Growth Performance of Laboratory-Scale Chaetoceros calcitrans in Different Containers

M Jannah¹, M F Ulkhaq²*, M H Azhar², Suciyono² dan W Soemarjati³
1Student of Aquaculture Study Program, PSDKU Faculty of Fisheries and Marine Universitas Airlangga Banyuwangi
2Aquaculture Study Program, PSDKU Faculty of Fisheries and Marine Universitas Airlangga Banyuwangi
3Brackiswhater Aquaculture Development Center Situbondo

*Corresponding author: m-faizalulkhaq@fpk.unair.ac.id

Abstract. This study aims to compare the growth performance of Chaetoceros calcitrans cultured in different containers. The stages of this study consisted of tools and materials preparation, tools and materials sterilization, making culture media and vitamin, Chaetoceros calcitrans inoculant preparation. The result of this study shows that exponential phase in both containers occurred in day 7 with cell density in the glass jar (9.4 x 10⁵ cells/ml) being higher than that in the plastic one (8.17 x 10⁵ cells/ml).

Keywords. Growth, chaetoceros calcitrans, different containers.

1. Introduction

Fish and shrimp hatchery business keeps going forward and developing, causing the need in natural feeds, especially phytoplankton, to keep increasing. One of the types of phytoplankton commonly used as natural feeds for fish and shrimp is Chaetoceros calcitrans. Chaetoceros calcitrans is the type of phytoplankton from diatoms groups that can be used as natural feeds for larvae due to the content of 35% protein, 6.6& carbohydrate, 28% ash and 6.9% fatty acid which are potential for larvae development (Isnansetyo dan Kurniastuty, 1995). According to Sutomo (2005), aside of being natural feed for fish and shrimp larvae, Chaetoceros calcitrans can also be used as rotifer feeds since they are easy to digest and are favoured by rotifers.

Plankton can be used as natural feeds after gradual culture improvement, such as semi-mass culture by moving the outcome of pure culture that has reached final exponential phase as inoculant into a new medium with bigger volume. The culture of Chaetoceros calcitrans plankton is required by silicate for its growth (2008). Silicate in Chaetoceros calcitrans culture is required as additional nutrition since Chaetoceros calcitrans have shells containing silicate to support the growth. The culture of Chaetoceros calcitrans can be conducted in laboratory-scale or pure culture. Plankton pure culture is aimed to obtain pure species (monospecies) and it is conducted in a laboratory with the stages of tools and materials sterilization, isolation, agar media culture and seed storage (2007). Laboratory-scale culture is the making of phytoplankton conducted in a laboratory in order to obtain monospecies plankton used as culture cultivation stock in intermediate and mass scales.
Many studies on *Chaetoceros calcitrans* culture technique have been conducted, including by Banerjee et al. (2011); Sureshkumar et al. (2014); Velasco et al. (2016). However, there has not been any study on growth performance of *Chaetoceros calcitrans* cultured in different containers and therefore this study aims to compare the growth performance of *Chaetoceros calcitrans* cultured in different containers.

2. **Methods**

2.1. **Tools and materials preparation**

This study was conducted in Natural Feed Laboratory, Brackishwater Aquaculture Development Center (BPBAP) Situbondo. The tools used are petri dishes, erlenmeyer, glass jars, plastic jars, inoculation needles, volumetric pipettes, beaker cups, Bunsen burner, autoclaves, ovens, filter bags, pans, gas stoves, neon lights, aerators, hoses and aeration stones. The materials used are *Chaetoceros calcitrans* inoculum, bacto agar, seawater, freshwater, aqueduct, diatom fertilizer, silicate, vitamin, chlorine and sodium thiosulfate.

2.2. **Tools and materials sterilization**

The tools such as petri dishes, Erlenmeyer, volumetric pipes, and beaker cups were sterilized using autoclave. Other tools such as pans, glass and plastic jars, hoses, and aeration stones were sterilized using washing soap scrubbed with scoring bag, rinsed with freshwater and then dried. The culture media (diatom fertilizer) was sterilized using autoclave. Meanwhile, the seawater was sterilized using filter bag, boiling, or soaking in chlorine of 10-20 ppm for 24 hours, and then neutralized using sodium thiosulfate of 5-10 ppm for 3 hours.

2.3. **Producing culture media and vitamin**

The culture media used was diatom fertilizer with composition of 75 g of KNO₃; 5 g of NaH₂PO₄; 5 g of Na₂EDTA; ad 3.15 g of FeCl₃. The materials were put into boiling aquadest one by one, stirred until they dissolved, and then sterilized using autoclave for 30 minutes at temperature of 121°C; 1 atm. After sterilization, solutions containing 100 mg of vitamin B1 and 5 mg of vitamin B12 was added into 1 liter of aquadest, and solution containing 30 mg of silicate was added into 1 liter of sterilized aquadest.

2.4. *Chaetoceros calcitrans* inoculant preparation

*Chaetoceros calcitrans* inoculant used in this study was the collection of BPBAP Situbondo. The inoculum was planted into Bacto Agar media (15 g of Bacto Agar in 1000 ml of aquadest, added with 0.1 ml of vitamin in 100 ml of aquadest) with streaking method, and then incubated for 1 week at 28 °C. Grown *Chaetoceros calcitrans* colony was moved into a reaction tube containing diatom fertilizer solution, and then incubated again at temperature of 28 °C for 1 week, shaken on a daily basis to avoid sedimentation. Afterwards, *Chaetoceros calcitrans* inoculum was moved into an erlenmeyer tube containing sterile diatom fertilized with the volume of 250-500 ml, and then incubated once more at 28 °C for 1 week in a shaker incubator. Incubation outcome was then moved into a glass jar with the volume of 1000-3000 ml and a plastic jar with the same volume.

2.5. *Chaetoceros calcitrans* culture

*Chaetoceros calcitrans* in the glass and plastic jars were maintained for 10 days by exposing them to aeration and lighting from a 40-watt fluorescent lamp. Density observation was conducted on a daily basis until the end of maintenance by taking 5 ml of samples every morning and counting them using haemocytometer. During the culture, water quality in terms of temperature, pH, and salinity was measured when taking the samples.

3. **Result and discussion**

3.1. *Chaetoceros calcitrans in glass and plastic jars*
Chaetoceros calcitrans in the glass jar (Figure 1) showed normal growth pattern with good quality and therefore can be used as natural feed for fish larvae. Adaptation phase occurred on day 1 to day 2. In this phase, Chaetoceros calcitrans grew a little due to adapting towards its environment. On day 3 to 7, a very rapid increase in density (exponential phase) occurred at 940,000 cells/ml. Optimum density of phytoplankton is affected by nutrition, initial stocking density and environmental factor such as temperature, pH and salinity (Suminto and Hirayama, 1996).

Death phase occurred on day 8 to 10, in which the number of Chaetoceros calcitrans decreased due to the lack of nutrition in the media. The death phase occurred on day 8 to day 10 with cells amounted of 225,000 cells/ml. Death phase is a phase where a higher death rate occurs as marked by the continuously decreasing population density (Pelczar and Chan, 1986).

Figure 1. Growth graph of Chaetoceros calcitrans in glass and plastic jars.

Chaetoceros calcitrans in carboy media showed normal growth. Adaptation phase occurred on day 0 to 3 with 187,500 cells/ml. Death phase occurred on day 8 to 10 with of 62,500 cells/ml. On day 4 to 7, occurred a very rapid increase in density (exponential phase) with 817,500 cells/ml. This corresponds to the argument of Daume and Ryan (2004), stating that diatom’s adaptation phase occurs on day 0 to 2 and a very rapid increase (exponential phase) occurs on day 2 to 6. Nutrition availability is crucial for population growth rate and the lack of nutrition in media materials will lead to decreased growth rate. According to Pelczar et al., (1986) in culture, nutrition availability is crucial for population growth rate, where the lack of nutrition in media materials will decrease population growth rate.

Chaetoceros calcitrans in glass and plastic jars showed different growth rates. The plastic jar is made of plastic, while the glass one is made of glass, which enables the obtained light to permeate optimally through the culture media and therefore can be used by Chaetoceros calcitrans to support their growth. Utami (2012) argued that decreased density of phytoplankton cells can be caused by reduced nutrition that hinders their growth and limited light due to the increased amount of phytoplankton. It is also reported that Spiruline sp. culture in glass container showed a more rapid cell growth than that in plastic one. It is because the difference of light intensity that permeates through the container affects the cell growth rate.

3.2. Water quality measurement

The observation result of water quality during the maintenance is presented in Table 1.

Table 1. Measurement of the water quality of Chaetoceros calcitrans culture in glass and plastic
Temperature is a physical parameter that affects metabolism activity. *Chaetoceros calcitrans* are able to grow at temperature of around 20-30°C and will be optimum at 28-30°C (Isnansetyo dan Kurniastuty, 1995). Based on the measurement result of laboratory-scale culture, the temperature obtained was 26-28°C and this data is in line for the growth of *Chaetoceros calcitrans*, in which when reduced media temperature will decrease *Chaetoceros calcitrans* growth rate, and increased temperature that exceeds the maximum limit will cause death (Taw, 1990).

Salinity is a highly influential factor towards aquatic organisms, which is useful for maintaining osmotic pressure between the organisms’ protoplasm and water as their living environment. Salinity was measured using refractometer. The optimum salinity for the growth of *Chaetoceros calcitrans* is around 30-35 ppt (Sulisatyowati and Amini, 2009). The measurement of salinity in *Chaetoceros calcitrans* culture media was 33 ppt, and the data implies that the seawater used in this study has met the need of the growth of *Chaetoceros calcitrans*. According to Fajrin (2012), salinity affects aquatic organisms in maintaining the osmotic pressure of their environment. Photosynthesis process of algae will be hampered when the medium’s salinity is higher or exceeding the tolerance limit.

pH value indicates the level of hydrogen ion concentration in a solution. Water solubility to bind or release a number of hydrogen ion will tell if the solution is acidic or basic. pH is a crucial factor for organisms’ life. The measurement result in laboratory-scale culture was 8. Renaund et al., (1991) stated that the optimum pH for the growth of *Chaetoceros calcitrans* is between 7-9, implying that the pH used in the culture media has met the need of the growth. The decrease and increase of pH in the media will affect metabolism and growth of microalgae in changing nutrition balance (Suriawiria, 2005).

4. Closing

*Chaetoceros calcitrans* in glass jar showed different growth performance from those in plastic jar. Exponential phase in both containers occurred on day 7 with cell density in glass jar (9.4 x 10^5 cells/ml) being higher than that in the plastic one (8.17 x 10^5 cells/ml).

5. References

[1] Banerjee, S., Hew, W.E., Khatoon, H., Shariff, M., Yusoff, F.M. 2011. Growth and Proximate Composition of Tropical Marine *Chaetoceros calcitrans* and *Nannochloropsis oculata* Cultured Outdoors and Under Laboratory Conditions. African Journal of Biotechnology 10(8): 1375-1383.

[2] Daume, S and S. Ryan. 2004. Nursery Culture of The Abalone *Haliotis laevigata*: Larval Settlement and Juvenile Production Using Cultured Algae of Formulated Feed Journal of Shellfisherie Research.

[3] Fajrin, Y. R. 2012. Kultur Murni Skala Laboratorium *Nannochloropsis oculata* Di Balai Budidaya Air Payau Panarukan, Situbondo Jawa Timur. Laporan Praktek Kerja Lapang. Program Tudi Manajemen Sumberdaya Perairan. Univerisita Brawijaya. Malang.

[4] Isnansetyo, A dan Kurniastuty. 1995. Teknik Kultur Phytoplankton dan Zooplankton. Yogyakarta: Kanisius.

[5] Manurung, A. I. 2008. Karakterisasi Awal Protein Diatom *Chaetoceros gracilis* yang Terlibat dalam Pembentukan Biosilika. Fakultas Pertanian. Medan: Universitas Darma Agung.

[6] Pelezar, Michael J dan Chan. 1986. Dasar-Dasar Mikrobiologi. Jakarta: Universitas Indonesia.

[7] Renaund, S., Parry, D., Thinh L-V., Kuo, C., Padovan, A. and Sammy, N. 1991. Effect of Light
Intensity on the Proximate Biochemical and Fatty Acid Composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in Tropical Aquaculture. Journal of Applied Phycology 3(1); 43 – 53.

[8] Rusyani, E. 2007. Budidaya Fitoplankton Skala Laboratorium Dalam Budidaya Fitoplankton Dan Zooplankton. Balai Budidaya Laut Lampung. Direktorat Jendral Perikanan Budidaya Departemen Kelautan Dan Perikanan.

[9] Sulistyowati, R., dan Amini, S. 2010. Produksi biodiesel dari mikroalga *Botryococcus braunii*.

[10] Suminto and K. Hirayama. 1996. Effect of Bacteria Coexistence on the Growth of a Marine Diatome *Chaetoceros gracilis*. Fish. Sci., 62: 40-43.

[11] Sureshkumar, S., Jasmin, B., Rahiman, K.M.M., Mohammed, A.H. 2014. Growth Enhancement of Micro Algae, Chaetoceros calcitrans and Nannochloropsis oculata using Selected Bacterial Strains. International Journal of Current Microbiology and Applied Science 3(4): 352-359.

[12] Suriawiria, U. 2005. Mikrobiologi Air Dan Dasar-Dasar Pengolahan Buangan Secara Biologis. PT. Alumni, Bandung.

[13] Sutomo, R. K. E. T. Wahyuni dan M. G. L. Panggabean. 2007. Pengaruh Jeni Pakan Mikroalga Yang Berbeda Terhadap Pertumbuhan Populai Rotifer (*Brachionus rotundiformis*). Jakarta: Oceonology Dan Limnology di Indonesia. Volume 33:159-176. ISSN 0125-9830.

[14] Taw, N. 1990. Petunjuk Pemeliharaan Kultur Murni dan Massal Mikroalga Proyek Pengembangan Udang. United Nations Development Programme, Food and Agriculture Organizations of the United Nations.

[15] Utami NF, Yuniarti MS, Kiki H. 2012. Pertumbuhan *Chlorella* sp. yang dikultur pada perioditas cahaya yang berbeda. Perikanan dan Kelautan. 3 (3):237-244.

[16] Velasco, L.A., Carrera, S., Barros, J. 2016. Isolation, Culture and Evaluation of *Chaetoceros muelleri* from the Carribbean as Food for the Native Scalars, *Argopecten nucleus* and *Nodipecten nodosus*. Latin American Journal of Aquatic Research 44(3): 557-568.

[17] Wahyuni, N., Masithah, E.D., Soemarjati, W., Suciyono, Ulkhaq, M.F. 2018. Pola Pertumbuhan Mikroalga *Spirulina* sp. Skala Laboratorium yang Dikultur Menggunakan Wadah yang Berbeda. Majalah Ilmiah Bahari Jogja 16(2): 89-97.