also a low total density (Fig. 3). The growth of *Oithona* sp. closely related with the population of bacterial contamination in the phytoplankton cells culture. The higher population of bacterial contamination in phytoplankton culture thus the lowers the quantity of *Oithona* sp. (Table 2).

Table 1. Growth performance of *Oithona* sp. fed the different phytoplankton in semi-mass condition

Diets	Density (ind · mL^{-1})	Population growth rate (d^{-1})	Eggs production (eggs · ind^{-1})
Chaetoceros calcitrans	6.96 ± 0.38^{a}	0.121 ± 0.003^{a}	16.50 ± 2.74
Chlorella vulgaris	1.13 ± 0.30^{b}	0.005 ± 0.19^{b}	9.08 ± 0.15
Nannochloropsis oculata	1.08 ± 0.09^{b}	0.005 ± 0.004^{b}	0.00 ± 0.00
Isochrysis galbana	6.28 ± 0.25^{a}	0.115 ± 0.003^{a}	15.76 ± 1.55

[a,b] Values (Mean ± S.E.) within a column with different superscript letters were highly significantly effect ($P < 0.01$).

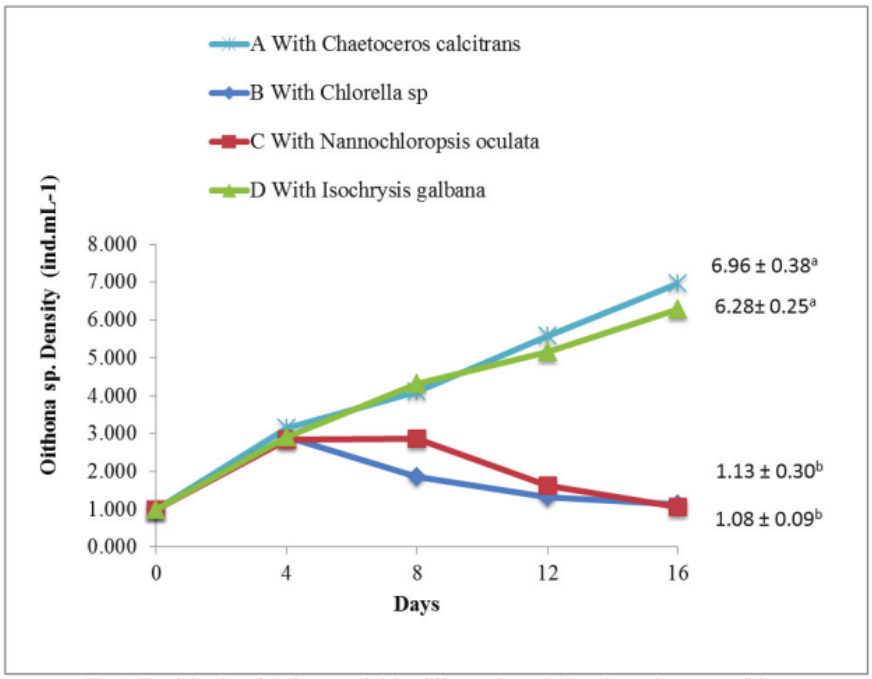

Fig. 1. Total density of *Oithona* sp. fed the different phytoplankton in semi-mass condition.

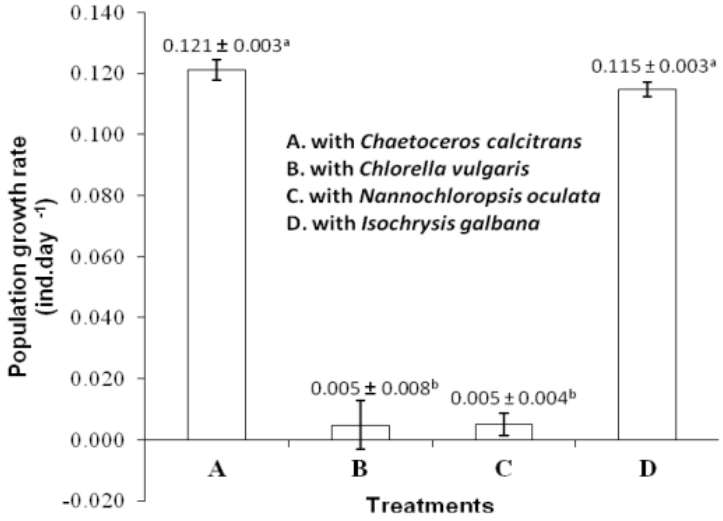

Fig. 2. Population growth rate of *Oithona* sp. fed the different phytoplankton in semi-mass condition

Tabel 2. Population of bacterial contamination in the phytoplankton culture

Diets	Density of bacterial contaminan (cfu) *)
Chaetoceros calcitrans	6.85 ± 0.80
Chlorella vulgaris	7.51 ± 0.94
Nannochloropsis oculata	7.48 ± 0.75
Isochrysis galbana	6.40 ± 0.68

*Value = mean ± SD

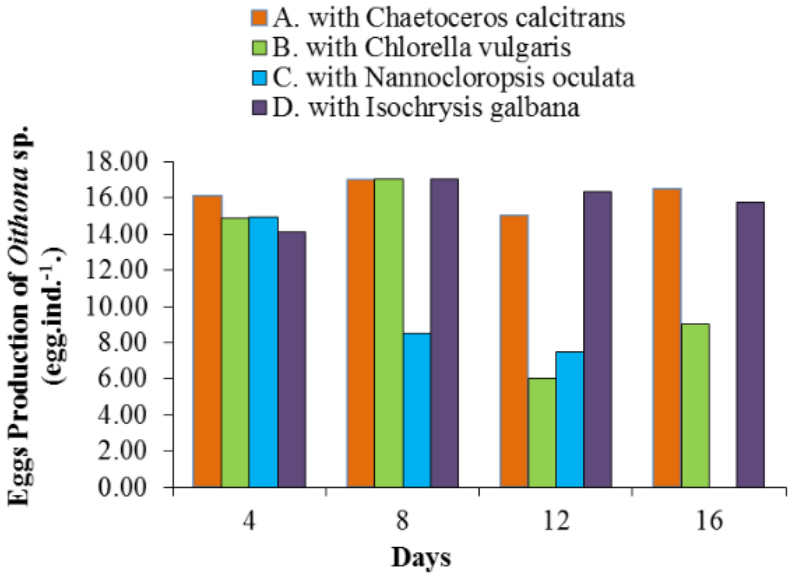

Fig. 3. Eggs production of *Oithona* sp. fed the different phytoplankton in semi-mass condition

3.2. Discussion

The fourth species of phytoplankton showed the high significantly different ($p < 0.01$) of *Oithona* sp. growth performance. There were phytoplankton treatment that were not suitable as a single diets for the culture of *Oithona* sp. There were low result on population growth rate of *Oithona* sp. It observed in *N. oculata* and *C. vulgaris*. treatment. Both of them were also had a low total density and egg production. The result showed that on *N. oculata* and *C. vulgaris* were thought to inhibit the nauplii did not develop into adults. They could not develop into adult, so on *N. oculata* treatment they had not produce egg on the last day of the culture. As a single diet, they could not fulfill the nutrient requirement of *Oithona* sp. Although *N. oculata* and *C. vulgaris* have small diameter, they have a thick cell wall (Puello-Cruz et al., 2009). Nauplii will have difficulty on diggesting both of the cell. The same condition had showed by *Paracyclopina nana* that fed by Marine *Chlorella ellipsoidea* (Lee et al., 2006). It showed lower fecundity, low egg production and mortality of nauplii, and these diets showed a lower population growth rate and negative growth in the community cultures. The performance of a phytoplankton to the growth performance of *Oithona* sp. depends on its digestibility, composition and available energy content (Lee et al., 2006). The poor performance of *N. oculata* and *C. vulgaris* might be explained by the toughness and poor digestibility of its cell wall.

The best population growth performance of *Oithona* sp. was performed by *Oithona* sp. fed by diatom *C. calcitrans*. Among the other phytoplankton, *C. calcitrans*. was a kind of diatom that had a high calcium (Ca: 0.59 %) and phosfor (P: 0.57 %) (Lee et al., 2006; Puelo-Cruz et al., 2009). The mineral nutrition was important to the growth and reproduction of *Oithona* sp. Diatoms have always been considered as a suitable source of energy for zooplankton, and in particular for copepods, to sustain secondary production in terms of reproduction (Payne and Rippingale, 2000). There were many copepod diet experimental result that showed the same thing. *Pseudodiaptomus euryhalinus* showed the best production was fed by monoalgal culture of *Chaetoceros muelleri* (Lee et al., 2006). Copepods fed by *C. calcitrans*. tended to have a shorter time to maturity and produce more nauplii than those fed by *Dunaliella* (Payne and Rippingale, 2000).

There was no high significantly different ($p > 0.05$) between population growth performance of *Oithona* sp. fed by *C. calcitrans*. and *I. galbana*. The experimental diets fed to G. Imparipes, *I. galbana*. was clearly the most efficacious (Payne and Rippingale, 2000). The highest ratio of DHA:EPA supporting the highest egg production, and it showed on *I. galbana* (Payne and Rippingale, 2000). The growth of *Oithona* sp. seems to be closely related with the population of bacterial contamination in the phytoplankton cells culture.

4. Conclusion

Different diet of phytoplankton cells were highly significantly different ($p < 0.01$) on *Oithona* sp. growth performance. *Chaetoceros calcitrans*. gave the best *Oithona* sp growth performance, where were 6.96 ± 0.38 ind · mL^{-1} of total density, 0.121 ± 0.003 d^{-1} of population growth rate, and the eggs production of 16.50 ± 2.74 eggs · ind^{-1}.

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