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Diet influence on egg production of the copepod *Acartia tonsa* (Dana, 1896)

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ABSTRACT

Egg production in the copepod *Acartia tonsa* was evaluated using different densities of the microalgae *Thalassiosi weissflogii*, *Chaetoceros muelleri* and *Isochrysis galbana*. Male and female were kept under controlled conditio (salinity 30, 20°C, photoperiod 12L:12D), acclimated to the experimental conditions and left over a period of 24 h allow copulation. Algal densities tested were equivalent in biovolume and corresponded to 0, 2.5, 5, 10, 20, 40 a 60.10^3 cells.mL^{-1} of *T. weissflogii*. Ten acclimated female were separated, transferred to glass bottles and exposed f further 24 h to the corresponding experimental medium. After this period, the eggs were fixed and counted. Copep egg production reached a threshold value when *T. weissflogii*, *C. muelleri* and *I. galbana* were supplied at 10.1 140.10^3 and 640.10^3 cells.mL^{-1}, respectively. Mean egg production corresponded to 28.0 $\pm$ 0.5, 20.1 $\pm$ 1.0 a 22.0 $\pm$ 3.5 eggs.female^{-1}.day^{-1}, respectively. Copepods fed *T. weissflogii* showed the highest mean egg producti while those fed *I. galbana* reached a maximum egg production when the algae were supplied at a density two- to fou fold higher, considering the biovolume of *T. weissflogii* and *C. muelleri*. These differences are explained consideri the different sizes of the microalgae used to feed the copepods.

Key words: egg production, *Acartia tonsa*, microalgae.

INTRODUCTION

Copepods from the genus *Acartia* play an important role in the food webs of estuaries in both tropical and subtropical areas (Björnberg 1981, Mauchline 1998). Generally, they show the higher biomass values in most shallow enclosed bays and estuaries (Azaiteiro et al. 2005, Leandro et al. 2007). This fact may be related to their omnivorous feeding behavior, being able to survive and 2007). Such characteristic makes *Acartia* speci tively easy to cultivate at small- and large-scale laboratory conditions, providing enough biomass as an alternative live food in marine aquaculture (S and Nosker 1997, McKinnon et al. 2003) and a ical model in toxicological tests (Bielmyer et al. Pedroso et al. 2007, Pinho et al. 2007).

The increased use of copepods as live food i

of different sizes. The high nutritional value of copepods, which is characterized by a rich content in phospholipids, highly unsaturated fatty acids (HUFA), and natural antioxidants (Watanabe et al. 1983, Kraul et al. 1992, Sargent et al. 1997, Støttrup and Nosker 1997, Støttrup 2000, Helland et al. 2003), can also explain their actual broad use in aquaculture.

The success of a copepod culture depends on survival and fecundity rates of the cultivated animals, which are limited by food availability (Checkley 1980), temperature (Gaudy et al. 2000), and water salinity (Cardozo 2004). Egg production estimation is commonly used as an indicator of the nutritional quality of the food employed to feed the cultivated copepods (Butler and Dam 1994, Ceballos and Ianora 2003). Also, some studies have been performed to characterize the influence of different natural diets on copepod growth and egg production (Jakobsen et al. 2005, Kaminski and Montú 2005). All the information generated from these studies is important to select feeding regimes in order to improve copepod culture in laboratory (Støttrup and Jensen 1990, Castro-Longoria 2003, Cardozo 2004).

In light of the above, the aim of the present study was to evaluate the influence of the source and amount of food available on egg production in the copepod *A. tonsa*, using three different species of marine microalgae at different densities.

MATERIALS AND METHODS

The marine microalgae *Thalassiosira weissflogii* (Ø 13.2 μm), *Chaetoceros muelleri* (Ø 7.3 μm) and *Isochrysis galbana* (Ø 4.7 μm) were used in the present study as food source for copepods. They were grown in 5-L bottles, using the Guillard F/2 adapted method (Guillard and Ryther 1962).

Copepods used in the tests were isolated from field samples collected in the Patos Lagoon estuary (Rio Grande, RS, Southern Brazil) and kept under laboratory conditions for approximately 5 generations. The culture was carried out in non-toxic plastic tanks (70 L) containing saltwater (salinity 30). Tanks were kept in a experimental room with fixed temperature (20 ± 1°C) and photo-

For each feeding regime (food source and density) adult copepods (50 males and 50 females) were acclimated for 24 h in glass bottles (1 L) containing filtered (1 μm) saltwater (salinity 30) and the microalgae to be tested at the desired density. For each feeding regime, 6 different microalgae densities were tested, including one control condition where no food was added to the experimental medium. Therefore, 19 glass bottles were used, each one containing 100 copepods (50 males and 50 females). Bottles containing copepods and the experimental medium were kept under fixed temperature (20 ± 1°C) and photoperiod (12L:12D) and gently aerated to prevent algae sedimentation.

Algae densities were selected based on the saturation curve of egg production for *A. tonsa* fed on *Rhodomonas baltica* (Kiørboe et al. 1985). These authors have shown that *A. tonsa* achieved a stabilization on egg production at ~ 500 μg C.L^{-1}. Considering the amount of carbon and the volume of *R. baltica*, the equivalent in biovolume required to achieve ~ 500 μg C.L^{-1} would be 20.10^3, 280.10^3 and 320.10^3 cells.mL^{-1} for *T. weissflogii*, *C. muelleri* and *I. galbana*, respectively. The other 5 algae densities were selected to bracket that giving ~ 500 μg C.L^{-1} and were calculated based on the *T. weissflogii* densities (Table I), using the equivalent biovolume approach (Hillebrand et al. 1999).

TABLE I
Algal densities used in the experiments to measure egg production in the copepod *Acartia tonsa*. Algae densities are equivalent in biovolume.

Densities (10^3 cells.ml^{-1})		
Thalassiosira weissflogii	*Chaetoceros muelleri*	*Isochrysis galbana*
0	0	0
2.5	35	40
5	70	80
10	140	160
20	280	320
40	560	640
60	840	960

The first 24-h period of test was used to acclimate

domly collected and transferred to 300-mL glass bottles (10 female copepods per bottle) containing the corresponding experimental medium. Therefore, each experimental condition (food source and density) was tested in triplicate, totalizing 63 experimental units. Female copepods were then kept for a further 24 h under the same experimental conditions (feeding regime, salinity 30, temperature 20 ± 1°C, and photoperiod 12L:12D). Experimental medium were gently aerated to prevent algae sedimentation. Every 4 h, bottles were carefully agitated to keep algae cells in suspension.

After the second 24-h period of test, the experimental medium from each bottle was filtered (20-μm mesh filter). Eggs retained in the filter were transferred to 20-mL bottles, fixed in 4% formaldehyde solution, and counted in squared Petri dishes under a stereomicroscope. Egg production was expressed as eggs.female^{-1}.day^{-1}, considering the number of living females after the 48-h period of test.

Data from each experimental condition were expressed as mean ± standard error (n = 3). Considering the facts that food availability is a natural factor limiting *A. tonsa* egg production and that the relationship between egg production and food quantity measured as algal density follows a saturation curve (e.g. Berggreen et al. 1988), the maximum egg production for each microalgae was determined through non-linear regression analysis (exponential with saturation), using SigmaPlot 2001 for Windows version 7.0 (SPSS Inc., USA).

RESULTS

For the three microalgae species used, mean egg production rate was dependent on the density of food supplied to copepods (Fig. 1). Mean egg production in copepods fed on *T. weissflogii*, *C. muelleri* and *I. galbana* increased as the algae cell density increased, until reaching 10.10^3, 140.10^3 and 640.10^3 cells.mL^{-1}, respectively. In all cases, egg production did not change at higher cell densities. In fact, data obtained fitted well to a saturation-type kinetic model, especially for *T. weissflogii* and *C. muelleri*, where very high regression coefficients were observed (R^2 = 0.99 and 0.95, respec-

This lower R^2 value would be associated with an ent outlier mean value observed at the density of 1 cells.ml^{-1} (Fig. 1).

Copepod egg production was also depend food source provided to animals tested (Fig. 1). on the data regression model employed, the ma mean egg production obtained was 28.0 ± 0.5, 3.5, and 20.1 ± 1.0 eggs.female^{-1}.day^{-1} with *T. flogii*, *I. galbana* and *C. muelleri*.

DISCUSSION

Data from the present study shows an influence microalgae species and density on the egg produc the copepod *Acartia tonsa*. At low microalgae de a low egg production was observed for the three algae tested. This fact could be explained by a dec efficiency of food capture at low microalgae de as observed in *A. tonsa* fed with *T. weissflogii* (hofer and Stearns 1988). At high microalgae de egg production became stable was obtained for th microalgae tested. Considering the equivalent ume approach (Hillebrand et al. 1999), density *weissflogii* and *C. muelleri* showing stabilization production corresponded to half of that using *monas baltica* ($\sim$ 500 μg C.L^{-1}). On the othe it corresponded to double for *I. galbana* (Kiø al. 1985). These findings suggest that different concentrations are required to obtain stabilization production in *A. tonsa* when different food sour employed.

For the three microalgae species, mean va saturation in egg production (20 to 28 eggs.fem day^{-1}) were always higher than those measured field (1 to 16 eggs.female^{-1}.day^{-1}) for *A. tonsa* pel et al. 1998, Kleppel and Hazzard 2000). Fo pods fed on the same algae (*T. weissflogii*) und trolled conditions, the mean value observed for *A* in the present study (28 eggs.female^{-1}.day^{-1}) was ly higher than that reported for *A. clausi* (21 to 2 female^{-1}.day^{-1}) by Richardson and Verheye (19

A. tonsa showed a higher egg production wh on *T. weissflogii* than on *C. muelleri* or *I. galba*

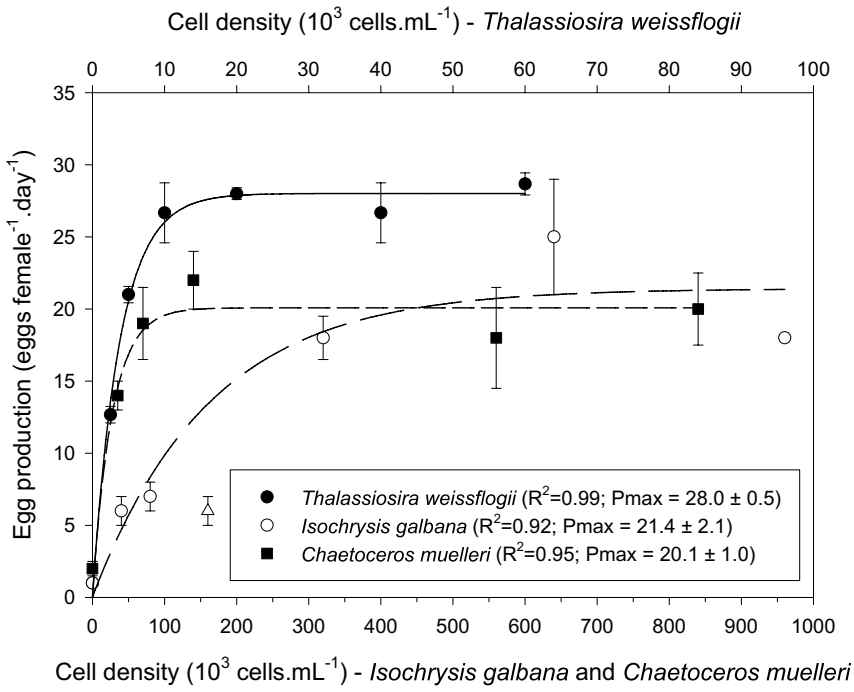

Fig. 1 – Egg production in the copepod *Acartia tonsa* using three different food sources: the microalgae *Thalassiosira weissflogii* (closed circles; solid line; top x axis), *Chaetoceros muelleri* (closed squares; short dashed line; bottom x axis) and Isochrysis galbana (open circles; long dashed line; bottom x axis). Different cell densities were tested for each microalgae species to give an equivalent biovolume value at each experimental condition. Copepods were acclimated to fixed salinity (30), temperature (20°C) and photoperiod (12L:12D) before tests. Tests were performed under the same acclimation conditions. Results are expressed as mean ± standard error. Data were analyzed by non-linear regression analysis (exponential with saturation). Pm = maximum egg production expressed as eggs.female^{-1}.day^{-1}. R^2 = regression coefficient. Egg production data with *I. galbana* at 160.10^3 cells.mL^{-1} (open triangle) was considered as an outlier.

was needed. These findings could be explained by the reduced food capture efficiency in *A. tonsa* when particles are between 2 and 4 μm (Irigoien et al. 2003, Katechakis et al. 2004). In this context, it is important to emphasize that *I. galbana* has a smaller size ($\sim$ 5 μm) than *T. weissflogii* ($\sim$ 13 μm) and *C. muelleri* (5-10 μm) (Berggreen et al. 1988). According to the size preference for food capture, *T. weissflogii* shows a more ideal size ($\sim$ 13 mm) for the adult stage of the copepod *A. tonsa* (Berggreen et al. 1988). Furthermore, *A. tonsa* can have low sensitive chemoreception mechanisms (Paffenhofer and Stearns 1988), requiring a higher biomass of smaller algae (e.g. *I. galbana*) than that of larger ones (e.g. *T.* with *T. weissflogii*. It is important to note that the sloppy feeding was not considered when comparing feeding efficiency among the different microalgae tested. According to Møller and Nielsen (2001), *A. tonsa* does not show sloppy feeding when capturing algae of sizes similar to those employed in the present study ($\sim$ 10 μm or less).

Ismar et al. (2008) reported that both *T. weissflogii* and *C. muelleri* allow the development of all life stages of *A. tonsa*. In the present study, *A. tonsa* fed on *T. weissflogii* and *C. muelleri* reached a saturated egg production at concentrations equivalent in biovolume. However, copepods fed on *T. weissflogii* produced in average 40% more eggs (28 eggs.female^{-1}.day^{-1}) than

higher fecundity observed with *T. weissflogii* suggests a preference for use of this microalgae under laboratory conditions. The fact that higher survival rates and better physiological responses and development of various stages of copepods are observed with *T. weissflogii* than with *I. galbana* under controlled conditions (Tirelli and Mayzaud 2005, Ismar et al. 2008, Koski et al. 2008) also supports this choice.

Despite the fact that a maximum egg production was obtained in the present study with a single food source, especially for *T. weissflogii*, a combination of at least two microalgae species could enhance *A. tonsa* egg production. For example, a higher egg production (32 eggs.female^{-1}.day^{-1}) was obtained in *A. tonsa* fed with a mixture of *I. galbana* and *Rhinomonas reticulata* (Medina and Barata 2004) than in those fed only with *I. galbana* (22 eggs.female^{-1}.day^{-1}) at an equivalent biovolume concentration. In other copepod species, better results were also obtained with plurialgal diets (Buttino et al. 2009).

In summary, data reported in the present study indicate that the feeding regime (microalgae species and density) influences egg production in the copepod *Acartia tonsa*. Differences in egg production with the different feeding regimes tested in the present study were explained considering the different sizes of the microalgae employed to feed the copepods.

RESUMO

A produção de ovos do copépode *Acartia tonsa* foi avaliada utilizando diferentes densidades das microalgas *Thalassiosira weissflogii*, *Chaetoceros muelleri* e *Isochrysis galbana*. Machos e fêmeas foram colocados sob condições controladas (salinidade 30, 20°C, fotoperíodo 12L:12D), aclimatados às condições experimentais e mantidos juntos por 24 h para permitir a copula. As densidades de algas foram equivalentes em biovolume e corresponderam a 0, 2,5, 5, 10, 20, 40 e 60,10^3 células.mL^{-1} de *T. weissflogii*. Dez fêmeas aclimatadas foram separadas, transferidas para frascos de vidro e expostas por mais 24 h ao meio experimental correspondente. Após este período, os ovos foram fixados e contados. A produção de ovos alcançou um valor limiar quando *T. weissflogii*, *C. muelleri* e *I.* ovos.fêmea^{-1}.dia^{-1}, respectivamente. Copépodes al dos com *T. weissflogii* mostraram a maior produção média enquanto os alimentados com *I. galbana* alca uma produção de ovo máxima quando as algas foram p a uma densidade de duas a quatro vezes maior, consi o biovolume de *T. weissflogii* e *C. muelleri*. Estas di podem ser explicadas considerando os diferentes tamar microalgas utilizadas para alimentar os copépodes.

Palavras-chave: produção de ovos, *Acartia tonsa*, algas.

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