

## Feeding Adult of *Artemia salina* (Crustacea-Branchiopoda) on the dinoflagellate *Gyrodinium corsicum* (Gymnodiniales) and the Chryptophyta *Rhodomonas baltica*

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### ABSTRACT

Experiments were carried out on feeding performance and survival rates of adult *Artemia salina* exposed to no axenic strains of the dinoflagellate *Gyrodinium corsicum* and of the Chryptophyta *Rhodomonas baltica*. Filtration rates on *R. baltica* and *G. corsicum* varied from 3.35 to 7.14 ml.artemia<sup>-1</sup>.h<sup>-1</sup> and from 2.97 to 15.86 ml.artemia<sup>-1</sup>.h<sup>-1</sup>, respectively. The ingestion rates observed for *A. salina* did not indicate any digestive dysfunction or physiological impairment for organisms fed on *G. corsicum* and their functional response were similar to those observed for other organisms like copepod fed on different food concentrations. Mortality rates oscillated from 2.5% to 100% when *A. salina* was fed on *R. baltica* or *G. corsicum*, respectively. Highest mortality rates observed for organisms fed on *G. corsicum* indicated that this dinoflagellate presented a hazard effect on *A. salina* that was not possible to confirm if it was related to toxin production or to nutritive inadequacy of this dinoflagellate as food for organisms of this species.

**Key words:** Feeding, *Artemia salina*, *Rhodomonas baltica*, *Gyrodinium corsicum*

### INTRODUCTION

Blooms of harmful algae increased in coastal waters around the world during the last decades (Anderson, 1989; Hallegraeff et al., 1988; Sournia et al., 1991; Smayda, 1992; 1997) which evidently would increase more in the future, given the increasing eutrophization (Lam and Ho, 1989; Okaichi, 1989; Hallegraeff, 1993) and global warming perspectives (Hallegraeff, 1993). Data available in the literature indicate that zooplankton

grazing could generate a substantial impact on wild populations of toxic dinoflagellates (Turner and Anderson, 1983; Watras et al., 1985). However, other authors consider that production and liberation of toxic compounds would interfere with the feeding processes of some zooplanktonic organisms like copepods (Dutz, 1998; Teegarden, 1999; Maneiro et al., 2000). The potential reduction of toxic blooms and the transference of dinoflagellate toxins through the marine food webs by some zooplanktonic organisms must be

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considered of great importance when dealing with feeding experiments that involve PSP toxin producers. Hence, it would be important to know whether dinoflagellates are only toxic to fish or also toxic to organisms at lower trophic levels in the pelagic food webs.

*Gyrodinium corsicum* Paulmier is an unarmoured dinoflagellate that was first reported for the salt-water lake of Diana in Corsica from which originates its denomination. This new species belongs to the order Gymnodiniales (family Gymnodiniaceae) and it was responsible for the development of outbreaks related with the mortality of important fishes such as gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), caged in intensive aquaculture systems during massive blooms in Diana Lake (Paulmier et al., 1995) and other fish species in Alfacs Bay (Garcés, 1998). These authors did not discard the possibility of ichthyotoxin (haemolysins or allotoxins) production by organisms of this species although negative results were observed for samples collected in Corsica.

*Rhodomonas baltica* was used in our work as a non-toxic control because it presented similar dimensions of those observed for *G. corsicum* and has been commonly offered as food supply in aquaculture systems. The present study aimed to determine the feeding patterns of adult *Artemia salina* (Branchiopoda - Crustacea) exposed to variable cell concentrations of *Gyrodinium corsicum* (toxic dinoflagellate) and to compare the results with those obtained for *Rhodomonas baltica* (non-toxic algae control).

## MATERIAL AND METHODS

### Food supply

*G. corsicum* and *R. baltica* were selected due to similar cell size and their toxic and non-toxic nature, respectively. The used stocks were obtained from the Institute of Marine Sciences of Barcelona (I.C.M. Barcelona, Catalonia- Spain). The algae selected were strains of the dinoflagellate *G. corsicum* Paulmier, GCORS1 (I.C.M. - Barcelona) and the non-toxic Cryptophyta *R. baltica* Karsten (CCAP 979/9 - UK from I.C.M. - Barcelona). The equivalent spherical diameter (ESD) of the used species were 12.61 µm to *G. corsicum* and 7.53 µm to *R.*

*baltica*. The algae were batch-cultured at  $15 \pm 1$  °C in an incubator under controlled temperature, light regime of 12:12 h light/dark cycle and a irradiance of  $200 \mu\text{mol. m}^{-2}.\text{s}^{-1}$  (day-light fluorescence tubes). The cultures were maintained in 200 mL flasks containing f/2 medium (Guillard, 1975) in seawater at 35 ‰ salinity.

### Animal preparations for feeding

Dried eggs of *Artemia salina* (L.) were obtained from New Technology Laboratories LTD, United Kingdom. A stock culture of *Artemia* was reared on *R. baltica* at  $27 \pm 1$  °C. The stocks were then transferred to the same controlled environmental chamber cited for the culture of phytoplankton and maintained with this cell food during one-generation time. All the *Artemia* used in the experiments were reared from adult females cultured under laboratory conditions. Before the experiments, adult organisms were sorted and placed in 1 liter beakers containing 800 ml of 0.45 µm filtered seawater, rinsed with filtered seawater and then transferred to the experimental bottles.

### Resistance to starvation

To study the tolerance of adult individuals to the lack of food, 15 adult *A. salina* were transferred to 250 ml bottles filled with 0.45 µm Millipore filtered seawater and fixed to a rotate grazing wheel adjusted to 1 rpm. The bottles were examined every day and the dead organisms were recorded and discarded using a Pasteur pipette. All determinations were carried out with 4 replicates. *A. salina* adults were defined as dead when no movements were registered within 60 s. Mortality rates were calculated as:

$$M24H = (NMC/TC) \times 100 \quad (1),$$

where: M24H is the mortality rate after the 24 h trials, NMC is the number of non-motile *Artemia* in each flask after the 24 h trials and TC is the total number of organisms in each bottle. The M<sup>n</sup>X<sup>m</sup>H index was calculated in the same way for 48 h, 72 h and so on.

### Feeding experiments

Fixed 6h grazing trials were carried out at  $15 \pm 1$  °C and low light levels to limit algae growth.

Initial cell concentrations were performed using a Coulter electronic particle counter model TA II equipped with a 140 µm aperture. Dilutions were carried out in 0.45 µm filtered seawater to provide food suspensions that varied between 100 and 2000 cell.ml<sup>-1</sup> (Table 1). Experiments consisted of three bottles with grazers for each of the species (*R. baltica* or *G. corsicum*) and cell concentrations used, three growth control bottles for *R. baltica* (non-toxic algae) and three growth control bottles for *G. corsicum*. At the beginning of the experimental period the bottles were capped and affixed to a rotate grazing wheel, and rotated at 1 rpm until the end of the experiment. At the end of the experiment, containers were removed from the wheel and artemias were separated from the algae suspensions with mesh nets of 200 µm and transferred to Millipore 0.45 µm filtered seawater.

Filtration and ingestion rates were calculated according to Fernández (1979):

$$F = (\ln C_c - \ln C_e) / t \cdot (V/N) \quad (2),$$

$$I = (C_c - C_e) \cdot (V/Nt) \quad (3),$$

where:

F= filtration rate (ml of medium swept clear.artemia<sup>-1</sup>.h<sup>-1</sup>);

I= ingestion rate (number of cells ingested.artemia<sup>-1</sup>.h<sup>-1</sup>);

C<sub>c</sub>= final algal concentration in control flasks;

C<sub>e</sub>= final algal concentration in the experimental flasks;

t = duration of the experiment in hours;

V and N = the respective volume (ml) and number of *Artemia* in the experimental flasks.

**Table 1** - Experimental conditions employed during the bioassays carried out with *A. salina* fed on *R. baltica* and *G. corsicum*.

Algae species	n*	F.C.** (Cell.ml <sup>-1</sup> )	Time (h)
<i>R. baltica</i>	5	150, 200, 350, 500, 700, 900, 1500, 2000	3
<i>G. corsicum</i>	5	150, 200, 350, 500, 700, 900, 1500, 2000	3

\* n = Number of organisms/bottle

\*\* F.C. = Food concentration

### Statistical analysis

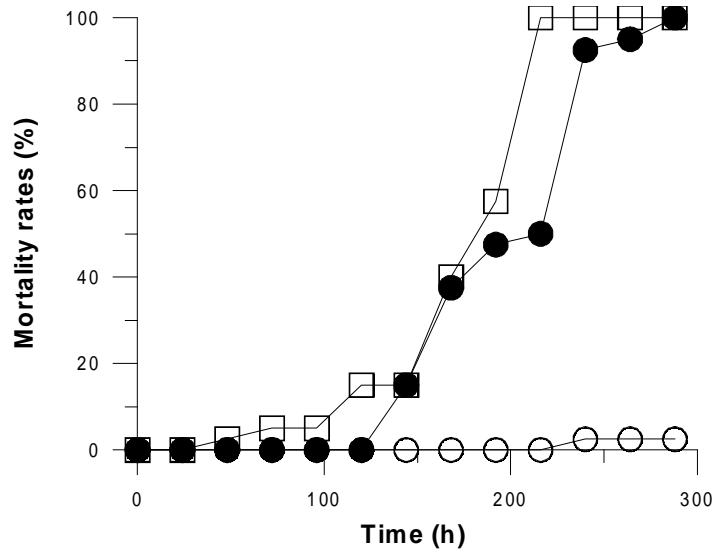
One-way ANOVA was employed to verify the existence of significant differences between ingestion rates of *A. salina* on the different algae concentrations. Fisher's PLSD test was used to analyse differences between treatments using a significance level of 0.05.

## RESULTS

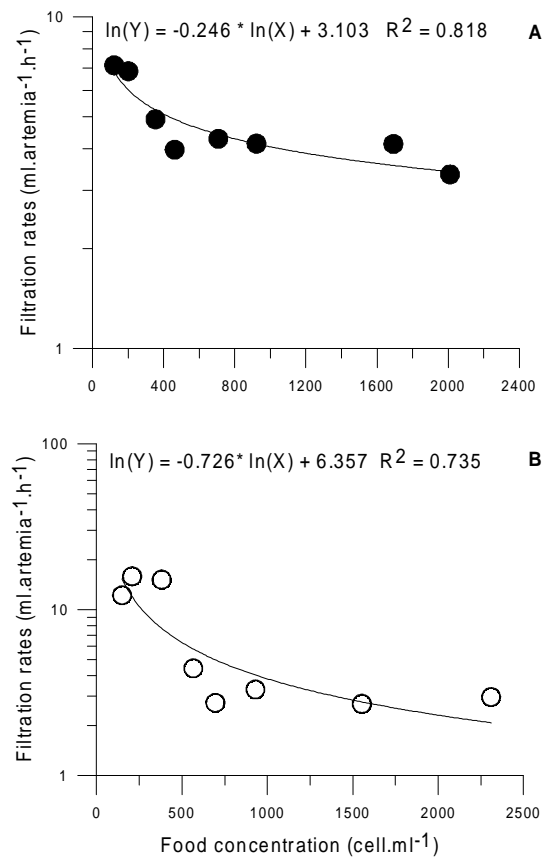
Starved adults of *A. salina* showed total mortality rates after 288 h of incubation in 0.45 µm Millipore filtered seawater. Incubations with adult organisms fed on *R. baltica* demonstrated

mortality rates of 2.5 % after the same incubation period (Fig. 1) showing that this alga was appropriated to maintain *Artemia* cultures under laboratory conditions. On the other hand, it was not possible to complete the life cycle of *A. salina* with *G. corsicum* as food. *A. salina* fed on this dinoflagellate showed 100 % mortality before attain adult stage.

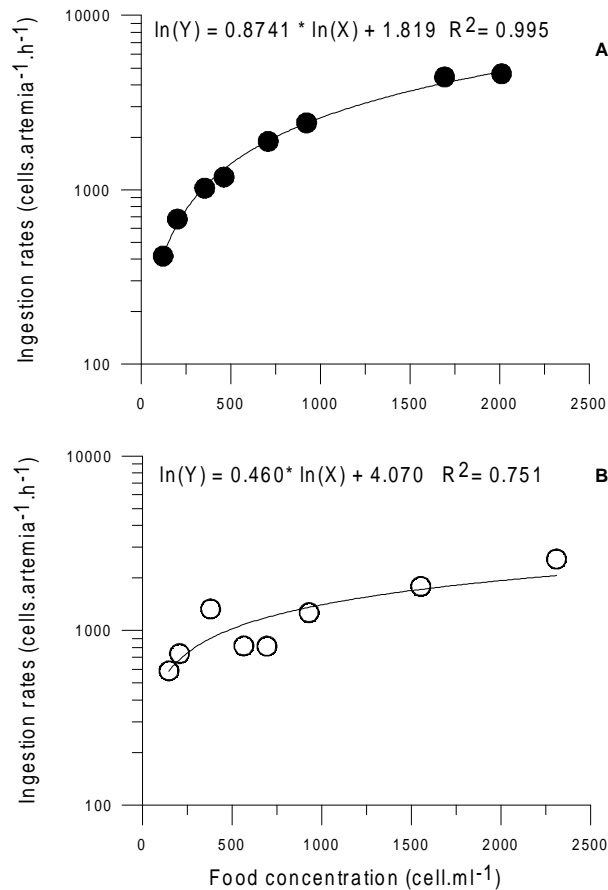
Filtration rates obtained for *A. salina* fed on *R. baltica* and *G. corsicum* are given in Figures 2 A and 2 B. Filtration rates on *R. baltica* varied from 3.35 to 7.14 ml.artemia<sup>-1</sup>.h<sup>-1</sup>. For organisms fed on *G. corsicum*, filtration rates oscillated from 2.97 to 15.86 ml.artemia<sup>-1</sup>.h<sup>-1</sup>.



**Figure 1** - Mortality rates of starved adult *A. salina* (dashed circle) and organisms fed on *R. baltica* (open circle) and *G. corsicum* (open squares).



**Figure 2** - Filtration rates (ml.artemia<sup>-1</sup>.h<sup>-1</sup>) of *A. salina* fed on different concentrations of *R. baltica* (A) and *G. corsicum* (B).



**Figure 3** - Ingestion rates (cells.artemia<sup>-1</sup>.h<sup>-1</sup>) of *A. salina* fed on different concentrations of *R. baltica* (A) and *G. corsicum* (B).

Maximal ingestion rates on *R. baltica* ( $5.45 \times 10^3$  cell.ml<sup>-1</sup>) and *G. corsicum* ( $5.70 \times 10^3$  cell.ml<sup>-1</sup>) were observed at the maximal food concentrations employed during the experiments ( $\cong 2000$  cells.ml<sup>-1</sup>). Significant differences ( $p < 0.05$ ) were found between ingestion rates on *R. baltica* and *G. corsicum* at low concentrations although it was not observed at high concentrations, except for 1500 cell.ml<sup>-1</sup>. Saturating concentrations were not observed neither for *R. baltica* or *G. corsicum* (Figs. 3 A and 3 B).

## DISCUSSION

Highest mortality rates observed for organisms fed on *G. corsicum* indicated that this dinoflagellate presented a hazardous effect on *A. salina* that was not possible to confirm if it was related to toxin production or to nutritive inadequacy of this

dinoflagellate as food for organisms of this species. Results reported by Costa and Fernández (2002) using *G. corsicum* as food for *Acartia grani* and *Euterpina acutifrons* (Copepoda) showed no haemolytic activity for extracts of this dinoflagellate as it was suspected by other authors (Paulmier et al., 1995) indicating that the toxic effects of *G. corsicum* could be due to the presence of other toxins. The functional response of *A. salina* was similar to that observed for other organisms like copepod (Uye and Takamatsu, 1990; Liu and Wang, 2002) with higher filtration rates when fed on lower food concentrations ( $\cong 8 \times 10^2$  cell.ml<sup>-1</sup>) and low filtration rates when exposed to high cell concentrations ( $\cong 4 \times 10^3$  cell.ml<sup>-1</sup>). Comparisons between *Artemia* filtration rates on both algae species were not carried out due to differences between alga dimensions. Some authors reported that differences in alga dimensions and shape (Frost, 1972; Nival and

Nival, 1976) could interfere in the filtration efficiency of some organisms (copepod) capturing large cells efficiently than those of low dimensions.

For *A. salina* fed on planktonic algae, Reeve (1963) did not observe saturating ingestion rates until concentrations higher than  $1 \times 10^4$  cell.ml<sup>-1</sup>. Saturating ingestion rates were also not observed in the present work where maximal cell concentrations were  $2 \times 10^3$  cell.ml<sup>-1</sup>. This functional response was similar to those registered for copepods where maximal ingestion rates were obtained when organisms were fed on the highest food concentrations employed in the experiments (Dutz, 1998; Frangópulos et al., 2000).

The ingestion rates observed for *A. salina* did not indicate any digestive dysfunction or physiological impairment for organisms fed on *G. corsicum*. Reduced ingestion rates of adult *A. salina* on *G. corsicum* could be an artefact related to differences of cell dimensions that implies in a higher carbon concentration in this species. So, to satisfy carbon requirements *A. salina* needs to ingest a low number of *G. corsicum* cells than it could be expected to *R. baltica* cells.

In summary it was possible to confirm that *A. salina* fed on *R. baltica* and *G. corsicum*. However, consequences of the ingestion of the later could be the responsible for the death of organisms in few days. On the other hand, *R. baltica* showed to be very efficient as food supply for adult *A. salina* giving survival rates of 97.5 % after twelve days of incubation.

## RESUMO

Experimentos foram desenvolvidos para estudar as taxas de alimentação e de sobrevivência de *Artemia salina* alimentada com cepas não tóxicas do dinoflagelado *Gyrodinium corsicum* e da Chryptophyta *Rhodomonas baltica*. As taxas de filtração sobre *R. baltica* e *G. corsicum* variaram entre 3,35 e 7,14 ml.artemia<sup>-1</sup>.h<sup>-1</sup> e 2,97 e 15,86 ml.artemia<sup>-1</sup>.h<sup>-1</sup>, respectivamente. As taxas de ingestão observadas para *A. salina* não indicaram disfunção digestiva ou prejuízo fisiológico nos organismos alimentados com *G. corsicum*, sendo a resposta funcional destes organismos similar a observada em copépodos alimentados com diferentes concentrações de alimento. As taxas de mortalidade de *A. salina* oscilaram entre 2,5 e 100% quando alimentada com *R. baltica* e *G.*

*corsicum*, respectivamente. As maiores taxas de mortalidade observadas para os organismos alimentados com *G. corsicum* indicam que este dinoflagelado apresenta algum efeito nocivo sobre *A. salina*, embora não tenha sido possível confirmar se sua origem está relacionada com a produção de toxinas ou com a inadequação nutritiva deste dinoflagelado para alimentação de organismos desta espécie.

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