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Laboratory Culture of the Caspian Sea Calanoid Copepod *Acartia clausi* (Giesbrecht, 1889) at Different Salinity Levels

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Abstract: The Caspian Sea calanoid copepod, *Acartia clausi*, has good potential for mass culture as a live feed for mariculture. This study was carried out to investigate the effects of salinity on density of *A. clausi* at four salinities of 13, 20, 35 and 45 ‰ in 15 L carboys container and mass culture at two salinities of 13 and 35 ‰ in 100 L fiberglass culture tanks over a two weeks period. *A. clausi* was cultured at temperature 25-26 °C and fed with two algal diets including *Isochrysis galbana* and *Chaetoceros calcitrans* at food concentration 40×10^3 cells mL^{-1}. Results showed that salinity had significant effects on density in 15 L container. With an initial stocking density of 5 adults L^{-1}, maximum and minimum mean density reached to 435.13±75.05 and 103.96±12.56 individuals L^{-1} at salinities of 13 and 45 ‰ with significant ($P\$0.05$) differences between treatments, respectively. Population density was not significant at 13 and 35 ‰ in 15 L containers ($P\#0.05$). In 100 L tanks with an initial stocking density of 18-20 adults L^{-1}, the mean density was not significant ($P\#0.05$) at two salinities of 13 and 35 ‰. Total mean copepod production reached to densities of 1048.15±186.4 and 1402.26±270.7 individuals L^{-1} with the dominance of nauplius stages at salinities of 13 and 35 ‰, respectively. Maximum density was recorded 3437 individuals L^{-1} (3267 nauplii, 73 copepodites (C1-5) and 97 adults L^{-1}) at salinity of 35 ‰. The results of this study recommended that *A. clausi* is a euryhaline species and can tolerate different salinities in culture conditions; however, it is better to be cultured at salinity of 35 ‰ for maximum population density growth.

Key words: Copepod % Salinity % *Acartia clausi* % Density % Caspian Sea % Live Feed

INTRODUCTION

Copepods constitute a major part of the diet of fish larvae in the natural pelagic food chain; in which, the three main orders including Calanoida, Harpacticoida and Cyclopoida have each been investigated for their suitability for larval and juvenile fish that each order has its advantages and disadvantages [1].

In aquaculture, copepods are generally considered to be nutritionally superior live feeds, as they are a valuable source of protein, lipid (especially highly unsaturated fatty acids contents, 20:5n-3 and 22:6n-3), carbohydrates, enzymes (amylase, protease, exonuclease and esterase), vitamins (C and E), small size, digestibility and their swimming motion [2-4], which are essential for larval survival, growth, digestion and metamorphosis [5-8]. Different studies showed that copepods diets show more significant benefits to increase larval marine fish growth and development better than rotifers *Brachionus* spp. and Artemia [6, 9-16]. Despite these findings, enriched rotifers and Artemia will probably continue to be the live feeds of choice in commercial hatcheries [15-17].

In general, copepods are difficult to culture at sufficient densities to be economically efficient on a commercial scale, because they require high water volumes for cultivation in captivity and this is perceived

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to be too expensive and unreliable for most intensive hatcheries. Therefore, only a few species of copepods have been successfully reared at near commercial scale in extensive systems. Most copepod rearing trials have been small scale and lasted only a few weeks or months [8,18]. According to the review by [6,8], a reliable system for the continuous large-scale indoors intensive culture of calanoid, harpacticoid or cyclopoid copepods has not yet been developed.

The most frequently cultured calanoid species belong to the genera found in coastal waters, such as those of the genera Acartia, Centropages, Eurytemora and Temora. These copepods are small, with relatively short generation times, a wide thermal and salinity tolerance and are easily adaptable to laboratory conditions and when reared in outdoor multispecies cultures, they tend to dominate with time [19]. Calanoid copepods of the genus Acartia is subtropical and temperate coastal marine, estuarine areas and semi-enclosed systems with a cosmopolitan distribution [20-22]. Acartia clausi is a free-spawning with more abundant outside the estuary and shows maximum densities during spring-autumn in the Caspian Sea. This species was acclimated to salinity of 13 ‰ in the southern waters of the Caspian Sea.

Salinity is one of the most important environmental parameters affecting the seasonal and spatial distribution of marine copepods in the wild and can affect the spawning, incubation, survival rate, growth, respiration and subrogation of the dominant species in nature [23]. Few studies, however, exist on the density of A. clausi and its mass culture for aquaculture purposes in laboratory or hatchery conditions at different salinities. Therefore, this paper discusses with the aim to study the effects of different salinity levels on density of A. clausi in laboratory conditions.

MATERIALS AND METHODS

Algal Culture: Microalgal cultures used for experiments were two algal diets including *Isochrysis galbana* (Prymnesiophyceae) and *Chaetoceros calcitrans* (Bacillariophyceae) that batch cultured in the SANRU phycolab laboratory at 26 ^{i}C, 14L: 10D (Light: Dark) photoperiod, 5,000-6,000 Lux illumination and fertilized with f_2 medium at different salinities *i.e.*, 13, 20, 35 and 45 ‰ [24].

Copepod Culture: Adult *A. clausi* were collected using 200-µm mesh size plankton nets from northern coast (Mahmoud Abad) of the Caspian Sea (13 ‰). The samples were immediately transported to the laboratory and thoroughly rinsed to reduce contamination by unwanted organisms. After rinsing, the zooplanktons were screened to isolate the size fraction containing predominantly adult copepods of *A. clausi* [21]. Adults (75-100 individuals LG^{1}) were divided randomly into four parts (each salinity level), which were reared with *A. clausi* in five 1 L beakers. Before the experiments, the five groups of adult copepods were acclimated gradually from 13 ‰ to 20, 35 and 45 ‰ and increased five units per day during 2 to 6 days period [23]. Healthy adults (survival rates approximately were 80-85 % at different salinities) from the groups were selected for the experiments. Various salinity conditions were obtained by diluting method with the salinity of 55 ‰ obtained from the Gomishan Shrimp Center ponds (Golestan province) with dechlorinated freshwater. Salinity was measured by a hand refractometer.

Then, the copepods were separately cultured under four salinity levels (13, 20, 35 and 45 ‰) in 15 L carboys containers and mass cultured under two salinity levels (13 and 35 ‰) in 100 L fiberglass tanks over two weeks culture period. The copepods fed daily with a mixture of two algal diets including I. galbana and C. calcitrans in a ratio of 1:1 to give a final density at concentration 40×10^{3} cells mLG^{1} that measured by haemocytometer. Copepods were cultured at 25-26 °C and 12h L: 12h D (Light: Dark) photoperiod. Aeration was provided for small and large experimental treatments. For 15 L containers, the aeration was provided centrally near the bottom of the carboys, using a glass pipette with an aperture of 1.5 mm controlled to give a bubble of air one to three times per second. For 100 L tanks, aeration was provided by an air stone and its intensity was strong enough to circulate the water in the tanks without causing excessive turbulence. Oxygen concentrations were regulated at 5.8-6.1 mg LG^{1}. For water exchange, 25-30 % of water volume was approximately exchanged every three days using a siphon with a 20 µm mesh attached to the end and new seawater that had been pre-adjusted to desired salinity was added [21]. For all experiments, three replicates were set up for each treatment.

The copepod densities were sampled every day in 15 L and every other day in 100 L cultures, respectively. After collection, clove oil was used to immobilize the copepods before they were counted using a stereo microscope (Model, Nikon-SMZ 1500-Japan). Total counts of representative samples from the condensed populations were repeated three times for each treatment and size group, recording the proportion of the population

in each life stages (egg and nauplius, copepodite and adult). Therefore, the population densities of copepods were estimated from counting the total number of copepods in multiple three subsamples and the numbers of nauplius instars (N_I-N_{VI}), copepodid instars (C_I-C_V) and adults (C_{VI}) recorded [15].

Statistical Analysis: Prior to analysis, the data were arcsin transformed for homogeneity and analyzed by one way ANOVA. When a significant difference (P#0.05) was found, Duncan's multiple comparisons test was used to discern significant differences between treatments. All statistical analyses were conducted using SPSS program (Version 17). Data are presented as mean ± standard error (SE).

RESULTS

The density of adult, copepodites, egg and nauplii and total copepods of all stages were compared between treatments. In 15 L containers, the maximum density of copepod was attained during 10 to 13th days of culture at different salinities. It was noticed that the copepod density decreased from the 14th days onwards (Fig. 1, 2 and 3). There were no significant differences in total copepod density between salinities of 13 and 35 ‰ (P$0.05); however, the observed differences between these treatments were significant (P#0.05) compared to the 20 and 45 ‰ (Table 1). Maximum and minimum of total copepod density per liter were recorded at 13 and 45 ‰ (P#0.05), respectively. Total copepod density was higher

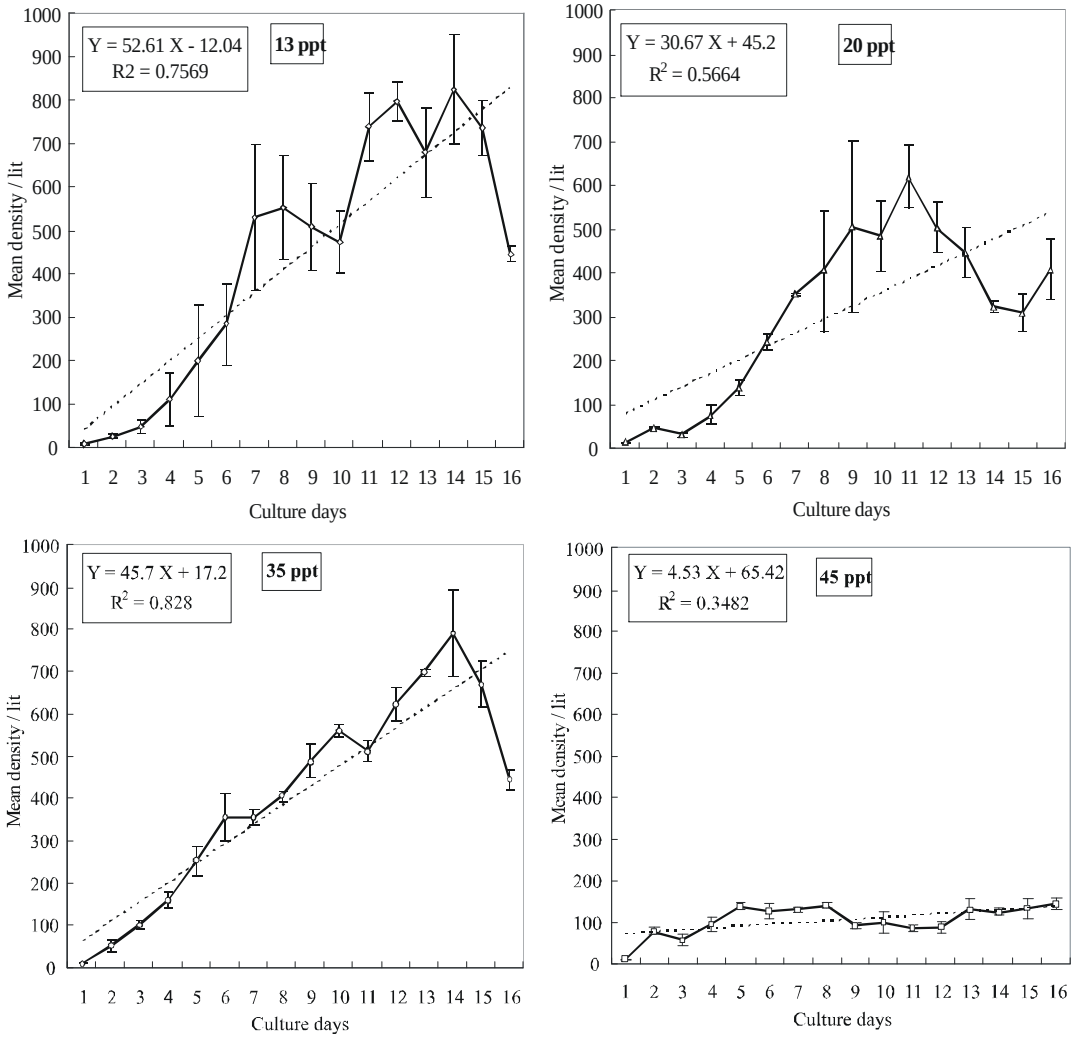

Fig. 1: Total production (Mean density per L ± SE) of copepod, *A. clausi*, at different salinity levels over two weeks culture in 15 L container (Dashed line represented regression linear).

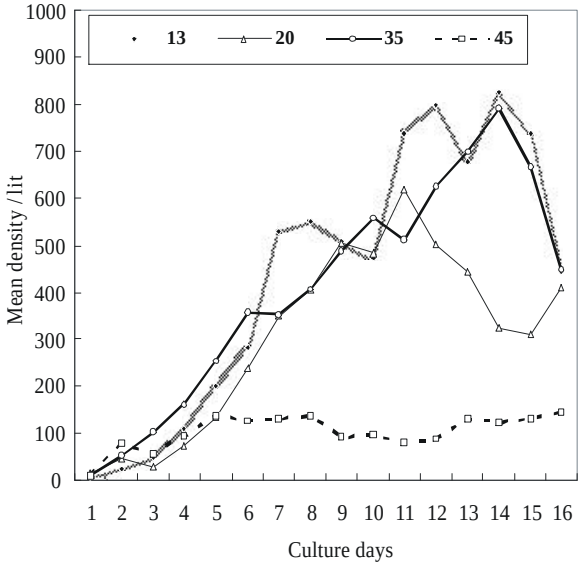

Fig. 2: The comparisons of total production (Mean density per L) of copepod, *A. clausi*, at different salinity levels over two weeks culture in 15 L container.

at 2th week compared to the 1th week in all treatments (Table 1), however, an opposite trend has been observed in nauplii percentage in all treatments. Moreover, during 15 days operation, the systems produced an average of 1663 to 6962 individuals LG^{1} of copepod at salinity of 45 and 13 ‰, respectively (P#0.05). The maximum density was recorded 1008 individuals LG^{1} at 13 ‰ (Table 1); however, higher regression coefficient (R^2) observed at 13 and 35 ‰ (P$0.05) ($R^2$=75.69 for 13 ‰ and R^2=82.80 for 35 ‰) (Fig. 1).

In 100 L tanks, there were no significant differences in total copepod density between two salinities levels (P$0.05). Mean copepod density (with initial stocking density of 18-20 adults LG^{1}) reached to 1048 and 1402 individuals LG^{1} at salinities of 13 and 35 ‰, respectively. Maximum density were recorded 3437 and 2248 individuals LG^{1} at 35 and 13 ‰, respectively (Table 2). After two weeks of experiment, the produced biomass of nauplius was relatively higher (P#0.05) in second week in comparison with the first week at different salinities.

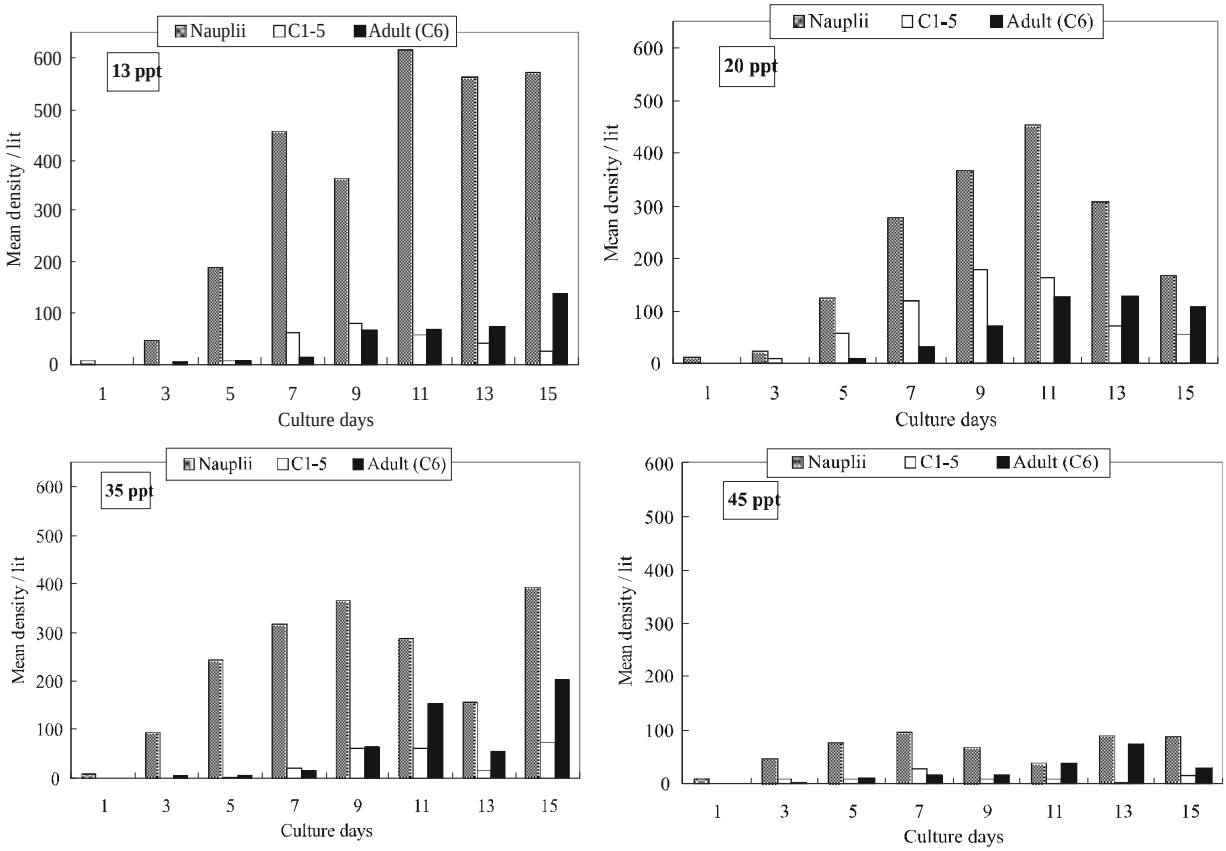

Fig. 3: Population composition of different life stages (nauplii, copepodites and adults) of copepod, *A. clausi*, from an initial stocking density of 5 adults LG^{1} at different salinities over two weeks culture in 15 L container.

Table 1: Total production (Mean density per L ± SE) of copepod, *A. clausi* at different salinities in 15 L containers over two weeks culture period

	Salinity (‰)			
Parameters	13	20	35	45
Total organism per L	435.13±75.05 [a]	305.94±49.77 [b]	405.65±29.31 [a]	103.96±12.56 [c]
Mean density per L in first week	219.29±74.28 [a]	162.29±26.21 [a]	212.13±20.41 [a]	96.5±9.92 [b]
Mean density per L in second week	650.96±75.81 [a]	449.58±73.34 [b]	599.17±38.21 [a]	111.42±15.20 [c]
Nauplius percentage in first week	83.32±6.06 [a]	81.65±5.98 [a]	84.53±4.52 [a]	66.84±7.37 [b]
Nauplius percentage in second week	76.90±3.55 [a]	53.43±3.28 [b]	59.70±5.04 [b]	58.54±4.62 [b]
Min-Max density per L	18-1008	24-784	24-996	40-178

Table 2: Total production (Mean density per L ± SE) of copepod, *A. clausi*, at two salinities of 13 and 35 ‰ in 100 L tanks over a two weeks period

	Salinity (‰)	
Parameters	13	35
Total organism per L	1048.15±186.39 [a]	1402.26±270.69 a
Mean density per L in first week	670.4±125.29 a	839±181.39 a
Mean density per L in second week	1520.3±262.77 a	2106.34±382.32 a
Nauplius percentage in first week	85.21±11.84 [a]	85.63±14.07 [a]
Nauplius percentage in second week	91.37±2.23 [a]	92.74±2.54 [a]
Min-Max density per L	141-2248	164-3437

Table 3: Culture methods and productivity for calanoid copepods used as a live feed for marine fish larviculture [1,6,8]

Species	Culture size (L)	Densities	Productivity	Culture period	Food conditions	Ref.
Acartia tonsa	1890	232 /L	2-75 nauplii adultG^{-1}	16 days	Natural phytoplankton, Extensive	[41]
Acartia tonsa	200-450	50-100 /L	200-220 eggs LG^{-1}	28 days	*Rhodomonas baltica Isochrysis galbana*	[36]
Gladioferens imparipes	500, Automated, batch culture	Stocked at 1000 nauplii LG^{-1}	878 nauplii.LG^{-1}.dG^{-1}	420 days	*Rhodomonas baltica Isochrysis galbana*	[40]
Acartia clausi	100, Recirculation	-	#40 per L	14 months	*Rhodomonas baltica* (50×10^3 cells mLG^{-1}) *Isochrysis galbana* (50×10^3 cells mLG^{-1})	[34]
Acartia tonsa	100, Recirculation	-	#40 per L	10 months	*Rhodomonas baltica* (50×10^3 cells mLG^{-1}) *Isochrysis galbana* (50×10^3 cells mLG^{-1})	[34]
Acartia tonsa	1890, Circular outdoor tanks	-	11-95 per L	6 months	Natural phytoplankton blooms	[41]
Acartia clausi	20	-	300-350 adults LG^{-1}	1 year	*Tetraselmis suecica*	[35]
Acartia clausi + *Tisbe furcata*	40, Multiple generations	-	1000 individuals LG^{-1} mixed species	-	*Tetraselmis suecica*	[35]
Eurytemora affinis	2000	-	3000 individuals LG^{-1}	-	*Nanochloris* sp.	[47]
Temora longicornis	40		100 adults LG^{-1}	2 months	*Tetraselmis suecica*	[35]
Temora longicornis	100, Multiple generations	-	#40 per L	-	*Rhodomonas baltica* ($2\text{-}4\times10^4$ cells mLG^{-1}) *Isochrysis galbana* ($3\text{-}8\times10^4$ cells mLG^{-1})	[48]
Parvacalanus crassirostris	20	-	6000 individuals LG^{-1}	-	*Isochrysis/Rhodomonas/Tetraselmis/Heterocapsa*	[42]
Acartia clausi	15,Batch culture	Initial stocked at 5 female LG^{-1}	103-435 organism LG^{-1}, Max: 1,008 individuals LG^{-1} at 13‰	2 weeks	*Isochrysis galbana Chaetoceros calcitrans* (1:1 ratio; 4×10^4 cells mLG^{-1})	The present study
Acartia clausi	100,Batch culture	Initial stocked at 18-20 female LG^{-1}	1048-1402 organism LG^{-1}, Max: 3,437 individuals LG^{-1} at 35‰	2 weeks	*Isochrysis galbana Chaetoceros calcitrans* (1:1 ratio; 4×10^4 cells mLG^{-1})	The present study

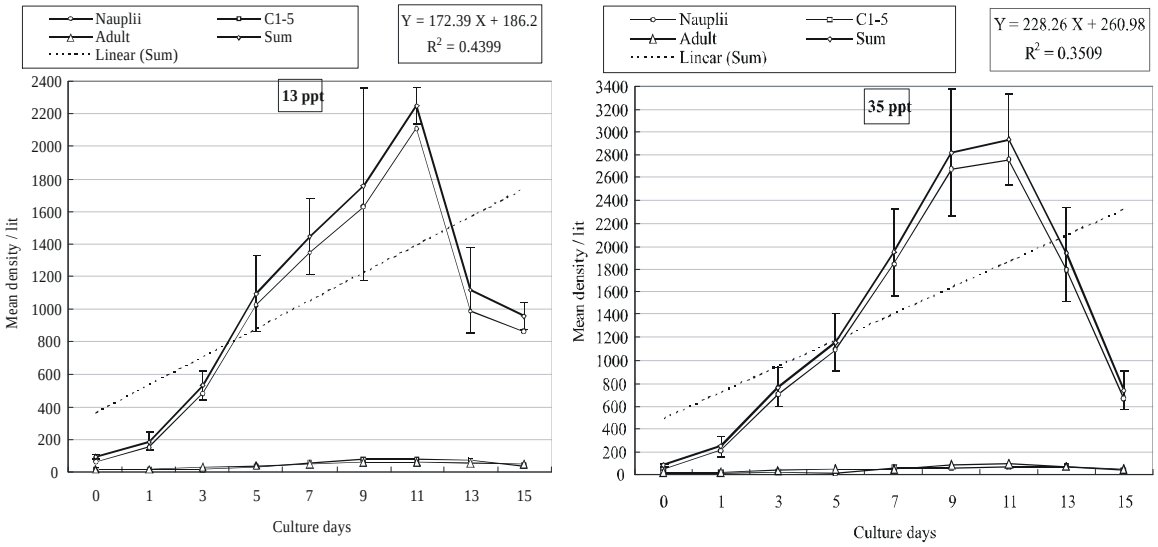

Fig. 4: Total production (Mean density per L ± SE) and population composition of different life stages (nauplii, copepodites and adults) of *A. clausi* at two salinities of 13 and 35 ‰ over a two weeks culture in 100 L tanks (Dashed line represented regression linear).

The nauplius densities of higher than 1000 individuals L^{-1} were achieved on day of fifth of culture at two above mentioned salinity levels; however, higher densities were observed at 35 ‰ during 6 to 11th days of culture; whereas, in terms of 13 ‰, similar densities achieved during 8 to 11th days of experiments (Fig. 4).

DISCUSSION

Calanoid copepods are often well adapted to cope with seasonal fluctuations in salinity variations under natural conditions. Maximizing copepod productivity is the major goal for aquaculture purposes and salinity conditions used for culture are likely to affect productivity [20]. It is often difficult to define salinity threshold for estuarine species, as different species may adapt to different salinities at various stages of their life history due to their habitat variations and it is likely to be species specific, or life-stage specific [20,25].

Fecundity of copepods is influenced by the density of breeding females and nauplii, food quality and quantity, temperature, salinity, turbulence, stocking density, cannibalism of nauplii by adult and higher copepodite stages, culture tank size and shape, water quality, sex ratio and female longevity [26-31]. Free-spawning calanoid copepods such as various *Acartia* species may produce between 11-50 eggs.female^{-1}.day^{-1}, producing a total of up 1200 from one single spawning in laboratory or field conditions [32, 29]. These data are higher than the egg production rate of the Caspian Sea copepod, *A. clausi*. [33] found that within the three salinities levels (13, 20 and 30 ‰), maximum egg production of *A. clausi* was obtained at 30 ‰; however, salinity had no significant effect on egg production rate, which remained at 6-9 egg.female^{-1}.day^{-1} at salinity of 13 and 30 ‰, respectively.

Although *A. clausi* completely adapted to the Caspian Sea brackish water (13 ‰), our results clearly revealed that it is a euryhaline species and can tolerate salinity fluctuations and even reproduce in such a condition. An increase in salinity caused a sharp reduction in nauplius percentage and increased the copepodite (C_{I-V}) and adult density after 2nd weeks, kept in 15 L carboys (apart from 45 ‰ treatment) (Fig. 2). As it was expected, higher densities were recorded in 100 L tanks at two salinity levels of 13 and 35 ‰ (Fig. 4). Salinity had no significant effects in itself on copepod production at two salinities 13 and 35 ‰ in 100 L tanks; however, the salinity of 35 ‰ had better results in final copepod production over two weeks culture. According to the results of the present study, assuming no culture harvesting, a batch culture of 15 days period has been found to be desirable under laboratory conditions and sufficient for obtaining maximum copepods densities for *A. clausi*. It seems that *A. clausi* had a development period of 8-10 days at 25-26 °C in our experiments.

The obtained densities in our study were higher than [34] and [35]. [34] obtained a density lower than 40 individuals LG^{1} at a 14 months period in 100 L recirculation systems for *A. clausi* that fed twice weekly with *Rhodomonas baltica* and *Isochrysis galbana* at algal density 1×10^{5} cells mLG^{1} in a ratio 1:1. Also, [35] cultured the same species in one year period in 20 L carboys systems and obtained densities approximately 300-350 adults $LG^{1}$with *Tetraselmis suecica* as a sole diet (mean density in 15 L carboy culture was 405 individuals LG^{1} at 35 ‰ in the present study).

Several attempts to mass-culture copepods in intensive systems have been undertaken with varying success and have resulted in the development of different systems for particular species of copepods. Although *Acartia* species have been cultured successfully for many generations in the laboratory [36] and in large outdoor tanks [37], the low densities likely make calanoids inappropriate for intensive or super-intensive mass cultivation (Table 3). The mass culture of *Acartia* spp. is related to the available water volume, rather than the available substrate area as in harpacticoids [27]. The different obtained densities in our study can be possibly attributed to different water volume for culture of *A. clausi* in 15 and 100 L cultures, which is in agreement with the results of some other researchers [1,36].

The maximum density of *A. clausi* was 3437 individuals LG^{1} (3267 nauplii, 73 copepodids (C_{I-V}) and 97 adult per L) at 35 ‰ in 100 L tanks in our experiment, which was lower than the production of calanoid copepod, *Acartia southwelli* and *A. centrura* with the maximum daily production of 4185 nauplii, 2145 copepodids and 1,285 adults LG^{1} for *A. southwelli* and 3547 nauplii, 1714 copepodids and 1,142 adults LG^{1} in *A. centrura* during a 15 days period in 25 L tanks [38]. These discrepancies may be attributed to the differences in species, culture methods, culture size, initial stocking density, productivity and food quality and quantity. [38] fed the copepods at a ratio of 60000 cells mLG^{1} which were higher than our experiments (40000 cells mLG^{1}). Our production figures exceed those reported for temperate *Acartia* species. For example, the maximum densities reported for *Acartia tsuensis* cultured in outdoor tanks were 1136 nauplii, 588 copepodids and 280 adults LG^{1} [39]. Mean densities of lower than 2000 individuals LG^{1} also obtained in an 8-days cycle for *Acartia* spp. in Darwin center marine fish [15]. However, a daily productivity of 95,000 eggs correspond to 1500 nauplii LG^{1} with 45 percent hatching success reported for *A. tonsa* in 200 L culture tanks [36]. Also, over 7 weeks of operation on *Acartia* spp. (Australian strain), [21] started with an inoculums of around 50-100 adults and 150-250 copepodites LG^{1}, the culture an average produced around 2,000 nauplii, 750 copepodites and 300 adults LG^{1} after 7 days (maximum output was 1,100 copepodids and 729 adults LG^{1}) and a mean peak density of nauplii was almost 2,000 individuals LG^{1} (the highest was 5,150 individuals LG^{1}) in 1,000 L tanks; which were higher than our data about *A. clausi*. They noted that an 8-day culture cycle seems an appropriate period for mass culture of *Acartia* spp. with a generation time of 5-7 days at 28-32 ^{i}C, 30-34 ‰ and 20×10^{3} cells LG^{1} of three algal diets including *Isochrysis*, *Rhodomonas* and *Tetraselmis* [21]. [40] were able to produce 878 nauplii LG^{1} $dayG^{1}$ of the calanoid species, *Gladioferens imparipes* in 500 L automated batch cultures for 420 days.

Most calanoid copepods can be grown at densities of only 100-200 adults lG^{1} [41, 36]. [27] discussed possible causes of sex ratio regulation (the female percentage). Crowding may cause changes in the sex ratio, so that low female percentages occurring at high stocking densities. Also, cannibalism of nauplii by adults and later stage copepodids is a problem when trying to maintain high densities of nauplii in copepods cultures [33,36-37]. However, obtained densities in Parvocalanus crassirostris stock cultures have been averaged about 7 nauplii and 3 adult/late stages copepodites per mL after nine days in 20 L culture tanks [15]. Cannibalism may also be a potential contributing factor to the sharp decline in nauplii numbers from the 11^{th} days onwards in 100 L tanks in the current study.

Within the population in 15 L carboys, there were some differences in the distribution of the various life-stages (nauplii, copepodites and adults) of *A. clausi* cultured at different salinities. At the lower salinities (13 and 20 ‰), a relatively higher proportion of the population was at the nauplius stage, while there was a more even distribution of different life-stages at the higher salinity of 35 ‰ (Fig. 3). These could be due to the necessity of calanoid copepods to higher volume water for maximum egg and nauplius production. It has already been stated that fecundity decreases with increasing density; in which, population density can affect population growth by modulating survival, development and fecundity [42]. It has been suggested that complex chemical compounds may be produced by the animals as a result of crowding, allowing them to perceive and respond to different crowding levels [27]. Alternatively, it has also been hypothesized that direct close encounter may change the behavior of copepods and even their development.

The optimal food concentrations for culture of calanoid copepods were adjusted to ranging from 6-8×10^4 to 1.2-1.4×10^5 cells mL^{-1} day^{-1} [44]. The lower densities in the present study may also be attributed to the lower food concentrations compared to other studies. It seems that fecundity has been decreased in lower food concentrations [30].

The intensive production of the harpacticoid and cyclopoid copepods were reported in different literatures and resulted with a higher density production compared to calanoid copepods. Harpacticoids such as species of genus of Nitokra, Tisbe, Euterpina, Tigriopus and Amphiascoides are the preferred organisms for the development of an intensive copepod culture system [1,6,8,17,45]. For example, [45] harvested a daily average yield of 300,000 nauplii per tray or 125 nauplii cm^{-2} d^{-1} for harpacticoid copepod, Tisbe spp. [17] also reported harvests of 70 copepods cm^{-2} d^{-1} from a marine harpacticoid copepod in mass culture of Amphiascoides atopus in a recirculating system culture. In general, harpacticoids can be grown in densities up to 115,000 individuals L^{-1} [1, 46].

CONCLUSION

From the results of this study, it can be concluded that *A. clausi* could survive at a very broad range of salinities (13-45 ‰ as achieved in the current study). This study was conducted at laboratory scale over relatively short periods of time; therefore the results may not be fully reproducible in large-scale cultures with capacity of 1-10 m^3 tanks. However, it clearly served the purpose of identifying the optimum salinity conditions for culturing of *A. clausi*, which are likely to be applicable in larger-scale cultures. Based on the findings of this study, to achieve maximum density of *A. clausi* culture for aquaculture purposes, it could be kept and cultured at a salinity of 35 ‰.

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