

Effects of salinity, temperature, light intensity and light regimes on production, growth and reproductive parameters of *Apocyclops dengizicus*

Farhadian, O.^{1*}; Md Yusoff, F.² and Arshad A.²

Received: May 2012

Accepted: December 2012

Abstract

The effects of salinity, temperature, and light conditions on production and development, longevity, survival and sex ratios of the cyclopoid copepod, *A. dengizicus* were determined. Seven different salinity levels (5, 10, 15, 20, 25, 30, 35 psu), four temperatures (20, 25, 30, 35 °C), three different light intensities (33.3, 85.3, 162.1 $\mu\text{mol photons/ m}^2/\text{s}$) and light regimes (24:0, 0:24, and 12:12 h light:dark regime) were employed. The highest production was achieved under 20 psu salinity. The optimum temperature required for the maximum reproduction and shortest development time was 35 °C. The production was highest ($p<.05$) and development rate of *A. dengizicus* was shortest ($p<.05$) under the lowest light intensity (33.3 $\mu\text{mol photons/ m}^2/\text{s}$). Continuous light (24:0 h LD) showed positive effects on growth and production. Light regimes 24:0 h and 12:12 h LD yielded the highest total production and growth ($p<.05$), with highest ($p<.05$) survival percentage. This study demonstrated that *A. dengizicus* can tolerate wide range of environmental conditions and can be cultured for commercial live feed purposes as well as toxicity studies.

Keywords: Environmental parameters, *A. dengizicus*, Cyclopoid copepod, Tropical, Production, Longevity, Sex ratio

1-Department of Natural Resources, Isfahan University of Technology, 84156-83111 Isfahan, Iran,

2-Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia

* Corresponding author's email: farhadyo@yahoo.com, omfarhad@cc.iut.ac.ir

Introduction

Copepods are an important link between phytoplankton, microzooplankton and higher trophic levels such as fish and shrimp (Kleppel, 1993). Accurate estimation of growth and reproduction of cyclopoid copepods in the field is difficult. However, laboratory culture of organisms under similar natural ranges of environmental parameters is one of the methods to determine production, growth, and reproductive parameters. Such information is important for the successful culture of copepods for use as live feed in the aquaculture industry (James and Al-Khars, 1986; Hernandez Molejon and Alvarez – Lajonchere, 2003). In addition, information on water quality requirements is necessary for toxicological studies of copepods (Bengtsson, 1978) and help to find good candidates for controlling mosquitoes (Rey et al., 2004). In spite of most live food, *A. dengizicus* maintenance is easy and when rotifer or protozoa contaminate it can easily subcultured by separation. Then, this species can be used as good copepod stock for long time without special care due to high tolerance to environmental parameters as well as to different food items (Cano et al., 2004). This species has six naupliar (85-240 μm size) and six copepodid (320-680 μm size) stages before become adult (Alvarez Valderhaug and Kewalramani, 1979; Farhadian, 2006). The high nutritional values of *A. dengizicus* compared to newly hatched *Artemia* nauplii and improved survival and growth rates of *Penaeus monodon* postlarvae (PL1-PL15) make it as new live food for aquaculture marine shrimp industry. The availability of *A.*

dengizicus in Malaysian coastal tropical waters and its occurrence in marine shrimp ponds (Farhadian, 2006) makes it as prey for larviculture. The purposes of this study were to determine the population growth and production of *A. dengizicus*, and to estimate survival, and developmental rates, fecundity (egg production), longevity and sex ratio under different levels of salinity, temperature, light intensity and light regimes.

Materials and methods

Apocyclops dengizicus was collected and isolated from a shrimp pond in Kuala Selangor ($03^{\circ} 17'$ N, $101^{\circ} 17'$ E) and the culture was maintained at the Aquatic Research Laboratory at Universiti Putra Malaysia (UPM). They were fed with mixed microalgae including *Nannochloropsis oculata* (2-4 μm , 0.500×10^{-5} μg d.w./ cell), *Isochrysis galbana* (4-7 μm , 7.097×10^{-5} μg d.w./ cell), *Chaetoceros calcitrans* (6-9 μm , 1.313×10^{-4} μg d.w./ cell), and *Tetraselmis tetrathele* (10-16 μm , 1.566×10^{-4} μg d.w./ cell) at least two weeks before the experiments. Dry weights of algal cells were determined by filtering and drying algae from aliquots of culture of known concentration according to method described by Lavens and Sorgeloos (1996). Since ingestion rates of nauplii, copepodids and adults stages of *A. dengizicus* differed for each other (Farhadian, 2006), the mixed microalgae prepared for better population growth and other reproductive parameters. During stocks maintenance each culture was examined daily,

any dead individuals were removed and up to 30 % of water was also changed daily.

Four algal species : *N. oculata*, *I. galbana*, *C. calcitrans*, and *T. tetrathele* were grown in Conway medium (Tompkins et al., 1995), at $29 \pm 1^{\circ}\text{C}$ ($\pm \text{SE}$), salinity 30 psu, 12:12h light : dark cycle and 40 μmol photons / m^2/s light intensity in 10-L carboys separately with mild continuous aeration for 10 days. The phytoplankton concentration in each beaker was determined using an improved Neubauer hemocytometer ($0.25 \text{ mm}^2 \times 0.1 \text{ mm}$) under a phase contrast microscope (Nikon / Eclipse 600) according to Martinez and Chakroff (1975) after the samples were fixed in lugol's iodine solution (0.1 mL for 3 mL sample).

Algal cells were collected by centrifugation at 8,000 rpm for 10 minutes when the microalgae growth reached the stationary phase. The algal density controlled during copepod feeding to prevent any contamination as well as preparing same algal feed for both set of experiment.

In addition, the microalgae concentrated before using in order to remove salt and Conway medium. A laboratory centrifuge Sigma 4-15 (10.380 g) using 250 mL bottle at 8000 rpm for 10 minutes used for algal harvesting. The microalgal pellet was then stirred in autoclaved filtered seawater and then re-centrifuge harvested microalgae were chilled to 4 or -20°C for *T. tetrathele* and stored for one week before an experiment (Heasman et al., 2000).

Every week during the experiment a quaternary, equal ration (by number) mix of the concentrated microalgae was prepared and

stored at 5°C . This algal mix ($5 \times 10^6 \text{ cells mL}^{-1}$) was fed twice a day in the morning and evening. This high numeric mixed algal concentration guarantee growth and production of *A. dengizicus* and also reduces possible effects of algal depletion and lack of nutrient during centrifuging. Therefore, it was suitable for population growth and production. On the other hand, weekly algal preparation allowed using the best algal growth phase which seems to be very suitable for copepod growth and reproduction. *Apocyclops dengizicus* easily grow and reproduce in microalgae which prepared in mentioned conditions (Farhadian, 2006).

To examine the effects of environmental parameters on the life cycle of *A. dengizicus* including salinity (0, 5, 10, 15, 20, 25, 30 and 35 psu), temperature (20, 25, 30, and 35°C), light intensity (33.3, 85.3, 162.1 μmol photons / m^2/s), light regime (24:0 12:12, 0:24 h, light: dark); two sets of experiments were run under controlled conditions for each parameter. In the first set, five *A. dengizicus* gravid females were reared in each of six glass beakers at different levels environmental parameter for 20 day period to determine population growth and production rates with 200 mL working volume (7.5 cm diameter). In the second set of each experiment, one *A. dengizicus* gravid female was reared at similar environmental conditions in 40 mL vial (4.5 cm diameter) to determine development, survival, fecundity, longevity and sex ratio. In addition, a second series of experiments continued until all gravid females died. For both sets of experiments, autoclaved filtered (0.47 mm Wattman) natural sea water

(collected from Port Dickson station) was partially replaced every two to three days or as needed to maintain the algal density and salinity levels. Both set of experiments carried on same conditions for effects of each parameter. The effects of examined parameters on population and reproductive parameters of *A. dengizicus* carried out respectively and results obtained in each part were used for next experiment. For salinity experiment, the three remaining parameters were kept close to habitat (27.5 °C, 45 μmol photons/ m^2/s and 12:12 h LD). After obtaining results from salinity experiment, the best salinity overall was used for other remaining experiments. Respectively, salinity, temperature, light intensity and light regimes experiments were carried out. The light regimes experiment was carried out at 20 psu, 30 °C, 33.3 μmol photons/ m^2/s). The above mentioned method allowed minimizing interaction between parameters, enough time to measurements, and practical suitable place to constant parameters because both set of each experiment incubated in the same time with similar *A. dengizicus* stock.

All gravid females (carrying egg sacs) were selected from one batch and placed in each experimental unit (beaker and vial). Every other day, at least two 10-mL samples from each beaker were removed and all the copepods were counted and staged. In the second set of the experiments, the females were removed from vials when they exuded their egg sacs. Egg sac development was monitored in the morning, afternoon and evening every day. After each brood hatched, the females were transferred by disposable

glass Pasteur pipettes (23 cm length) to a new vial. The average naupliar density in each vial was 1 nauplius mL. The developmental stages of brood were monitored daily. The brood was considered to have completed the naupliar stage when 50% of individual was copepodids. When the brood matured, each vial was fixed with a few drop of 10% formalin, sexed and counted. The sex ratio was expressed as ratio male to female in a brood.

The specific population growth rate (*SGR*) (*K*) of *A. dengizicus* copepods was calculated as $K = (\ln N_t - \ln N_0)/T$, where N_t and N_0 were total number and initial number of copepods after T days (Omori and Ikeda, 1984). In addition, doubling time was calculated by dividing \log_2 by the specific population growth rate (*K*) according to James and Al-Khars (1986).

Salinity was measured using a hand refractometer (ATAGO, Japan), temperature with a mercury thermometer (Strengthened, England) and light intensity with light meter (model LI-COR Light Sensors LI-189). All the experiments were performed and maintained inside a growth cabinet (Versatile Environmental Test Chamber, Sanyo MLR). Cabinet exterior and interior dimensions were 760 (width) \times 700 (depth) \times 1835 (height) mm and 520 (width) \times 490 (depth) \times 1135 (height) mm, respectively. 5 fluorescent lamps and 5 glow lamps are incorporated to light the chamber. The control panel has settings for temperature and light program and alarm.

All of the experiments were conducted using a completely randomized design with six replicates in each treatment. Differences in treatment means were compared by Duncan's

New Multiple Range Test (DNMRT). The percentage of survival rates were arcsine-square root transformed to ensure a normal distribution (Zar, 1984) and tested for statistical significance ($p < .05$) by one-way analysis of variance (ANOVA). All statistical analysis was carried out using Statistical Package for Social Science (SPSS, 2002).

Results

The highest *A. dengizicus* production (including nauplii, copepodids and adults), and specific population growth rate and the lowest doubling time of were found at a salinity of 20

psu (Table 1). The development from egg to the onset of sexual maturation and observation of a female with an egg sac was significantly shortest (9.38 days) at 30 psu while it was longer at 35 psu (11.93 days) and 5 psu (11.70 days). The total survival rates from nauplius I to adult was significantly higher at 20 psu (91.5 %) and 25 psu (88.0 %). Longevity of *A. dengizicus* females varied between 43 to 71 days, with the maximum at 30 psu and the minimum at 5 psu (Table 1). The sex ratio (male to female) in the cultured broods ranged from 0.90 to 1.17.

Table 1:Effects of different salinities on *A. dengizicus*

Parameters	Salinity levels (psu)						
	5	10	15	20	25	30	35
Production(ind./female)	115.3 \pm 6.6 f	158.7 \pm 1.1 e	422 \pm 14. b	535.3 \pm 30.0 a	282.7 \pm 4.6 c	240 \pm 10.9 d	118 \pm 12.0 f
Production(ind./day)	5.8 \pm 0.3 f	7.9 \pm 0.6 e	21.1 \pm 0.7 b	26.8 \pm 1.5 a	14.1 \pm 0.2 c	8.8 \pm 0.5 d	5.9 \pm 0.6 f
Specific population growth rate (K) (SGR)	0.237 \pm 0.003f	0.25 \pm 0.003e	0.302 \pm 0.002b	0.314 \pm 0.003a	0.282 \pm 0.001c	0.259 \pm 0.003d	0.239 \pm 0.004 f
Doubling time (days)	2.92 \pm 0.03 a	2.74 \pm 0.04 b	2.30 \pm 0.02c	2.20 \pm 0.01 c	2.45 \pm 0.01 c	2.67 \pm 0.02 b	2.90 \pm 0.04 a
Developmental time (NI-NVI) (days)	6.56 \pm 0.24 a	5.68 \pm 0.22 bc	5.90 \pm 0.12b	4.21 \pm 0.13cd	5.15 \pm 0.13 d	5.13 \pm 0.14 d	4.63 \pm 0.42 e
Developmental time (CI-CVI) (days)	3.79 \pm 0.25 c	3.67 \pm 0.22 c	3.23 \pm 0.12d	5.11 \pm 0.19 b	4.18 \pm 0.16 b	3.39 \pm 0.20 d	6.39 \pm 0.36 a
Developmental time (egg-adult) (days)	11.70 \pm 0.23 a	10.60 \pm 0.26 b	10.20 \pm 0.10b	10.35 \pm 0.06 b	10.27 \pm 0.18 b	9.38 \pm 0.19 c	11.93 \pm 0.39 a
Hatching time (hours)	32.4 \pm 1.1 a	29.4 \pm 1.9 a	25.6 \pm 0.7 b	24.8 \pm 0.6 bc	22.6 \pm 1.0 bc	21.3 \pm 0.7 c	22.2 \pm 1.2 bc
Total survival(NI-adult) %	45.4 \pm 4.0 d	65.6 \pm 2.6 c	81.3 \pm 2.3 b	91.5 \pm 1.9 a	88.0 \pm 1.6 a	76.8 \pm 2.5 b	48.7 \pm 3.5 d

Continue Table 1:

Spawning duration (days)	22.5±0.9 d	24.1±1.5 d	33.6±0.5bc	35.2±0.7 ab	35.6±1.0 ab	38.3±1.3 a	30.9±1.8 c
Longevity (days)	43.4±1.0 d	43.8±0.6 d	54.6±0.8 c	66.8±1.5 b	66.1±1.3 b	70.8±1.8 a	44.5±1.0 d
Sex ratio (M/F)	1.17±0.04 a	1.15±0.02 ab	1.17±0.03 ab	1.05±0.01 c	0.90±0.03 d	0.92±0.04 d	1.08±0.03 bc
Number of eggs/ sac	6.5±0.6 e	7.8±0.7 cd	8.3±0.3 bc	8.3±0.2 bc	9.3±0.2 ab	9.6±0.2 a	7.0±0.5 de
Number of egg sacs/ female lifespan	17.0±0.5 c	16.0±0.0 d	17.3±0.4 c	18.0±0.0 c	20.7±0.4 a	17.7±0.3 c	19.3±0.4 b

Data are mean \pm S.E., n=6, Means in the same row sharing a common superscript are not significantly different ($P>.05$, ANOVA following Duncan multiple range test, $\alpha = 0.05$).

The mean total production and specific population growth rate of *A. dengizicus* at 35 °C was significantly higher than cultures at 30, 25, and 20°C ($p<.05$, Table 2). The population doubling time at different temperature levels was lowest at 35°C (2.32 days) and 30 °C (2.43 days). The development time at 25°C took significantly longer compared to that at 30 and 35 °C (Table 2). The development time from eggs to adult stages was 7.40, 8.96 and 11.70 days at 35, 30 and 25 °C respectively. The total survival rates

from nauplii I to adults was significantly ($p<.05$; $F_{4, 24} = 8.45$) highest at 30 and 25 °C compared to 35 °C. The spawning period (time between first and last spawning) of *A. dengizicus* was significantly longer at 25 °C (33.9 days) compared to 30 °C (30.4 days) and 35 °C (22.1 days). The greatest longevity of females was found at 30 °C (60.7 days). The sex ratio was less than 1 at 30 °C while at 25 and 35 °C were increased respectively (Table 2).

Table 2 : Effects of different temperature on *A. dengizicus*

Parameters	Temperature levels (°C)			
	20	25	30	35
Production (ind./female)	6.3 ± 1.1 d	208.4 ± 25.9 c	300 ± 45.4 b	386.3 ± 60.3 a
Production (ind./day)	0.3 ± 0.1 d	10.4 ± 1.3 c	15.0 ± 2.3 b	19.3 ± 3.0 a
Specific population growth rate (K) (SGR)	0.092 ± 0.008 d	0.267 ± 0.006 c	0.285 ± 0.007 b	0.298 ± 0.007 a
Doubling time (days)	7.5 ± 0.57 a	2.6 ± 0.06 b	2.43 ± 0.05 c	2.32 ± 0.04 d
Developmental time (NI-NVI) (days)	14.58 ± 0.22 a	5.22 ± 0.16 b	4.41 ± 0.12 c	2.93 ± 0.42 d
Developmental time (CI-CVI) (days)	nd	4.85 ± 0.72 a	3.59 ± 0.13 b	3.67 ± 0.27 b
Developmental time (egg-adult) (days)	nd	11.70 ± 0.13 a	8.96 ± 0.14 b	7.40 ± 0.23 c
Hatching time (hours)	52.8 ± 4.2 a	33.5 ± 1.6 b	23.0 ± 0.9 c	19.3 ± 1.1 c
Total survival (NI-adult) %	nd	90.35 ± 1.68 a	94.03 ± 1.95 a	84.00 ± 3.83 b
Spawning duration (days)	nd	33.9 ± 1.4 a	30.4 ± 0.8 b	22.1 ± 1.3 c
Longevity (days)	nd	48.3 ± 1.3 a	60.7 ± 0.8 b	38.6 ± 1.9 c
Sex ratio (M/F)	nd	1.12 ± 0.02 a	0.97 ± 0.04 b	1.08 ± 0.05 a
Number of eggs/ sac	3.5 ± 0.4 c	7.5 ± 0.2 b	8.8 ± 0.4 a	8.2 ± 0.3 ab
Number of egg sacs/ female lifespan	6.0 ± 0.5 b	19.7 ± 0.6 a	20.7 ± 0.8 a	20.3 ± 0.6 a

nd: not determine, data are mean ± S.E., n= 6, Means in the same row sharing a common superscript are not significantly different ($P>.05$, ANOVA following Duncan multiple range test, $\alpha = 0.05$).

The mean total production of *A. dengizicus* was 523, 217, and 182 ind./female in 33.3 (low), 85.3 (medium), and 162.1 (high) $\mu\text{mol photons/m}^2/\text{s}$ light intensity, respectively. However, increasing the light intensity did not significantly affect the growth and production ($p < .05$). In addition, the maximum specific population growth rate (K) at low level (0.313 /day) was significantly greater than that medium (0.269 /day) and highest levels (0.260 /day). The development time required from egg to adult at low level was significantly shorter compared to medium and high light levels. The mean total survival rates from nauplii to adults were 95.1 %, 86.8 %, and 84.2 % at low, medium, and high levels light intensity respectively (Table 3). The duration of the spawning period (33.3 days) and longevity (62.9 days) were significantly greater ($p < .05$) when females were maintained at a light intensity of 33.3 $\mu\text{mol photons/m}^2/\text{s}$ compared to the two higher light intensities.

Light intensity did not significantly influence affect the male to female ratio ($p > .05$).

The maximum total production, maximum specific population growth rate and minimum doubling population time after two reproduction peaks were observed at continuous light period (24:0 h LD) (Fig. 1D, Table 4). The average development time from egg to the production of gravid females at 12:12, 24:0, and 0:24 h LD were 10.8, 10.9, and 11.1 days respectively (Table 4). The mean total survival rate was significantly higher under continuous illumination at 97.7 % compared to 90.5 % and 88.7 % at 12:12 and 24:0 h LD respectively. Spawning period and longevity of *A. dengizicus* were also significantly greater under continuous illumination with 35.1 days and 58.2 days at respectively (Table 5). The number of *A. dengizicus* females that carry egg sacs at continuous light were significantly higher than the other two treatments ($p < .05$, Table 4).

Table 3:Effects of different light intensities on *A.dengizicus*

Parameters	Light intensity levels ($\mu\text{mol photons/m}^2\text{s}$)		
	Low (33.3)	Medium (85.3)	High (162.1)
Production (ind./female)	523.2 \pm 37.4 a	216.8 \pm 36.6 b	181.7 \pm 21.9 b
Production (ind./day)	26.2 \pm 1.9 a	10.8 \pm 1.8 b	9.1 \pm 1.1 b
Specific population growth rate (K) (SGR)	0.313 \pm 0.003 a	0.269 \pm 0.008 b	0.260 \pm 0.006 b
Doubling time (days)	2.21 \pm 0.01 b	2.57 \pm 0.06 a	2.66 \pm 0.05 a
Developmental time (NI-NVI) (days)	5.05 \pm 0.15 c	7.03 \pm 0.27 b	8.17 \pm 0.08 a
Developmental time (CI-CVI) (days)	4.73 \pm 0.28 a	3.75 \pm 0.28 b	3.48 \pm 0.38 b
Developmental time (egg-adult) (days)	10.83 \pm 0.27 b	11.97 \pm 0.28 a	12.53 \pm 0.31 a
Hatching time (hours)	24.6 \pm 0.9 ab	28.3 \pm 1.4 a	21.1 \pm 1.4 b
Total survival (NI-adult) %	95.1 \pm 1.3 a	86.8 \pm 1.8 b	84.2 \pm 1.7 b
Spawning duration (days)	33.2 \pm 0.9 a	26.1 \pm 0.7 b	20.4 \pm 0.6 c
Longevity (days)	62.9 \pm 0.9 a	51.9 \pm 1.7 b	40.0 \pm 1.6 c
Sex ratio (M/F)	0.95 \pm 0.03 a	1.00 \pm 0.03 a	0.99 \pm 0.03 a
Number of eggs/ sac	8.7 \pm 0.2 a	8.5 \pm 0.2 a	6.8 \pm 0.3 b
Number of egg sacs/ female lifespan	21.0 \pm 0.4 a	17.3 \pm 0.4 b	18.7 \pm 0.4 c

Data are mean \pm SE., n= 6, Means in the same row sharing a common superscript are not significantly different ($p>.05$, ANOVA following Duncan multiple range test, $\alpha = 0.05$).

Table 4: Effects of different light regime levels on *A. dengizicus*

Parameters	Light regime (Light: Dark)		
	12 : 12	24: 0	0 : 24
Production (ind./female)	468.7 ± 39 ab	647.5 ± 144 a	266.4 ± 60.4 b
Production (ind./day)	23.4 ± 2.0 ab	32.4 ± 7.2 a	13.3 ± 3.0 b
Specific population growth rate (K) (SGR)	0.307 ± 0.004 ab	0.324 ± 0.010 a	0.279 ± 0.010 b
Doubling time (days)	2.25 ± 0.02 b	2.14 ± 0.06 b	2.48 ± 0.08 a
Developmental time (NI-NVI) (days)	4.76 ± 0.22 a	5.25 ± 0.12 a	4.85 ± 0.18 a
Developmental time (CI-CVI) (days)	5.06 ± 0.16 a	4.55 ± 0.12 a	4.78 ± 0.24 a
Developmental time (egg-adult) (days)	10.83 ± 0.16 a	10.85 ± 0.15 a	11.05 ± 0.26 a
Hatching time (hours)	24.5 ± 1.0 b	24.7 ± 1.0 b	33.7 ± 2.1 a
Total survival (NI-adult) %	90.5 ± 2.5 b	97.7 ± 1.1 a	88.7 ± 1.6 b
Spawning duration (days)	26.0 ± 1.1 c	35.1 ± 1.2 a	30.3 ± 1.0 b
Longevity (days)	49.3 ± 1.4 c	58.2 ± 1.0 a	52.8 ± 0.7 b
Sex ratio (M/F)	1.03 ± 0.03 b	0.95 ± 0.36 b	1.23 ± 0.06 a
Number of eggs/ sac	8.3 ± 0.2 b	9.5 ± 0.2 a	7.2 ± 0.3 b
Number of egg sacs/ female lifespan	19.7 ± 0.9 a	21.0 ± 0.4 a	19.0 ± 0.5 a

Data are mean ± S.E., n= 6, Means in the same row sharing a common superscript are not significantly different ($p>.05$, ANOVA following Duncan multiple range test, $\alpha = 0.05$).

Discussion

Apocyclops dengizicus can easily grow (all life stages, both adult sexes) and reproduce in a wide range of salinities from 5 to 35 psu. The maximum population growth and overall reproduction parameters were observed at a salinity of 20 psu. The findings in this study showed that *A.dengizicus* prefers brackish waters to marine ones. These results are supported by findings of other authors (Dexter, 1993; Hsu et al., 2001). The salinities of 5 and 35 psu were not suitable for *A. dengizicus*

population and reproduction, probably due to the high physiological stress at these low and high salinities (Dexter, 1993).

The differences in survival, development, longevity, number of egg sac and sex ratio this study compared to Dexter (1993) and Hsu et al. (2001) maybe due to interactions between water quality parameters and also quality and quantity of food organisms. Dexter (1993) reared *A. dengizicus* at salinities 0.5 to 68 psu at 23-25 °C, 60-70 $\mu\text{mol photons/ m}^2/\text{s}$ light

intensity and they were fed with phytoplankton which were collected from the Salton Sea (wild habitat) plus fish food pellets. The current study on salinity levels was carried out at 27.5 °C, 45 $\mu\text{mol photons/m}^2/\text{s}$ and 12:12 h LD cycle. In addition, *A.dengizicus* at all stages were fed with mixed algal diet including four algae *N.oculata*, *T.tetrathele*, *C. calcitrans* and *I.galbana* in concentrated form. The female longevity of *A.dengizicus* increased with salinity.

The sex ratio for *A.dengizicus* ranged from 0.90 to 1.23. Goswami (1978) found male to female ratios of 1:2 in laboratory cultures but 1:6 in natural populations of copepod *Pseudodiaptomus binghami*.

The present results showed that *A.dengizicus* was able to reproduce, survive and develop over a temperature range from 25 to 35°C throughout its life stages. Hsu et al. (2001) showed that survival rate and production of copepod *Apocyclops royi* were not affected by increasing temperature range from 25 to 35°C. They stated that at 35°C, females of *A. royi* matured in 6.7 days and the fecundity was 14.2 nauplii / female / day. They also noted that the temperature mainly affected the growth rate. Therefore, high water temperature can increase growth rate and shorten the maturation time. On the other hand, studies have suggested that temperature could affect reproductive parameters by controlling metabolic activity (Hirche et al., 1997). Many of researchers showed that the maximum number of clutches produced during the complete lifespan of a female copepod is affected by both temperature and food because female longevity depends on temperature and

food (Abdullahi, 1990; Hall and Burns, 2002). Temperature may also affect metabolic processes linked to oxygen consumption (Roddie et al., 1984), development (Ban, 1994) and reproduction (Abdullahi, 1990). Temperature controls body length through development speed; high temperatures decrease development time and thus reduce the final organism body size (Mauchline, 1998). In general, maximum growth and production, normal development, minimum mortality and high fecundity were obtained at 35 °C for *A.dengizicus* in the present study.

According to Milio (1992) constant light generally inhibits growth, maturation and reproduction of aquatic invertebrates. In the same way, constant light was found to be least favorable for the harpacticoid copepod *Tisbe holothuriae* (Milio, 1992). Changing illumination affects endocrine activity. Omori and Ikeda (1984) showed that endocrine activities affect mating, ripening and release of eggs or broods, hatching, molting and death in copepods. Milio (1992) explained that crustacean light sensitivity and vision depend on the naupliar eye, on the compound lateral eyes and on a dermal light sense. In this study, *A. dengizicus* grown under low light intensity showed significantly maximum production compared to medium and high light levels. One of the important reasons to interpret these results is related to stress and energy consumption by copepods for living under intense light conditions. Velasquez et al. (2001) reported that maximum density of nauplii (21 / mL) and copepodids (5 / mL) of *A.distans* was obtained in the dark and natural temperature (34°C) on third day of culture

using *Tetraselmis chuii*. They showed that the highest average density of adult females was obtained on the sixth day under 34 °C and the maximum average density of eggs (5.4 / mL) was produced at natural temperature. Velasquez et al. (2001) in their experiment used the light intensity of 2500-3000 Lux which was equal to 250-300 μmol photons/ m^2/s which it seems that this level of light intensity is too much for culture of *A.distans*.

During the culture of *A.dengizicus* at different light regimes, it was found that in the first 10 days (first reproductive peaks), alternating light regime has maximum number of copepod production followed by continuous light and full dark period (Figure 1). However,

in the next 10 days (second reproductive peak), results showed that *A. dengizicus* production was maximum at continuous light period due to high survival rate of copepodid to adult, and the number of females carrying egg sac increased significantly compared to other treatments ($p<.05$, Figure1). Although a number of studies stated that constant light generally has inhibitory role in growth and reproduction of copepods (Segerstral, 1970; Milio, 1992), they did not mention the values of light intensity. In this study also it was found that *A. dengizicus* could grow and develop very well at all different light regime but better production was observed in continuous light.

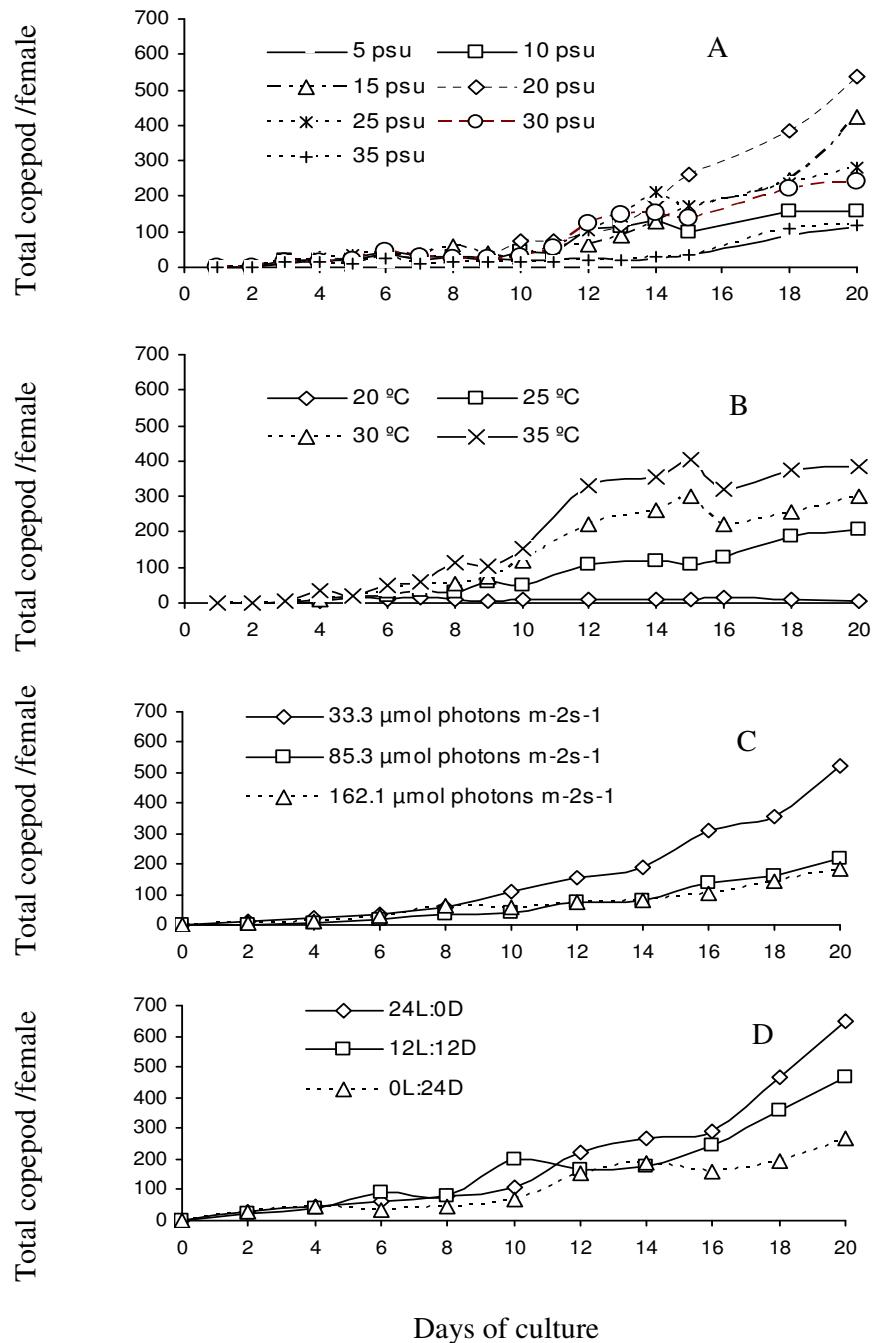


Figure 1: Total *A. dengizicus* production per female under different salinity levels (A), temperature levels (B), light intensity levels (C) and light regime levels (D) during days of culture. Data are mean (n=6).

The total densities of *A. dengizicus* fed on mixed microalgal diets under obtained conditions ranged from 9.7 to 16.2 ind. / mL. The densities for *A. distans* (Velasquez et al., 2001); *Acartia tsuensis* (Ohno and Okamura, 1988); *Acartia* sp. (Schipp et al., 1999); *A. royi* (Cheng et al., 2001) and with *Paracyclops nana* (Lee et al., 2006) were 6.5, 2.0, 7.0, 33.0 and 96-119 ind. / mL, respectively.

As conclusion, environmental parameters are highly important for the well being of live feed production. In this study, it was established that *A. dengizicus* under different levels of salinity, temperature, light intensity and light regime can survive and reproduce very well. The salinity of 20 psu, 35 °C, 33.3 μ mol photons/ m^2 / s and continuous light provide better growth, production and suitable reproductive parameters which could make *A. dengizicus* as good live food for aquaculture industry.

Acknowledgments

This study was supported by the Government of Malaysia through Project no. 01-02-04-0392 awarded to Prof. Fatimah Md. Yusoff. The Iranian Ministry Science, Research and Technology (IMSRT) gave scholarship to the main author is duly acknowledged.

References

Abdullahi, B. A., 1990. The effects of temperature on reproduction in three species of cyclopoid copepods. *Hydrobiologia*, 196, 101-109.

Ban, S., 1994. Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinis*. *Journal of Plankton Research*, 16, 721-735.

Bengtsson, B. E., 1978. Use of a harpacticoid copepod in toxicity tests. *Marine Pollution Bulletin*, 9, 238-241.

Bodmer, W. F. and Edwards, A. W., 1960. Natural selection and the sex ratio. *Annals of Human Genetics*, 24, 239-244.

Farhadian, O., 2006. Culture of planktonic cyclopoid *Apocyclops dengizicus* (Lepeshkin, 1900) and its suitability as live feed for giant black tiger shrimp, *Penaeus monodon* Fabricius (1798), postlarvae. Doctoral Thesis, Faculty Science, Universiti Putra Malaysia, 301P.

Cheng, S., Chen, H., Chang, S., Chen, T. and Liao, I., 2001. Study on the optimal density of mass culture in copepod *Apocyclops royi*. The 6th Asian Fisheries Forum, p. 58, Book of Abstract, 29th November 2001, Kaohsiung, Taiwan.

Dexter, D. M., 1993. Salinity tolerance of the copepod *A. dengizicus* (Lepeschkin, 1900), a key food chain organism in the Salton Sea, California. *Hydrobiologia*, 267, 169-171.

Goswami, S. C., 1978. Developmental stages, growth and sex ratio in *Pseudodiaptomous binghami* Sewell (Copepoda: Calanoida). *Indian Journal of Marine Sciences*, 7, 103-109.

Hall, C. J. and Burns, C. W., 2002. Effects of temperature and salinity on survival and egg production of *Gladioferens pectinatus* Barly (Copepoda: Calanoida). *Estuarine, Coastal and Shelf Sciences*, 55, 557-564.

Heasman, M. P., Diemar, J. A., Sushames, T. M., O'Connor, W. A. and Foulkes, L. A., 2000. Development of extended shelf life microalgae concentrate diets harvested by centrifugation for biovalue mollusks – a summary. *Aquatic Research*, 31, 637-659.

Hernandez-Molejon, O. G. and Alvarez-Lajonchere, L., 2003. Culture experiments with *Oithona oculata* Farran, 1913 (Copepoda: Cyclopoida), and its advantages as food for marine fish larvae. *Aquaculture*, 219, 471- 483.

Hirche, H. J., Meyer, U. and Niehoff, B., 1997. Egg production of *Calanus finmarchicus* –effect of food, temperature and season. *Marine Biology*, 127, 609-620.

Hsu, C. H., Su, H. M. and Chen, I. M., 2001. Effects of food types on the development and reproduction of *Apocyclops royi* (copepoda, cyclopida). In : Larvi 2001, Fish and Shellfish Larviculture Symposium. C. I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (eds.). Gent, Belgium.

James, C. M. and Al-Khars, A. M., 1986. Studies on the production of planktonic copepods for aquaculture. *Syllogeus*, 58, 333-340.

Kleppel, G. S., 1993. On the diets of calanoid copepods. *Marine Ecology Progress Series*, 99, 183-195.

Lavens, P. and Sorgeloos, P., 1996. Manual on the production and use of live food for aquaculture. F.A.O Technical Paper, 361, 295P.

Lee, K. W., Park, H. G., Lee, S. M. and Kang, H. K., 2006. Effects of diets on the growth of the brackish water cyclopoid copepod *Paracyclopsina nana* Smirnov. *Aquaculture*, 256, 346-353.

Martinez, M. P. and Chakroff, J. B. P., 1975. Direct phytoplankton counting technique using the hemacytometer. *Philippine Agricultural Scientist*, 59, 43-50.

Mauchline, J., 1998. The biology of calanoid copepods. In : Advances in Marine Biology, Blaxter J. H. S. (ed.), Academic Press, 707P.

Milio, H., 1992. Effects of light (photoperiod, spectral composition) on the population dynamics of *Tisbe holothuriae* Humes (Copepoda: Harpacticoida). *Hydrobiologia*, 232, 201-209.

Mirabdullayev, I. and Stuge, T. S., 1998. Redescription of *Apocyclops dengizicus* (Lepeshkin, 1900) from Central Asia. *Spixiana*, 21, 173-178.

Ohno, A. and Okamura, Y., 1988. Propagation of the calanoid copepod, *Acartia tsuensis*, in outdoor tanks. *Aquaculture*, 70, 39-51.

Omori, M. and Ikeda, T., 1984. Methods in Zooplankton Ecology. John Wiley and Sons Inc., New York, 332P.

Rey, J. R., O'Connell, S., Suarez, S., Menendez, Z., Lounibos, L. P. and Byer, G., 2004. Laboratory and field studies of *Macrocyclops albidus* (Crustacea: Copepoda) for biological control of mosquitoes in artificial containers in a subtropical environment. *Journal of Vector Ecology*, 29, 124-134.

Roddie, B. D., Leakey, R. J. G. and Berry, A. J., 1984. Salinity-temperature tolerance and osmoregulation in *Eurytemora afinis* (Poppe) (Copepoda: Calanoida) in relation to its distribution in the zooplankton of the upper reaches of the Forth Estuary. *Journal of Experimental Marine Biology and Ecology*, 79, 191-211.

Schipp, G. R., Bosamans, J. M. P. and Marshall, A. J., 1999. A method for hatchery culture of *Pontoporeia affinis* Lindstrom (Crustaceana: Amphipoda). *Journal of Experimental Marine Biology and Ecology*. 5, 272-275.

SPSS, 2002. Statistical Pakage for Social Science. Version 11.5, SPSS Inc., Michigan Avenue, Chicago, Illinois, USA.

Tompkins, J., DeVille, M. M., Day, J. G. and Turner, M. F., 1995. Catalogue of Strains. Culture Collection of Algae and Protozoa (CCAP), UK, 204P.

tropical calanoid copepods, *Acartia* spp. *Aquaculture*, 174, 81-88.

Velasquez, A., Rosas, J., Carbrera, T. Millan, J. and Hernandez, H., 2001. Effects of *Tetraselmis chuii*, *Nannochloropsis oculata* and *Dunaliella salina* on the population growth of *Apocyclops distans* (Copepoda: Cyclopoidae) at different temperature and light conditions. *Revista de Biología Marina y Oceanografía*, 36, 189-197.

Zar, J. H., 1984. Biostatistical Analysis, 2nd edition. Prentice Hall Inc., Engewood Cliffs, New York, 718P.