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Developing High-Density Culture System of Pseudodiaptomus annandalei (Copepoda: Calanoida) with Various Microalgae Concentrate

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Abstract- Pseudodiaptomus annandalei **rearing high-density culture system require further development productivity. The feeding copepod with different species of microalgae concentrate could present opportunity of improves production for *P. annandalei* rearing in high-density culture system. This study was carried out to investigate the population growth (copepod density and specific growth rate) and egg rate (number of female carrying egg) of *P. annandalei* when fed 3 mono- and 4 combination microalgae concentrate, *Chaetoceros* sp. (Chae), *Isochrysis* sp. (Iso), and *Nannochloropsis* sp. (Nan) Chae+Iso, Chae+Nan, Iso+Nan, and Chae+Iso+Nan in high-density culture system for 10 days. Three replicates, with an initial 1,000 adult *P. annandalei* per 1 mL (1,250 L fiberglass tank), were set up for each treatment. In all the treatments water temperature and salinity were maintained at 28-29°C and 30-31 ppt. Pure oxygen was used in each treatment in order to maintain adequate dissolved oxygen concentrations under high copepod density culture conditions. The results showed that microalgae concentrate significantly affected population growth. The highest copepod density was 2,820±27 copepod/ mL on *P. annandalei* fed Chae+Iso+Nan, which was significantly higher (P<0.05) than all other microalgae concentrate tested. The lowest copepod density was 1,520±35 copepod/mL on *P. annandalei* fed Nan (P<0.05). The highest specific growth rate was 0.29±0.03 of *P. annandalei* fed Chae+Iso+Nan, and lowest specific growth rate was 0.18±0.02 of *P. annandalei* fed Nan (P<0.05). Microalgae concentrate also had a significant effect (P<0.05) on egg rate, though the high egg rate was recorded with eggs produced by *P. annandalei* fed Chae+Iso+Nan (80%), this was not significantly different (P<0.05) from that of eggs produced by *P. annandalei* fed either Chae+Iso (76%). The results of this study suggest that the combination of Chae+Iso+Nan was the best for the culture of *P. annandalei* in high-density culture system.**

Keywords- Pseudodiaptomus annandalei**; *copepod;* calanoida; microalgae concentrate; high-density culture system**

I. INTRODUCTION

As natural diets of fish larvae, a number of calanoid copepod species are being investigated for use as live feed in aquaculture hatcheries. One of these, the tropical calanoid copepod, *Pseudodiaptomus annandalei*, has good potential as a live feed for marine finfish and shrimp larvae culture. The *P. annandalei* widely distributed in coastal and estuarine waters of the tropics and subtropics. This species is considered to be most adaptive to the estuarine ecosystems in tropics and subtropics and frequently occur in polluted and eutrophicated waters [1]. It shows clear sexual dimorphism, and mating has been observed to be essential for successive fertile clutch production [2]. Copepod diets have been shown to increase larval marine fish growth and development better than a diet of rotifers *Brachionus plicatilis* and *Artemia* [3]. However, some technology to culture marine live feed as rotifer at high enough yields is already developed such high-density culture system, microalgae concentrate diet, it is not investigation for copepod until now.

Among microalgae species commonly used as a diet for the culture of copepods, *Chaetoceros* sp. is often reported as being effective as a sole diet or as part of a mixed microalgae diet for Acartia species, including *A. sinjiensis* [4], [5], [6]. That related to it contains substantial levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [7], [8]. The other microalgae species *Isochrysis* sp. is often favored in copepod culture. *Isochrysis* sp. contains a high concentration of DHA and medium to high amounts of 18:4n-3 and 18:5n-3 [9], [10], the fatty acids commonly associated with promoting high growth rates in aquatic larvae [11]. However, reliance upon microalgae can be problematic because it is relatively unstable in culture and is prone to suddenly dying off [4]. Therefore, finding alternative microalgae species that are more reliable to culture and are equally successful as diets for *P. annandalei* could represent a significant improvement in feeding protocols when culturing this species in high-density culture system. Algae concentrate or paste has become more popular in hatcheries recently because it provides 'off the shelf' convenience and it can be used as a back-up food source in the case of microalgae culture crashes, which are not unusual occurrences in hatcheries. Furthermore, microalgae concentrate of *Nannochloropsis* sp. has generally been successful as an alternative to live

algae for the high-density culture system of rotifers [12], this option appears not to have been tested for copepod.

One of the most important information in sustainable copepod culture in high-density system is the kind of microalgae concentrate diet needed for optimum population growth and egg rate. Previous studies suggested that many calanoids are specific in their choice of microalgae diets either for growth and reproduction [13], [8]. As a potential species for aquaculture, it is important to determine the population growth and egg rate of *P. annandalei*. Thus the objective of this study was to determine the effect of different microalgae concentrate diet on the growth population and egg rate of this specie in high-density culture system.

II. MATERIAL&METHOD

A. Microalgae culture and concentrate

All microalgae used for the diet experiments were cultured outdoors in 10,000 L tanks at Coastal Aquaculture Research and Development Regional Center 5 (Phuket) (PCARDRC 5), Phuket, Thailand. The strain codes of these microalgae were: *Chaetoceros* sp. (Chae), *Isochrysis* sp. (Iso), and *Nannochloropsis* sp. (Nan). The Nan was used commercial fertilize. The others were indoor-grown algae that there were inoculated into 1 µm filtered and UV irradiated seawater of salinity 30 ppt and vigorously aerated. Nutrients used for all species were f/2 concentration [14]. Cultures were maintained at 25 °C, on a 12 h:12 h light:dark cycle. After that, there were cultured outdoor with commercial fertilize. Microalgae concentrate of Chae, Iso and Nan concentrate were produced by using centrifuge at PCARDRC 5, Phuket, Thailand. There were centrifuged at 8,400 rpm spin speed and 2,000 L per hour spin rate.

B. P. annandalei stock culture

P. annandalei used in current experiments was initially obtained from a culture kept at PCARDRC 5, Thailand. The copepods were kept in 20 L carboys filled with 1 µm-filtered and UV-irradiated seawater with gentle aeration. Salinity was 30±1 ppt, and the culture temperature was 27±1 °C. Light intensity was 1000 lux on a photoperiod of 12 h :12 h light:dark cycle. Seventy to eighty percent of the culture water was exchanged daily using a siphon with a 38 µm mesh attached to the end to prevent the removal of the copepods. The carboys were completely drained every 10 days to remove the buildup of detritus while the copepods were retained on a 150 µm sieve. The carboys were then cleaned and sterilized in autoclave before the cultures were restarted again.

C. Experimental design and setup

The study consisted of the experiment examined population growth and egg rate. The experiment was seven diet treatments with three replicates with an initial 1,000 adult of *P. annandalei* per 1 mL for each treatment. The seven diet treatments include 3 mono-microalgae concentrate diets (Chae, Iso and Nan) and 4 combination microalgae concentrate diets (Chae+Iso, Chae+Nan, Iso+Nan, and Chae+Iso+Nan). In this experiments were carried out using 1,250 L fiberglass tank in high density culture system. Each tank was equipped with a central nylon screen to retain copepod in the tank and facilitate water changing. Additional aeration was provided through an aeration collar located on the bottom of the screen in order to maintain homogeneous distribution of copepod and microalgae concentrate in the water column. Water flow rate was 150 L/hour. The cleaning arm was used to remove residue from culture every hour. In all the treatments water temperature and salinity were maintained at 28-29°C and 30-31 ppt. Pure oxygen was used in each treatment in order to maintain adequate dissolved oxygen concentrations under high copepod density culture conditions (5.5-6.5 mg/L or 90% oxygen saturated). The pH values were maintained using NaOH solution and did not drop below 7.0. For combination microalgae concentrate diets, Chae+Iso, Chae+Nan, Iso+Nan = 1:1 and Chae+Iso+Nan = 1:1:1 at the biomass ratio. One milliliter of the concentrated microalgae diet contains 3.2 $\times 10^9$ cells/mL. In each experiment the daily ration was fed 1 mL per minute whole day using automatic feeder. The copepod feeding rate in each experiment was 4 L per day.

D. Data collection

For the growth population, copepod densities were determined based on the mean of duplicate samples (1 mL) taken from the culture unit. A third sample was taken if there was >10% difference between the first 2 samples. Copepod was fixed using Lugol's solution, and then was counted under a light microscope. The specific growth rate (SGR) was calculated using the equation:

$$\text{specific growth rate} = (\ln Nt - \ln N0)/t$$

where N0 is the initial copepod concentration, Nt is the copepod concentration at time t, and t is the culture period (days).

The number of females carrying eggs was also determined and reported as the egg rate.

E. Statistical analysis

Data for both population growth and egg rate were analyzed using one-way analysis of variance. When significant differences ($P<0.05$) were found, Tukey's multiple comparisons test was used to determine specific differences among treatments. All statistical analyses were conducted using SPSS program. Data are presented as mean ± standard deviation (SD).

III. RESULT

A. Population growth

The mean copepod density, including nauplii, copepodites and adults, for each microalgae concentrate diet after 10 days of culture. The results showed that microalgae concentrate diet had a significant effect on the copepod density of *P. annandalei* ($P<0.05$). Mean copepod density of *P. annandalei* after being fed Chae+Iso+Nan was highest 2,820±27 copepod/mL ($P<0.05$). The *P. annandalei fed Chae+Iso was lower m*ean copepod density *than* Chae+Iso+Nan ($P<0.05$), but it was higher *m*ean copepod density *than Chae+Nan and Iso+Nan (P<0.05).* The copepod densities of Chae and Iso mono-microalgae concentrate diets were significantly lower ($P<0.05$) than all the other treatments except for the Nan

mono-microalgae concentrate diet, which also had a significantly lowest copepod density (P<0.05). The SGR results showed that *P. annandalei* fed Chae+Iso+Nan (0.29±0.1) gave highest SGR (P<0.05). The *P. annandalei fed Chae+Iso Chae+Nan and Iso+Nan were lower SGR than* Chae+Iso+Nan (P<0.05), but it was higher SGR *than Chae and Iso* mono-microalgae diets *(P<0.05).* The Nan mono-microalgae concentrate diet had a significantly lowest copepod SGR (P<0.05). (Table 1)

TABLE I
MEAN POPULATION GROWTH OF *P. ANNANDALEI* FED WITH DIFFERENT MICROALGAE CONCENTRATE DIET

Microalgae concentrate diet*	Population growth	
	Copepod density (copepod/mL)	SGR**
Chae	1,825±25[d]	0.17±0.12[c]
Iso	1,710±40[d]	0.16±0.08[c]
Nan	1,520±34[e]	0.12±0.10[d]
Chae+Iso	2,470±34[b]	0.22±0.06[b]
Chae+Nan	2,070±37[c]	0.21±0.13[b]
Iso+Nan	2,020±20[c]	0.20±0.14[b]
Chae+Iso+Nan	2,820±27[a]	0.29±0.09[a]

* Chae = *Chaetoceros* sp., Iso = *Isochrysis* sp., Nan = *Nannochlorops* sp., Chae+Iso = *Chaetoceros* sp. + *Isochrysis* sp., Chae+Nan = *Chaetoceros* sp. + *Nannochloropsis* sp., Iso+Nan = *Isochrysis* sp. + *Nannochloropsis* sp., Chae+Iso+Nan = *Chaetoceros* sp. + *Isochrysis* sp. + *Nannochloropsis* sp.
** SGR = Specific growth rate
Means in the column with the different superscript are significantly different (P<0.05).

B. Egg rate

For the seven remaining microalgae concentrate diets, there were significant differences in egg rate (P<0.05). The high mean percentage egg rates were not significantly different among two of the microalgae concentrate diets, i.e. the combination diet Chae+Iso (76.0±5.2%) and Chae+Iso+Nan (80.0±4.0%) (P>0.05). The Chae+Nan and Iso+Nan microalgae concentrate diets gave higher mean percentage egg rates of copepod than Chae and Iso microalgae concentrate diets (P<0.05). The lowest egg rate was Nan mono-microalgae concentrate diet (P<0.05). (Table 2)

TABLE II
MEAN EGG RATE OF *P. ANNANDALEI* FED WITH DIFFERENT MICROALGAE CONCENTRATE DIET

Microalgae concentrate diet*	Egg rate (%)
Chae	54.0±4.3[c]
Iso	58.0±5.2[c]
Nan	45.0±8.6[d]
Chae+Iso	76.0±5.2[a]
Chae+Nan	61.0±4.0[b]
Iso+Nan	63.0±4.3[b]
Chae+Iso+Nan	80.0±4.0[a]

* Chae = *Chaetoceros* sp., Iso = *Isochrysis* sp., Nan = *Nannochloropsis* sp., Chae+Iso = *Chaetoceros* sp. + *Isochrysis* sp., Chae+Nan = *Chaetoceros* sp. + *Nannochloropsis* sp., Iso+Nan = *Isochrysis* sp. + *Nannochloropsis* sp., Chae+Iso+Nan = *Chaetoceros* sp. + *Isochrysis* sp. + *Nannochloropsis* sp.
Means in the column with the different superscript are significantly different (P<0.05).

IV. DISCUSSION

The results of this study showed that microalgae concentrate diets can significantly affect both the population growth and egg rate of *P. annandalei* and it confirms that both combination diets used supported the best results overall: *P. annandalei* fed combination diet of Chae+Iso+Nan produced the highest mean population growth. Meanwhile, the highest egg rate was obtained from the combination diets of Chae+Iso+Nan and Chae+Iso. Copepods fed combination microalgae diets often exhibit higher egg rate compared to those fed mono-microalgae diets [4], [15]. The combination microalgae diets are thought to provide a better balance of nutrients than a mono-microalgae diet [9], [16]. In fact, in a study on the effects of microalgae diets on the survival, development and egg production of copepod showed that a binary microalgae diet of *Isochrysis* sp. and *Tetraselmis* sp. produced better results for nauplii development of *A. sinjiensis* than fed either *Isochrysis* sp. or *Tetraselmis* sp. alone [4]. The mono-microalgae concentrate diets used in this study, *Chaetoceros* sp., *Isochrysis* sp. and *Nannochloropsis* sp., all produced substantially lower population growth and egg rate than the combination microalgae concentrate diets.

Studies have shown that microalgae concentrate diet may perform well for one particular parameter of copepod productivity but not for another one. The copepod fed with Chae+Iso+Nan gave high copepod density and SGR than other microalgae concentrate diets in this study due to highly unsaturated fatty acids (DHA and EPA) enhanced growth of copepod. Since *N. oculata* contains EPA, which is an important element for the growth of copepod, copepod fed with *N. oculata* still was able to survived until the nauplii from first egg sac was able to hatch its own egg. The microalgae *Isochrysis* sp., for instance, contains high DHA but it has low levels of EPA, while *Nannochloropsis* sp. contain high levels of EPA, but it has virtually no DHA [17]. The *Nannochloropsis* sp. has been found to perform well in other studies as a binary microalgae diet for copepods. Copepod fed with *C. calcitrans*+*N. oculata* gave high copepod survival and maximum specific growth rate [8]. Similarly, a combined diet of *Isochrysis* sp. and *Nannochloropsis* sp. is likely to compensate each other on their essential fatty acid profile to achieve a balanced EPA to DHA ratio [18]. On the other hand, copepod fed with Chae+Iso gave good egg rate as copepod fed with Chae+Iso+Nan due to highly unsaturated fatty acids (DHA) enhanced egg production of copepod. According to the ineffectiveness of *N. oculata* related to its lack of DHA [18], [8]. *Chaetoceros* sp., *Isochrysis* sp. supported high egg production rates of *A. tonsa* [13]. Likewise, *Chaetoceros* sp. and *Isochrysis* sp. and have been conspicuously successful as a food for rearing copepod species [15], [19].

Population growth and egg rate results were lowest for Nan mono-microalgae concentrate diet. The green algae *Nannochloropsis* sp. has been found to perform poorly in other studies as a mono-microalgae diet for calanoid copepods. According to [20], [21], algae are considered to be of low quality if they possess thick cell wall or gelatinous sheaths, which the zooplankton cannot ingest or

digest. Similar investigation, using *N. oculata* as the sole algal food source for *Paracalanus parvus* and *Gladioferens imparipes* [18] and [22], support the present study.

V. CONCLUSION

The present study showed that all the microalgae concentrate diets (*Chaetoceros* sp., *Isochrysis* sp., *Nannochloropsis* sp. and their combinations) tested were able to support the population growth and egg rate of *P. annandalei*. However, the effect of each diet item on the population growth and egg rate depended on the nutritional profile of the microalgae, especially DHA and EPA. The present study revealed that amongst the microalgae offered, combination microalgae concentrate of Chae+Iso+Nan was the best diet item as indicated by highest population growth and egg rate of the *P. annandalei*. In addition, this study illustrated that Chae+Iso combination microalgae concentrate was suitable and effective for the egg rate of *P. annandalei*.

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