Chapter 4

Sustainable Technique for Selected Live Feed Culture

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Additional information is available at the end of the chapter

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1. Introduction

Sustainability in the aquaculture industry depends on several factors including the minimum production cost in comparison to the yield, unexpected environmental conditions which affect the farm and practices in the farm management itself. These factors are inter-connected and always incur a synergistic effect on the issue of sustainability. Live feeds as the fundamental needs for larval rearing and fry production have to be prioritised for sustainable farming activity. Dependency on imported sources of live feeds or inert feed will increase the production cost. Thus, the continued activity of screening, stocking and maintaining some local species as an option for live feed production is economically necessary.

Live feeds are an important basic diet for newly-hatched fish and shrimp larvae as they still have an incomplete digestive system and are lacking in enzymes. They are still at a very young stage to generate their own required nutrients or convert them from any pre-cursor obtained from a diet. They need a ready-made diet with readily available nutrient to be absorbed through their digestive system. There have been many species suggested or tested for their potential as live feed. All test animals were mostly zooplankton in nature and must meet the requirement as live feed. They must be in a compatible size with the mouth size or gape of the larvae predator, or they cannot be swallowed. Since larvae are still weak to track down the food, the wave created by the prey will be a great help, thus 'active' swimming prey is preferred. The most important role of a prey is the ability to supply energy and other nutrients which are essential for the larval survival and growth. Live feeds, as the starter diet in larval rearing and fry production must be continuous in supply. Good, nutritious and compatible-size prey must be able to reproduce fast to meet the requirement and adaptable to a simple mass-production technique.

1.1. Copepods as live feeds

The conventional live feed, brine shrimp and rotifers, are considered unsuitable as live feed if compared to copepods in term of nutritional value. *Artemia* sp. is deficient in polyunsaturated fatty acids (PUFAs), thus it needs to be enriched before feeding [1, 2]. Similarly, rotifer have poor nutritional value and are small in size [3]. On the other hand, copepod diets were proven to increase the growth of larval marine fish compared to diet of rotifers *Brachionus plicatilis*, [4, 5] or *Artemia* [4]. The potential use of copepods as live feed due to their excellent fatty acid content has been highlighted by using an example, a paracalanid [6]. They improved the quality of the cultured organism, particularly the larval stages. The superiority of copepods over other live feed such as brine shrimp and rotifers was further confirmed [7]. They have the appropriate ratio of docosahexaenoic acid (DHA) to eicosapentaenoic acid (EPA) which will improve the growth and survival of fish larvae if compared to the conventional live feeds [8]. Nonetheless, it is reminded that copepods could be better used as supplement rather than a sole diet in larval rearing, unless they are used for some high-valued commercial reef fish due to the high operation cost [9]. The possibility of using copepods, particularly the harpacticoids as alternative live feed in fish larval rearing has been stressed [10] and it is confirmed that the nutritional quality of harpacticoid copepods as live feed is extremely high [11,12]. *Macrosetella gracilis*, a planktonic harpacticoid copepod, is also reported to have better diet quality when compared to *Artemia* [13].

Despite these positive findings, rotifers and *Artemia* continue to be the live feeds of choice in commercial hatcheries, because copepods are not currently cultured at sufficient densities to be economically efficient on a commercial scale [14, 15, 16]. In term of culture condition, it was found that the optimum condition for the high production of a tropical harpacticoid copepod, *Pararobertsonia* sp., was at salinity 35psu and temperature of 25^{o}C [17]. The fluctuation in salinity, pH and temperature in the culture vessel would definitely influence the reproduction and population growth of copepods such as the harpacticoids if kept in small containers [18]. Nonetheless, a strategy to produce harpacticoid in large quantities for hatchery use by using a tray-culture method has been suggested [19].

Another copepod group, a Cyclopoida, *Apocyclops dengizicus* was found to increase the survival and growth of *Panaeus monodon* postlarvae when used as live feed [20]. Cyclopoids are omnivorous, and can be fed a mixture of feeds, mainly phytoplankton or a combination of phytoplankton, yeast or other feeds [7]. As for *Apocyclops panamensis*, there is a report on a successful technique for outdoor ponds [15]. Information on the use of copepods in aquaculture, particularly from the tropical *Apocyclops* sp., is still scarce. The species reported in abundance and potentially exploited as live feeds for shrimp post-larvae in Malaysia for example is *A. dengizicus*. A new species, *Apocyclops ramkhamhaengi*, has been described [21] and added to the present report of 3 species of *Apocyclops* recognised from Asia: *A. dengizicus* (Lepeshkin), *A. royi* (Lindberg), and *A. borneonensis* (Lindberg). This new species is found in abundance in eastern Thailand water and has yet to be determined for its potential in aquaculture. Planktonic copepods such as cyclopoids feed on other plankton including planktonic microalgae. To maintain planktonic copepods in the hatchery or aquaculture ponds, a continuous supply of their diet, particularly the microalgae, will definitely be required.

1.2. Microalgae

Microalgae are a diverse group of unicellular autotrophs inhabiting almost all aquatic water bodies. Microalgae are rich in many specific and attractive compounds [22] and their nutritional values for aquaculture had been highlighted [23]. Production of microalgae is mandatory in the hatchery as it is a basic and nutritious diet for live feed, specifically the zooplankton. However, its mass production is generally costly due to huge manpower, space requirements and operation which usually related to the cost of the energy used. A good strategy in manipulating the culture environment, particularly during the production process of microalgae would scale down the operational cost.

Light plays a fundamental role in the development of microalgae through photosynthesis. It is one of the major environmental factors which control the performance of microalgae phototrophic growth and productivity [24, 25, 26]. Light may either be natural or supplied by fluorescent tubes giving the maximum effective radiation which can be absorbed by the pigments of the microalgae. Light intensity plays a vital role, but the requirements vary with the culture depth or volume as well as the density of the algae in the culture. At a higher volume, light intensity must be increased to enable it to penetrate through the culture. However, an extreme light intensity may result in photo-inhibition which reduces the photosynthetic rates and growth [27,28]. Furthermore, overheating due to artificial or natural illumination should be avoided in microalgal culture. The most often employed light intensity is 1000 lux which is suitable for Erlenmeyer flasks but 5000-10000 lux is needed for a greater volume of microalgal culture [29, 30]. The duration of illumination can be varied where photosynthesis of microalgae can be enhanced or increased in the light/dark (LD) cycle (usually 12:12 or 14:10 LD, maximum 16:8 LD). For some microalgae, photosynthesis rate could also be increased exponentially with increasing light/dark frequencies where a longer period of dark in relation to the light period can further increase photosynthetic efficiencies but not vice versa [31]. The illuminations also affect the nutrient utilisation in the culture vessel [32].

A cost-effective and nutritionally-adequate alternative to costly maintenance of live microalgae is the production of moist-microalgae concentrates. It is seen to simplify hatchery procedures and has shown promising potential in the aquaculture industry [33,34].The storing of microalgae concentrates in moist form under low temperature can preserve their high nutrient composition and excellent cell viability [35,33]. Juvenile pacific oyster (*Crassostrea gigas*) fed with different algal pastes had shown significant improvement in growth rate than oyster fed with other diets [36]. Concentrates of *Chaetoceros muelleri* and *Tetraselmis pseudonana* refrigerated for 6 weeks at 4°C were found to promote similar survival rates of the tiger prawn *Penaeus monodon* larvae fed with live microalgae [37]. Supplementation of microalgae concentrates to bivalves, oysters and scallops have also recorded the same extent of growth rates as live microalgae [38,34]. It has been documented that most of the demand for mariculture feed in Japan is supplied with live and fresh microalgae which is thickly concentrated and readily stored at 2-4ºC for 1-8 weeks with good shelf life [39].

Preparation of concentrated condition of microalgae usually involves centrifugation technique. Nonetheless, although this technique has been successfully applied and utilised for preparing microalgae concentrates, it poses some limitations. First, the process involves

exposure of cells to high gravitational and shear forces deteriorating the cell structure with the leaking of nutritional contents. Second, centrifuging large volumes of cultures is time-consuming and requires expensive equipments. Several alternative procedures, less damaging to the cells, which can be applied are filtration [40], foam fractionation [41] and flocculation [33, 34, 38]. Previous studies have observed the excellence of ultrafiltration technique in preserving and retaining the cellular structure and properties of fragile algal cells with little loss of material [42, 43].

The level of natural resources exploitation for aquaculture purposes is commonly high. Coastal land and mangroves forests always become the target area for brackish-water aquaculture ponds. The water source of this area, which is always from the nearby river estuary and lagoon, is also used as the live feeds (zooplankton and microalgae) source. Nonetheless, the supply is always seasonal and could become unavailable unexpectedly due to many factors and natural phenomena. This chapter aims to discuss the possible ways to produce local live feeds, a marine microalgae species and a planktonic copepod, sustainably using a simple technique for larval-rearing purposes. Maintaining local species is hypothesised to be more economical and practical. The usage of the microalgae as an enrichment element for live feed copepods will be proved.

2. Methodology

Experiment 1: Production of *Chlorella vulgaris* Concentrate Isolated from Bidong Island and Assessment as Copepod Diet

Seawater samples were obtained from Bidong Island, Terengganu. The collection was made by lowering a Niskin water sampler to a required depth, following the light-penetration depth. Concentrated water samples were then transferred into chilled, white-plastic containers and brought back to the laboratory for microalgae isolation process. Successive plating out on agar plates was performed in order to select the desired marine *Chlorella* colonies. Monospecific colonies were then transferred into trial culture tubes before scaling up into larger volumes of Erlenmeyer flasks.

The microalgae was then cultured for the preparation of moist concentrates in the temperature controlled room (20±2°C) using the standard batch culture method. Triplicate of actively-growing starter cultures were inoculated into 30 litres acrylic tanks enriched with Conway medium under constant illumination (cool-white type; 110 watts). All cultures were started with an initial inoculum of $2x10^6$ cells mL^{-1}. Cultures were aerated continuously using humidified filtered air. Evaporation in the culturing tanks was kept to a minimum by covering the top of the tanks. Cellular density of microalgae cultures was monitored daily using a Neubauer haemocytometer [29]. Scanning electron microscopic observation was also done to determine the ultra structure of the cell. Measurement of radius and height of the target microalgae cells was done under the advanced research microscope (Model Nikon Eclipse 80-i, Japan) and twenty individual cells were measured for the calculation of cell biovolume to

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avoid biasing results. Cell biovolume was calculated as assumed round-shape volume with the following formula proposed by Sun and Liu [44]:

$$\text{Cell volume} = 4/3\ \pi R^3 \tag{1}$$

Where, π= 3.142, R= radius of cell

Specific growth rate was calculated from the expression as proposed [45] which is shown below:

$$\text{Specific growth rate } (\mu) = \ln(F_1/F_0)/t_1\text{-}t_0 \tag{2}$$

Where, μ = specific growth rate, F_1= biomass at time harvest, t_1 and F_0= biomass at time zero, t_0.

Doubling time was computed based on the formula as proposed [45] which is shown below:

$$\text{Doubling time}(ò) = \log(2)/\mu \tag{3}$$

Where, T = doubling time, μ = specific growth rate.

All microalgae cultures were grown to late-logarithmic phase for the preparation of concentrates via ultrafiltration technique. The concentrated aqueous suspensions of microalgae were filtered through a membrane filter (0.1μm pore size) to remove access water from the suspension without rupturing the microalgae, thereby obtaining the microalgae concentrate or paste. Cell viabilities of microalgae concentrates were assessed using Eosin dye as a viability assay on the basis of its penetration into non viable-cells based on the expression as proposed [46]:

$$\text{Cell viability}(\%) = \frac{\text{Viable cells x 100}}{\text{Total cells}} \tag{4}$$

The harvesting efficiency or percentage recovery (%) was evaluated by comparing the remaining total number of cells in the concentrate with the total number of cells before filtration with the following expression:

$$\text{Harvesting efficiency/Percentage recovery } (\%) = C_B/C_A \text{ x } 100 \tag{5}$$

Where, C_B = total number of cells before filtration, C_A = total number of cells after filtration

Microalgal concentrates were compared to live cultures of the same algae as food for marine copepods. Copepods were obtained from existing culture in UMT's laboratory. Two different sets of cultures were done using a Petri dish where each of them was fed with live and

microalgae concentrate respectively. Individual copepods were counted daily under the Leica stereo microscope before being fed (1 drop). The maximum specific growth rate (K) was calculated [47] as shown below:

$$K=\ln\left(X_1/X_0\right)/t_1\text{-}t_0 \quad (6)$$

Where, K = specific growth rate, X_1= the number of copepods at harvest time, t_1 and X_2= the number of copepods at time zero, t_0

The doubling time was computed as:

$$\text{Doubling time}\left(\grave{o}\right)=\log\left(2\right)/K \quad (7)$$

Where, τ = doubling time, K = specific growth rate.

Experiment 2: Effects of Photoperiod and Culture Size on *Chlorella vulgaris* Stock Growth

Pure strains of *Chaetoceros* sp. and *C.vulgaris* were obtained from the microalgae maintenance laboratory at Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia, while the pure strain of *Nannochloropsis* sp. was obtained from the Fisheries Research Centre, Pulau Sayak, Kedah, and was maintained in autotrophic conditions in liquid and semisolid agar with Conway media [48]. This axenic culture was stored at 25±2^{0}C for 3 days which served as an inoculum for further experiments.

Microalgae were grown in autotrophic conditions as a monospecific axenic culture in different volumes (250mL, 500mL and 2000mL) containing Conway media. 25mL of pure strain with the cell density of ~2 x 10^6 cells mL^{-1} were transferred to each Erlenmeyer culture flask and kept at complete illumination provided by luminescent tubes (1000 Lux). Carbon source was provided by bubbling sterile 2% (v/v) CO_2 in air through the cultures. Culture flasks were maintained at a constant temperature (22°C± 1°C) with the pH range of 7-8 and salinity of ~35ppt in an air-conditioned laboratory over 2-3 weeks. Daily cell count was calculated using a haemocytometer. To determine the effect of different photoperiods, microalgae cultured in a 2 litre flask containing Conway media was treated at different photoperiods (light/dark) (24:0, 12:12 and 8:16) in replicates and cell count was achieved as mentioned above. Growth curve for each species of algae was constructed and One-way ANOVA with Dunnett's post-test was performed using Graph-Pad Prism.

Mean cell count and specific growth rate were calculated using the formula $X=\frac{\Sigma\chi_i}{n}$ and $SGR=Ln\ \frac{W_2/W_1}{t_2-t_1}$ respectively (where, X = mean cell count; χi = total number of cells; 'n'= number of cell counts; SGR = specific growth rate; W_1= Initial cell density, $cell^{-1}$; W_2= Cell density at late exponential phase, $cell^{-1}$; t_1 = Time at initial cell density, $cell^{-1}$; t_2 = Time at late exponential phase, $cell^{-1}$).

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Experiment 3: Low-Cost Commercial Fertiliser for Mass Culture of Marine *Chlorella vulgaris*: Manipulation of N:P:K Ratio

An investigation was made to see the adaptability of the local marine *C. vulgaris* to the natural conditions in an aquaculture farm. This means that they need to adapt to different fertilisers other than Conway media, different salinity regimes and uncontrolled temperatures. Preparation of NPK-based fertiliser was made by manipulating the ratio of nitrogen, phosphorus and potassium source as summarises in Table 1. Each of the different N:P:K ratio treatments was prepared in triplicate. Source of nitrogen was obtained by using urea fertiliser.

Culture containers were well-cleaned with bleach and rinsed thoroughly before filling up with 1L of the farm water (salinity of between 20-25ppt). The marine *C.vulgaris* concentrate was prepared and 1mL of it was inoculated into the container and 1mL of the fertilizer was added. The containers were vigorously aerated to provide required quantity of oxygen and to keep cells and media in suspension. The containers were kept in the open under 100% outdoor light exposure.

N:P:K ratios			Type and fertiliser used		
N	**P**	**K**	**Urea (g)**	**P^+ (mL)**	**Potash$^+$(g)**
1	**1**	**1**	**0.98**	**0.98**	**0.98**
15	15	15	14.7	14.7	14.7
8	8	2	7.84	7.84	1.96
16	8	6	15.68	7.84	5.88
12	6	4	11.76	5.88	3.92
12	8	4	11.76	7.84	3.92

Table 1. Type and fertiliser used in N:P:K ratio for mass culture of marine *Chlorella vulgaris*

Sampling of microalgae cells was done daily and counting was carried out using a Neubauer Hemocytometer covered with glass slide under a compound microscope.

The growth rate, divisions per day, and generation time or doubling time was calculated following [49]

$$\text{Growth rate; } K' = \text{Ln}\left(N_t/N_o\right)/\left(t_2 - t_1\right) \tag{8}$$

$$\text{Divisions per day; } \text{Div.day}^{-1} = K'/\text{Ln}2 \tag{9}$$

$$\text{Generation time}\left(\text{days}\right); \text{ Gen't} = 1/\text{Div.day}^{-1} \tag{10}$$

$$\text{Generation time } (\text{hours});\ \text{Gen't} = 24\left(1/\text{Div}.\text{day}^{-1}\right) \tag{11}$$

Where, No and Nt = final and initial populations at time t1 and time t2, respectively.

Since sample was collected daily, therefore, t2 – t1 = 1.

Experiment 4: Egg Production, Growth and Development of *Apocyclops ramkhamhaengi* Fed on Marine *Chlorella vulgaris*

Detailed observation on the reproduction performance of a zooplankton depending solely on a *C.vulgaris* diet was planned to prove the important role played by this local microalgae in live-feed production. Samples of copepods were collected from Sungai Semerak (N 05° 51.737, E 102° 30.809'), Tok Bali, Kelantan using a zooplankton net. This area receives sea water from the South China Sea, which is near to the Thailand coast where the copepod species was first identified and reported. Live copepods were maintained and adapted to the laboratory environment. Sand-filtered sea water from the Marine Hatchery, Universiti Malaysia Terengganu was diluted with deionised water to be at salinity of 25ppt and was further filtered through a GFC membrane filter and then autoclaved at 121°C for 15 minutes [11]. Salinity was measured using a portable hand-refractometer (ATAGO, Japan). Microalgae diet for the copepod was prepared from the marine algae *C.vulgaris* stocked at the Marine Hatchery. The microalgae were cultured in 29-31ppt Conway medium with 24h-light, room temperature of 25-27°C and continuous aeration for 7days. The cell concentration in each 500ml conical flask was determined by using Neuber haemocytometer (0.25mm^2 x 0.1 mm) under a compound microscope. The algal production was done weekly and supplied to *A. ramkhamhaengi* culture.

The investigation on the reproduction performance started with fifteen gravid females of *A. ramkhamhaengi* placed into two sets of triplicate of 250mL beakers. The diet constituted,1mL of Baker's yeast (0.02g/L) and 1mL of *C.vulgaris* at density 1x10^{6}cells/mL which were introduced into both sets of the beakers and covered with parafilm layer to avoid contamination. Three subsamples (approximately 1mL) from each beaker of the cultures were observed daily. The number of the copepods at all stages, including nauplii, copepodite, adult and gravid female, were counted under a dissecting microscope (Leica ZOOM 2000) and then returned to the culture. Changing of approximately 80% of the culture medium was done every alternate day by passing the copepods culture through 100 and 40 microns nylon net which would retain all stages of copepods (the smallest size of 60 microns) but remove most of the waste.

The population growth of *A. ramkhamhaengi* was studied for 30 days. The specific growth rates (K) of all stages of the copepods in both diets given were calculated by using the formula [50]:

$$K = \frac{\ln Nt - \ln No}{t} \tag{12}$$

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Where, t is the culture days, No and Nt is the number of copepods at the initial and final selected time interval. The doubling time (Dt) was calculated by dividing $\log_e 2$ by the population growth rate (K) of all stages of *A. ramkhamhaengi* in both diets given:

$$Dt = \frac{\log_e 2}{K} \tag{13}$$

Although cyclopoid copepods are known to suspend in water column, *A. ramkhamhaengi* showed its adaptability to swim on the near bottom of its culture vessel. The culture for this experiment was started by introducing a gravid female on the experimental petri dish. The adult was removed after the eggs hatched and the nauplii were monitored until they reached copepodite-v stage and were ready to mate. Adult females and males from the culture were prepared for the experiment. A pair of male and female was put into each set of glass Petri dish filled with 15mL sea water. The use of a Petri dish instead of a beaker eased the daily observation of different stages of the copepod in the population. The cultures were maintained at room temperature of 25-27°C without additional oxygen supply or aeration. Observation was done twice per day under a dissecting microscope (Leica ZOOM 2000) before feeding to avoid the disturbance of the diet materials during individual or population counting. The culture medium was changed approximately 80% daily, and culture containers were subsequently changed every 4days. Daily feeding was done in the morning and evening by dropping 1mL of 1 x 10^6 cells/ml *C.vulgaris* into the culture. The time taken for the females to become gravid was based on the observation recorded twice per day (morning/evening). Once the females become gravid, the male broodstocks were removed, and the female were left alone inside the Petri dish in order to determine the number of eggs per female from its first copulation. Observation on the development time from nauplii to adult, maturation time and generation time of *A. ramkhamhaengi* were recorded coupling with the numbers of offspring produced and percentage of hatching.

3. Result and discussions

Experiment 1: Production of *Chlorella vulgaris* Concentrate Isolated from Bidong Island and Assessment as Copepod Diet

The ultra-structure of the *C.vulgaris* isolated and cultivated in this study is shown in Figure 1. The scanning electron micrographs displayed the characteristic features of green single cells with spherical shape and possession of rigid cell wall. There are some differences found in the present specimen if compared to some other established species. The outer shell is rough if compared to the latest SEM of *C.sorokiana* [51].The feature is almost the same as found in SEM of *C.vulgaris* [52]. In terms of size, the specimen was found to be in between the size of marine *C.vulgaris* (2.1μm) and estuarine *C.vulgaris* (2.3μm) from Korean waters [53].

Kingdom PLANTAE
Phylum CHLOROPHYTA
Class CHLOROPHYCEAE
Order CHLORELLALES
Family CHLORELLACEAE
Genus *CHLORELLA*

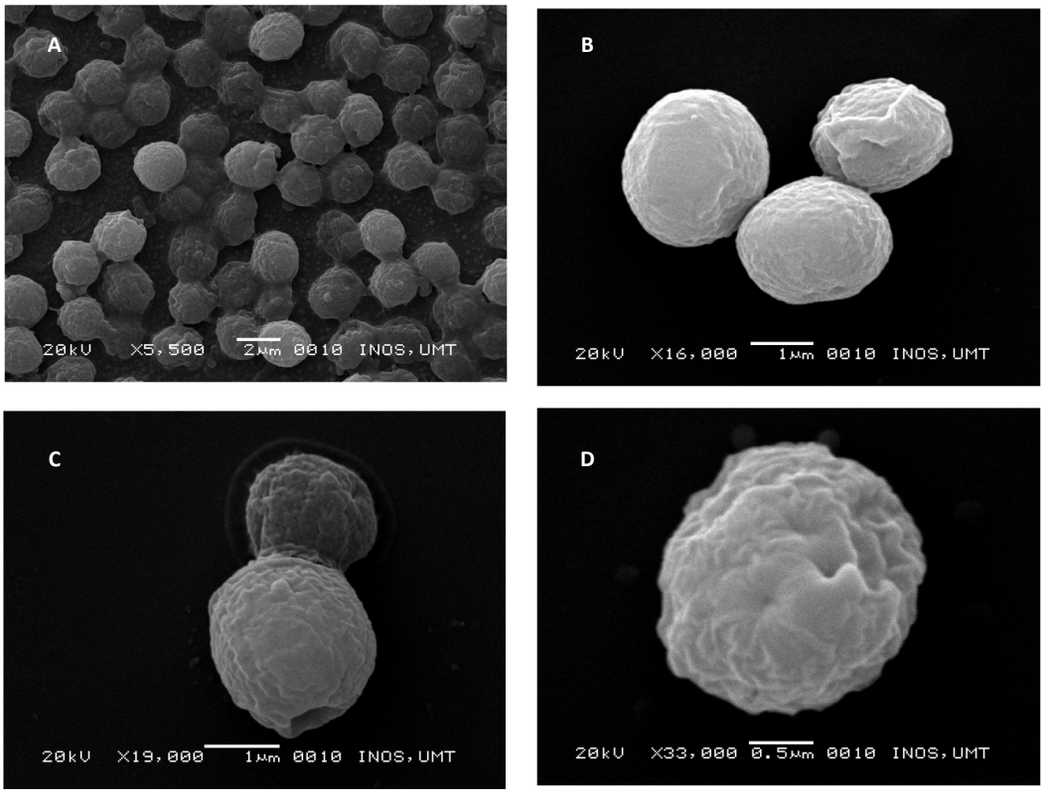

Figure 1. The scanning electron micrographs of *C.vulgaris* under different views and various magnifications. A, Cell aggregation. B, *C.vulgaris* cells under different magnification C. Cell divison in *C.vulgaris*. D, Single cell and cell wall structures.

The cell has an average cell biovolume of 5.26±0.87 μm^3. The cell densities changed following the culture period in both culture of concentrates (paste) and live condition (although they both started at the same density). Nonetheless, they followed more or less the same growth patten. The variation in cell densities during the experimental period is shown in Figure 2. Cell density of *C.vulgaris* increased rapidly to 227.22±0.87 $x10^6$ cells mL^{-1} prior to stationary phase. After that, the cell densities maintained at this point for ten days before decreasing significantly thereafter (Figure 2). The average specific growth rate (SGR, μ) achieved during the exponential phase was 0.660±0.001 day^{-1} with the doubling time (T) of 0.580±0.004 hour which then decreased drastically to 0.126±0.001 day^{-1} during the retardation phase with the doubling time of 2.420±0.019 hour before the death phase. Based on cell density and growth rate observed, the following growth phase is described:

i. Exponential (log) phase (days 0-6),

ii. Declining of relative growth rate phase (days 6-12),

iii. Stationary phase (days 12-22),

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iv. Death phase (days 22-26).

C.vulgaris paste was successfully concentrated from the pure culture isolated from Bidong Island. This concentrate contains cell density of approximately 58.46±2.44 $x10^9$ cells mL^{-1} - 227.22±0.82 $x10^6$ cells mL^{-1}. The present result also showed that this *C.vulgaris* concentrate can still be inoculated after refrigeration for a duration of six weeks and exhibited similar growth characteristics as the live culture (Figure 2). The cells had very high viability even after 6 weeks of storage in chilling conditions (4ºC) as shown in Figure 3. It is interesting to note that the paste had recorded the highest cell viabilities of 99.51±0.57% and continued to display slow and steady decrement of cell viabilities to 83.28±0.58% on the sixth week of storage. Microscopic examination also indicated that the cells were in single forms without any aggregation occurring and can be readily dispersed in seawater medium as single suspension of cells upon inoculation (Figure 1A). The harvesting efficiency of the ultrafiltration technique using membrane filter had recorded a very high percentage recovery of 93.14±1.35% showing the effectiveness of this technique for harvesting and concentrating the microalgae biomass.

Copepod species, *Apocyclops* sp., showed a higher population density when fed with *C.vulgaris* paste (60±4.36 individual mL^{-1}) than with the live culture (14.33±0.58 individual mL^{-1}) (Figure 4). In addition, the copepod populations fed with this concentrate exhibited a higher instantaneous growth rate, K=0.455±0.008 day^{-1} and faster doubling time (0.662±0.012 hour) than live culture which recorded an instantaneous growth rate of 0.296±0.005 day^{-1} and doubling time of 1.108±0.016 hour. Results from one-way ANOVA test has shown that there is a significant difference (P<0.005) between the copepod population densities fed with the *C.vulgaris* concentrate and live culture.

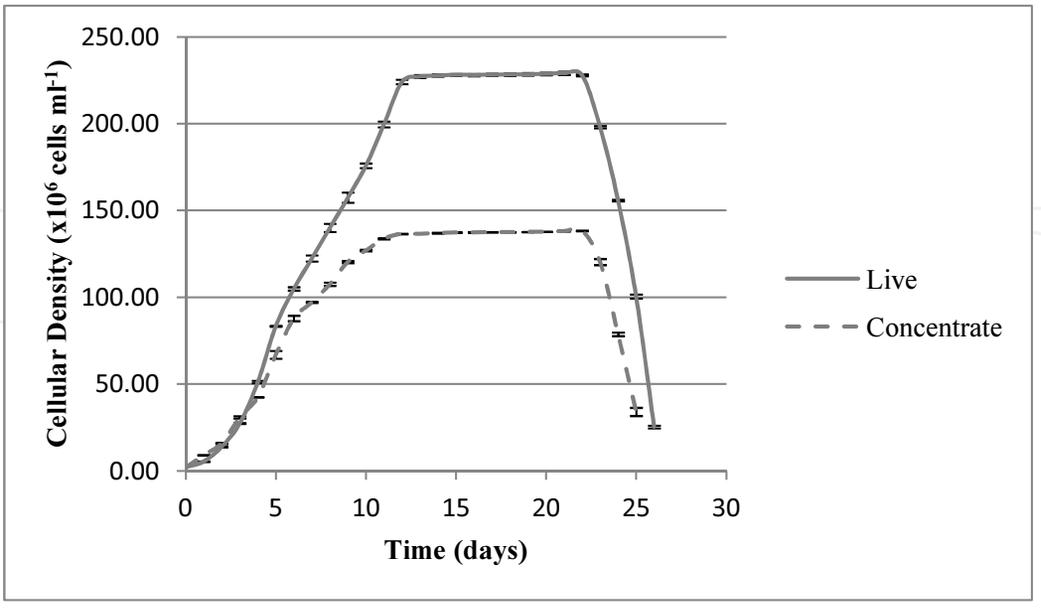


Figure 2. Cellular densities of live *C.vulgaris* (30L) and concentrate/paste (after reinoculation in 5L) cultured under laboratory conditions with Conway medium. Data are mean value and standard deviation of 3 repetitions.

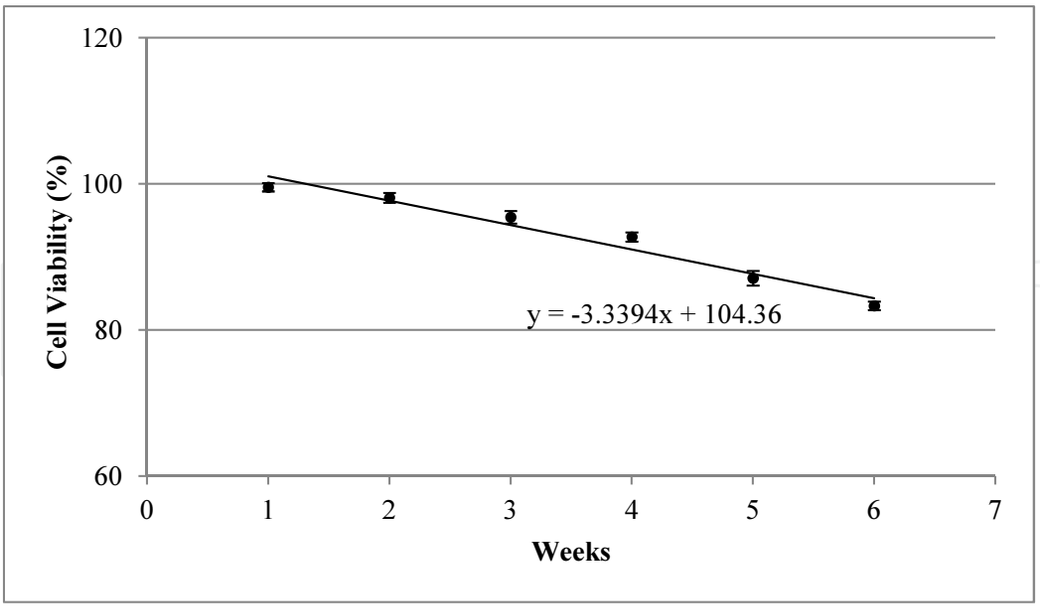
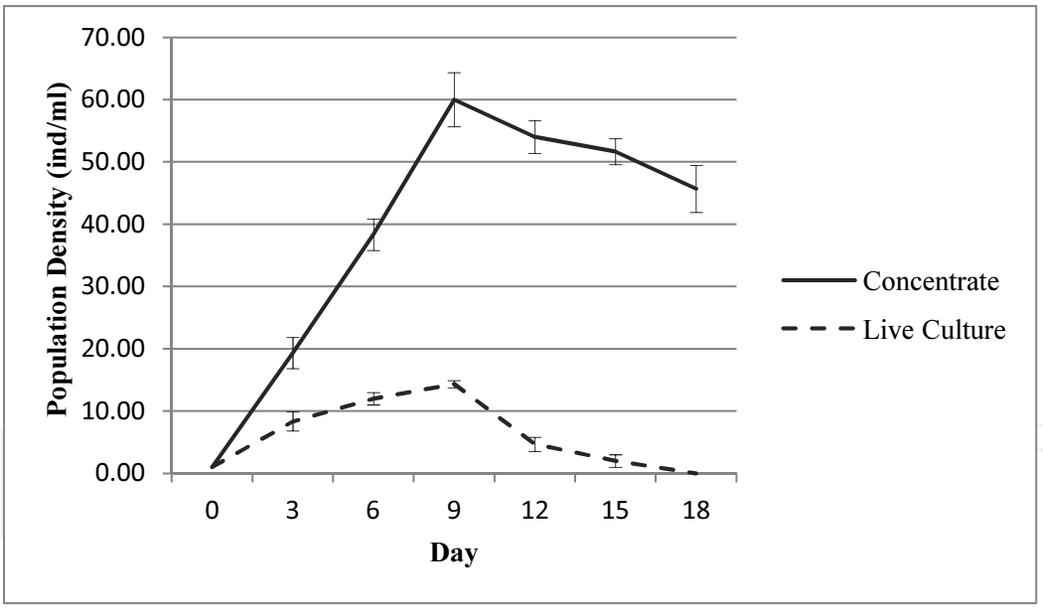


Figure 3. Variations in the cell viabilities of *C.vulgaris* microalgae concentrate over 6 weeks of storage in chilling condition at 4° C. Data are mean value and standard deviation of 10 repetitions.



Data are mean value and standard deviation of 3 repetitions.

Figure 4. Population densities of *Apocyclops* sp. fed with live culture and microalgae concentrate of *C.vulgaris*.

C.vulgaris isolated from Bidong Island had exhibited an extremely rapid growth rate. This might be attributed to its relatively small cell volume with an average of 5.26±0.87 μm^3. This is indeed much smaller than the cell sizes recorded for some other species; *Chaetoceros calcitrans* (8μm diameter, volume 276.95 μm^3) and *Isochrysis galbana* (4μm diameter, volume

33.49 μm^3) [54]. Small size species of microalgae grow faster with a rapid growth rate. This is due to the reason that the greater surface-to-volume ratio of smaller size cells facilitates assimilation of nutrients at a relatively faster rate. In addition, the smaller size cells may achieve high density because they occupy less space. Apart from that, it was also cultured with optimal values for all environmental factors in the laboratory, thus promoting favourable environmental conditions for the cells to grow to extremely high density. In this high-density culture, the possibilities of contamination were excluded. The sudden collapse in the growth rate after day six could be mainly the result of the depletion of the nutrient in the culture. Growth rate declines and growth of microalgae ceases when the nutrient in shortest supply relative to the metabolic needs of algal population [27,55]. The populations of *C.vulgaris* cells then entered the stationary phase of the growth cycle and collapsed after day twenty two. The long stationary phase of this culture might indicate that contamination was absent during the culture period. It has been reported that this stationary phase can last for several weeks if there is no contamination in the culture [30].

The ultrafiltration technique which was used to concentrate the *C.vulgaris* cells in this study can be applied to concentrate a range of other microalgae species used as aquaculture feeds. Concentrating and storing the microalgae concentrate in moist form preserves its high nutritional value through maintaining excellent cell viability [33, 35]. The cells were readily re-suspended upon dilution in sea water with high cell viability which was proven by their ability to be inoculated even after storage for a duration of 6 weeks. The efficiency of ultrafiltration through this study was ≥90% which is very comparable to the reported efficiency of ≥80% for flocculation technique by Knuckey *et al*. [34]. There has been no comparative assessment of concentrates prepared by ultrafiltration with those prepared by centrifugation. However, from a practical and theoretical point of view, it is proven that the centrifugation method possesses some disadvantages due to its exertion of shear gravitational forces rupturing the microalgae cell structure during harvesting procedure. This reduces their nutritional values due to leaking of nutritional contents. On the other hand, microalgae concentrates prepared by ultrafiltration are not subjected to the same gravitational forces during harvesting. As reported earlier [38], the major production cost of centrifuged concentrates may exceed US$10,000 (RM32,620) which is unaffordable for small-scale hatcheries and is likely to be limited to larger hatcheries with specialised equipments or facilities specifically set up to produce microalgae concentrates to hatcheries. Advantages of the ultrafiltration technique used in this study is that it is a relatively simple, inexpensive and volume-independent process which can be readily adopted by small-scale hatcheries to prepare their own microalgae concentrates on site.

The use of *C.vulgaris* concentrate as diet for cyclopoid copepods increases population density, instantaneous growth rate as well as doubling time and it was proven as a better diet than the *C.vulgaris* live cultures. This might be possibly due to the significantly higher cellular density of the microalgae concentrates. The rates of ingestion and egg production in copepods are dependent on the quantity of the provided microalgae [56, 57] implying that quantity of food is the most important factor regulating the productivity of copepod culture. Other studies have also demonstrated that the rate of egg production of calanoid copepod, *Acartia tonsa*, increases

with increasing food concentrations [58, 59]. Essential substances such as cholesterol, HUFA and PUFA are present or exist abundantly in microalgae, and, copepod production is positively related to the lipid levels or DHA: EPA ratio in the diet [60]. Thus, microalgae concentrate could be useful as a replacement for live or fresh microalgae. This is extremely important as a stable and continuous supply of live feed for aquaculture hatcheries must always be provided.

Experiment 2: Effects of Photoperiod and Culture Size on *C.vulgaris* Stock Growth

It is very important for hatcheries to be able to maintain the stock for microalgae for their sustainable live-feeds supply. Batch cultures need to be maintained under optimal environmental conditions and in a suitable culture vessel which will not affect the cell density and quality. Comparison on the effect of photoperiod and culture sizes between *C.vulgaris* and other microalgae was made to investigate the adaptability of the species to simple stock handling in the laboratory or hatchery conditions. No significant difference in the cell density was noted in *Nannochloropsis* sp. (Figure 5A) and *C.vulgaris* (Figure 5C) cultures grown in different volume flasks while *Chaetoceros* sp. Figure 5B showed significant variation in cell-density level at similar culture conditions ($P < 0.001$). However, stationary phases of all cultured species were achieved earlier in 250mL flask compared to the cultures in 500mL and 2L flasks. All cultures showed greater response towards daylight variations whereby higher cell density was noted in culture flasks exposed to continued illumination (24:0 L/D), and it was followed by 12:12 L/D and 6:18 L/D condition. *Nannochloropsis* sp. (Figure 5D) responded less towards the treatment compared to the other 2 species (Figure 5E & F) which clearly showed a specific response towards culture conditions.

It is well-documented that, in natural conditions, microalgae growth is not curtailed by ambient environmental conditions because the growth rate is just enough for species survival. However, their multiplication rate is highly influenced by various environmental parameters. In an *In vitro* setup, the proper maintenance of optimum culture condition triggers the metabolic pathway of target species in a unidirectional fashion to achieve high cell density. In this study, a higher cell density of microalgae in low volume flask culture together with early stationary phase was observed could be used to obtain continuous harvest of selected microalgae.

Highest cell density and specific growth rate were recorded in selected species cultured in 250mL culture flask compared to the cultures in 500mL and 2000mL flasks (Table 2). The highest cell density was achieved during the end of the log phase. Cell density of early stationary phase, which is the end of the log phase for *Nannochloropsis* sp., *Chaetoceros* sp. and *C.vulgaris* was achieved on the 10^{th} day of culture in 250mL. After the 10^{th} day, density decreased and the lowest level was different for different species and different culture volumes. Similar results were also noted for the specific growth rate values. Significant variation in cell density and specific growth rate was observed between the cultures in different sizes of culture flask ($P < 0.05$ or $P < 0.001$). This observation might probably be due to the light-penetration efficiency in the culture flask. Similar observation was noted for the culture of *Nannochloropsis* sp. in 2000mL flasks which produced greater cell density compared to the culture in 20L carboys [61]. The effect of light saturation could decrease in the denser culture and the average irradiance in the culture reduced due to absorption from other cells [62]. The

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large volume culture needed higher light intensity to allow light penetration while the smaller volumes were less affected by the light penetration.

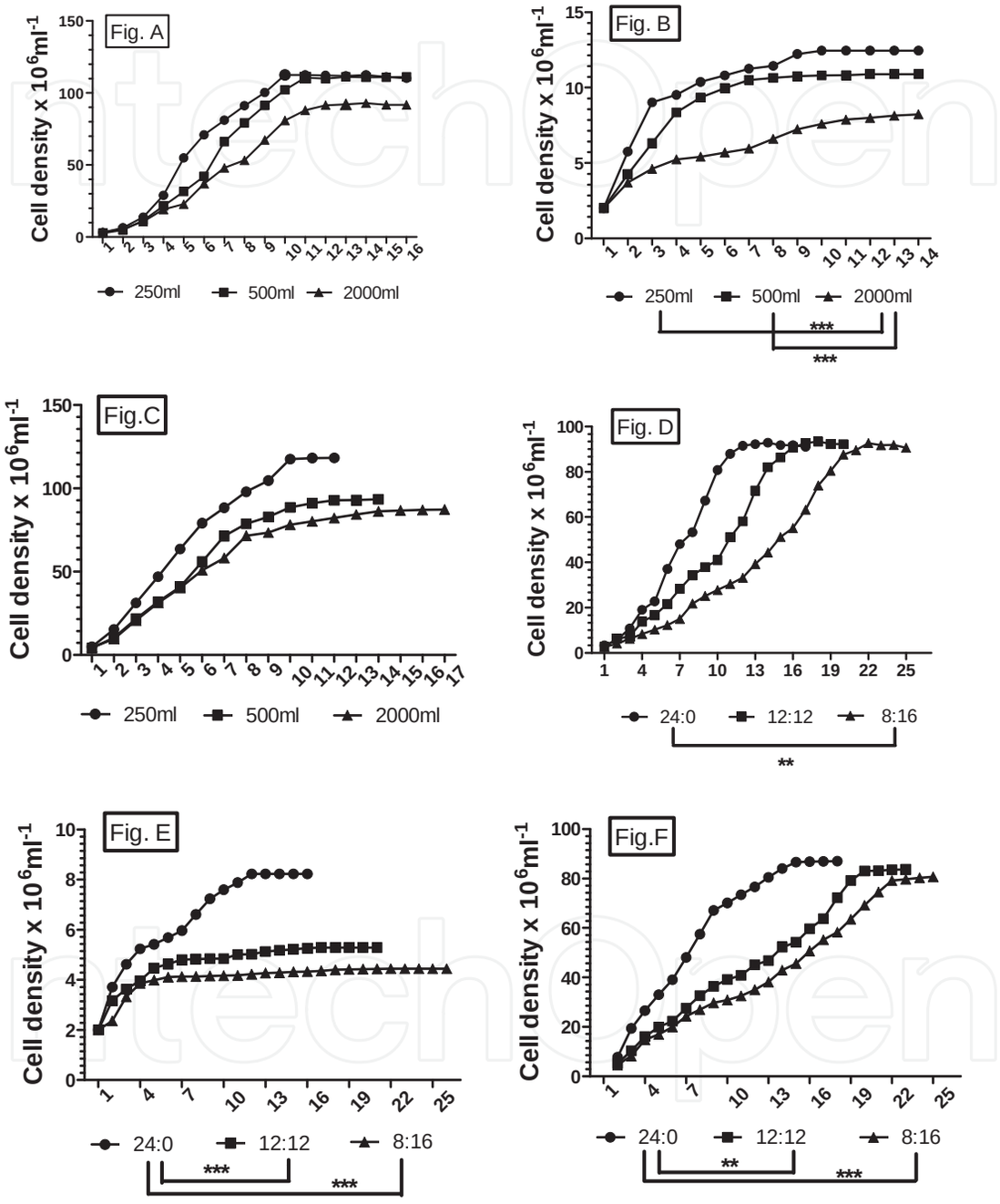

Figure 5. Influence of different photoperiods and culture flask volumes on the cell density of *Nannochloropsis* sp. (Fig A & D), *Chaetoceros* sp. (Fig B & E) and *C.vulgaris* (Fig C & F) respectively. X-axis shows the days of culture. Data represented as 250ml, 500ml and 2000ml are culture flask volumes while 24:0, 12:12 and 8:16 are photoperiods (Light : Dark phase). [***] and [**] are significantly different at $P < 0.001$ and $P < 0.05$ level in the culture conditions respectively.

Microalgae	Flask volume (mL)	Early stationary phase	Cell density (x10^{6}cells mL^{-1})	Specific growth rate (K')
Nannochloropsis sp.	250	Day 10	112.5±2.36^a	0.52±0.01^a
	500	Day 11	110.17±1.77^a	0.46±0.04^b
	2000	Day 13	92.2± 0.87^a	0.34±0.01$^{c*\ (ac)}$
Chaetoceros sp.	250	Day 10	12.460±0.018^a	0.203±0.002^a
	500	Day 12	10.889±0.013$^{b**\ (ab)}$	0.145±0.001$^{b*\ (ab)}$
	2000	Day 14	8.225±0.001$^{c**\ (ac\ \&\ bc)}$	0.037±0.003$^{c**\ (ac),\ *\ (bc)}$
C.vulgaris	250	Day 10	117.53± 0.84^a	0.4749±0.0007^a
	500	Day 11	91.0± 0.55^a	0.4081±0.0002^b
	2000	Day 14	86.13±0.81^a	0.3166±0.0007$^{c*\ (ac)}$

Note: Data represented in Mean ± SD. [*] and [**] indicates significant difference at P < 0.05 and P < 0.001(respectively) level between different superscripts depicted for each species.

Table 2. Cell density and specific growth rate of selected microalgae cultured at different flask volumes.

Microalgae	Photo period (Light : Dark phase) in hours	Early stationary phase	Cell density (x10^{6}cells ml^{-1})	Specific growth rate (K')
Nannochloropsis sp.	24:0	Day 13	112.5±2.36^a	0.34±0.01^a
	12:12	Day 17	110.17±1.77^a	0.25±0.01^b
	8:16	Day 23	92.2± 0.87^a	0.19±0.02$^{c*\ (ac)}$
Chaetoceros sp.	24:0	Day 12	8.225±0.001^a	0.129±0.003^a
	12:12	Day 17	5.293±0.009$^{b**\ (ab)}$	0.061±0.002$^{b*\ (ab)}$
	8:16	Day 22	4.453± 0.003$^{c**\ (ac)}$	0.037±0.003$^{c**\ (ac)}$
C.vulgaris	24:0	Day 14	86.60 ± 0.17^a	0.3170±0.0001^a
	12:12	Day 19	83.04 ± 0.19$^{b**\ (ab)}$	0.2313±0.0001^b
	8:16	Day 21	79.23 ± 0.21$^{c**(ac)}$	0.2010±0.0001$^{c*\ (ac)}$

Note: Data represented in Mean ± SD. [*] and [**] indicates significant difference at P < 0.05 and P < 0.001 (respectively) level between different superscripts depicted for each species.

Table 3. Cell density and specific growth rate of selected microalgae cultured at different photo periods.

Highest cell density and specific growth rate were recorded in all cultured species that were exposed to continued illumination (24:0. L/D) followed by 12:12 and 6:18 L/D respectively. Early stationary phases differed for *Nannochloropsis* sp. (day 13), *Chaetoceros* sp. (day 12) and *C.vulgaris*. (day 14) respectively while the corresponding specific growth rate was also highest under 24hours illumination. Significant variation in both growth parameters was observed between the cultures exposed to different photoperiods (P < 0.05 or P < 0.001) (Table 3). Photosynthetic efficiency of microalgae can be enhanced by sudden alteration between light

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and dark phase [63]. During this process, the fast reduction of e-acceptors, Qa and Qb, associated to photosystem II (PSII) followed by their oxidation in the dark period will take place that will ultimately maximise the proton-accepting capacity of PSII during sudden irradiant of light [64].

C.vulgaris proved its adaptability to different culture volumes and lighting periods with good growth performance comparable to *Nannochloropsis* sp. and better than *Chaetoceros* sp. The cells responded positively towards continuous illumination of light by producing higher cell density and specific growth rate in the culture media. It was also noted that the culture in the low-volume flask produced an early stationary phase due to high penetration of light and continuous sharing of available nutrients in the media for faster growth and survival. On the other hand, *C.vulgaris* consistently grew at significant cell densities even in larger volume containers and shorter period of illumination than dark condition (comparable to *Nannochloropsis* sp. and better than *Chaetoceros* sp.). In another study to analyse the effect of photoperiod to the cellular essential fatty acid in these species, the photoperiod of 12:12h L/D regime is recommended for the fast and economical technique for batch culture production [65]. A better ratio of essential fatty acid accumulated in *C.vulgaris* exposed in the 12:12h if compared to 24:0 or 8:16 L/D photoperiod.

Experiment 3: Low-cost Commercial Fertiliser for Mass Culture of Marine *Chlorella vulgaris*: Manipulation of N:P:K Ratio

C.vulgaris showed its adaptability to grow well when fertilised with a low-cost commercial N:P:K plant fertiliser (Figure 6). Duration of the log phase for *C.vulgaris* varied among treatments. The 12:6:4 and 12:8:4 ratios had a result of 3 days while the longest period was in the 15:15:15 treatment. Combined applications of urea, P+ and K+ (N:P:K; 12:6:4) produced the highest cell number (4.0×10^6 cells mL^{-1}) during log period at 5 days while N:P:K; 15:15:15 (control) produced highest cell number (4.16×10^6 cells mL^{-1}) at 7 days of log period. Different ratios of N:P:K, 12:8:4, 8:8:2 and 16:4:6 resulted in decrease of cell density, 3.3×10^6, 3.0×10^6 and 2.7×10^6 cells mL^{-1}, respectively.

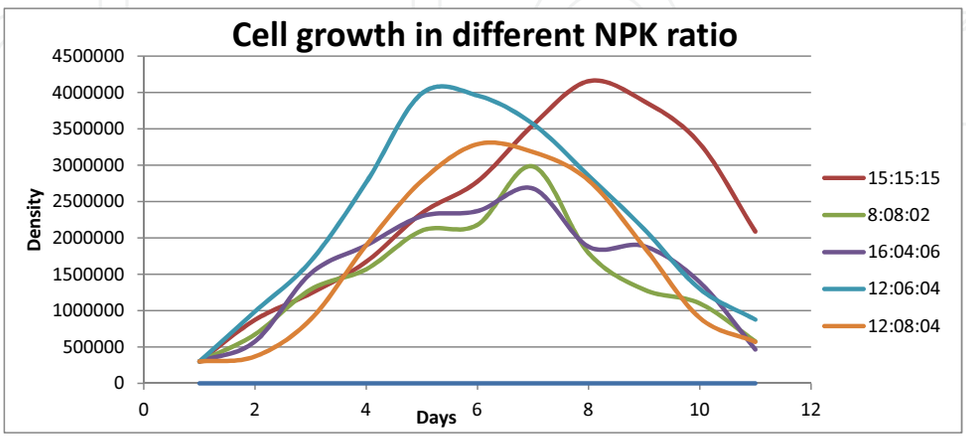

Figure 6. Density (cells mL^{-1}) of *C.vulgaris* cultured with commercial fertiliser of different N:P:K ratios

Days in Log Phase							
NPK ratio	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	
		Growth rate; K'					**Average**
15:15:15	1.07	0.35	0.30	0.34	0.17	0.25	0.41
8:08:02	0.81	0.65	0.20	0.29			0.49
16:04:06	0.65	0.96	0.23	0.19			0.51
12:06:04	1.19	0.53	0.50				0.74
12:08:04	0.20	0.87	0.77				0.61

Table 4. Growth rate of *C.vulgaris* cultured with commercial fertiliser of different N:P:K ratios

Days in Log Phase							
NPK ratio	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	
		Division/ Day; Div. day-1					**Average**
15:15:15	1.54	0.50	0.44	0.48	0.25	0.36	0.59
8:08:02	1.16	0.94	0.28	0.42			0.70
16:04:06	0.94	1.39	0.33	0.28			0.73
12:06:04	1.72	0.77	0.72				1.07
12:08:04	0.29	1.25	1.11				0.89

Table 5. Division per day of *C.vulgaris* cultured with commercial fertiliser of different N:P:K ratios

The 12:6:4 ratios showed the best average (74%) growth rates of natural increase at log phase. The second was 12:8:4 with 61% average growth rate.15:15:15 NPK ratio showed the lowest average growth rate of 41% (Table 4). The *C.vulgaris* cell in 12:6:4 NPK ratio recorded an average division per day by 107% which was the best compared to others. In 12:8:4 ratios the average cell division was 89% and decreasingly followed by 16:4:6 and 8:8:2 for 73% and 70% respectively. The control ratio which was 15:15:15 showed the lowest average division which was 59% (Table 5).

Measurement of generation time for *C.vulgaris* is summarised in Table 6 and Table 7. *C.vulgaris* cultured with fertiliser of the ratio 12:6:4 only took 1.09 days (26.22 hour) to complete one generation of replication, the shortest time compared to other treatments. The longest generation time was when using the15:15:15 ratio which was completed in 2.31 days (55.49 hour). The other three intermediate treatments recorded 1.70 (40.82 hour), 1.96 (47.11 hour) and 2.1 days (50.63 hour) for 12:8:4, 8:8:2 and 16:4:6 respectively. When comparing the performance by using all of the growth parameters, N:P:K; 12:6:4 ratio gave the best result with average

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Days in Log Phase							
NPK ratio	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	
		Generation time (days); Gen't					**Average**
15:15:15	0.65	2.00	2.28	2.06	4.07	2.80	2.31
8:08:02	0.86	1.06	3.55	2.38			1.96
16:04:06	1.06	0.72	3.04	3.62			2.11
12:06:04	0.58	1.31	1.39				1.09
12:08:04	3.41	0.80	0.90				1.70

Table 6. Generation time (days) for *C.vulgaris* cultured with commercial fertiliser of different N:P:K ratios

Days in Log Phase							
NPK ratio	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	
		Generation time (Hour); Gen't					**Average**
15:15:15	15.56	47.99	54.84	49.55	97.66	67.32	55.49
8:08:02	20.60	25.55	85.22	57.05			47.11
16:04:06	25.44	17.28	73.01	86.79			50.63
12:06:04	13.97	31.34	33.37				26.22
12:08:04	81.76	19.14	21.54				40.82

Table 7. Generation time (hour) for *C.vulgaris* cultured with commercial fertiliser of different N:P:K ratios

growth rate per day (74%), maximum growth rate day^{-1} (107%), maximum cell density (4.0×10^6 cell/mL), division's day^{-1} (107%) and generation time (1.09 day; 26.22 hour). *C.vulgaris* in control treatment (15:15:15) exhibited the poorest growth performance. Nonetheless, it is interesting to note that they experienced longer log period which could give more time for reproduction activity, thus the density did not decrease drastically as when cultured using other ratios. The fluctuation of temperature and different salinities could be the reason why cell densities were not as high as the first and second experiment.

Numerous nutrient media have been use for the culture of pure *Chorella* sp. Most of those were for laboratory use and/or for low-grade production of algae. Majority of these media are composed of pure nutrients (N-8). Commercial fertilisers are least considered for *Chlorella* culture because of the conception that they do not provide required nutrients for algal growth and are mostly suitable for crop (land) agriculture. Nevertheless, it has been proved that the commercial plant fertiliser could support a freshwater *Chlorella* [66]. The use of N:P:K fertiliser could be a better choice if compared to the organic fertiliser. Organic matter has its own limitations and depends on the microbial activity to release the inorganic nutrient and it cannot

be compared to the performance of pure nutrients. Despite good growth performance, the short period of the log phase when *C.vulgaris* is cultured using N:P:K; 12:6:4 need specific and efficient up-scaling or harvesting method, indicating that other ratios such as 15:15:15 could be a better choice.

Experiment 4: Egg Production, Growth and Development of *Apocyclops ramkhamhaengi* Fed on *C.vulgaris*

Different diets gives significantly ($P<0.05$) different densities of *A. ramkhamhaengi*. The mean gravid production of *A. ramkhamhaengi* fed on *C.vulgaris* and Baker's yeast was highest on 23rd day with 1.11ind./ml and 0.67ind./ml respectively. The production peaked on 11th, 23rd, and 26th day and on 17th, 20th and 23rd day when fed with *C.vulgaris*. and Baker's yeast respectively (Figure 7). In this 30 days culture, the highest mean population density of *A. ramkhamhaengi* fed with *C.vulgaris* was recorded on the 9th day with 3.31ind./ml and when fed on Baker's yeast was on day 19th with 1.83ind./ml (Figure 8). *A. ramkhamhaengi* fed on *C.vulgaris* showed the higher instantaneous growth rate (K) than when fed on Baker's yeast (Table 8). The period taken to double their population (Dt) was shorter in *A. ramkhamhaengi* fed *C.vulgaris* (8days) than Baker's yeast (11days).

Diets	Instantaneous growth rate (K)	Doubling Time(Dt)(day)
C.vulgaris	0.1150	8
Baker's yeast	0.0756	11

Table 8. The instantaneous growth rate and doubling time of *A.ramkhamhaengi* fed on different diets.

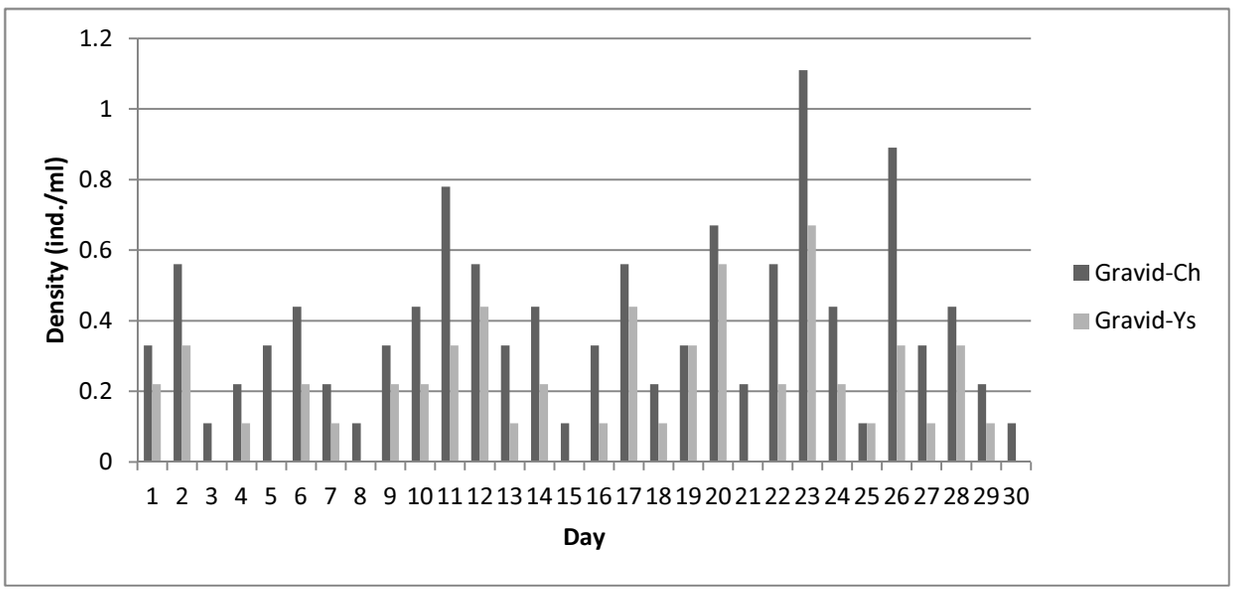

Figure 7. Mean density of gravid female of *A.ramkhamhaengi* fed with *C.vulgaris* (Gravid-Ch) and Yeast (Gravid-Ys) in 30 days.

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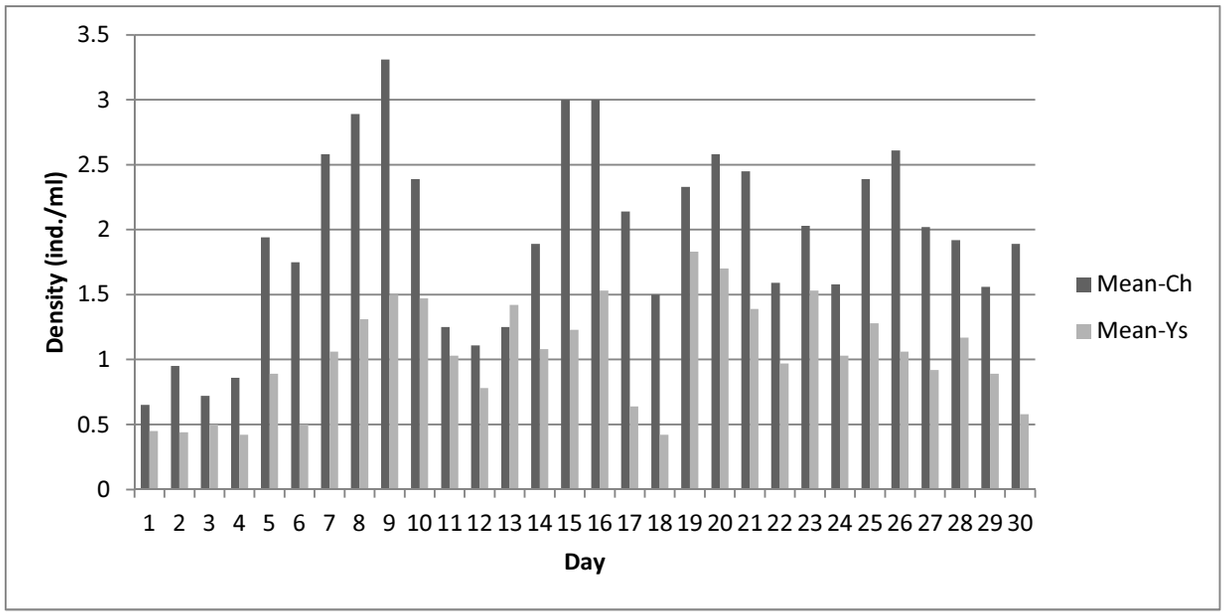

Figure 8. Mean total density of *A.ramkhamhaengi* fed with *C.vulgaris* (Mean-Ch) and Yeast (Mean-Ys) in 30 days.

The development times for nauplii, copepodite, adult and gravid female were observed separately using the copepod culture fed on *C.vulgaris*. The longest period was at copepodite stage (7.33 ± 2.08days) and the shortest period was the naupliar stage which needed only 1.33 ± 0.58 days (Figure 9). The mean number of eggs produced was 21.33 ± 1.53. Hatching percentage of the three individuals of *A. ramkhamhaengi* was 96.82 ± 2.77 % (Table 9). Maturation time which is the time between the appearance of eggs and their hatching time was 1.33 ± 0.58 days. The time taken to become gravid female from the produced nauplii was about 20.67 ± 3.51days and it is known as generation time.

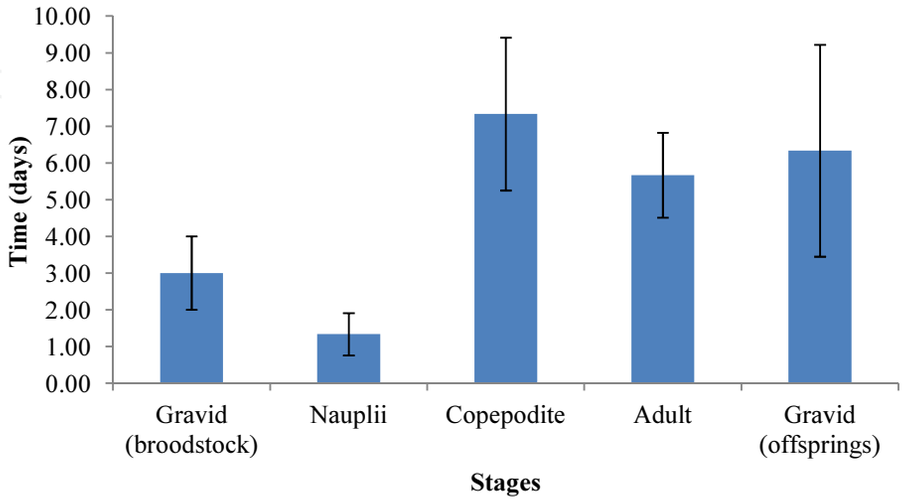

Figure 9. Mean development time in each stage in *A.ramkhamhaengi* life cycle

Parameter	N	Mean ± SD	Minimum	Maximum
Number of eggs	3	21.33 ± 1.15	20	22
% hatching	3	96.82 ± 2.77	95	100
Maturation time(days)	3	1.33 ± 0.58	1	2
Generation time(days)	3	20.67 ± 3.51	17	24

Table 9. The number of observation (N), mean and standard deviation (SD), minimum and maximum of total number of eggs, percentage of hatching of *A.ramkhamhaengi*, maturation time and generation reared in laboratory-controlled condition.

Production of the gravid females and the population density obviously increased when *A. ramkhamhaengi* was fed on *C.vulgaris* as compared to Baker's yeast. This finding is in agreement with the previous study [20] on the population growth and production of *A. dengizicus* fed on different diets. Population reached its peak in term of total density for several periods in the 30day culture condition indicates the existence of different populations. These populations reached their peak density in accordance with the diet taken where *Chlorella*-fed population were found to grow faster than those fed on Baker's yeast. It seemed that *A. ramkhamhaengi* has the potential to become more nutritional when enriched, thus growing faster when fed on a good-quality diet such as microalgae if compared to Baker's yeast. The nutritional value has been shown to increase when cyclopoid nauplii stage such as in *A. panamensis* was offered an enriched diet [15]. Although other microalgal diets such as *Tetraselmis* sp. and *Isochrysis* sp. could be a better choice for *Chlorella* sp. [67], at least the present finding is able to prove the potential of the species to reproduce and grow when fed on the marine *C.vulgaris* as used in this study.

A female of *A. ramkhamhaengi* fed on *C.vulgaris* could produce between 20 and 22 eggs with about 97% hatching success. This is more than what has been reported before for *A. panamensis* [68], and it could be related to many environmental factors and culture procedure. Environmental parameters such as temperature, food availability and predation were reported to influence the life-history strategy in copepods [69]. Binary diet of *Nannochloropsis* sp. and T-ISO improved the hatching rate by 88.1 ±2.1 % in a calanoid copepod, *Acartia sinjiensis* [70]. The brackish water cyclopoid, *A. ramkhamhaengi* has shown its potential to be cultured and reproduced under controlled conditions. The population adapted very well to the introduced diet, a marine *C.vulgaris* and a common Baker's yeast. *Chlorella*-fed population of *A. ramkhamhaengi* grow faster and need fewer number of days to double its population than those fed on Baker's yeast.The number of eggs produced was 21.33 ± 1.15 eggs at the maturation time of 1.33 ± 0.58 days and generation time was 20.67 ± 3.51 days. The species show great potential to be cultured together with *C.vulgaris* for hatchery and farm use. A more comprehensive study is essential to investigate the reproductive biology of this species, particularly in a large-scale production system to verify its suitability in aquaculture.

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4. Conclusion

C.vulgaris isolated from Bidong Island exhibited a rapid growth rate under optimum environmental conditions in the laboratory culture and was able to achieve an extremely high density when cultured in bigger containers. The photoperiod of 24:0 proved to be the best condition for cells growth but 12:12 L/D photoperiod could be the more economical. The high-density culture could be harvested using a relatively cheap, inexpensive and simple ultrafiltration technique for other use or reinoculation. This will save the space and long period of maintaining live algae for unexpected use. The cells collected using a ultrafiltration technique showed high viability and long shelf life when kept in 4^{o}C refrigerator. The product is called as *C.vulgaris* paste or concentrate which could be used to enrich or maintain the zooplankton live feeds for aquaculture purposes. The *C.vulgaris* also showed its best growth performance when cultured using a common commercial plant fertiliser with certain ratio of N:P:K. This was shown by their ability to perform cell division and grow and easily adapted to certain ratio such as 15:15:15 and 12:6:4. Nonetheless, the cells density is very much lower than those cultured with the specific chemical fertilizer, Conway media. This problem could be overcome by further investigation on their ion requirement when cultured openly in hatchery or ponds. The suitability of C.vulgaris as enriched diet for a zooplankton potentially used as live feed, A. ramkhamhaengi was proved by the population increase and reached high individual density with good reproduction performance. Maintaining local species of microalgae and zooplanktons in hatchery and ponds will definitely support the continued supply of live feeds for larval rearing and the aquaculture industry.

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