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Effects of different diets on population growth and fatty acids composition in cyclopoid copepod, *Acanthocyclops trajani* (Mirabdullayev and Defaye, 2002): A potential supplementary live food for freshwater fish larvae

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Abstract

This study compared the efficacy of different diets on the population density, specific growth rate, doubling time, and fatty acids composition of the freshwater cyclopoid copepod *Acanthocyclops trajani*. The experimental diets consisted of fresh binary microalgae (*Scenedesmus obliquus*+*Spirulina maxima*), dry binary microalgae (*S. obliquus*+*S. maxima*), dry *Gracilaria corticata*, and mixed dry vegetables (including spinage, coriander, and parsley). Results have showed that the highest population growth rate (0.145), mean density (1445 ind L^{-1}), and the shortest doubling time (4.76 d) have been obtained in copepods fed on dry binary microalgal diet. The amounts of fatty acids in *A. trajani* could be modified by feeding copepod with different diets. The amount of C22: 6n-3 (DHA) was significantly higher in copepods fed dry binary microalgae (3.75±1.08 %) compared to the other diets. This cyclopoid copepod had higher levels of EPA and DHA than those in corresponding diets, suggesting that the *A. trajani* is probably able to accumulate EFA (essential fatty acid). This study indicates the potential of using specifically dry microalgae to improve the nutritional composition in copepods as a suitable supplementary live food for freshwater fish larvae.

Keywords: *Acanthocyclops trajani*, Specific growth rate, Doubling time, Fatty acid, Live food.

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Introduction

Copepods are ubiquitous and often abundant in freshwater bodies, where they play an important role in freshwater food webs (Blaha, 2010). Copepods offer a great variety of sizes, species and qualities (Delbare *et al.*, 1996), and have high levels of protein, highly unsaturated fatty acids (HUFA), carotenoids and other essential compounds (Krual *et al.,* 1992; Evjemo *et al.,* 2003) and because of their high PUFA (poly unsaturated fatty acid) content are compared to *Artemia* or rotifers which have been used widely in the culture of fish larvae (Mckinnon *et al.,* 2003; Satoh and Takeuchi, 2009). The differences in HUFA levels in biomass of cladocerans and copepods are related with their ability to synthesize these long chain fatty acids by conversion from short chain precursors, such as α-linolenic acid (ALA) (18:3n-3) (Goulden and Place, 1990; Von Elert, 2002).

The ability of copepods to synthesize some PUFAs is limited (Kainz *et al.,* 2004; Burns *et al.,* 2011). Several copepods for example Harpacticoids and Cyclopoids are able to convert unsaturated fatty acids between fatty acid families, but most calanoid copepods cannot elongate and desaturate α-linolenic acid (ALA) (18:3n-3) to produce significant amount of longer chain PUFA (Støttrup and Jensen, 1990; Bell *et al.,* 2007; Jonasdottir *et al.,* 2009).

Namely copepods, which have the ability to either selectively retain certain HUFA from the diet or to synthesize them, are known to be nutritionally beneficial to optimize growth, survival and metamorphosis of fish larvae and to reduce the incidence of disease (Caramujo *et al.,* 2008). Therefore, the cultivation of copepods as alternative or supplemental live feed has received more attention from a number of researchers (Lavens and Sorgeloos, 1996; Rippingale and Payne, 2001; Evjemo *et al.,* 2003; McKinnon *et al.,* 2003; Lee and O'Bryen, 2005; Chen *et al.,* 2006; Castonguay *et al.,* 2008; Camus *et al*., 2009; Ohs *et al.,* 2010; Alajmi and Zeng, 2015).

In copepods, egg production is linked to the quality of the diet and it is influenced by how much the nutritional needs of the copepod are met (Kleppel and Burkart, 1995; Ianora, 2005; Milione and Zeng, 2007). Maternal nutrition has a profound influence on the fecundity of copepods and on the reproduction in the next generation (Ohs *et al.,* 2010). Accordingly, the population density increase depends on egg production and egg hatching success, both of which are affected by the copepod diet (Tang and Taal, 2005). Unlike rotifers and *Artemia*, the nutrient content in copepods cannot be manipulated through existing enrichment techniques (Rasdi and Qin, 2014). Therefore, the change in copepod nutrition has to be done through feeding copepods with different types of diet.

The response of copepods growth, productivity, and specially their ability in accumulation or bioconversion of essential fatty acids from mono, binary or mix algal diets in fresh form have been experienced by several researchers

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(Rajkumar and Kumaraguru, 2006; Farhadian and Arshad, 2008; Farhadian *et al.*, 2014; Liu and Xu, 2009; Ljubobratovic *et al.*, 2016; Kumar *et al.*, 2014). There is a motivation to identify which kind of algae can be used to enrich or change the content of copepod nutrition. Most previous studies have been utilized fresh algae, but there is limited data for using dry microalgae to feed copepods (Mostary *et al.*, 2010). Previous researchers have been found that copepods fed the dry diatom (*Melosira* sp.) have produced higher contents of EPA, DHA and ARA than those fed other dry microalgae. There was no difference on the essential fatty acids (EFAs) contents in copepods fed either dry *Nannochloropsis oculata* or fresh *N. oculata* (Rasdi *et al.*, 2016). Also, the rotifers fed dry or frozen *Nannochloropsis* sp. produced similar EFA as those fed live microalgae (Mostary *et al.*, 2010).

The *Acanthoicyclops trajani* (85-941 µm size) is a common planktonic species occurring in fish ponds, lakes, and reservoirs as well as pools and pond channels (Mirabdullayev and Defaye, 2004). Types and concentrations of diets are two important factors of *Acanthocyclops* rearing experiments because they affect mostly on population growth, density, population doubling time, as well as the copepod fatty acid composition (Farhadian and Arshad, 2008). Accordingly, the population growth and fatty acids composition of the freshwater cyclopoid copepod, *A. trajani*, has been studied across a variety of algal and non-algal diets in dry and fresh forms in the recent study. Therefore, the main objectives of this study were to compare the population growth, doubling time, and fatty acids composition of *A. trajani*, as well as to understand if the nutritional composition in *A. trajani* is affected by dietary food and whether copepods are able to accumulate certain fatty acids from the diets when fed separately on different fresh or dry diets.

Materials and methods

The copepod *A. trajani* has obtained from an earthen freshwater fish pond of Sari Agriculture Sciences and Natural Resources University (SANRU) in Mazandaran province (September 2017) in Iran. Several gravid females were separated with a zooplankton net (mesh size, 400 µm) and individually cultured in different 1 L chambers under determined temperature (22 $^{\circ}$C) and food (live *Scenedesmus obliqqus* and green water) conditions and then transferred to 5, 10 and 20 L carboys in a batch culture system. Then, a laboratory population have been maintained at 20–22 $^{\circ}$C at an irradiance level of 115 µE m^{-2} s^{-1} with a 12 L: 12 D h (Light: Dark) photoperiod. The water culture medium was prepared from well water. The *A. trajani* has been fed the same amount of fresh *S. obliquus* and green water at least for four weeks prior to the feeding experiment.

Four types of diets (one fresh and three dried forms) with different composition of fatty acids have been separately fed to *A. trajani* in the feeding trials each with three replicates

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(Table 1). The dry microalgae (*S. obliquus* and *Spirulina* (*Arthrospira*) *maxima*) have been provided by Schipperus (2014) in Urmia University.

The uniform laboratory population of *A. trajani* has been distributed between 12 zoug tanks with volume of 120 L, filled by well water (pH: 6.5, DO: 4.8 mg L^{-1}, NH_3: 0.38 mg L^{-1}, Total hardness: 276 mg L^{-1}). The diets have included: 1-fresh *S. obliquus*+fresh *S. maxima*; 2-dry *S. obliquus*+dry *S. maxima*; 3-dry *Gracilaria corticata*; and, 4-mixed dried vegetables (including spinage, coriander, and parsley). Each type of diets have been fed to the copepod at a daily ration of 4.18 µg dry weight ml^{-1}, which is equivalent to 3.16 µg C ml^{-1} and 1.67×10^5 algal cells ml^{-1} (Rasdi, 2015). The microalgal diets *S. obliquus* and *S. maxima* have been cultured using the BBM and Zarrouk mediums, respectively (Lavens and Sorgeloos, 1996). The experimental tanks have been exposed to an irradiance of 115 µE m^{-2} s^{-1} provided with fluorescent tubes on a 12 L: 12 D h. Mean water temperature was 22–24 °C, and a continuous aeration has also provided to the experimental tanks to ensure homogeneous distribution of foods and oxygen supply.

Table 1. Fatty acids compositions of four different diets.

Fatty Acids	Diet types			
	Mix fresh algae	Mix dry algae	Dry *Gracilaria corticata*	Mix dry vegetables
Saturated				
C14:0	1.56±0.84	1.38±0.89	1.97±1.75	2.23±1.19
C16:0	34.59±17.46	28.05±11.04	29.60±16.52	8.47±6.18
C18:0	9.37±5.71	10.40±7.72	4.00±2.75	13.39±6.71
C20:0	10.84±5.44	0.51±0.25	0.17±0.17	5.57±5.57
C22:0	−	1.17±0.59	4.68±4.68	−
C24:0	0.23±0.15	0.75±0.37	0.05±0.05	−
Monounsaturated				
C14:1n5	1.55±0.96	1.26±0.63	7.85±6.58	4.27±3.25
C16:1n7	3.48±1.74	4.41±2.21	1.59±1.42	6.32±6.32
C18:1n9	2.66±2.49	5.61±2.81	0.67±0.67	0.33±0.33
C18:1n7	7.18±3.60	1.58±0.79	0.38±0.38	−
C20:1n9	1.91±0.96	0.31±0.16	0.18±0.18	12.34±12.34
C22:1n9	0.75±0.75	−	0.31±0.31	−
C24:1n9	−	0.02±0.01	−	−
Polyunsaturated (PUFA, HUFA)				
C18:2n6 *CIS*	11.86±7.33	22.43±11.23	−	6.24±6.24
C18:3n3 (ALA)	3.33±1.78	19.05±9.53	0.16±0.16	−
C20:2n6	0.62±0.37	0.11±0.05	0.27±0.27	−
C20:4n6 (ARA)	1.07±0.70	2.20±1.10	−	−
C20:3n3	0.67±0.67	0.68±0.68	5.19±5.19	−
C20:5n3 (EPA)	1.17±0.92	0.33±0.16	0.11±0.11	−
C22:6n3 (DHA)	0.20±0.20	0.25±0.13	0.07±0.07	−
n- 3	5.37±2.70	20.30±10.16	5.53±5.53	−
n- 6	13.56± 7.90	24.74± 12.38	0.27± 0.27	6.24±6.24

All values are mean ± S.E. (n=3)
Value of − indicates that FA amounts were trace.
ALA, alpha-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

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Population growth rate of *A. trajani* has conducted for a 30-d period (Farhadian and Arshad, 2008). Every 3 days (Farhadian and Arshad, 2008), three samples from each tank have removed and number of copepods in each sample have been counted under a dissecting microscope and then returned to culture. The specific population growth rate (K) of *A. trajani* has calculated using the following formula (Omori and Ikeda, 1984; Hada and Uve, 1991):

$K= (Ln\ Nt – Ln\ N0)/t$

Whereas t is the culture days and N0 and Nt are the initial and final (the highest) density of copepods, respectively.

In addition, doubling time (Dt) has calculated according to the following formula (James and Al-Khars, 1986):

$Dt = (Ln\ 2)/K$

Fatty acids analysis in algae and copepods

The overall fatty acids composition have been determined by heating samples at 80 ^{o}C in 1 ml of 2.5% (v/v) H_2SO_4 in methanol for 90 min in screw capped tubes. After the addition of 1.5 ml of 0.9% NaCl solution and 1 mL of hexane, fatty acids have been extracted into the organic phase by shaking and the tubes have been centrifuged at low speed. Samples of the organic phase have been separated by gas chromatography on a 15-m×0.53-mm supelcowax column and quantified using a flame ionization detector. The results have been expressed as a percentage of total fatty acids (FAME) 150-200 mg wet weight of sample (Miquel and Browse, 1992).

Copepods have been collected on a 100µm mesh net and transferred to new jars. Copepods have been starved for 24 h to allow for the clearance of dietary products present in the gut and rescreened to remove any accumulated waste products (Nanton and Castell, 1999). Subsequently, the copepods were captured and transferred to microtubes and then -80 ^{o}C freezer for fatty acids analysis. The rest procedures for fatty acids analysis were the same to those used in algae.

Data analysis

Results are given as mean±standard error (S.E.). All statistical analyses have conducted using SPSS version 22.0 (SPSS, Chicago, IL, USA). Comparisons of population growth rate, doubling time and also fatty acids in different diets and copepods were made using one-way ANOVA. Differences were considered significant at the $p<0.05$ level. When the main treatment effect was significant, post hoc comparisons were made using LSD test. All the data were tested for normality, homogeneity and independence to satisfy the assumptions for ANOVA.

Results

Population density, population growth rate, and doubling time of A. trajani fed on different diets

The analysis showed that different diets had significant effects on population growth rate (K) of *A. trajani* as well as population doubling time (Dt) ($p<0.05$). The average density of *A. trajani* in different diets during treatment period is shown in Fig. 1. The average density

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of *A. trajani* ranged from 221 to 1445 ind L^{-1} on 30th culture day (Fig. 2) in different diets trials. The highest and the lowest densities have been determined with dry binary microalgal diet (2023 ind L^{-1}) and dry *G. corticata* diet (214 ind L^{-1}), respectively. The population growth rate (K) and doubling time (Dt) for *A. trajani* fed on different diets had shown in figures 3 and 4. The population growth rate (K) ranged from 0.098 to 0.145, and the lowest and the highest rates have been observed in dry *G. corticata* and dry binary microalgal diet, respectively. The *A. trajani* fed on fresh binary microalgal, dry binary microalgal, dry *G. corticata*, and dry mix vegetables required 5.53, 4.76, 7.00 and 7.05 day, respectively, to double their population (Dt). *Post hoc* comparisons using LSD demonstrated that dry binary microalgal diet was significantly different in population growth rate (K) and doubling time (Dt) (p<0.05).

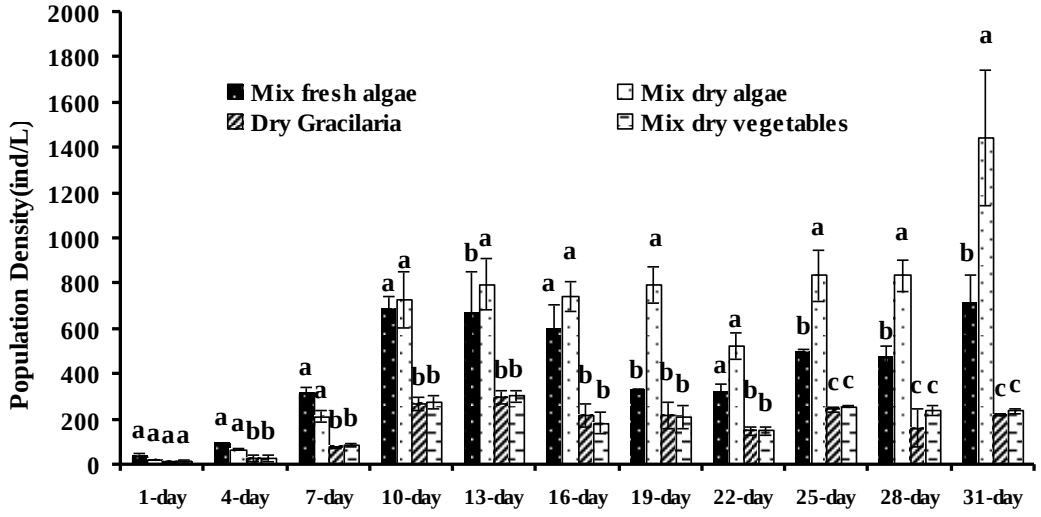


Figure 1: Mean (±S.E.) population density of *Acanthocyclops trajani* in different diets during treatment period (Different letters represent significant differences (p<0.05).

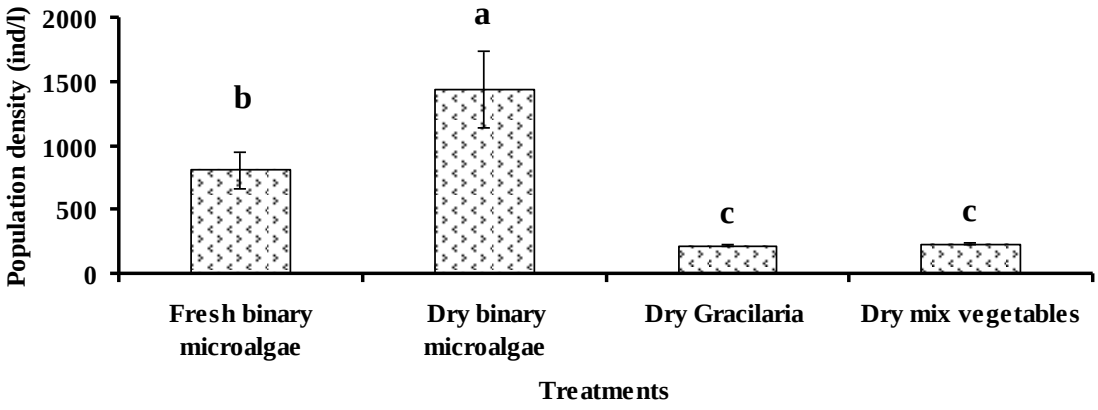


Figure 2: Mean (±S.E.) population density of *Acanthocyclops trajani* in different diets on 30th culture day (Different letters represent significant differences (p<0.05).

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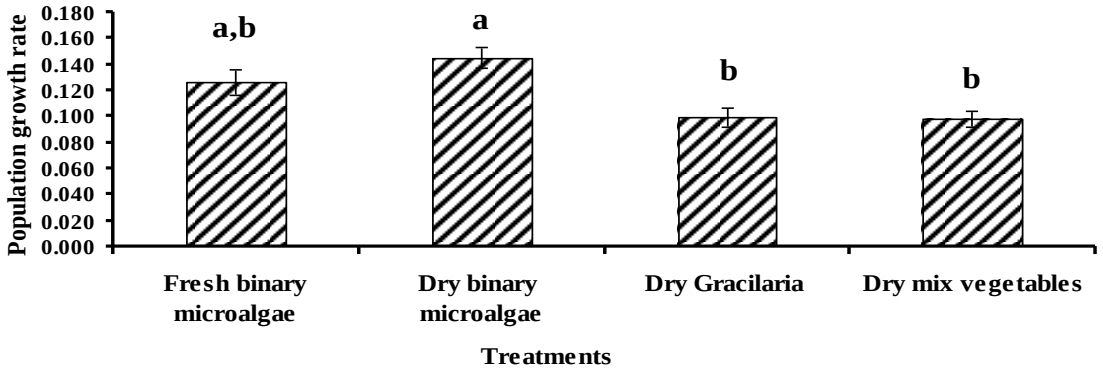


Figure 3: Mean (±S.E.) population growth rate (K) of *A. trajani* in different diets (Different letters represent significant differences (p<0.05).

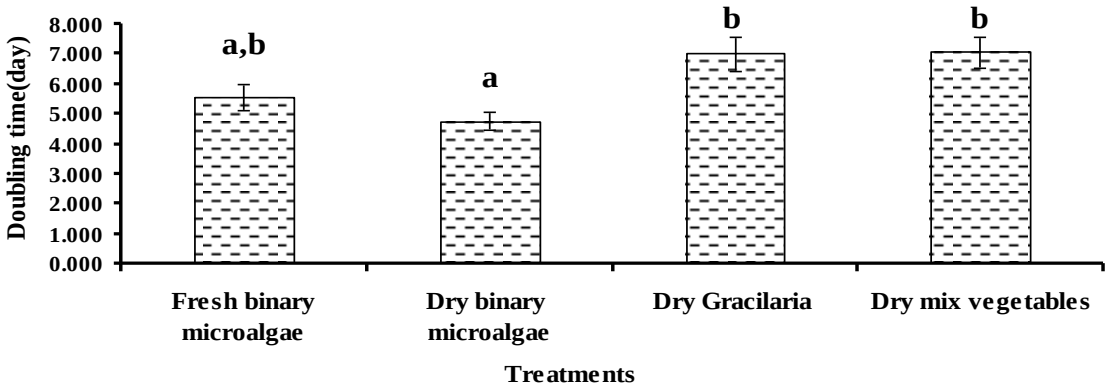


Figure 4: Mean (±S.E.) population doubling time (Dt) of *A. trajani* in different diets (Different letters represent significant differences (p<0.05).

Fatty acids composition

Six types of saturated fatty acids have been found in *A. trajani* fed different diets (Table 2). The highest amount (29.57±15.60) of palmitic acid (C16:0) was obtained in the *A. trajani* fed the fresh binary microalgal diet. The amount of C24:0 (8.24±2.72) was higher in the *A. trajani* fed the dry form binary microalgal diet in comparison with the fresh form (p<0.05). Also, there was significant difference in C24:0 fatty acid amount of diets and *A. trajani* fed of these diets (p<0.05).

Seven types of monosaturated fatty acids have been detected in *A. trajani* (Table 2). The amount of C14:1n-5 in the *A. trajani* fed the fresh binary microalgal diet (19.66±6.37) was higher than others (p<0.05). There was significant difference in C22:1n-9 amount between *A. trajani* fed fresh and dry binary microalgae composition and their diets (p<0.05). The C24:1n-9 fatty acid was only detected in copepods fed dry binary microalgae and also in latter diet (p<0.05).

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Table 2: Fatty acid compositions of *A. trajani* fed different diets

Fatty Acids	Diet types			
	Mix fresh algae	Mix dry algae	Dry *Gracilaria corticata*	Mix dry vegetables
Saturated				
C14:0	0.62±0.17	0.74±0.31	4.61±4.42	2.38±1.27
C16:0	29.57±15.60	10.61±6.57	17.68±22.24	8.38±5.79
C18:0	4.55±2.30	5.66±0.37	7.63±5.24	14.31±7.17
C20:0	4.49±0.87	5.48±1.34	−	7.28±3.64
C22:0	−	1.54± 0.78	2.77±2.40	−
C24:0	5.16±2.94[b]	8.24±2.72[a]	−	−
Monounsaturated				
C14:1n5	19.66±6.37[a]	10.68±1.32[b]	4.22±2.21[c]	1.24±0.68[c]
C16:1n7	5.83±1.00	12.97±3.23	5.19±2.99	1.75±0.89
C18:1n9	5.99±0.30	2.91±0.79	2.31±1.87	1.01±0.57
C18:1n7	1.48±0.79	6.18±4.72	4.63±3.93	−
C20:1n9	−	0.08±0.08	−	12.10±6.10
C22:1n9	4.19±2.70[a]	3.73±1.57[b]	−	−
C24:1n9	−	6.86±4.26	−	−
Polyunsaturated (PUFA, HUFA)				
C18:2n6 *CIS*	0.26±0.26	0.59±0.34	−	11.0±5.87
C18:3n3 (ALA)	3.11±0.78[a]	3.37±0.94[a]	0.54±0.54[b]	−
C20:2n6	1.47±0.77	1.64±0.31	−	−
C20:4n6 (ARA)	0.20±0.20	1.91±1.28	0.93±0.93	−
C20:3n3	7.95±2.30[b]	11.59±4.31[a]	−	−
C20:5n3 (EPA)	3.49±3.40[a]	2.48±1.84[a]	0.38±0.30[b]	−
C22:6n3(DHA)	2.01±1.01[b]	3.75±1.08[a]	1.7±1.7[c]	0.68±0.34[c]
n – 3	16.57±2.75	21.19±3.59	2.62±1.47	0.68±0.34
n – 6	1.93±0.99	4.14±1.47	0.93 ±0.93	11.00 ±5.87

All values are mean (± S.E.) (n=3).
Value of − indicates that FA amount was trace. Different letters indicate significant difference between treatments (a,b,c)
ALA, alpha-linolenic acid; ARA, Arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Among seven types of PUFA in copepods, ALA, ARA, EPA and DHA were the major EFAs. The amount of linolenic acid (C18:3n-3) (ALA) was significantly different between fresh and dry binary microalgal diets (p<0.05) but the ALA content was not significantly different in copepods fed of these diets (p>0.05). There were significant differences on the some of PUFAs (C20:2n-6, C20:3n-3 and C22:6n-3) contents in copepods and their diets composition (p<0.05). The highest amount (11.59±4.31) of C20:3n-3 was detected in copepods fed dry binary microalgae (p<0.05). The copepods fed fresh binary microalgae contained, not significantly, higher C20:5n-3 content (EPA) than copepods fed dry binary microalgae (3.49±3.40) (p>0.05). The amount of C22:6n-3 (DHA) has been significantly higher in copepods fed dry binary microalgae (3.75±1.08) (p<0.05) compared to the other diets. Also, the DHA content was significantly different between fresh and dry binary microalgae and copepods composition (p<0.05). In spite of the low contents of EPA and DHA in all of diets, the copepods fed these diets contained the higher EPA and DHA contents (Figs. 5 and 6). The

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highest amount of *n*-3 PUFA fatty acids was obtained in copepods fed dry binary microalgae (Table 2).

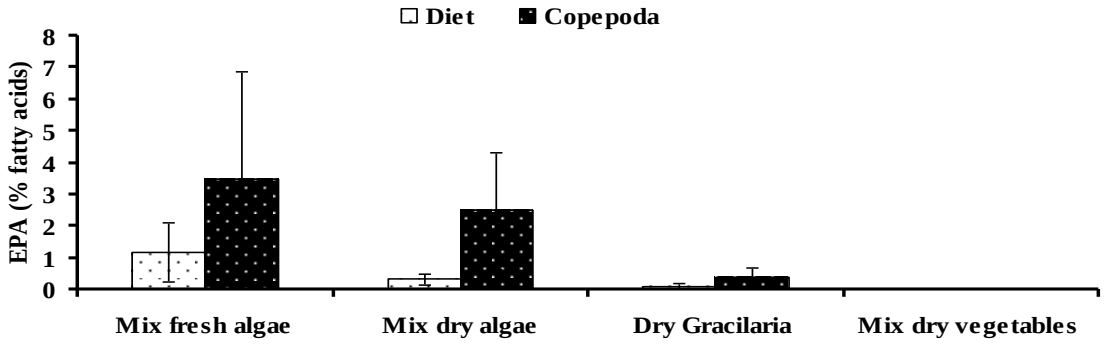


Figure 5: Production of EPA by *Acanthocyclops trajani* corresponding to different diets.

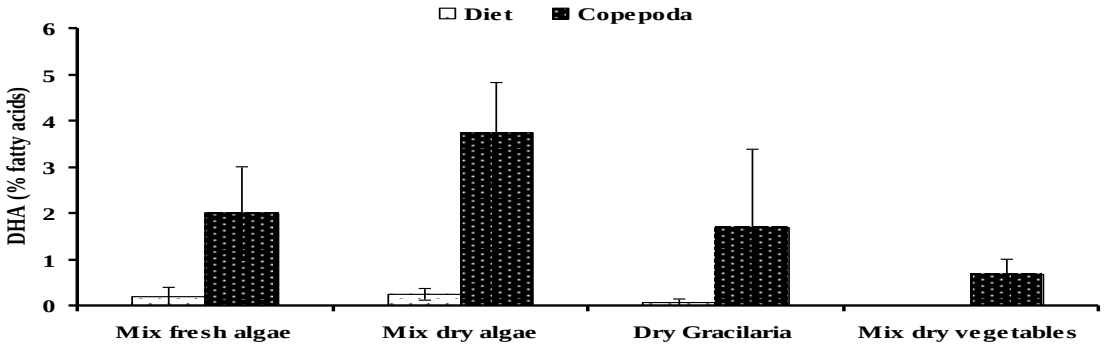


Figure 6: Production of DHA by *Acanthocyclops trajani* corresponding to different diets.

Discussion

The results of the present study clearly demonstrated that the food diet type has been crucial to the culture success of *A. trajani* in terms of the population growth. The highest population growth rate have been found in copepod fed by the binary microalgal diet in dry form (0.145±0.008). The growth rate of *A. trajani* was higher than some other cyclopoid species reported previously such as *Apocyclops royi* (0.096±0.013) and *Tigriopus japonicas* (0.08±0.014) (Lee *et al.*, 2006; Lee *et al.*, 2013). Also, *A. trajani* required 4.76 d to double their population which was consistent with doubling time in previous study on fresh water cyclopoid *A. robustus* (4 d) (Sharafi *et al.*, 2014). In another study, Farhadian and Arshad (2008) had mentioned that cyclopoid copepod *Apocyclops dengizicus* fed on binary fresh microalgae (*Chaetoceros calcitrans+Tetraselmis tetrathele*) required 3.14 d at high algal density to double their population. The highest mean density of *A. trajani* in this research has been observed in copepods fed dry binary microalgae (1445 ind L^{-1}) which was more than the highest mean density in another species, *A. robustus* (1282 ind L^{-1}) fed on fresh *Scenedesmus quadricauda* and soil (Sharafi *et al.*, 2014).

The food types (quality) have significant effects on the profiles of saturated, monosaturated, and PUFAs contents of this cyclopoid copepod. The

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n-3 PUFA amount was higher in copepods fed the dry binary microalgal diet and also they produced higher contents of ALA, ARA and DHA than those fed other diets which are consistent with previous studies (Veloza *et al.,* 2006; Mostary *et al.,* 2010). There was only significant difference on the DHA content from EFAs between copepods fed dry and fresh binary microalgal diets. In the current experiment, the fatty acid composition was different between fresh and dry binary microalgal diets unlike previous studies (Albentosa *et al.,* 1997; Cnudde *et al.,* 2011). Other than EPA, the dry form contained higher proportions of essential fatty acids (EFA). In general, dried microalgae are subject to reduction in food quality, but the nutritional value of dried algae for copepods is still controversial (Dobberfuhl and Elser, 1999; Rasdi, 2015). Cano *et al.* (2004) stated that some of cyclopoid copepods could not digest the hard cell wall of microalgae. So, in dry microalgae due to the fragile hard cell wall following drying, the accessibility of copepods to microalgae cell compounds is more.

The better population growth rate, the highest density (2023 ind L^{-1}) and the shortest doubling time in copepods fed on dry binary microalgal diet could be related to microalgal higher dietary value (Stottrup, 2000). Moreover, there was significant difference on the some of PUFAs (C20:2n-6, C20:3n-3, and C22:6n-3) contents in copepods and their diets composition. Many copepod species are known to synthesize substantial amounts of EPA and especially DHA from dietary ALA (18:3n-3) (Desvilettes *et al.,* 1997; Nanton and Castell, 1998; Anderson and Pond, 2000; Von Elert and Stampfl, 2000; Caramujo *et al.,* 2008). The presence of the HUFAs and the continued reproduction of the animal when fed a diet deficient in HUFAs suggested that copepod was able to bio-convert the shorter chain 18:3n-3 and 18:2n-6 into EPA, DHA and ARA to meet its physiological requirements for these fatty acids (Lee *et al.,* 2005).

In the current study, the ALA (18:3n-3) composition was significantly higher in fresh and especially dry binary microalgal diet and there has been significant difference on DHA content in these diets and copepods fed on them. *Tisbe holothuriae* and another *Tisbe* sp. of Harpacticoida have been shown to synthesize significant amounts of EPA and DHA when fed *Dunaliella tertiolecta*, which is HUFA limited, but contains large amounts of the precursor ALA (Nanton and Castell, 1998; Lee *et al.,* 2005). The accumulation of EPA and DHA from ALA has been reported in other copepods species. For example, Desvilettes *et al.* (1997) have suggested that the cyclopoid copepod *Paracyclopina nana* was able to convert ALA to EPA and DHA when fed on *Tetraselmis suecica*, an ALA rich green microalga, which coincides with our finding on *A. trajani*.

The better growth performance in *P. nana* fed on *T. suecica* could be attributed to biosynthesis DHA from linolenic acid (18:3n-3) in the study of Lee *et al.* (2006). This is also observed

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in the study on the harpacticoid copepod *Attheyella trispinosa* where EPA and DHA have been detected in the copepods despite the negligible amounts of these PUFA in the dietary algae *Leptolyngbya foveolarum* (Caramujo *et al.*, 2008).

The fatty acids profile of two freshwater cyclopoid copepods, *Thermocyclops hyalinus* and *Mesocyclops aspericornis* have been improved by providing mixed algal diets because their combined nutrient contents are more likely to meet the nutritional requirements of the target species (Kumar *et al.*, 2014). Also, Rasdi (2015) indicated that the absence or a low of DHA and EPA contents in dry *Melosira* sp. resulted in higher EPA and DHA in Cyclopoid copepod *Cyclopina kasignete*, suggesting that copepods can accumulate these PUFAs. In the recent study, despite the low contents of EPA and DHA in all of the diets, copepods had showed higher EPA and DHA when fed of these diets. Also, in monosaturated fatty acids, C24:1n-9 was only significantly detected in copepods fed the dry binary microalgae which have been detected in latter diet in low content. Marine algae are major producers of PUFA, while freshwater algae predominantly produce saturated or monosaturated fatty acids (Rasdi, 2015). As shown from out results, the copepods have higher EPA and DHA after ingesting freshwater algae with high ALA content, suggesting that copepods might have the ability to accumulate and bio-convert ALA in the diets to EPA and DHA. In our study this trend has clearly been observed in fresh and dry binary microalgae diets.

Although non-living microalgae are unlikely to provide adequate nutrition for zooplankton growth and reproduction (Bear and Goulden, 1998; Mostary *et al.*, 2007), a dry microalga is still a good option to feed copepods when the supply of fresh algae is insufficient. In the present experiment, preparation the dry weight biomass content of algal cultures has been done by standard operating procedure which was clearly useful in keeping the nutritional quality of dried microalgae (Schipperus, 2014). Another issue with fresh algal diets was the difficulty in maintaining an adequate supply due to algal culture crash (Mostary *et al.*, 2007). Algae need to be carefully maintained in order for it to reach substantial biomass to feed copepods. Therefore, there is a need to further consider the use of dried algae in the place of fresh algae especially during the time of algae culture crashes (Mostary *et al.*, 2010).

Since, the use of freshwater copepods as supplemental live feed can improve the survival rate for a variety of freshwater fish larvae (Drillet *et al.*, 2006; Sipaúba-Tavares and Pereira, 2008; Camus *et al*., 2009), so stored algae such as dried algae could be used as substitute.

In conclusion, the amounts of fatty acids in *A. trajani* can be modified by feeding copepod with different diets. The copepod fed dry binary microalgal diet had higher PUFA and HUFA (ALA, ARA, and DHA) than those fed other diets. Also, the *A. trajani* had the

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best population growth rate and the shortest doubling time when fed on the latter diet. Furthermore, this cyclopoid copepod had higher levels of EPA and DHA than those in corresponding diets, suggesting that the *A. trajani* is probably able to accumulate EFA. This study indicates the potential of using specifically dry microalgae to improve the nutritional composition in copepods as a suitable supplementary live food for freshwater fish larvae.

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