Growth and survival rates of early developmental stages of *Acartia grani* (Copepoda: Calanoida) in relation to food concentration and fluctuations in food supply

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ABSTRACT· The scales of temporal and spatial variability of food resources in marine systems are determinant factors in the control of zooplankton populations. For adult copepods, egg production rates are dependent on the frequency of fluctuating food availability. However, very little is known about the effects of food fluctuations on the life-history parameters for early developmental stages of copepods. The growth and survival rates for naupliar stages of the marine copepod *Acartia grani* were studied in relation to food concentration and to experimentally induced fluctuations in food availability. Growth rates of early nauplii were highly dependent on food abundance, and were comparable to simultaneously estimated rates of adult production (egg production rates). Tolerance to starving conditions (survival time) increased through the cohort development. For short-time fluctuations in food abundance (alternating, 12 h high food / 12 h low food conditions), growth rates of naupliar stages depended on the light conditions at which food was available. Lower frequency fluctuations (alternating, 24 or 48 h high food / low food conditions) significantly reduced both naupliar growth and survival rates. The sensitivity of growth rates to food fluctuation for naupliar stages, and their low tolerance to starving conditions, are further evidence to explain the confinement of *A. grani* to coastal habitats.

KEY WORDS: Growth rates · Food availability · Copepods · Nauplius · *Acartia grani*

INTRODUCTION

The dynamics of zooplankton populations is strongly dependent on the conditions of food availability (Holling 1959, Dagg 1977, Kleppel et al. 1996), which are mediated by the rate of temporal variability and patchy nature typical of planktonic communities (Holligan 1984, Mackas et al. 1985, Le Fèvre 1986, Cushing 1989). In this variable food environment, herbivorous zooplankton would perceive the succession of phytoplankton pulses or biomass patches as fluctuations in the amount of food available (Dagg 1977, Le Fèvre & Frontier 1988, Calbet & Alcaraz 1996).

Food fluctuations are known to play a significant role in the control of copepod populations, mainly through changes in mortality and female fecundity rates (Dagg

1977, Nival et al. 1990, Davis & Alatalo 1992, Calbet & Alcaraz 1996). Nevertheless, most of the available data have been concentrated on adult copepods. Studies on the life-history parameters for early developmental stages refer in general to the control exerted by predation (Landry 1978) or food concentration (Paffenhöfer 1970, Klein Breteler & Gonzalez 1982, Berggreen et al. 1988, Tsuda 1994), but the effects of fluctuations in food availability for early developmental stages of copepods are less well known.

In this work, we have evaluated the functional effects of food abundance and fluctuation on growth and mortality rates for early developmental stages of the marine pelagic copepod *Acartia grani*. The objectives were: (1) to determine the growth rates of early naupliar stages under different food concentrations, and to compare naupliar production with simultaneous estimations of adult production (female egg production

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rates); (2) to estimate the tolerance of the different developmental stages to starving conditions; and (3) to evaluate the effects of different frequencies of food fluctuations on naupliar growth rate and mortality as compared with an equivalent concentration of food continuously supplied.

METHODS

Experimental organisms and general procedures. Naupliar stages of the planktonic marine copepod *Acartia grani* were obtained from a laboratory culture reared at $18 \pm 1°C$ in a temperature-controlled room, in a 12 h light-dark cycle, and fed ad libitum a suspension of *Rhodomonas baltica* (6.9 to 7.3 µm diameter, 4 to 5 ppm by volume). The algae were maintained in exponential growth in order to avoid changes in nutritional quality by the daily addition of f/2 medium (Guillard 1975). The experimental nauplii were collected from eggs hatched during 12 to 24 h intervals in order to obtain a maximum degree of homogeneity in initial naupliar stage and size. All the experiments were conducted under the same light and temperature regime as described for the culture conditions, except 1 case in which one of the variables was the light-dark periodicity.

Algal concentrations (volume) were measured by means of a Multisizer Coulter Counter, and the conversion to carbon contents was done according to the volume-carbon relation given by Berggreen et al. (1988).

Stage-specific biomass in *Acartia grani* was estimated from the length-dry weight equations given by Durbin & Durbin (1978) for *Acartia clausi*. Egg dry weight (DW) came from values of Kiørboe et al. (1985) for *Acartia tonsa*. Organism size was measured with an Image Analysis System (dissecting microscope with camera) and NIH Image software on organisms fixed with acidic Lugol. Instantaneous specific growth rates of nauplii (*g*) were calculated from the exponential change in biomass (*DW*):

$$g = \frac{1}{t} \ln \frac{DW_{t1}}{DW_{t0}}$$

Functional response to food concentration. About 500 nauplii II to III (average size 172.98 ± 2.100 µm length, equivalent to 0.13 ± 0.004 µg DW) were placed into 1.25 l Pyrex bottles filled with a suspension of *Rhodomonas baltica* at different concentrations (0, 0.5, 1, 2, 4 and 6 ppm, equivalent to 0, 135, 270, 540, 1081 and 1620 µg C l^{-1} or 0, 2850, 5700, 11400, 22800 and 34200 cells ml^{-1} respectively). Experiments were run in triplicate. A slow-rotating Ferris wheel (0.2 rpm) was used in order to avoid algal sedimentation. The experiment lasted 3 d, and the animals were transferred to a new

algal suspension every day. An aliquot of the bottle contents, calculated to obtain about 30 to 40 nauplii per replicate, was filtered daily through a 20 µm mesh submerged sieve. Subsequently the nauplii were fixed with acidic Lugol and measured under a dissecting microscope by means of the described image analysis system.

In order to compare the naupliar growth (production) with adult female egg production rates, groups of 7 adult females were incubated in 625 ml Pyrex bottles filled with concentrations of *Rhodomonas baltica* similar to those for naupliar growth rates. The experiments were run in triplicate. After 3 d of acclimation to food concentrations the following 24 h eggs were collected in a 20 µm submerged sieve and counted under a dissecting microscope.

Survival to starving conditions. Eggs obtained from the laboratory culture of *Acartia grani* were hatched and grown under excess food (5 to 6 ppm *Rhodomonas baltica*, equivalent to 1350 to 1620 µg C l^{-1}), and the evolution (length and stage) of the cohort was monitored from nauplius I to adult. During the development of the cohort, about 50 individuals of the successive naupliar or copepodite stages were placed individually in 2.5 ml chambers filled with filtered sea water and checked once a day for mortality. The control consisted of a parallel group of individuals of the same developmental stage incubated in similar receptacles but filled with a suspension of *R. baltica* at excess food concentration. Two-thirds of the culture suspension in the controls and of the filtered sea water in the experimental chambers were changed daily. Survival time to starvation for each developmental stage was estimated as the days elapsed in filtered sea water until mortality reached 50% of the initial population.

Effects of fluctuating food availability in naupliar growth and survival. The experimental design to test the effects of different frequencies of fluctuating food concentration on naupliar growth rates consisted of providing the copepods with equivalent average amounts of food under 2 conditions: fluctuating, high food-low food concentrations, simulating the food variability of natural, variable systems; and continuous supply of food (controls) at a concentration equivalent to the average food experienced by the organisms in fluctuating conditions (Calbet & Alcaraz 1996). The chosen concentrations were maintained in the range at which growth rates were proportional to the food concentration. The maximum food concentration was always maintained below saturating conditions for growth.

About 500 recently molted nauplii II were placed in each experimental chamber. These chambers consisted of a perspex cylinder (15 cm long, 10 cm diameter) with the bottom covered with 40 µm nitex mesh.
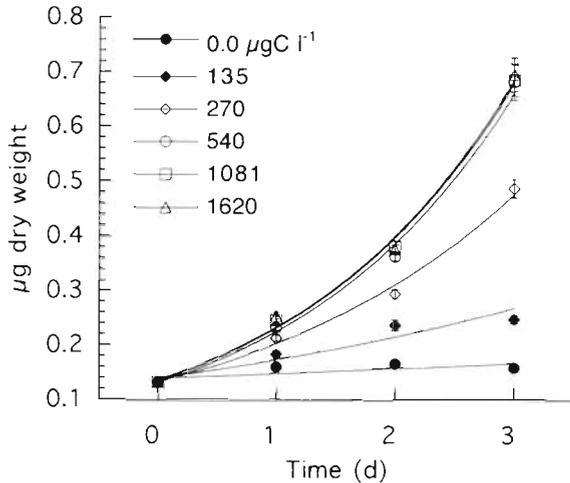
Fig. 1 Temporal evolution of naupliar dry weight (*DW*) in relation to food concentration of *Rhodomonas baltica* (in µg C l⁻¹). Error bars represent ±SE

Fig. 2. Specific growth rate (± SE) of nauplii and adult (egg production rate) *Acartia grani* as function of food concentration (µg C l⁻¹ of *Rhodomonas baltica*). Lines correspond to the Holling type II equation (see text). The fitted parameters were: for adults, $a = 1.57 \times 10^{-3}$ and $b = 1.73$; for nauplii, $a = 4.43 \times 10^{-3}$ and $b = 1.89$

Experimental chambers were suspended in 15 l plastic containers (6 chambers per container) with filtered sea water and algae at the chosen concentration. In order to avoid algal sedimentation and to provide water flow through the egg-laying chambers, water was circulated with a peristaltic pump at a rate of 0.4 l h⁻¹ in each chamber. Food concentration was monitored by means of a Coulter Multisizer 4 times a day and corrected if necessary. Every day the culture medium was exchanged for a new suspension of *Rhodomonas baltica* at exponential growth rate.

Fluctuations in food concentration were simulated by gently changing the experimental chambers from the high food to low food containers and vice versa at the required frequencies, and an aliquot of the nauplii were collected once a day. Control chambers were also periodically removed in order to uniformize the treatments. All the experiments were run in triplicate.

Three frequencies of food fluctuations were tested: (1) 12F (12 h low food, 0.1 ppm equivalent to 27 µg C l⁻¹, and 12 h high food, 1.2 ppm equivalent to 324 µg C l⁻¹), combining 2 light situations: high food supplied at night (high food-dark, 12FN) and high food supplied during the day (high food-light, 12FD); (2) 24F (1 d low food / 1 d high food); and (3) 48F (2 d low food / 2 d high food).

The control consisted of a food concentration equivalent to the average low food-high food concentration (0.65 ppm, 175.5 µg C l⁻¹) continuously supplied.

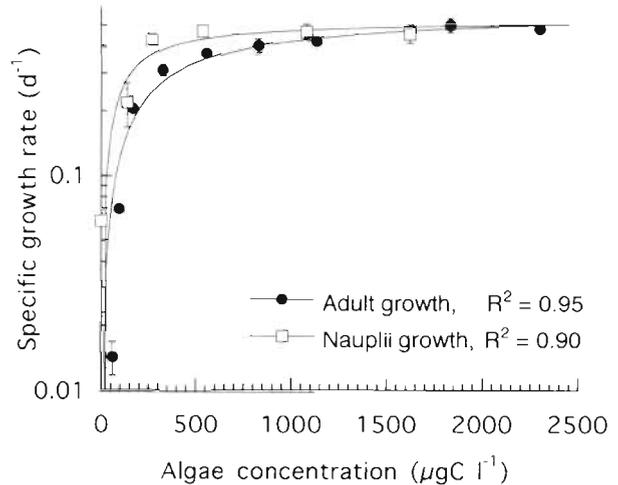Parallel experiments were run in order to estimate the effects of 24F and 48F food

fluctuations on the naupliar survival rates. The experimental setup was the same as for the study of growth rates under fluctuating food conditions. After 4 d of running the experiment, the number of surviving individuals was counted.

RESULTS

The temporal evolution of naupliar dry weight (*DW*) in relation to food concentration is represented in Fig. 1, and the corresponding values of instantaneous specific growth rates (*g*, d⁻¹) are shown in Table 1. The stage composition of the cohorts for each food concentration at the end of the experiment is also indicated. Naupliar growth saturated at around 430 µg C l⁻¹,

Table 1. *Acartia grani*. Functional growth responses of early naupliar stages to food concentration. *F*: food concentration, in ppm and µg C l⁻¹; *g*: instantaneous specific growth rates (d⁻¹ ± SE); r² coefficients of determination; %: demographic composition at the end of the experiment (3 d), as percentage of developmental stages (N: nauplius; C: copepodite). Experiments started at nauplius II-III

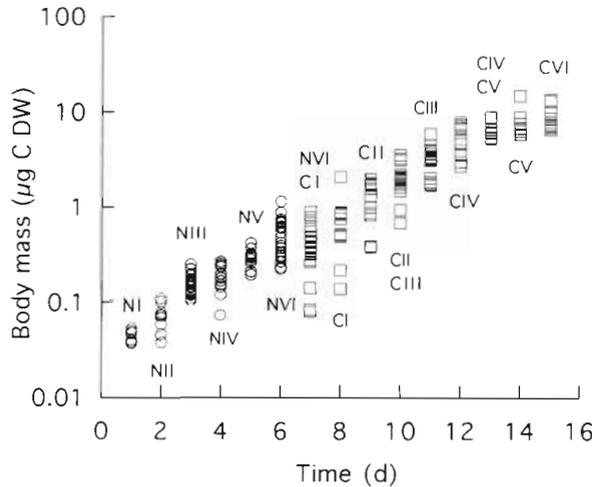| F		*g*	r²	Stage (%)
ppm	µg C l⁻¹			
0.0	0.00	0.060 ± 0.038	0.49	NIV (100)
0.5	135	0.218 ± 0.049	0.89	NV (45) – NVI (55)
1	270	0.427 ± 0.029	0.99	NVI (89) – CI (11)
2	540	0.470 ± 0.033	0.99	NVI (60) – CI (40)
4	1081	0.466 ± 0.026	0.98	NVI (58) – CI (42)
6	1620	0.458 ± 0.038	0.98	NVI (54) – CI (46)

Fig. 3. *Acartia grani*. Cohort development expressed in dry weight. The most abundant stages present for each day are also given
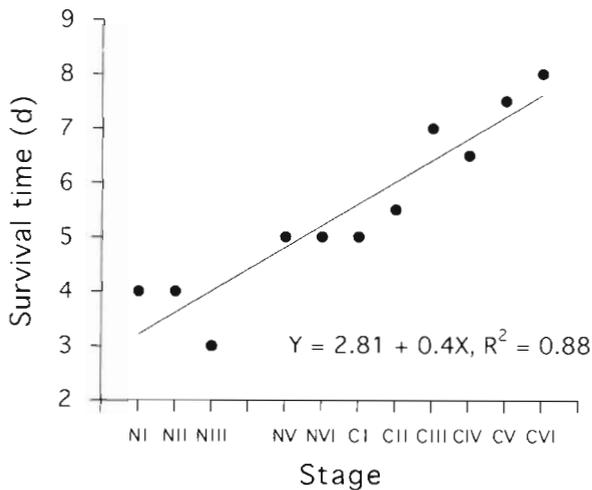

Fig 4. *Acartia grani*. Starvation tolerance of the different stages. In abcissae: development stage. In ordinates: time elapsed (in days) under starving conditions to reach 50% mortality

1.6 ppm (Fig. 2). The comparison of specific adult production rates (as egg production rates) and naupliar growth rates is represented in Fig. 2. Data have been fitted to the Holling type II equation (Holling 1959):

$$g = \frac{aC}{1 + abC}$$

where g is the instantaneous growth rate, C is the food concentration and a and b are constants.

To determine saturating concentrations, we have arbitrarily chosen a value of 2‰ for the rate of change of the curve. This value of 2‰ was close to the asymptotic stabilization of the rate of change of the curve.
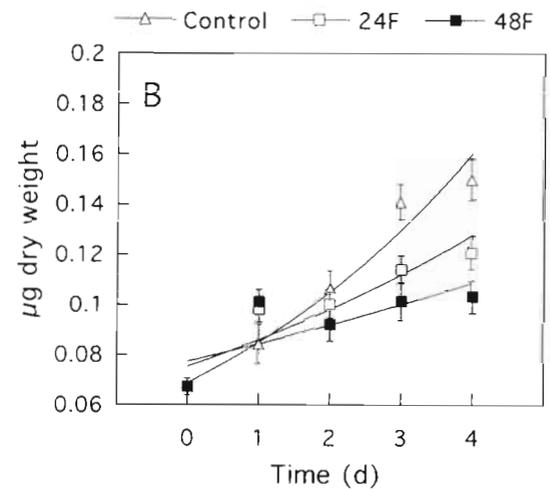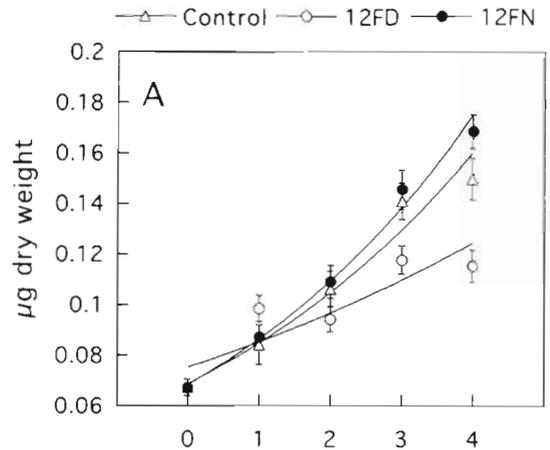

Fig. 5. Temporal evolution of naupliar dry weight (±SE) in relation to the frequency of food fluctuations. (A) 12 h food fluctuations. Control: continuous average supply; 12FD: high food supplied during the day; 12FN: high food supplied at night. (B) 24 and 48 h food fluctuations. Control: continuous average supply; 24F: 1 d low food / 1 d high food; 48F: 2 d low food / 2 d high food

Food saturation for adult egg production occurs at around 650 µg C l^{-1}, 2.4 ppm. The cohort development under saturating food conditions is shown in Fig. 3. The time needed to reach adult stage under saturating food conditions and 18°C is 15 d, about 1 stage per day. The instantaneous rate of growth corresponding to the cohort development (from NI to adult) coincided with the values obtained for naupliar stages and adult production at saturating food concentration (cohort g: 0.43 ± 0.004; NII-CI g: 0.45 ± 0.01; adult g: 0.45 ± 0.024).

The survival time of the different developmental stages to starvation is represented in Fig. 4. In general, there was a progressive increase in the tolerance to starving conditions according the cohort development, except for nauplius III. Survival rate in control animals was 100%.
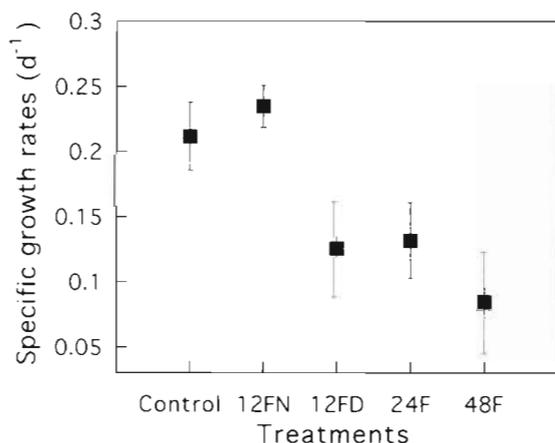
Fig. 6. Effects of the different frequencies of food fluctuations on specific growth rates (± SE) in the fluctuating treatments. Control: continuous average supply, 12FD: high food supplied during the day, 12FN: high food supplied at night, 24F:1 d low food / 1 d high food, 48F: 2 d low food / 2 d high food

The temporal evolution of naupliar DW in relation to the different frequencies of food fluctuations is represented in Fig. 5A, B. The effect of short-term fluctuations (12 h) on growth rate depended on the light conditions at which the food was available (Fig. 5A). When high food concentrations were supplied at night (12FN), naupliar growth rates were slightly higher than for those feeding continuously on average food concentration (controls), although differences were not statistically significant. However, high food concentrations supplied during daylight hours (12FD) resulted in significantly lower naupliar growth rates (Tukey test, $p < 0.05$).

Under longer-term food fluctuations (24F and 48F; Fig. 5B), naupliar growth rates were significantly lower ($p < 0.05$) than for controls or 12FN, and similar to 12FD fluctuations (Fig. 6). Although 48F food fluctuations gave the lower growth rates, differences with respect to 24F or 12FD were not statistically significant.

Mortality rates (% of nauplii alive at the end of the experiment) induced by 24 or 48 h food fluctuations (34 and 29% respectively) were significantly higher than for the control (11%, $p < 0.005$), but did not differ between them.

DISCUSSION

A precise knowledge of the effects of different food concentrations on life-history parameters of copepods is a prerequisite to adequately design laboratory experiments related to food fluctuations. The food concentration threshold at which growth rates are saturated, and the capacity to survive, should be carefully

taken into account in order to scale both the intensity and frequency of food oscillations to the response range of the organisms (Calbet & Alcaraz 1996).

The effects of food concentration on specific growth rates for early naupliar stages of *Acartia grani* were comparable to those observed for other congeneric species (*A. tonsa*; Berggreen et al. 1988) at similar temperatures and feeding of the same algal species. Maximum specific growth and growth-saturating food concentration coincided for both species.

The similar response of early nauplii and adult *Acartia grani* to food concentration appears to confirm the feasibility for translating production estimates based on definite age classes to the whole community (Berggreen et al. 1988, Fryd et al. 1991). This assumption is reinforced by the similarity in the values of instantaneous specific growth rates for naupliar stages, total cohort development and adult production. However, the differences in the food concentration at which adult copepod production (as egg production rates) and naupliar growth are saturated could induce minor errors in estimating copepod production for the whole population in the range at which growth is not saturated. Similar results were found *in situ* for several species by Peterson et al. (1991). They suggested that in food limited areas it is necessary to determine age-specific growth rates to obtain unbiased estimates of copepod production.

The increase in survival time to starving conditions along the copepod development coincided with a similar tendency observed by Burns (1985) for 3 *Boeckella* species, by Borchers & Hutchings (1986) for *Calanoides carinatus*, and by Tsuda (1994) for *Pseudocalanus newmani*, although in all these cases the tolerance to starvation was significantly higher than for *Acartia grani*. In our experiments, survival time to starvation increased linearly with age. The unexpected high survival rates to starvation of NI and NII as compared with NIII could probably be due to the persistence of embryonic reserve lipids in these early developmental stages, which would have disappeared in NIII. The significant differences in naupliar growth rates observed in short-term food fluctuations (12 h), in relation to the light conditions at which high food concentrations are available, confirm previous results obtained by Calbet & Alcaraz (1996). As for egg production rates, naupliar growth is significantly higher when high food concentrations occur during the night. This can only be explained by higher nocturnal feeding rates in nauplii, similar to those described for adults (Bautista et al. 1988).

Acartia grani is a clear example of a copepod with low capacity for dampening food variability and high egg production rates (Dagg 1977). The negative effects of relatively short (a few days) food fluctuation periods

on naupliar growth are compensated by its high repro-
duction rates in favourable environments, like marine
areas where phytoplankton variability occurs mainly at
micro and fine scales (Walsh 1976, Owen 1989). This
would explain its abundance in the innermost parts of
harbours and estuaries (Alcaraz 1983).

The control exerted by food availability conditions
(abundance and frequency of fluctuations) on life-his-
tory parameters of copepods confirm the importance of
match-mismatch mechanisms between extensive
properties (i.e. phyto- and zooplankton biomass and
distribution) and rate processes in plankton systems
(Cushing 1989, Calbet et al. 1996).

A better understanding of the ecological implications
for zooplankton dynamics of the scales of variability by
the different trophic groups would require a global
trophodynamic approach, and its study in relation to
the scales of physical variability in natural systems.

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