

ISSN: 1679-3013

D.O.I.: 10.5914/to.2011.0065

AN EVALUATION OF DIFFERENT MICROALGAL DIETS FOR THE CULTURE OF THE
CALANOID COPEPOD *Temora turbinata* (Dana, 1849) IN THE LABORATORYXiomara Franchesca García DÍAZ¹Bárbara BARKOKEBAS¹Renata P. S. CAMPELO¹Maria Luise KOENING¹Ralf SCHWAMBORN²Sigrid NEUMANN-LEITÃO¹

Recebido em: 10/01/2012

Aceito em: 11/08/2012

ABSTRACT

Temora turbinata (Dana, 1849) is an exotic plankton species copepod with a wide distribution in Northeastern Brazil and a high potential to be cultured for ecotoxicological tests and as a resource of live food for aquiculture. With the aim of determining the best diet for the maintenance of this species in the laboratory, an 8-day experiment was performed. The adult survival, egg production rate and egg hatching success of this species were evaluated. Adult *T. turbinata* copepods were fed by three different diets that consisted of two monoalgal diets (*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle and *Tetraselmis chuii* (Butcher)) and a mixed algal diet combining the two microalgae. The monoalgal diets or the mixed algal diet were provided as food to the copepods in the same biomass proportion (1 mg C L⁻¹ day⁻¹). Among the three analyzed diets, the diatom *T. weissflogii* was found to be the best diet to maintain a high egg production rate and a high percentage of adult survival. The *T. turbinata* that were fed by *T. chuii* had a high recruitment of nauplii, but this species decreased the production of *T. turbinata* eggs and adult survival when it was added to the diet.

Key words: exotic copepod, survival, egg production rate, egg hatching success, *Thalassiosira weissflogii*, *Tetraselmis chuii*.

RESUMO

Temora turbinata (Dana, 1849) é uma espécie de copépodo planctônico exótico de ampla distribuição no Nordeste brasileiro, com potencial para ser usado em testes ecotoxicológicos e como recurso de alimento vivo na aquicultura. Com o objetivo de determinar a melhor dieta para a manutenção dessa espécie em laboratório foi realizado um experimento no período de 8 dias. Foram avaliadas a sobrevivência dos adultos, a taxa de produção diária de ovos e a taxa de eclosão dos náuplios dessa espécie. Copépodos adultos de *Temora turbinata* foram alimentados com três dietas diferentes, duas monoalgais (*Thalassiosira weissflogii* (Grunow) Fryxell & Hasle e *Tetraselmis chuii* (Butcher)) e uma mista combinando as duas espécies de microalgas. As dietas monoalgais e mista foram ofertadas aos copépodos na mesma proporção de biomassa (1 mg C L⁻¹ dia⁻¹). Entre as três dietas analisadas, o tratamento com *T. weissflogii* foi considerado a melhor opção por manter maiores taxas de produção de ovos e maiores porcentagens de sobrevivência dos adultos de *Temora turbinata*. Apesar do maior

recrutamento de náuplios nos organismos alimentados com *T. chuii*, esta microalga diminuiu a produção de ovos e a sobrevivência dos adultos quando adicionada na dieta.

Palavras chave: Copépodo exótico, sobrevivência, taxa de produção diária de ovos, taxa de eclosão de ovos, *Thalassiosira weissflogii*, *Tetraselmis chuii*.

INTRODUCTION

Temora turbinata (Dana, 1849) is an epiplanktonic copepod with a wide distribution in coastal and oceanic waters, particularly in the tropical and subtropical waters of the Atlantic and Indian oceans and in the western region of the Pacific Ocean (BRADFORD-GRIEVE et al., 1999). In Brazil, *T. turbinata* has a wide distribution and is abundant in estuarine and coastal waters (SILVA, et al., 2004; ARAÚJO et al., 2008; CAVALCANTI; NEUMANN-LEITÃO; VIEIRA, 2008; SANTOS et al., 2009; PESSOA et al., 2009). *T. turbinata* is an exotic species that was likely introduced to Brazilian waters in the 1990's through ballast water (ARAÚJO AND MONTÚ, 1993). This copepod is found alongside the native species, *Temora stylifera* (Dana, 1849), and there are records of the displacement of the native species for the exterior of the continental shelf and the replacement of *T. stylifera* with *T. turbinata* in some places (ARA, 2002; CEPAN, 2009).

Temora turbinata is an omnivorous species, feeding on microalgae and detritus (ESKINAZI-SANT'ANNA, 2000). Similar to the majority of calanoids, *T. turbinata* creates currents in the water to facilitate the ingestion of suspended particles (HWANG AND TURNER, 1995; HWANG; CHEN; WONG, 1998; TURNER et al., 1998). However, the ingestion of these particles is selective, as shown by the predominance of diatom frustules in the fecal pellets of this species (ESKINAZI-SANT'ANNA, 2000). Defining the proper diet that favors *T. turbinata* growth and reproduction is fundamental for its culture. Few calanoid copepods have defined culture protocols. Although *Acartia tonsa* (Dana, 1849) is one of the most frequently cultured planktonic copepods to perform ecotoxicological tests, this species is not widely distributed in tropical areas and can be difficult to obtain in this region. *T. turbinata* may be a good candidate for culture due its dominance in coastal areas and estuaries of northeastern Brazil (Silva et al., 2004), its short life cycle and its high reproductive potential (Li AND Fang, 1986; Cunha, 2008). The identification of the best culture conditions for this species will facilitate the creation of a protocol for its maintenance in the laboratory.

The aim of this study was to compare the daily egg production, the egg hatching success and the survival of the planktonic copepod, *T. turbinata*, fed with three different microalgal diets (treatments): two monoalgal diets and one mixed algal diet. Monoalgal diets of either diatoms (*Thalassiosira weissflogii* = T.w.) or chlorophyceans (*Tetraselmis chuii* = T.c.) and a mixed algal diet including both microalgae (T.w.+T.c.) were tested. Because mixed algal diets favor the reproduction and development of adults (BUTTINO et al., 2009) and because diatoms are unfavorable for egg hatching success (MIRALTO et al., 1999; IANORA, 2005), the hypothesis was that the type of diet (microalgae) affects the daily egg production, the hatching eggs and the survival of *T. turbinata*.

MATERIAL AND METHODS

Microalgae culture

The phytoplankton species used as a food resource for the copepods were *Thalassiosira weissflogii* (Bacillariophyceae) and *Tetraselmis chuii* (Prasinophyceae). Three diets (treatments) were tested: two monoalgal diets (Treatment 1: *T. weissflogii* = T.w., Treatment 2: *T. chuii* = T.c.) and a mixed algal diet (Treatment 3: *T. weissflogii* and *T. chuii* = T.w.+T.c.). The cultures were kept in 500 mL Erlenmeyer flasks with F/2 medium Guillard (1975) prepared with sea water with a salinity of 36, a mean temperature of 21°C and a cycle of 24 h of light (fluorescent lamps of 20 W). The cultures were maintained in the exponential growth phase by dilution with the Guillard's F/2 medium every two days. The cell concentration offered to the copepods was adjusted in each diet to obtain an approximate concentration of $0.98 \mu\text{g C cop.}^{-1} \text{ mL}^{-1} = 98 \mu\text{gC cop.}^{-1} \text{ day}^{-1}$ (BUTTINO et al., 2009). The daily density and equivalent carbon content of

each microalga was calculated from the biovolume (HILLEBRAND et al., 1999; MENDEN-DEUER AND LESSARD, 2000). The biovolume (BV) and the biomass (BM) were determined from the mean of the diameter (d) and the height (h) of 30 cells of *T. weissflogii* and from the widest (h) and smallest (d) diameter of *T. chuii* (Tab. 1). The food concentration (cell mL⁻¹) of the mixed algal diet (Treatment 3) was estimated allowing for an input of carbon of 50% from each microalga. The density of the culture was estimated daily by counting a 1 mL sub-sample in a Neubauer chamber under an optic microscope with the aid of a manual counter.

Table 1 – The microalgae densities used to measure the daily egg production, egg hatching and adult survival of the copepod *Temora turbinata* in the laboratory.

Microalgae diets	Size (µm)		Biovolume (µm ³)	Carbon (10 ⁻⁵ µgC)	Food Concentrations	
	Diameter	Height			10 ³ cell mL ⁻¹	µgC mL ⁻¹
<i>T. weissflogii</i> (T.w.)	12.5	12.5	1534.0	11.04	9.06	1
<i>T. chuii</i> (T.c.)	7.5	12.5	552.2	9.05	11.05	1
<i>T. weissflogii</i> (T.w.)					4.53	0.5
<i>T. chuii</i> (T.c.)					5.52	0.5

Copepod sampling

The copepods were collected from the coastal region of Boa Viagem beach, Recife city (8° 3' S - 34° 54' W) (Northeastern Brazil). Successive vertical hauls were performed using a conic plankton net of 300 µm mesh size. To reduce the stress on the organisms, the net was adapted with a cod-end without holes. The organisms that accumulated in the cod-end were carefully transferred to black plastic bags (5 L) and placed in covered buckets. Each bucket contained a hose connected to a battery-powered air pump to provide oxygen to the samples during transportation to the Plankton Culture Laboratory at the Department of Oceanography of UFPE. Seawater samples were collected and the local temperature and salinity were measured.

Copepod culture

At the laboratory, healthy adults (complete antennae, good movements) of *T. turbinata* were selected, and males and females were housed separately in 200 mL beakers for a 24 hour acclimation period. Water from the sampling area that had been filtered through a 45 µm mesh to remove protozoans was added to the beakers. The environmental conditions in the laboratory were maintained at a salinity of 36, a temperature of 25°C and a photoperiod of 12:12 (light: darkness) with mild aeration.

After the period of acclimation (day 0), individuals were selected for an 8-day experiment to determinate the effect of the microalgal diet on the fecundity and survival of *T. turbinata*. Medium containing the corresponding microalgal diet was placed in 50 mL beakers. Four individuals (2 females and 2 males) were introduced into each beaker and were maintained in an incubator with a controlled temperature and photoperiod (BUTTINO et al., 2009). Each treatment was replicated 5 times.

The copepods were transferred daily to a new beaker containing medium with the corresponding diet. Following the copepod transfer, the empty beakers were examined under a stereomicroscope to count the eggs and to determinate the daily egg production per female (Eggs fem.⁻¹ day⁻¹). The eggs were transferred to small petri dishes and incubated for 48 hours. Following the incubation, the dishes were observed under a stereomicroscope to count the hatched nauplii and to determine the egg hatching success (%). The survival of both females and males was recorded daily during the experiment. Upon the death of an individual, it was replaced by a new individual (of the same gender) taken from the stock culture. If both females died, the sample was terminated (BUTTINO et al., 2009).

Data analysis

The pre-requisites for the application of parametric tests were examined (e.g., normal distribution, homogeneity of variance, independence of the treatments and randomness of the data). The effects of the diet and culture time on the daily egg production rate, the egg hatching success rate and the survival of adult *T. turbinata* were analyzed using a two-way ANOVA (with interactions). The Bioestat 5.0 program was used to perform the statistical analyses.

RESULTS

Daily egg production (DEP)

Significant variations in the daily egg production (DEP) of *T. turbinata* were observed among the diets and across the days of culture, and interactions between the two variables were confirmed (Tab. 2; Fig. 1). The mean value of the DEP was higher for the individuals that were fed *T. weissflogii* than for individuals that were exclusively fed *T. chuii* or individuals fed the mixed algal diet (Tab. 3). The copepods fed *T. weissflogii* had the highest DEP value, with a mean value of 12.4 ± 6.3 eggs fem.⁻¹ day⁻¹ on the third day of the experiment (Fig. 1). The maximum DEP values of individuals fed *T. chuii* or the mixed algal diet were 4.6 ± 7.0 eggs fem.⁻¹ day⁻¹ on the fifth day and 7.6 ± 8.8 eggs fem.⁻¹ day⁻¹ on the fourth day, respectively (Fig. 1). There was no production of eggs on the last three days of culture in the experimental unities kept with *T. chuii* (Fig. 1).

Table 2 – A summary of the Two-way ANOVA test (with interactions) to compare the influence of the microalgae diets and experiment days on the egg production, egg hatching success and adult survival in the copepod *Temora turbinata* in the laboratory. *Significant differences, $p < 0.05$.

	Eggs fem. ⁻¹ day ⁻¹			Egg hatching (%)		Adult survival (%)	
	f.d.	F	p	F	p	F	p
Diet	2.96	5.8275	0.0044*	0.2211	0.8044	1.0531	0.3539
Days	7.96	3.6427	0.0019*	0.6716	0.6975	2.8148	0.0105*
Interactions	14.96	3.2578	0.0005*	1.122	0.3488	0.9114	0.5496

Table 3 – The mean and standard deviations of each parameter calculated for *Temora turbinata* fed with different algal diets (*T. weissflogii* (T.w.), *T. chuii* (T.c.) and *T. weissflogii* + *T. chuii* (T.w.+T.c.)) in the laboratory.

Diet	Eggs fem. ⁻¹ day ⁻¹	Egg hatching (%)	Nauplii recruited f ⁻¹	Adult survival (%)
T.w.	$3.6 \pm 5,0$	12.3 ± 18	15.6	83.3 ± 16
T.c.	$1.5 \pm 4,2$	33.2 ± 46	20.3	69.4 ± 27
T.w.+T.c.	$1.8 \pm 3,6$	11.5 ± 14	7.6	79.4 ± 12

Egg hatching success

The percentage of *T. turbinata* eggs that hatched was low and unstable for the three tested diets (Fig. 2). The highest percentage of egg hatching success was obtained from the individuals that were fed *T. chuii*, and the lowest percentage was obtained from individuals that were fed the mixed algal diet (Tab. 3). The copepods that were fed *T. chuii* produced eggs for five days, and nauplii were recorded on the second and fourth days of the experiment resulting in an egg hatching rate of 92.3% and 73.7%, respectively (Fig. 2). The egg production and hatching events were more frequent in the copepods fed *T. weissflogii*, but the egg hatching success rate was lower (maximum

values of 50%) than the rate obtained from the copepods fed *T. chuii* (Fig. 2). The copepods fed with a mixed algal diet showed the lowest egg hatching success (Tab. 3; Fig. 2). There were no significant differences among the treatments and among the days of culture (Tab. 2).

To compare the reproductive production of *T. turbinata* fed the different diets during the experiment, the total naupliar recruitment rate was calculated (total number of eggs by each female during the experiment multiplied by the percentage of eggs that hatched). The results showed low rates of naupliar recruitment throughout the experiment with all three diets (Tab. 3). The copepods that were fed *T. chuii* showed the highest rate of naupliar recruitment of 20.3 nauplii fem^{-1} , and the individuals fed the mixed algal diet showed the lowest rate of 7.6 nauplii fem^{-1} (Tab. 3).

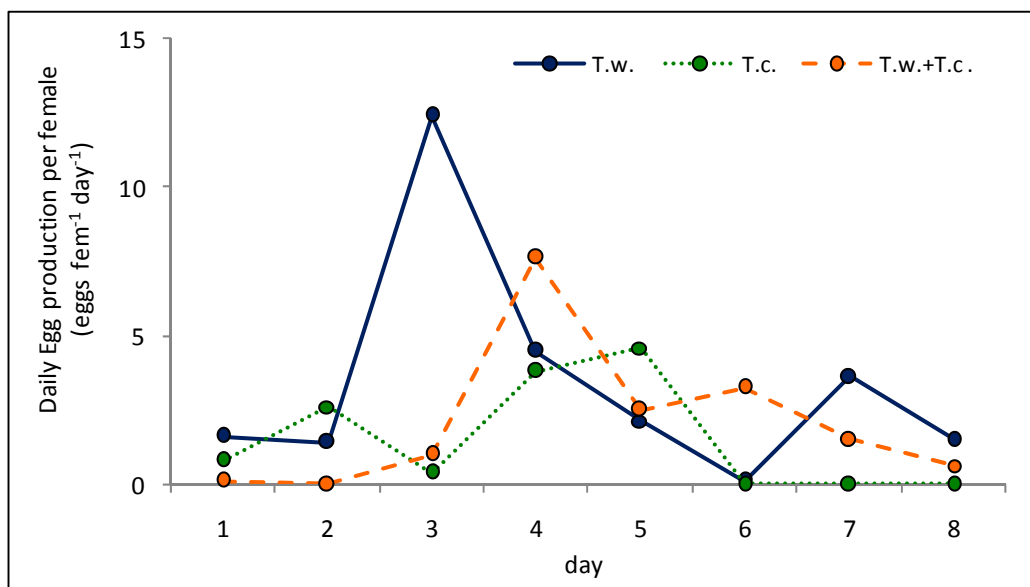


Figure 1 – *Temora turbinata* daily egg production per female that were fed a monoalgal or pluri-algal diet (*T. weissflogii* (T.w.), *T. chuii* (T.c.) and *T. weissflogii* + *T. chuii* (T.w.+T.c.)) in the laboratory.

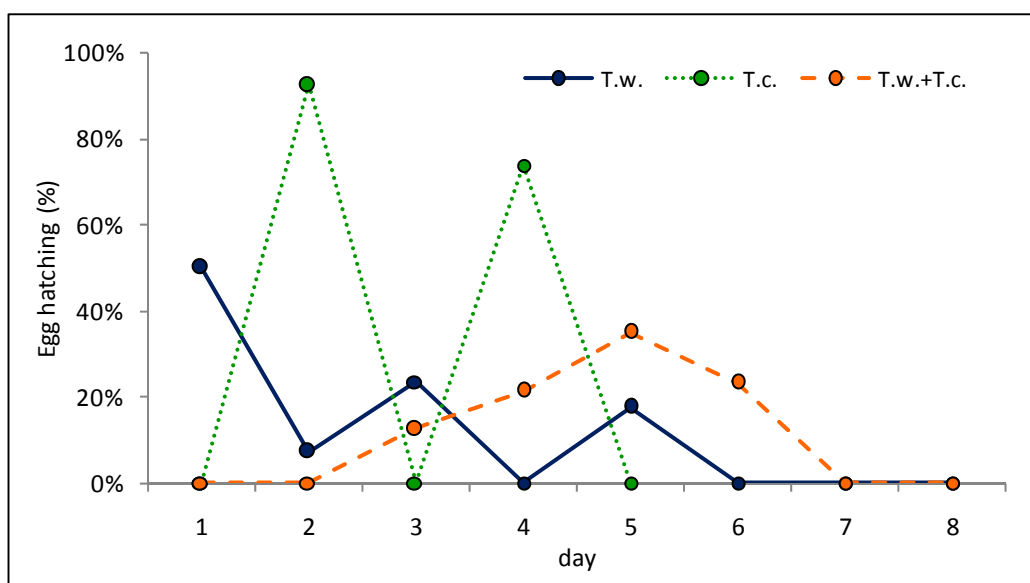


Figure 2 - *Temora turbinata* % egg hatching success in individuals fed a monoalgal or pluri-algal diet (*T. weissflogii* (T.w.), *T. chuii* (T.c.) and *T. weissflogii* + *T. chuii* (T.w.+T.c.)) in the laboratory.

Adult Survival

The highest percentage of *T. turbinata* adult survival was found in individuals that were fed *T. weissflogii* (Tab. 3). Significant differences were found among the days of the experiment (Tab. 2). The adult survival gradually diminished in individuals that were fed *T. weissflogii*; there was an initial decrease on the fifth day of the experiment, leading to a final survival rate of 75% at the end of the experiment (Fig. 3). A gradual decrease in survival was also observed in individuals fed the mixed algal diet; there was an initial decrease on the third day, and then survival remained relatively constant, resulting in an adult survival rate of 70% (Fig. 3). The lowest survival rates were observed in individuals fed *T. chuii*; survival was stable until the fifth day of the experiment and further decreased to a final value of 35% (Fig. 3).

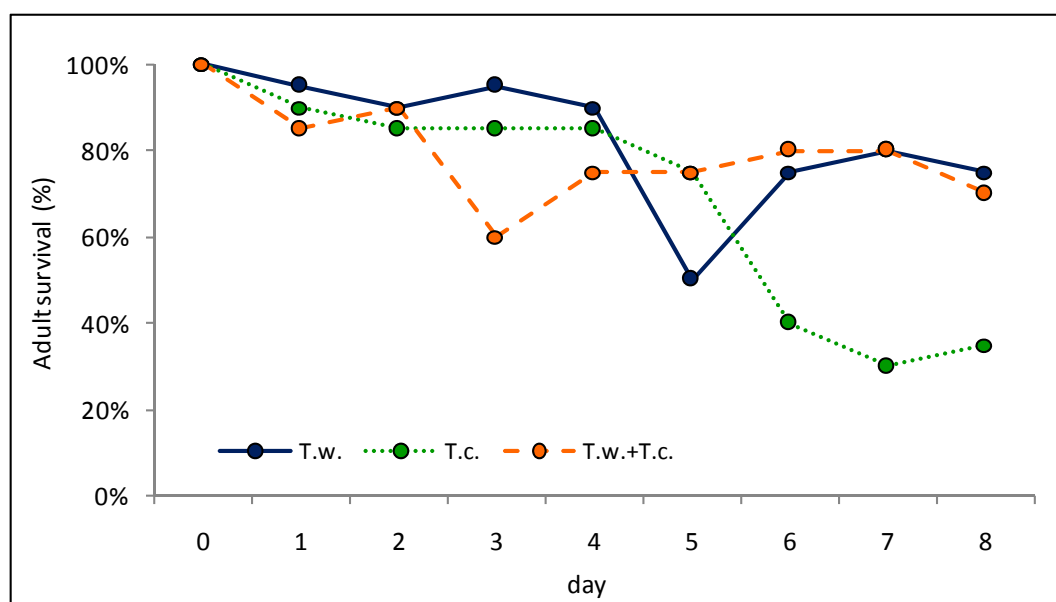


Figure 3 - *Temora turbinata* % adult survival in individuals fed a monoalgal or pluri-algal diet (*T. weissflogii* (T.w.), *T. chuii* (T.c.) and *T. weissflogii* + *T. chuii* (T.w.+T.c.)) in the laboratory.

DISCUSSION

In the natural environment, the copepods can obtain a variety of items in its diet, including diatoms, dinoflagellates, protozoans, bacteria and detritus (ESKINAZI-SANT'ANNA AND BJÖRNBERG, 2000), besides other animals. When food availability and diversity is high, many copepods have the ability to select the food items to be ingested (COWLESS, 1979). However, diatoms are at the base of the classic trophic model that includes copepods as the primary consumers and fishes as the final consumers (MIRALTO et al., 1999; IRIGOIEN et al., 2002). It has been shown that the type and quality of food strongly modifies the development processes and fecundity of copepods (IANORA, 2005). The food must include primary metabolites, such as proteins, fatty acids and vitamins, for proper copepod development. Monoalgal diets may contain deficiencies in essential nutrients (JONÁSDOTTIR AND KIØRBE, 1996; JONES AND FLYNN, 2005), minerals (URABE AND WATANABE, 1992), unsaturated fatty acids (MÜLLER-NAVARRA et al., 2000), sterols (KLEIN BRETELER et al., 1999) or amino acids (KLEPPEL; BURKHART; HOUCHIN, 1998). Thus, there are several studies using pluri-algal diets to increase the rates of copepod fecundity and development (MAUCHLINE, 1998). Alternatively, pluri-algal diets may increase the production costs and the effective culture time necessary for aquaculture (BUTTINO et al., 2009).

In this study, the *T. weissflogii* monoalgal diet resulted in the highest *T. turbinata* fecundity in comparison with the *T. chuii* monoalgal diet or the mixed algal diet. Generally, there is a directly positive relationship between the egg production rate in

copepods and the concentration of diatoms in both field and laboratory studies (BAN et al., 1997; IRIGOIEN et al., 2000). Experiments performed on *T. stylifera* fed with a monoalgal diet of *T. weissflogii* found high fecundity values but low egg hatching success (CEBALLOS AND IANORA, 2003). The use of diatoms has been avoided in some studies due to their negative effect on egg hatching success (MIRALTO et al., 1999; IANORA, 2005). Low fecundity and hatching rates were observed in the copepods *Acartia stueri* and *Centropages hamatus* when they were exclusively fed with *T. weissflogii* (BAN et al., 1997). Monoalgal diets of cryptophyceans (*Rhodomonas baltica* Karsten) and dinoflagellates (*Prorocentrum minimum* (Pavillard) Schiller) have shown good results in both the rate of egg production and in the egg hatching success of *T. stylifera* (BUTTINO et al., 2009), and these algae may be good alternatives to feeding diatoms.

While the best results were obtained from individuals fed with a monoalgal diet of *T. weissflogii*, the values of *T. turbinata* fecundity were similar (maximum value of 12.4 eggs fem⁻¹ day⁻¹) than other studies performed with the same species in the field (HOPCROFT AND ROFF, 1998; MELO JUNIOR, 2009) and in the laboratory (KAMINSKI AND MONTU, 2005). In studies performed in tropical waters (~28°C), mean values between 8 and 18 eggs fem⁻¹ day⁻¹ were recorded (HOPCROFT AND ROFF, 1998). In Brazilian subtropical waters (~20°C), the population production of *T. turbinata* was lower than that found in this work, with mean values of 2.6 ± 2.4 eggs fem⁻¹ dia⁻¹ (MELO JUNIOR, 2009). These values were not much different from the mean values (between 5 and 21 eggs fem⁻¹ day⁻¹) found in laboratory experiments under lower temperatures (~20°C) (KAMINSKI AND MONTU, 2005). *T. turbinata* has low rates of fecundity compared to other calanoid copepods in tropical waters (e.g., *Acartia tonsa*, 99 eggs fem⁻¹ day⁻¹) (HOPCROFT AND ROFF, 1998). In the laboratory, It is likely that the low rates of daily egg production were caused by the type of diatom that was offered; the best rates were obtained from individuals fed diets of *Nannochloropsis oculata* (Droop) and *Chaetoceros calcitrans* (Paulsen) (KAMINSKI AND MONTU, 2005). The nutritional content of each microalga is species specific and is described in the literature. The type and quantity of antimetabolic compounds in some diatoms may cause negative effects on copepod reproduction (CEBALLOS AND IANORA, 2003).

The egg hatching success rate of *T. turbinata* was very low. A discontinuous rate of production with interruptions in the egg hatching throughout the experiment was observed in copepods fed each of the three tested diets. The egg hatching success was high in copepods fed a diet of *T. chuii*, likely due to the absence of antimetabolic metabolites in these diatoms. *T. chuii* produce unsaturated aldehydes as a chemical defense prior to predation; this may result low egg viability, malformations in the nauplii and low rates of development in copepods that prey on *T. chuii* (IANORA, 2005). High fecundity and low egg hatching success have been recorded by many studies on copepods that were fed diatom diets, and they substantiate the negative effect of diatoms on the embryos but not in the adult individuals (MIRALTO et al., 1999). The utilization of *T. chuii* in this study was proposed as an alternative diet to avoid the deleterious effect of diatoms on egg hatching success. The chlorophyceans are considered to be an adequate source of food in cultures of zooplanktonic organisms due to their thin cell walls, which results in a low ash content and a high organic carbon to dry weight ratio (SIPAÚBA-TAVARES AND ROCHA, 1994). However, despite of the high egg hatching success and naupliar recruitment in copepods fed *T. chuii*, no significant differences among the treatment diets were observed.

The percentage of *T. turbinata* survival was very low in individuals fed a diet of *T. chuii*; however, the inclusion of *T. weissflogii* in the mixed algal diet increased survival compared to the monoalgal diet of this chlorophycean. These results show that the chlorophycean *T. chuii* was not favorable for daily egg production or adult *T. turbinata* survival, and despite the low viability of eggs, a monoalgal diet of *T. weissflogii* favored daily egg production and adult survival. A monoalgal diet may promote the reproduction and development of copepods due to the essential nutritional requirements for each species. Other monoalgal diets of diatoms (e.g., *Thalassiosira* spp.) and dinoflagellates, which have shown good results in previous laboratory experiments (CEBALLOS AND

IANORA, 2003; IANORA, 2005), can be used in future experiments with *T. turbinata* to examine the population recruitment. Thus, it remains necessary to test other diets that can increase the egg hatching success to improve the rates of fecundity and the survival of the species in laboratory conditions.

ACKNOWLEDGEMENTS

The authors are grateful to FACEPE for the scholarship that allowed the first author to perform the research and to PETROBRAS for partially financing the research.

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