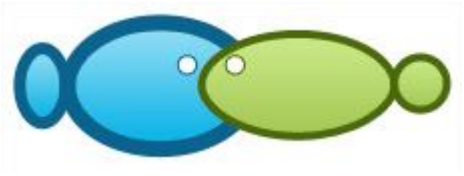

The attachment rate of microalgae *Pavlova* sp. and *Chaetoceros* sp. for food of abalone larva

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Abstract. Supply of live feeds becomes one of the most important factor in supporting the successful of abalone hatching. At larva phase, abalone fed with live feeds microalgae. Some of live feeds for mollusks are *Pavlova* sp. and *Chaetoceros* sp.. This research was aimed to know the attachment rate of microalgae *Pavlova* sp. and *Chaetoceros* sp. for abalone larva food. The result showed that cell of *Pavlova* sp. and *Chaetoceros* sp. had growth at both semi-mass and mass scale. The growth rate of both types of live feeds at semi-mass scale were valued 46 cell mL^{-1} day^{-1} for *Pavlova* sp. and cell mL^{-1} day^{-1} for *Chaetoceros* sp.. The growth rates at mass scale were divided into the rate at column water (25 cell mL^{1} day^{-1} for *Pavlova* sp. and 18 cell mL^{-1} day^{-1} for *Chaetoceros* sp.) and attachment media (42 cell mL^{-1} day^{-1} for *Pavlova* sp. and 27 cell mL^{-1} day^{-1} for *Chaetoceros* sp.). Based on the result, the rate growth for *Pavlova* sp. at attachment media showed greater value than the rate growth for *Chaetoceros* sp.. Both types of microalgae could be provided as live feeds for abalone larva by noticing the growth phase at attachment media which started to provide at third day of live feeds culture.
Key Words: live feeds, larva phase, attachment rate, abalone.

Introduction. Abalone (*Haliotis* sp.) is a kind of mollusk that is distributed in some regions of Indonesia seawaters, starting from Lampung, Sulawesi, Lombok, Bali, Maluku up to Raja Ampat. There are about 100 species of abalones distributed over the world which some of them are in Indonesia (Setyono 2006). Abalone is a herbivore that feeds diatom at larvae phase (Capinpin 2007) and macro algae at mature phase (Freeman 2001).

To increase the production of abalone seed, hatching is needed, either at natural waters or at hatchery. Food supply becomes one of the important factors that support the successful hatching. There are some types of macro algae that are able to be cultured to be live feeds for abalone, such as: *Pavlova* sp., *Isochrysis galbana, Tetraselmis chui, Nitzschia* sp., *Chaetoceros simplex, C. gracilis* (http://vedca.siap.web.id).

Some researches about using live feeds in abalone larvae rearing and attachment have been conducted (Ohgai et al 1991; Ishida et al 1995; Kawamura et al 1998; Roberts et al 1999). The trial in rearing abalone larvae showed that the need of food is changed along with the abalone's growth (Kawamura & Kikuchi 1992; Kawamura & Takami 1995; Kawamura et al 1995; Matthews & Cook 1995; Kawamura 1996). Daume et al (1999) reared larva of *Haliotis rubra* fed with a combination of microalgae *Amphora* sp., *Navicula* sp. and *Cocconeis* sp. and reported the attachment larva after 24 hours of rearing were as following: *Amphora* sp. resulting 7% of abalone larvae attachment, *Navicula* sp. and *Amphora* sp. (12%), *Amphora* sp. (13%) and combination of *Cocconeis* sp. and *Amphora* sp. (8%) while the control was about 1%.

Some types of microalgae generally used as live feeds in hatching of mollusk are *Pavlova* sp. and *Chaetoceros* sp. Both of them have good nutrition contents for growth of

mollusk. Both of those types are suited to be live feeds of abalone larvae due to the attachment ability at plates where the abalone larvae attached.

The hatching of abalone depends on the supply of live feeds in larvae rearing. The success of larvae feeding activity is related to the success of microalgae attachment. If the number of abalone larvae is not balanced with the attachment of the microalgae, the percentage of abalone larvae survival rate may be low. It is critical to supply the sufficient number of microalga that is intended for abalone larva rearing. The research is conducted to know the success of microalgae attachment in semi mass scale and mass scale.

Material and Method. The tools used in this research are listed in Table 1 below, and the materials used in this research are divided into the material used in general, the material used in semi mass culture, and the material used in mass culture as shown in Tables 2, 3, and 4.

Table 1

Tools used during the research

No.	*Name of tool*	*Model/Manufacture*	*The purpose*
1	Aquarium	30 x 20 x 20 cm^3	Container of semi mass culture
2	Fiber tank	130 x 130 x 60 cm^3	Rearing container, mass culture container
3	Digital thermometer	Microlife MT200	To measure the temperature
4	DO meter	Lutron 5510	To measure the dissolved oxygen
5	Refractometer	Brix	To measure the salinity
6	pH meter	Lutron PH-208	To measure the pH of water
7	Microscop	Binoculer	To observe the microalgae
8	Cell sedwig rafter	Gridded	To count the microalgae
9	Pipette	Laboratory Pipette	To take the samples
10	Measuring cup	10 mL	To measure the water volume
11	Scale	Digital	To scale the material

Table 2

The material used in general during research

No.	*Name of material*	*The purpose*
1	Plate	As attachment substrate
2	Sea water	Rearing media
3	Polyester rope/nylon rope	To hang the plate
4	*Chaetoceros* sp.	Testing material (live feed)
5	*Pavlova* sp.	Testing material (live feed)
6	Alcohol	For sterilization purpose
7	Aquades	For calibration purpose
8	Tissue paper	For calibration purpose

Table 3

Material used in semi mass scale culture

No.	*Name of material*	*The quantity*
1	KNO_3	5 g
2	NaH_2PO_4	0.75 g
3	$FeCl_3$	0.25 g
4	Na_2 EDTA	0.5 g
5	Vitamin B_{12}	1 tablet

Table 4

Material used in mass scale culture

No.	*Name of material*	*The quantity*
1	Urea	100 g
2	Ammonium Sulphate / $(NH_4)_2SO_4$ (Za)	50 g
3	Triple Super Phosphate / $Ca(H_2PO_4)$ (TSP)	30 g
4	Na_2 EDTA	5 g
5	$FeCl_3$	2.5 g
6	Vitamin B_{12}	1 tablet
7	Vitamin B_6	1 tablet

Pure stock culture of microalgae *Pavlova* sp. and *Chaetoceros* sp. were obtained from Balai Perikanan Budidaya Laut (BPBL) Ambon. The pure stocks were acquired in cold condition and directly transported to research location. The stocks were put in the sealed bottle. During transported, the bottle were shaked at times to maintain the stocks still in good condition. The research was conducted at hatchery of abalone in Hulaliu Village (about 3 hours from BPBL), consequently, the correct handling of pure stocks was required. The mass and semi mass scale culture were held at hatchery of abalone.

The vessel of larvae food preparation was a fiber tank sized of 130 x 130 x 60 cm^3 and equipped with two aeration spots. The attachment media or plates are sized 65 x 50 cm^2, and water level is about 55 cm. The plates were used as the attachment media for microalgae and abalone seed stocking as well. The media also functions as sheltering media. The plates were hung with nylon rope horizontally to facilitate the attachment process.

The sterilized seawater about 50 L is poured into cleaned and sterilized aquarium (volume 80 liter) as semi mass culture vessel. The fertilizers KNO_3 5 g, NaH_2PO_4 0.75 g, $FeCl_3$ 0.25 g, and Na_2EDTA 0.5 g were mixed in 100 mL of water, and a tablet of vitamin B_{12} was dissolved in 100 mL, then they are poured into the vessel. Thereafter, the 10 L of inoculums (from pure stock) were poured into the prepared vessel. The lighting relies on sun light. The harvesting process was conducted after 7 days of rearing and then was transferred into the mass culture vessel.

About 800 L of sterilized seawater was poured into cleaned and sterilized rearing tank (volume 1 ton). The seawater was sterilized with 10 g of chlorine and aerated for about 2 hours until the chlorine content is run out. The fertilizers (NH_2CONH) 100 g, ZA $(NH_4)_2SO_4$50 g, TSP (PO_2O_5+MgOCa) 30 g, $FeCl_3$2.5 g, and EDTA 5 g are mixed with 100 mL of water, and then two tablets of vitamin B_{12} and vitamin B_6, respectively, were dissolved, then the solution was poured into the rearing tank. Thereafter, the 50 L of inoculums (from semi mass culture) were poured into the prepared tank. The lighting relies on sun light. The harvesting process was conducted after 7 days of rearing. The harvesting of abundant microalgae may be conducted using chemical ingredient (flocculant) NaOH to form microalgae deposit which yielding the high density of microalgae. The deposits are able to be packaged and stored. The deposit can be used in case of the microalgae supply is unable to fulfill the needs of abalone larvae.

Data were collected by using sampling method, which the microalgae are picked in small portion from the plate. The samples were preserved with 4% of formaldehyde followed by the cells density counting, using a binocular microscope with 40x magnification. The frequency of sampling was conducted everyday during a week.

The cell increasing rate of microalgae cellis was counted based on formula suggested by Effendi (1997):

$$G = (Nt-No)/t$$

where: G - cell increasing rate (cell m^{-1});
Nt - cell density at the end of observation time (cell m^{-1});
No - cell density at the initial of observation time (cell m^{-1});
t - time of observation (day).

Data of microalgae cell density were expressed in graphic model by using MS. Excel program and descriptively interpreted.

Result and Discussion. Microalgae cell is increasing day by day which is implied to the density increasing of each type of microalgae. Figure 1 shows the value trend of microalgae cell increasing and density from both *Pavlova* sp. and *Chaetoceros* sp. at semi mass culture. Figure 1 describes that number of cell increasing and density of *Pavlova* sp. is greater than *Chaetoceros* sp.. Both of the type record cells increasing, however, *Pavlova* sp. showed greater. It may be allegedly related with the size of microalgae cell. The size of cell of *Pavlova* sp. is smaller than the cell size of *Chaetoceros* sp. Rehberg-Haas (2014) exposes the size of *Pavlova* sp. is 4-6 µm. *Chaetoceros* sp. observed during the research is quadrangular. Isnansetyo & Kurniastuty (1995) noted that *Chaetoceros* sp. composed of globular with smaller size (4-6 µm) and quadrangular with greater size (8-12 x 7-18 µm).

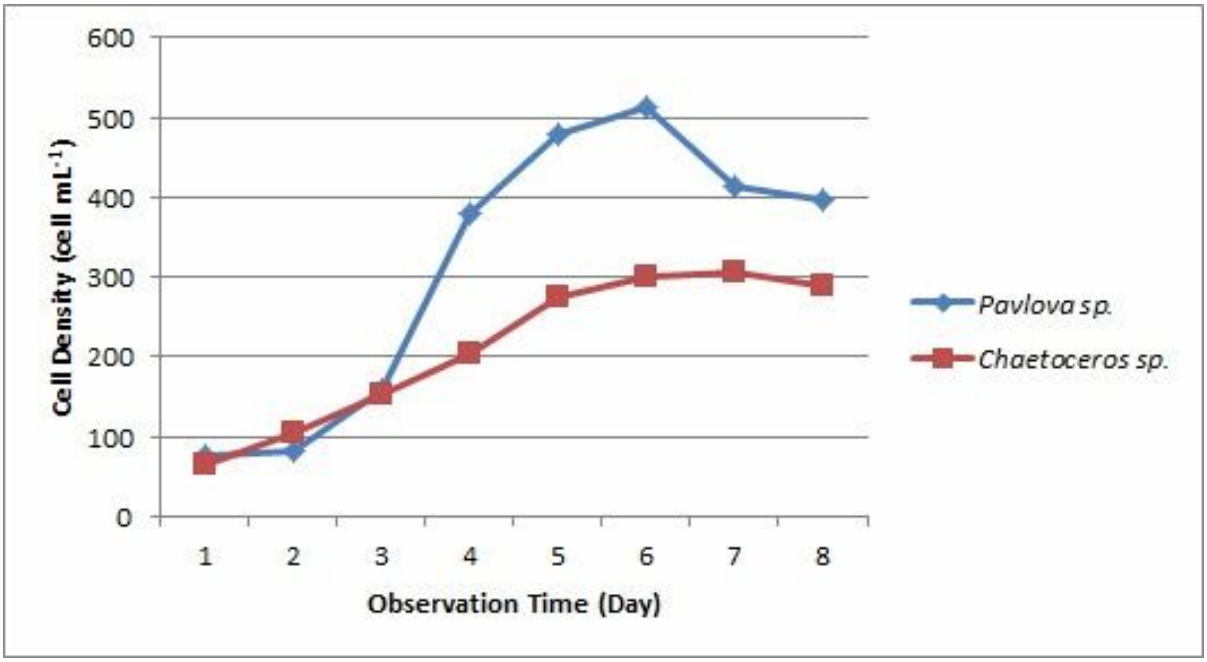

Figure 1. Graphic of cell increasing of microalgae *Pavlova* sp. and *Chaetoceros* sp. at semi mass scale culture.

The growth rate (related with cell increasing) depicts the rate of *Pavlova* sp. is about 46 cell mL^{-1} day^{-1} and *Chaetoceros* sp. is 32 cell mL^{-1} day^{-1}. The growth rate of both microalgae implies their densities at the end of observation. The cell density of *Pavlova* sp. is declining at sixth day of culture, while the cell density of *Chaetoceros* sp. is decreasing at seventh day.

At semi mass scale culture, both types of microalgae attain the cell increasing and attachment to plates previously equipped. Figure 2 expresses the cell density of microalgae *Pavlova* sp. and *Chateoceros* sp. at mass scale culture both in water column and at attachment media (plate). The figure describes that the number of cell are rising each day in water column and at plate. In spite of this, the trend is declining at seventh day in column water while is still continued increasing at plate. It assumes that the cells grown in water column are going to attach at next day.

The growth rate (related with cell increasing) in water column describes the rate of *Pavlova* sp. is about 25 cell mL^{-1} day^{-1} and *Chaetoceros* sp. is 18 cell mL^{-1} day^{-1}, while at attachment media represents greater result that the rate of *Pavlova* sp. is about 42 cell mL^{-1} day^{-1} and *Chaetoceros* sp. is 27 cell ml^{-1} day^{-1}.

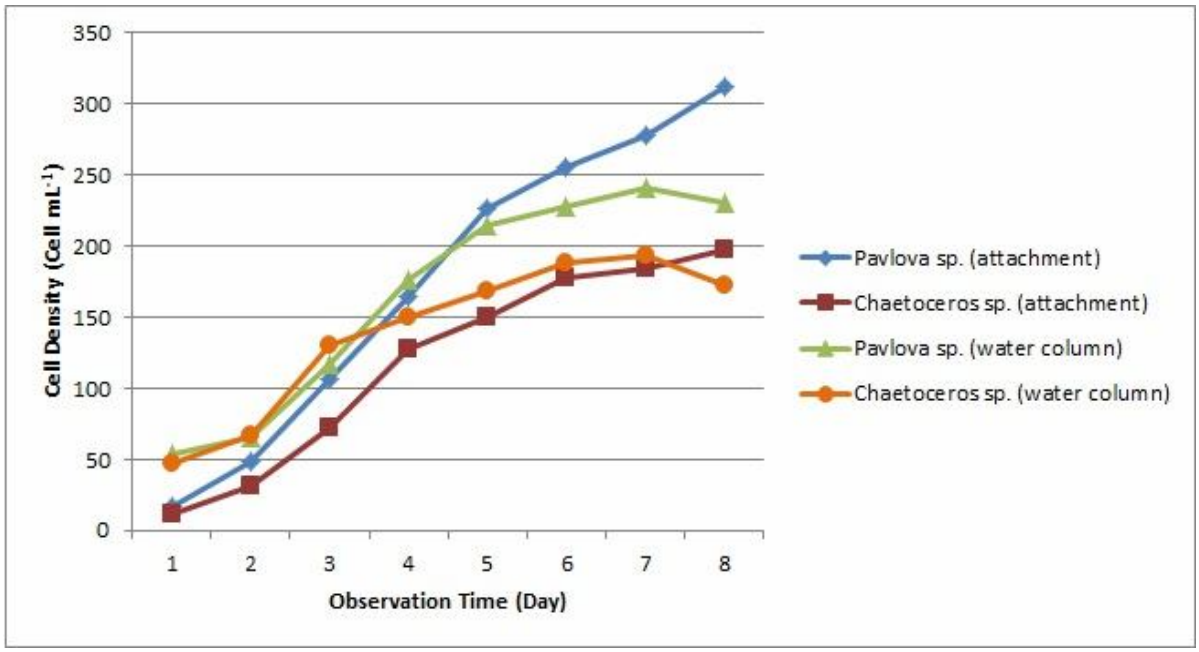

Figure 2. Graphic of cell increasing of microalgae *Pavlova* sp. and *Chaetoceros* sp. at mass scale culture in water column and at attachment media.

Cell growth of microalgae is generally indicated with five phases, which are lag or induction phase, exponential phase, phase of declining relative growth, stationary phase, and death phase (Madigan et al 2009). At initial time of observation, stocked microalgae are starting to adapt with culture media. During two days of culture, *Pavlova* sp. has not yet shown the significant cell growth and after the second day it establishes the exponential phase. It is different of *Chaetoceros* sp. where the significant cell growth occurs in several first days. Haryanto (2013) confirms that *Chaetoceros* sp. possesses high tolerance to culture media and does not require a large culture space and longer period to culture and harvest, hence, it can be assumed that cell increasing of *Chaetoceros* sp. that significantly occurs from first day due to the induction phase that is transpired very fast, whereas, the phases of cell growth normally occurs at the attachment media.

The rearing of microalgae using the attachment media is purposed to prepare live feeds for abalone larvae due to the characteristic of abalone larva. *Pavlova* sp. and *Chaetoceros* sp. are proved to attach to plate. Niu et al (2009) affirm that *Pavlova* sp. is a unicellular marine microalgae which has a haptonema that is a structure similar to flagellum which is utilized to collect food. In addition, the haptonema function is to avoid collision and attaching to substrate. *Chaetoceros* sp. is a planktonic diatom that is categorized in centrales diatom. Yet, Marufkasim (2005) affirms that *Chaetoceros* sp. possesses the ability to attach to substrate due to the gelatin content (gelatin extrusion) that provides attaching capacity to substrate. Siregar & Telaumbanua (2010) stated that the attachment ability is dependent on substrate texture which the more rough the more the microalgae is able to survive. Wetzel (1975) confirms that this ability is a form of adaptation to current and wavy water.

Conclusions. Based on the result, it is notable to arrange the time of larvae stocking appropriate to cell growth pattern at mass scale culture. It is recommended to start stocking the larvae at third day of microalgae rearing time due to the microalgae have exceeded induction phase and entered exponential phase where the cell is significantly increasing in several days. It is also important to preserve the water quality parameters in range of the abalone and microalgae are able to tolerate.

References

Capinpin E. C., 2007 Feeding, growth, and survival of post-larval abalone *Haliotis asinina* on different benthic diatoms. Science Diliman 19(2):49-59.

Daume S., Brand-Gardner S., Woelkerling W. J., 1999 Preferential settlement of abalone larvae: diatom films vs non-geniculate coralline red algae. Aquaculture 174(3-4): 243-254.

Effendi I. M., 1997 [Fisheries biology]. Yayasan Pustaka Nusantara, Bogor. [in Indonesian]

Freeman K. A., 2001 Aquaculture and related biological attributes of abalone species in Australia - a review. Fisheries Research Report No. 128, Department of Fisheries, Australia, 55 pp.

Haryanto N., 2013 [Utilization of *Chaetoceros* sp.] Available at: http://harry-vht.blogspot.co.id/2013/05/manfaat-mikroalga-chaetoceros.html. Accessed: July, 2016. [in Indonesian]

Ishida T., Akutsu T., Torisawa K., 1995 Effects of monocultured benthic diatoms on the metamorphosis of veliger larvae and on the growth of juveniles of abalone, *Haliotis gigantean*. Bull Shizuoka Pref Fish Exp Stn 30:17-21.

Isnansetyo A., Kurniastuty, 1995 [Culture techniques of phytoplankton and zooplankton as live feeds for marine organism hatching]. Kanisius, Yogyakarta, 57 pp. [in Indonesian]

Kawamura T., 1996 The role of benthic diatoms in the early life stages of the Japanese abalone *Haliotis discus* Hannai. In: Survival strategies in early life stages of marine resources. Watanabe Y., Yamashita Y., Oozeki Y. (eds), Rotterdam Balkema, pp. 355-367.

Kawamura T., Kikuchi T., 1992 [Effects of benthic diatoms on settlement and metamorphosis of abalone larvae]. Suisanzoshoku 40:403–409. [in Japanese]

Kawamura T., Takami H., 1995 Analysis of feeding and growth rate of newly metamorphosed abalone *Haliotis discus* Hannai fed on four species of benthic diatoms. Fisheries Science 61:357-358.

Kawamura T., Saido T., Takami H., Yamashita Y., 1995 Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus* Hannai. Journal of Experimental Marine Biology and Ecology 194:189-199.

Kawamura T., Roberts R. D., Takami H., 1998 A review of the feeding and growth of postlarval abalone. Journal of Shellfish Research 17:615-625.

Madigan M. T., 2009 Biology of microorganisms. 12th edition, Pearson Benjamin Cummings, San Fransico, California, 1061 pp.

Marufkasim, 2005 [Diatom identification]. Available at: http://marufkasim.blog.com/Mengenal+Diatom/. Accessed: May, 2017. [in Indonesian]

Matthews I., Cook P. A., 1995 Diatom diet of abalone post-larvae (*Haliotis midae*) and the effect of pre-grazing the diatom overstorey. Marine and Freshwater Research 46:545-548.

Niu Y., Kong J, Fu L., Yang J., Xu Y., 2009 Identification of a novel C20-elongase gene from the marine microalgae *Pavlova viridis* and its expression in *Escherichia coli*. Marine Biotechnology 11(1):17-23.

Ohgai M., Wakano M., Nagai S., 1991 Effect of attached microalgae on the settlement of larvae and growth of juveniles in abalone, *Haliotis discus* Hannai Ino. Suisanzoshoku 39:263-266. [in Japanese]

Rehberg-Haas S., 2014 Utilization of the microalga *Pavlova* sp. in marine fish nutrition. Dissertation der Agrar-und Ernährungswissenschaftlichen Fakultät, der Christian-Albrechts-Universität zu Kiel, 98 pp.

Roberts R. D., Kawamura T., Nicholson C. M., 1999 Growth and survival of post-larval abalone (*Haliotis iris*) in relation to their development and diatom diet. Journal of Shellfish Research 18:243-250.

Setyono D. E. D., 2006 Reproductive aspects of the tropical abalone, *Haliotis asinina*, from southern Lombok waters, Indonesia. Marine Research in Indonesia 30:1-14.

Siregar S. H., Telaumbanua K. S., 2010 [Variation of ephyphytic diatom (Bacillariophyceae) at trunk and pneumatophores of *Avicennia* sp. at Tanjung Buton Port Areal, Province of Riau]. Ilmu Lingkungan 1(4):11-24. [in Indonesian]
Wetzel R. R., 1975 Primary production. In: River ecology. Whitton B. A. (ed), Blackwell, Oxford, UK, pp. 230-247.
*** http://vedca.siap.web.id.

Received: 31 May 2017. Accepted: 04 July 2017. Published online: 10 August 2017.
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How to cite this article:
Latuihamallo M., Loupatty J. W., Tahalea A. H., Manuputty G. D., 2017 The attachment rate of microalgae *Pavlova* sp. and *Chaetoceros* sp. for food of abalone larva. AACL Bioflux 10(4):875-881.