Effect of two microalgae species (*Rhodomonas* sp. & *Thalassiossira weissflogii*) on the hatching success, moult and survival time of naupliar stages of *Acartia tonsa* Dana (1848).

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**Introduction**

Calanoid copepods are the most abundant and probably the most ecologically significant animals at the first consumer level of the marine plankton (Parrish & Wilson, 1978) and have been recommended for standard toxicity tests due to their wide distribution, short life cycle and high reproductive potential (Medina & Barata, 2004).

Dietary sterols are necessary for the growth and reproduction of copepods. Sterols of phytoplankton vary widely in both composition and concentration (Hassett, 2004). *Acartia tonsa* feed primarily on phytoplankton but also on detritus, its own eggs and nauplii, and ciliates, but it does not consume bacteria in significant quantities (Roman, 1984).

Berggren et al. (1998) suggest that particle size spectra of the diet of *A. tonsa* are bell-shaped. The low limit of particles retained is 2-4 \( \mu m \) in all stages.

Tester & Turner (1990) demonstrated that carbon ingested by adult females appears rapidly in eggs (<10 h) So changes in the biochemical composition of eggs as well as of can be observed adults during short-term experiments.

The effect of the diet of parents upon the development of early stages copepods is scarce or non-existent. Therefore, the aim of the present study was to evaluate the hatching success, nauplii survival time, death time and stage attained by non-fed individuals produced by females of *A. tonsa* fed under diets of microalgae with different chemical composition: *Rhodomonas* sp. and *T. weissflogii*.

The main working hypothesis for the present study was

The food regime (*Rhodomonas* sp. or *T. weissflogii*) during development of *Acartia tonsa* females affects their offspring in terms of hatching success, survival time and stage attained by non-fed nauplii.
Material and Methods

The experiment was carried out at the Kristineberg Marine Research Station.

*Maintenance of copepods culture and Experimental design*
A single batch of copepodid stages I and II of *A. tonsa* were taken from a large laboratory culture and kept in two 7 L containers and fed daily at a constant concentration either *Rhodomonas* sp or *T. weissflogii*. The water was aerated and kept at a constant temperature of 18°C. Food concentration was checked daily with an Elzone electronic particle analyzer and adjusted to 200 µg C L⁻¹ by the addition of the necessary amount of fresh algal suspensions.

After in vitro population reached the adult stage, 15 mature females and 5 male from each treatment were transferred to 1 L glass bottles with fresh algal suspensions of the respective species (2 bottles per treatment) and mounted on a rotating wheel for 24 h. Then females were collected on a sieve and placed individually on trays of 12 well-dish (ca. 2 ml) for three hours. Then eggs sheds by the more production females were individually placed in a 96 multi-well dish containing algae-free filtered sea water. These trays were kept at room temperature of ca 20°C and checked under inverted microscope for hatching, moult and survival, every 3 h and 6 h intervals (day and night, respectively).

The response variables recorded for every individual were:

- **Hatching time**
- **Ha-Mo time**
- **E-Mo time**
- **Ha-D time**
- **E-D time**

**Ha-Mo time** indicates the elapsed time between hatching and moult; **E-Mo time** indicates the elapsed time between egg spawning in trays of 96 multi-well dish and moult. **Ha-D time**
indicates the elapsed time between hatching and death and E-D time indicates the elapsed time between molting and death.

Passage of nauplii, still without food, from one stage to the next was indicated by the presence of moults on the bottom of the well. Death was indicated by lack of movement and unresponsiveness after mechanical stimuli.

**Statistical Analysis**

Comparisons between treatments (*Rhdomonas* sp. & *Talassiossira weissflogii*) were made by Kruskal-Wallis Test using the statistical program SPSS. In all cases a significance level of 5% was considered.
Results

Two pilot experiments were run but their results were not further evaluated due to different problems. In the first of them the sample size for one of the treatments (T. weissflogii) was too small (44 vs. 80 for Rhodomonas sp.); on the second there was contamination of algae during the incubation of eggs and some nauplii presented evidence of feeding activity (coloured guts).

Total egg numbers analyzed of A. tonsa fed with Rhodomonas sp or T. weissfloggi were 89 and 81, respectively. There was no significantly difference (p>0.05) between treatments in the amount of hatching-eggs, time of hatching and time elapsed between egg spawning and moult for eggs (Table 1). Nonetheless, females fed either T. weissflogii or Rhodomonas sp. differed in the fraction of nauplii that moulted (Fig. 1) and in the survival period (Fig 2).

Among Rhodomonas sp-fed females there was significant variability in fraction of eggs that hatched (Table 2) (Fig 3), egg-hatching time (Fig 4), nauplii- Ha-D time (Fig 5) and E-D time (Fig 6).

Among T. weissfloggi-fed females there were significant differences (Table 3) in the fraction of eggs that hatched (Fig. 7), hatching time (Fig. 8), fraction of nauplii that moulted (Fig. 9), Ha-Mo time (Fig. 10), Ha-D time (Fig. 11) and E-D time (Fig. 12).

Longer survival time of nauplii produced by females fed with T. weissflogii moulted to stage II; on the contrary, among those nauplii that did not reach stage II survival time was longer in those produced by females fed Rhodomonas sp. (Fig 13 y 14).
Two pilot experiments were run but their results were not further evaluated due to following problems. In the first of them the sample size for one of the treatments (T. Weissflogii) was too small (44 vs. 80 for Rhodomonas sp.); on the second there was contamination of algae during the incubation of eggs and some nauplii presented evidence of feeding activity (coloured guts).
Discussion

There are numerous laboratory studies on the effect of the quality and food concentration on the egg-production rates, growth and development on copepods (Berggreen 1988, Calliari & Tiselius 2005, Hassett 2004, Stottrup & Jensen 1990) However, the effect of food type on the development of early stages of copepod (Nauplii) is scarcely studied.

Present results showed that most eggs hatched within 24 h, at 20°C. Those were compared with results previous (Tester & Turner, 1990) for same species, but differed to reported by Marcus & Wilcox (2007) who found that hatching requires ca. 48 h.

Hatching success of eggs produced by females fed either Rhodomonas sp. or T. weissflogii (94% and 92%, respectively) were similar lack of effects of theses diets upon hatching success suggested also by Enderington et al (1995) for T. weissflogii. These results suggest that both algae, at the concentration here employed, do not have different effects on the egg hatching success of A. tonsa. These results were also valid when considering most response variables explored. Notable exceptions were stage attained by nauplii (higher in T. weissflogii) and death time (later for T. weissflogii).

Variability between females in most response variables was significant for T. weissflogii fed females and only for a few response variables for Rhodomonas sp. fed females. This implies that individual variability between females may be related to inorganic and organic components of T. weissflogii.
Bibliographical References


Tables

Table 1: Results of Kruskal-wallis test to assess significance differences among *Rhodomonas* sp. and *T. weissflogii* regarding different descriptors of naupliar development. n Rho = number of eggs or nauplii in *Rhodomonas* sp. treatment, *Thalassiosira weissflogii*, df = degree of freedom.

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<th>n Rho</th>
<th>n Tw</th>
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Table 2: Results of Kruskal-wallis test to assess differences between individual *Acartia tonsa* females fed *Rhodomonas* sp. regarding different descriptors of naupliar development. n Rho = number of eggs or nauplii in *Rhodomonas* sp. treatment, *Thalassiosira weissflogii*, df = degree of freedom.

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<td>E-D time</td>
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<td>0,036</td>
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Table 3: Results of Kruskal-wallis test to assess differences individual *Acartia tonsa* females fed *T. weissflogii*, regarding different descriptors of naupliar development. n Rho = number of eggs or nauplii in *Rhodomonas* sp. treatment, *Thalassiosira weissflogii*, df = degree of freedom.

<table>
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<td>E-D time</td>
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Figures

Fig 1: Percent of *Acartia tonsa* nauplii that moulted when females were fed *Rhodomonas* sp. or *Thalassiossira weissflogii*.

Fig 2: Survival period of *Acartia tonsa* nauplii produced by females fed *Rhodomonas* sp. or *Thalassiossira weissflogii*. 
Fig 3:
Fraction of eggs hatched produced by individual *Acartia tonsa* females fed *Rhodomonas* sp.

Fig 4:
Hatching time of eggs produced by individual *Acartia tonsa* females fed *Rhodomonas* sp.
Fig 5: Nauplii-Ha-D time of nauplii produced by individual Acartia tonsa females fed Rhodomonas sp.

Fig 6:
Nauplii-E-D time of nauplii produced by individual Acartia tonsa females fed Rhodomonas sp.
Fig 7:
Fraction of eggs hatched produced by individual Acartia tonsa females fed Thalassiossira weissflogii.

Fig 8:
Eggs-hatching time produced by individual Acartia tonsa females fed Thalassiossira weissflogii.
Fig 9: Fraction of *Acartia tonsa* moulted nauplii produced by females fed *Thalassiossira weissflogii* that moulted to stage II.

Fig 10: Ha-Mo time of *Acartia tonsa* nauplii produced by females fed *Thalassiossira weissflogii*.
Fig 11: Ha-D time produced for females Acartia tonsa fed with Thalassiossira weissflogii.

Fig 12: E-D time eggs hatching for females Acartia tonsa fed with Thalassiossira weissflogii.
Fig 13: Nauplii that did not moult under two regimens feeding (*Rhodomonas* sp. and *Thalassiossira weissflogii*).

Fig 14: Nauplii moulted under two regimens feeding (*Rhodomonas* sp. and *Thalassiossira weissflogii*).