EFFECTS OF CULTURE CONDITIONS ON THE GROWTH RATE AND POPULATION SIZE OF *APOCYCLOPS DENGIZICUS* (ARTHROPODA: COPEPODA)

Trinh-Dang Mau*, Tran-Nguyen Quynh Anh, Le Ba Nguyen Hung

The University of Danang - University of Science and Education, Danang, Vietnam

*Corresponding author: tdmau@ued.udn.vn

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Abstract - The study aimed to investigate the growth rate and the population size of the copepod *Apocyclops dengizicus* under different culture conditions of salinity, temperature, and diet. Five salinities, four levels of temperature, and four diet ratios of baker's yeast and the microalgae were employed for a 20-day experiment. The results showed that *A. dengizicus* could survive in every experimental concentration of salinity after 20 days of culture, of which the highest growth rate and the largest population size were recorded at a salinity of 15 ppt with values of 0.26 ± 0.01d^{-1} and 1221 ± 270 individuals, respectively. As for temperature, the *A. dengizicus* population thrived best at 34°C, with a growth rate reaching 0.29 ± 0.01d^{-1} and a maximum population size of 2097.0 ± 193.29 individuals obtained at the end of the experiment. Moreover, a diet with 25% *Saccharomyces cerevisiae* and 75% *Chlorella vulgaris* showed the best population development (0.25 ± 0.01d^{-1}, 997.5 ± 1 92.09 individuals).

Key words - Copepoda; Culture conditions; Temperature; Salinity; Growth rate

1. Introduction

Apocyclops dengizicus is a species of zooplankton belonging to the group of planktonic crustaceans (Copepods), living in brackish water environments. The copepodite of *A. dengizicus* has a small size of less than 200 µm, and high levels of EPA, DHA, amino acids, essential fatty acids, vitamins, and many digestive enzymes [1], which are very suitable for the nutritional needs of larvae of aquatic species [2]. Meanwhile, the lack of nutrition from live food sources has been identified as a main cause of high mortality rates in the larvae of fish and shrimp, which is a current major challenge of aquaculture [3]. As such, *A. dengizicus* could be a potential live food source for shrimp and fish larviculture [4], [5].

The nutritional value of *A. dengizicus* specifically and of copepod species generally, depends on their size and the type of food they are fed. Farhadian et al. showed that the protein and lipid contents in *A. dengizicus* differed when fed with different microalga types [6]. Specifically, when fed with *Chaetocerous calcitrans* (C), *Tetraselmis tetrathele* (T), and their combination in 1:1 ratio (CT), *A. dengizicus* accumulated protein contents of 46.8%, 60.5%, and 55.3%, and lipid contents of 19.0%, 17.8% and 19.1% of dry weight, respectively. The total essential amino acids without tryptophan measurement were 57.1%, 60.3%, and 67.8%, and total non-essential amino acids were 42.9%, 40.0%, and 32.2% of total amino acids, respectively in C, T, and CT culture media. In addition, the DHA: EPA: ARA ratios of *A. dengizicus* were 6.8:3:1; 14:5.8:1, and 11.6:2.6:1 when fed with C, T, and CT, respectively. Moreover, other environmental factors including salinity, temperature, and light conditions significantly impacted *A. dengizicus*'s production and development. According to the study of Altaff and Janakiraman, a temperature of 26 ± 1°C may be ideal for *A. dengizicus* from Adyar estuary, India as their density, body size, and egg diameter were all superior to those under a higher temperature of 31 ± 1°C [7]. Meanwhile, the optimum temperature for *A. dengizicus* isolated from a shrimp pond in Malaysia to reach the maximum reproduction and shortest development time was 35°C [8]. Moreover, other conditions for the best growth of this strain were under 20 psu salinity, a light intensity of 33.3 µmol photons/m^2/s, and a continuous light period (24:0 h light:dark).

In Vietnam, information on potential copepod species as food sources for rearing seafood has been still very limited even though the demand for aquatic feed is increasing due to the expanding scale of the aquaculture sector [9]. Ut and Vinh investigated some biological characteristics (the filtration rate and feeding rate, the growth in length and time at different development stages, and the reproductive characteristics) of the species *Schmackeria dubia* [10]. Ut et al. conducted experiments to determine the ability of using bread yeast and the optimal harvesting rate in mass culture of the species *Schmackeria dubia* [11]. Meanwhile, the effects of microalgae *Isochrysis galbana* density on the fertility and hatching success of a cyclopoid copepoda *Apocyclops royi* was investigated by Nguyen et al. [12]. Ngoc et al. determined the effects of salinity on the growth and development of the copepod *Apocyclops panamensis* isolated from an intensive white shrimp pond [13]. For *A. dengizicus*, Pham et al. investigated the life cycle and reproductive characteristics of the species at different temperatures ranging from 26 to 34°C [14]. Their results show that the maturation time, reproductive rhythm, and embryo development time of *A. dengizicus* reduced with the increase of the temperature while the fertility and life cycle did not show significant differences at different temperatures.

In this study, we examined the effects of three major factors: Salinity, temperature, and diet on the development of *A. dengizicus* population with the aim to determine the most suitable culture conditions for the multiplication of this species. The study results could contribute to the application of *A. dengizicus* as a valuable live food for aquaculture under actual conditions in Vietnam and other areas with similar environmental conditions.

2. Material and Methods

2.1. Copepod stock culture

The copepod species *Apocyclops dengizicus* was provided by the Laboratory of Plankton Research, Faculty of

Biology and Environmental Science, The University of Danang - University of Science and Education. The *A. dengizicus* population was maintained in laboratory conditions at a temperature of 25°C, a light intensity of 500 lux, and a light regime of 16:8 h light:dark. The culture medium has a salinity of 20 ppt, prepared by diluting seawater with distilled water to the desired salinity. The copepods were fed daily with the microalgae *Chlorella vulgaris* at a density of $5x10^6$ cells/mL.

2.2. Experimental design to investigate the effects of salinity, temperature, and diet on A. dengizicus population development

Experiment 1: Effects of salinity on the development of A. dengizicus

To limit the sudden effects on the population of *A. dengizicus* when transferred from the stock culture condition to the experimental environmental conditions, the *A. dengizicus* population was evenly divided from the culture flask and acclimatized to the new environmental conditions with different salinity concentrations (10, 15, 20, 25, and 30 ppt) for a period of 10 days. Then, three pairs of *A. dengizicus* (consisting of 3 egg-bearing females and 3 male adults) that have adapted to the new environment conditions were selected from each salinity concentration treatment to monitor the characteristics and development indicators every 5 days for 20 days. During the experiment, except for the salinity that was different from each treatment, the remaining factors were maintained as in the stock culture condition. The copepods were fed daily with Chlorella vulgaris microalgae at a density of $5x10^6$ cells/mL of copepods culture medium. Each salinity treatment was repeated four times.

Experiment 2: Effects of temperature on the development of A. dengizicus

Four levels of temperature (25, 30, 34, and 38°C) were selected for the investigation of temperature effects on the *A. dengizicus* population. Similar to the 1st experiment, *A. dengizicus* population was first reared in temperature treatment conditions for 10 days for acclimatizing. Afterward, three pairs of *A. dengizicus* were selected from each temperature treatment for continuing rearing in corresponding temperature treatments and monitoring the population development every 5 days for 20 days. During the experiment, *A. dengizicus* individuals were maintained at the optimum salinity concentration obtained from the previous salinity experiment results (Experiment 1), a light intensity of 500 lux, a light regime of 16:8 h light:dark, and daily fed with *Chlorella vulgaris* microalgae at a density of $5x10^6$ cells/mL. Each temperature treatment was repeated 4 times.

Experiment 3: Effects of diet on the development of A. dengizicus

In this experiment, baker's yeast (*Saccharomyces cerevisiae*) (Sac) and the microalgae *Chlorella vulgaris* (Chl) were used in combination as food for copepods. Three pairs of *A. dengizicus* were collected from the cultural stock to examine the effects of four diets with different mixing ratios (25%Sac : 75%Chl, 50%Sac : 50%Chl, 75%Sac : 25%Chl, and 100%Sac : 0%Chl), with a daily feed of 1 $g/10^6$ individuals. The experimental environmental conditions were maintained at the optimum temperature and salinity obtained from the results of previous temperature and salinity effects experiments while other parameters (light intensity and light regime) were kept similar as in the stock culture condition. Each diet was repeated four times and the characteristics and development of the *A. dengizicus* population were monitored every 5 days throughout a 20-day experimental period.

2.3. Determination of growth rate and doubling time

The growth rate K (d^{-1}) of the *A. dengizicus* population was calculated using the following formula [15], [16]:

$$K = \frac{(ln_{Nt} - ln_{N0})}{dt}$$

Where: N0 and Nt (individual/mL) are the initial and final density of *A. dengizicus*, respectively, and t is the rearing time.

The doubling time Dt (days) was calculated by dividing $ln2$ by the growth rate K [17]:

$$Dt = \frac{ln2}{K}$$

2.4. Data analysis

Statistical analysis was performed using R software. Comparison of mean values between treatments was performed by one-way analysis of variance (ANOVA). In addition, Tukey's post hoc test was used to evaluate the actual differences between treatments after ANOVA analysis. P-values of less than 0.05 were identified as statistical significance.

3. Results and discussion

3.1. Effects of salinity on the growth rate and population size of A. dengizicus

The results showed that the *A. dengizicus* population could survive in a wide range of salinity from 10 to 30 ppt; however, its growth rate was different among these salinities (Table 1, Figure 1). At the end of the experiment (day 20), the highest growth rates of *A. dengizicus* were recorded at salt concentrations from 15 ppt to 20 ppt with respectively 0.26 ± 0.011 d^{-1} and 0.25 ± 0.01 d^{-1}. Meanwhile, the lowest growth rate was observed at a salt concentration of 30 ppt with a value of 0.19 ± 0.01 d^{-1}.

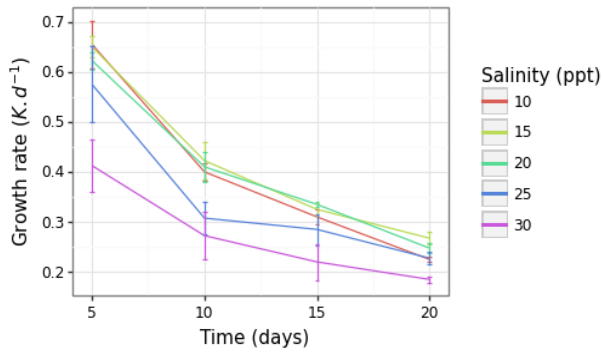

Figure 1. *Effects of different salinities on the growth rate of A. dengizicus population over 20 days of culture*

Table 1. *Effects of different salinities on the growth rate and doubling time of A. dengizicus population*

Day	Sanility level (ppt)	Growth rate K (d^{-1})	Doubling time Dt (days)
5	10 ppt	0.65 ± 0.05^{a}	1.06 ± 0.08^{a}
	15 ppt	0.65 ± 0.02^{a}	1.07 ± 0.03^{a}
	20 ppt	0.62 ± 0.02^{a}	1.12 ± 0.04^{a}
	25 ppt	0.57 ± 0.07^{a}	1.22 ± 0.15^{a}
	30 ppt	0.41 ± 0.05^{b}	1.69 ± 0.21^{b}
10	10 ppt	0.40 ± 0.02^{a}	1.74 ± 0.08^{a}
	15 ppt	0.42 ± 0.04^{a}	1.65 ± 0.15^{a}
	20 ppt	0.41 ± 0.03^{a}	1.83 ± 0.13^{a}
	25 ppt	$0,31 \pm 0,03^{bc}$	2.29 ± 0.26^{b}
	30 ppt	0.27 ± 0.05^{c}	2.60 ± 0.43^{b}
15	10 ppt	0.31 ± 0.01^{a}	2.24 ± 0.01^{a}
	15 ppt	0.32 ± 0.01^{a}	2.15 ± 0.01^{a}
	20 ppt	0.34 ± 0.01^{a}	2.06 ± 0.01^{a}
	25 ppt	0.29 ± 0.03^{a}	2.43 ± 0.03^{a}
	30 ppt	0.22 ± 0.03^{b}	3.17 ± 0.03^{b}
20	10 ppt	0.22 ± 0.004^{a}	3.05 ± 0.06^{a}
	15 ppt	$0.26 \pm 0,011^{bc}$	2.62 ± 0.11^{b}
	20 ppt	0.25 ± 0.01^{c}	2.81 ± 0.08^{ab}
	25 ppt	0.23 ± 0.01^{a}	3.07 ± 0.16^{a}
	30 ppt	0.19 ± 0.01^{d}	3.72 ± 0.12^{c}

Note: Data presented in the table are means and standard deviations (mean ± SD, n = 4). Values within the same column with different superscripts indicate statistically significant differences (p-value<0.05, ANOVA following Tukey's post hoc test)

The population doubling time showed an increasing trend from the beginning till the end of the experiment in all salt concentrations. The shortest population doubling time on day 5 was observed in treatments with the salinity from 10 ppt to 20 ppt (1.06 ± 0.08 days to 1.12 ± 0.04 days). At day 20, the shortest doubling time was found at a salinity of 15 ppt with a value of 2.62 ± 0.11 days, followed by 20 ppt (2.81 ± 0.08 days). The longest doubling time during the experiment always fell in the salinity of 30 ppt (1.69 ± 0.21 days on day 5 and 3.72 ± 0.12 days on day 20).

The population size of *A. dengizicus* under different salinities is shown in Figure 2. In general, after 20 days of the experiment, the largest population size was found at the salinity 15 ppt with 1221 ± 270 individuals.L^{-1}, followed by 20 ppt salinity with 842 ± 121 individuals.L^{-1}. However, the difference between these two treatments was not statistically significant (p-value> 0.05). The number of individuals in 10, 25, and 30 ppt were respectively 515 ± 52.4, 567 ± 124.1, and 252 ± 30 individuals.L^{-1}. A salinity of 30 ppt consistently resulted in the lowest total number of individuals in the *A. dengizicus* population during 20 days of the experiment. Regarding the population development over time, the population size of *A. dengizicus* showed an increasing trend from day 0 to day 20 at salinities of 15, 25, and 30 ppt. Meanwhile, at the salinities of 10 and 20 ppt, a decrease in population size was observed after day 15. However, this decrease was not significant (p-value > 0.05).

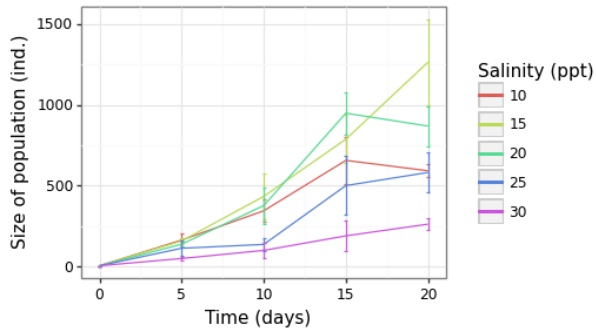

Figure 2. *Effects of different salinities on the size of A. dengizicus population over 20 days of culture*

Our results were similar to Farhadian et al.'s study, which reported that *A. dengizicus* can easily grow and reproduce in a wide range of salinities from 5 to 35 ppt but prefer brackish waters to marine and fresh waters [8]. Specifically, salinities of 15 ppt and 20 ppt gave the best growth rate (0.302 ± 0.002 d^{-1} and 0.314 ± 0.003 d^{-1}, respectively) and the shortest doubling time (2.30 ± 0.02 days and 2.20 ± 0.01 days, respectively) of *A. dengizicus* population while salinities of 5 ppt and 30 ppt caused negative effects its on population and reproduction. The good tolerance of *A. dengizicus* to salinity was also recognized by Dexter [18]. This species from the Salton Sea, California could tolerate salinities from 0.5 to 79 ppt and possibly up to 107 ppt; however, low salinities (0.5 - 1 ppt) and high salinities (68 - 79 ppt) led to its poorer performance, e.g., higher mortality, lower densities, fewer nauplii surviving to copepodite stage. The salt tolerance of *A. dengizicus* was similar to that of the other cyclopoid copepod species. The copepod *Apocyclops royi* also exhibited the highest productivity at a 20 ppt salinity (1917.25 ± 316.5 individuals) and lower population growth rates at the extreme salinities of 0 - 5 ppt (67.25 ± 12.8 and: 229.25 ± 58.6, respectively) and 30 - 35ppt (237.25 ± 23 and 85.25 ± 38 individuals, respectively) [19]. The optimal salinity for the population growth of the copepod *Oithona nana* was 20 ppt [20]. Moreover, a salinity of 20 ppt was pointed out to provide better growth and development rate of the copepod *Apocyclops panamensis* than 10 and 30 ppt of salinity [13]. These results demonstrated that *A. dengizicus* in particular and other cyclopoid copepod species in general are capable to tolerate a wide range of salinities, which is a favorable trait for applying in aquaculture. Even though, these species showed a tendency to favor a medium salinity, in this study is around 20 ppt. This is possible because too low or too high salinities can cause physiological stress that led to less population development and poorer performance. In addition, the salinity levels may also influence the osmoregulation and respiration demands of copepods, led to affect their survival and production [20]–[22].

3.2. Effects of temperature on the growth rate and population size of A. dengizicus population

The experimental results demonstrated that the growth rate of *A. dengizicus* was significantly affected by different temperature treatments after 20 days of culture, as shown in Table 2 and Figure 3. The treatment at 34°C resulted in

the highest growth rate, followed by 30v. Meanwhile, treatments at 25°C and 38°C led to the lowest growth rates. Specifically, the growth rate of *A. dengizicus* on day 5 was 0.80 ± 0.07 d^{-1} at 34°C, followed by 0.67 ± 0.04 d^{-1} at 30°C, 0.57 ± 0.03 d^{-1} at 25°C, and 0.35 ± 0.05 d^{-1} at 38°C. On day 20, the growth rate was 0.29 ± 0.01 d^{-1}, 0.28 ± 0.002 d^{-1}, 0.24 ± 0.02 d^{-1}, and 0.18 ± 0.03 d^{-1}, respectively under 34°C, 30°C, 25°C, and 38°C treatments. This difference was statistically significant (p-value < 0.05).

Relating to the population doubling time, on day 5, the shortest time was 0.87 ± 0.07 days recorded at 34°C whereas the longest time was 2.04 ± 0.31 days at 38°C. After 20 days of experiment, the doubling time gradually increased in all treatments with the shortest time (2.37 ± 0.04 days) observed in the 34°C treatment and the longest time (5.13 ± 1.75 days) was still at the 38°C treatment.

Table 2. *Effects of different temperatures on the growth rate and doubling time of A. dengizicus population*

Day	Temperature (°C)	Growth rate K (d^{-1})	Doubling time Dt (days)
5	25°C	0.57 ± 0.03^{a}	1.22 ± 0.07^{a}
	30°C	0.67 ± 0.04^{a}	1.03 ± 0.06^{ac}
	34°C	0.80 ± 0.07^{b}	0.87 ± 0.07^{bc}
	38°C	0.35 ± 0.05^{c}	2.04 ± 0.31^{d}
10	25°C	0.32 ± 0.01^{a}	2.15 ± 0.06^{a}
	30°C	0.47 ± 0.02^{b}	1.48 ± 0.08^{b}
	34°C	0.51 ± 0.04^{b}	1.37 ± 0.10^{b}
	38°C	0.32 ± 0.02^{c}	2.20 ± 0.11^{a}
15	25°C	0.30 ± 0.01^{a}	2.30 ± 0.10^{a}
	30°C	0.38 ± 0.03^{b}	1.81 ± 0.03^{b}
	34°C	0.38 ± 0.03^{b}	1.81 ± 0.02^{b}
	38°C	0.21 ± 0.03^{c}	3.94 ± 0.10^{c}
20	25°C	0.24 ± 0.02a	2.95 ± 0.19a
	30°C	0.28 ± 0.002^{b}	2.46 ± 0.02^{a}
	34°C	0.29 ± 0.01b^{c}	2.37 ± 0.04^{a}
	38°C	0.18 ± 0.03^{d}	5.13 ± 1.75^{b}

Data presented in the table are means and standard deviations (mean ± S.D., n = 4). Values within the same column with different superscripts indicate statistically significant differences (p-value<0.05, ANOVA following Tukey's post hoc test)

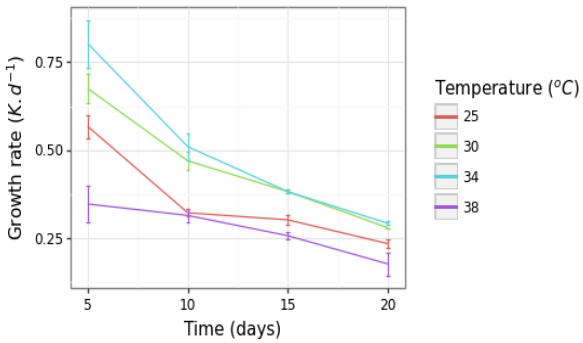

Figure 3. *Effects of different temperatures on the growth rate of A. dengizicus population over 20 days of culture*

The temperature factor had a significant impact on the size of the *A. dengizicus* population (Figure 4). From day 0 to day 15, the *A. dengizicus* population showed growth with the largest population sizes on day 15 were 1907 ± 110.54 and 1886 ± 185.02 individuals respectively under the treatments of 34°C and 30°C. The difference in population size between the two treatments 34°C and 30°C was not statistically significant (p-value > 0.05). Meanwhile, it is worth noting that there was a significant difference between treatments of 34°C and 38°C on day 15, where the population size at 34°C was more than 6 times higher than that at 38°C (291 ± 43.13 individuals) (p-value < 0.0001).

By the 20th day, the population size in the treatments of 30°C and 38°C tended to decrease gradually compared to day 15, with a population size of 1694 ± 77.67 and 222.8 ± 178 individuals, respectively. On the other hand, an increase in the number of individuals in the *A. dengizicus* population was observed in the treatments of 25°C and 34°C, with a population size of 689 ± 215.37 and 2097.0 ± 193.29 individuals, respectively. This resulted in a superiority in population size at 34°C over other temperatures on day 20, which was respectively 3 times, 2 times, and 9 times significantly higher than that under 25°C, 30°C, and 38°C treatments (p-value < 0.05).

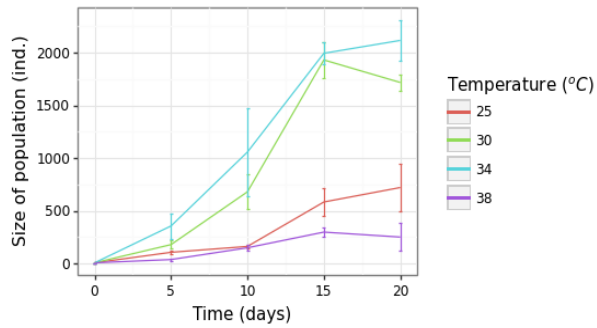

Figure 4. *Effects of different temperatures on the size of A. dengizicus population over 20 days of culture*

The temperature was reported to significantly influence the copepod species in many aspects including growth and reproduction parameters. High water temperature leads to an increase in growth rate and shortens the maturation time [8]. High temperature also decreases development time and results in reducing the final organism body size of *A. dengizicus* [14]. Furthermore, high temperature can inhibit their growth and reproduction as well as the sex ratio [23]. Pham et al. reported that the maturation time, reproductive rhythm, and embryo development time are also shortened when the temperature increases [14]. In contrast, low temperature causes eggs to fail to hatch or extends the hatching time [10]. In addition, daily nauplii production was reduced and growth retardation was observed at low temperatures (15 - 20°C) in the copepod *Paracyclopina nana* [24]. In our study, the copepod *A. dengizicus* can survive and develop at temperatures from 25°C to 38°C throughout its life cycle, but the optimal temperature was 34°C. Farhadian et al. [8] reported similar results with the treatment at 35°C resulting in the highest growth rate (0.298 ± 0.007 d^{-1}), followed by the 30°C treatment (0.285 ± 0.007 d^{-1}), 25°C treatment (0.267 ± 0.006 d^{-1}), and 20°C treatment (0.092 ± 0.008 d^{-1}). Moreover, the shortest development time was also obtained at 35°C with 2.32 ± 0.04 days, followed by

30°C with 2.43 ± 0.05 days, while the treatment at 20°C led to the longest doubling time of up to 7.5 ± 0.57 days. This suggests that a temperature from 30 to 35°C might be suitable for the development of *A. dengizicus*, temperatures above or below this threshold could cause a decrease in the population growth rate. The optimal temperature for *A. dengizicus* was similar to that for the cyclopoid copepod *Apocyclops royi* (32°C) [25], but relatively higher than that for Paracyclopina nana (25°C - 30°C) [24].

3.3. Effects of diet on the growth rate and population size of A. dengizicus population

The effects of different diets (25%Sac:75%Chl, 50%Sac:50%Chl, 75%Sac:25%Chl, and 100%Sac:0%Chl) on the growth rate and population doubling time of *A. dengizicus* were investigated over a 20-day experiment (Table 3). The treatment with 25%Sac:75%Chl showed the highest growth rate during the experiment, which was 0.62 ± 0.04 d^{-1} on day 5 and 0.25 ± 0,01 d^{-1} on day 20 (Table 3, Figure 5). Meanwhile, the treatment with 100%Sac:0%Chl led to the lowest growth rate with 0.44 ± 0.10 d^{-1} and 0.19 ± 0.004 d^{-1} on day 5 and day 20, respectively.

Table 3. *Effects of different diets on the growth rate and doubling time of A. dengizicus population*

Day	Diet (%Sac:%Chl)	Growth rate K (d^{-1})	Doubling time Dt (days)
5	100%Sac:0%Chl	0.44 ± 0.10^{ab}	1.60 ± 0.20^{a}
	75%Sac:25%Chl	0.52 ± 0.02^{a}	1.33 ± 0.06^{b}
	50%Sac:50%Chl	0.54 ± 0.02^{b}	1.29 ± 0.05^{b}
	25%Sac:75%Chl	0.62 ± 0.04^{c}	1.12 ± 0.08^{b}
10	100%Sac:0%Chl	0.29 ± 0.01^{a}	2.38 ± 0.11^{a}
	75%Sac:25%Chl	0.29 ± 0.03^{a}	2.39 ± 0.10^{a}
	50%Sac:50%Chl	0.34 ± 0.03^{b}	2.05 ± 0.19^{b}
	25%Sac:75%Chl	0.38 ± 0.01^{c}	1.83 ± 0.05^{b}
15	100%Sac:0%Chl	0.22 ± 0.01^{a}	3.08 ± 0.01^{a}
	75%Sac:25%Chl	0.31 ± 0.008^{b}	2.22 ± 0.06^{a}
	50%Sac:50%Chl	0.31 ± 0.01^{ac}	2.26 ± 0.04^{b}
	25%Sac:75%Chl	0.32 ± 0.004^{c}	2.19 ± 0.03^{b}
20	100%Sac:0%Chl	0.19 ± 0.004^{a}	3.60 ± 0.08^{a}
	75%Sac:25%Chl	0.24 ± 0.01^{b}	2.88 ± 0.07^{bd}
	50%Sac:50%Chl	0.24 ± 0.01^{b}	2.84 ± 0.08^{bc}
	25%Sac:75%Chl	0.25 ± 0.01^{b}	2.72 ± 0.11^{b}

Data presented in the table are means and standard deviations (mean ± S.D., n = 4). Values within the same column with different superscript indicate statistically significant differences (p-value<0.05, ANOVA following Tukey's post hoc test)

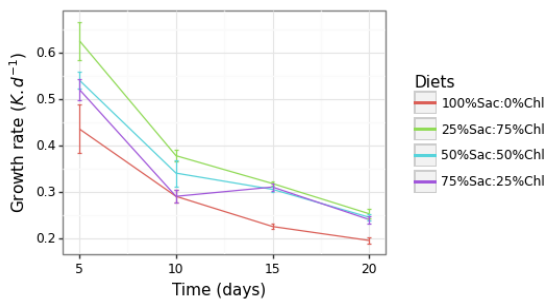

Figure 5. *Effects of different diets on the growth rate of A. dengizicus population over 20 days of culture*

Similar to the growth rate, the population doubling time was shortest under the treatment with 25%Sac:75%Chl (1.12 ± 0.08 days on day 5 and 2.72 ± 0.11 days on day 20) whereas the treatment with 100%Sac:0%Chl led to the longest doubling time of *A. dengizicus* population (1.60 ± 0.20 days and 3.60 ± 0.08 days on day 5 and day 20, respectively).

The population density of *A. dengizicus* showed an increasing trend over 20 days of the experiment for all treatments with different ratios of baker's yeast and *Chlorella vulgaris*. However, while three treatments with the presence of microalgae in the diet (25%Sac:75%Chl, 50%Sac:50%Chl, and 75%Sac:25%Chl) resulted in a sharp increase in population size, the treatment without microalgae (100%Sac:0%Chl) produced only a slight increase (Figure 6). This led to the population size of *A. dengizicus* in day 15 and 20 under these three treatments significantly higher over that in the treatment with 100%Sac:0%Chl (p-value < 0.05). Among these, the diet with 25%Sac:75%Chl led to the largest population size from the beginning till the end of the experiment. On day 20, the population density of *A. dengizicus* in this treatment was 997.5 ± 192.09 individuals whereas this value for treatments with 50%Sac:50%Chl, 75%Sac:25%Chl, and 100%Sac:0%Chl was respectively 801 ± 114.73, 739.5 ± 91.18, and 283.5 ± 23.17 individuals.

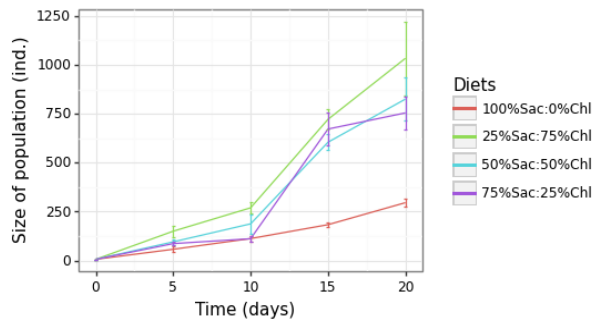

Figure 6. *Effects of different diets on the size of A. dengizicus population over 20 days of culture*

Farhadian et al. reported that the growth rate of *A. dengizicus* when fed with baker's yeast was lower than that when fed with algae, but baker's yeast can be used as an alternative food source when algae production is difficult [6]. In this study, the lowest growth and population size of *A. dengizicus* was observed on the diet with 100% yeast and the highest growth was on the diet with the highest ratio of microalgae (25%Sac:75%Chl). Our findings are similar to the results of Vu et al. [11], which showed that the combined ratio for the best growth of copepods was 75% algae and 25% baker's yeast. Yeast has the advantage of having a high protein content (45 - 52%) and low cost, thus, can reduce the cost of farming copepods [11]. However, it lacks essential nutrients (e.g., DHA, EPA, ARA fatty acids) that are important to the growth of the copepods. Therefore, if only yeast is used as the main food for copepods, it can not meet their nutritional needs and could affect their development and nutritional value [6], [26]. However, despite the lower growth rate than other algal supplementation treatments, the *A. dengizicus*

population in this study and the *Schmackeria dubia* in Ut et al.'s study were still able to grow under the conditions of feeding only baker's yeast [11]. This implies that yeast can completely serve as a food source for copepods when algae production is difficult.

4. Conclusions

The rearing conditions, here are salinity, temperature, and diet, greatly influence the population of *A. dengizicus*. Moreover, our experiment with various treatments also demonstrates that the copepods could exist, develop and reproduce in a wide range of temperatures, salinities, and food diversity. However, the best condition for the development and reproduction of *A. dengizicus* was found to be in an environment with a temperature of 34°C, a salinity of 15-20 ppt, and a diet of 25%Sac:75%Chl. These results contribute more useful information to making the production of copepods on a large scale for the aquaculture industry as a food source more convenient.

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