

Homeostasis in the essential amino acid composition of the marine copepod *Euterpina acutifrons*

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Abstract

The essential amino acid composition (EAA) of females and eggs of *Euterpina acutifrons* and their food was analyzed on copepods fed with different microalgae and collected from the field. There were not significant differences in the EAA of females, but the EAA of eggs varied according to food source. As predicted by homeostasis theory, a higher reproductive success was observed as the EAA of females and food became more similar. This chemical homeostasis explains the selective retention of amino acids observed in copepods, tells why conversion efficiencies of ingested nitrogen may vary according to the food source, and shows the importance of food quality on reproductive success in copepods.

The most important biotic factors affecting herbivorous copepod production are considered to be: food concentration, capture efficiency (mainly determined by particle size and relative retention abilities), presence of toxic or inhibitory substances, and food quality.

Food quality can be defined as the proportion and composition of biomolecules such as proteins, carbohydrates, lipids, etc., or elements. Although some specific fatty acids have been shown to be important for copepod production (Prahl et al. 1984; Jónasdóttir 1994; Jónasdóttir et al. 1995; Jónasdóttir and Kjørboe 1996; Pond et al. 1996), nitrogen (protein) also seems to be a limiting factor (Checkley 1980; Ambler 1986). This protein limitation could explain why copepods maximize nitrogenous ingestion by selectively consuming cells with a higher protein content (Cowles et al. 1988) and why amino acids are so efficiently digested (Cowie and Hedges 1996).

However, besides the importance of the proportion of protein in the diet, studies carried out with copepods fed algal species with similar C/N ratios, in which protein was the limiting factor for egg production, showed differences in conversion efficiencies of ingested nitrogen to egg nitrogen (Støttrup and Jensen 1990). This result could be explained if: (1) the intraspecific composition of amino acids is as rigid as has been observed with the C:N:P ratio in major members of zooplankton (Andersen and Hessen 1991), and (2) a higher production efficiency of herbivorous zooplankton is obtained as composition of the diet becomes closer to the composition of the consuming organism, as is predicted by the homeostasis theory (Sterner 1990).

To test these two hypotheses, experiments were conducted to determine whether reproductive success in the egg-sac carrier copepod *E. acutifrons* fed with different algal species and in copepods collected directly from the field varied ac-

ording to the similarity between the EAA of females and eggs with their food.

Methods

Laboratory studies—Laboratory-reared *E. acutifrons* females came from a long-established population cultured in our laboratory using *Tetraselmis suecica* as food. Different algal species were used as food: the diatom *Chaetoceros calcitrans* (mean \pm SD; $4.1 \pm 1.2 \mu\text{m}$), the prymnesiophytes *Isochrysis galbana* (mean \pm SD; $3.7 \pm 1.5 \mu\text{m}$) and *Pavlova lutheri* (mean \pm SD; $4.1 \pm 1.2 \mu\text{m}$), the prasinophyte *T. suecica* (mean \pm SD; $8.5 \pm 2.4 \mu\text{m}$), the eustigmatophytes *Nannochloropsis gaditana* (mean \pm SD; $2.3 \pm 0.6 \mu\text{m}$) and *Ellipsoidion* sp. (mean \pm SD; $2.0 \pm 0.5 \mu\text{m}$), and the chlorophyte *Chlorella autotrofica* (mean \pm SD; $2.9 \pm 1.0 \mu\text{m}$). For each experimental concentration, 20 gravid females were isolated and transferred to individual 20-ml beakers containing some of the following food concentrations of each microalgae: 5×10^3 , 1×10^4 , 5×10^4 , 1×10^5 , 2×10^5 , 3×10^5 , 4×10^5 , and/or 5×10^5 cells ml^{-1} . Females were fed algae at stationary phase because amino acid composition of microalgae is stable only at this stationary phase (Brown et al. 1993). Females were kept at 18°C under a 12:12 light:dark (LD) cycle. Each day, the copepods were gently transferred to fresh phytoplankton suspensions at the experimental concentration. After 5 d of acclimation, nauplii were collected daily and counted for an additional 3 d to estimate daily naupliar production per female.

EAA analyses of laboratory-reared *E. acutifrons* females and eggs were performed on individuals cultured for 7 d at a food concentration within the range at which maximum daily naupliar production is obtained for each algal species.

As the aim of the study was to compare the EAA of females, eggs, and food, total protein concentration was used as the indicator of food available for copepods both reared in the laboratory and collected from the field. Total protein analysis was performed on GF/C-filtered material of each experimental concentration.

Field study—To estimate naupliar production in the field, a sample was collected by vertically integrated tows in

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March 1998 at a field station 39 m deep located in Ría de Vigo, Spain (42°13.3'N, 8°47.7'W). From the sample, 20 gravid females were isolated within 4 h of collection and transferred to individual 20-ml containers of seawater, which had been collected from the sampling station and filtered through 20- μ m mesh. For 3 d, copepods were transferred daily to fresh suspensions of this seston fraction (<20 μ m), and nauplii were counted. The embryonic development time of *E. acutifrons* at 18°C is around 2.5 d (Zurlini et al. 1978); thus, it was necessary to provide 3 d for freshly laid eggs to hatch.

EAA analyses of females and eggs from the field were performed on gravid females isolated from the same sample collected by the vertically integrated tows in March 1998, as mentioned above.

The EAA of food available for the animals in the field was analyzed on the 3–20- μ m seston size fraction integrated from seawater samples collected at 2-, 5-, 10-, 15-, and 20-m depths at the field station. This size fraction is within the range of particle effectively captured by adult *E. acutifrons* (Kinne 1977). As depth distribution of copepods may vary during the day, it has been shown that food concentration integrated from concentrations at several standard depths is a good indicator of the average food concentration available for *E. acutifrons* over the entire water column (Guisande et al. 1996).

As mentioned above, total protein concentration was used as the indicator of food available for copepods in the field. Total protein analysis was performed on GF/C-filtered material of the 3–20- μ m seston size fraction integrated from seawater samples from the depths mentioned above.

Analytical methods—Analyses of the EAA of food were performed on GF/C-filtered material, and analyses of the EAA of females and eggs were performed on samples containing three adult females and three sacs, respectively.

Amino acids were analyzed from four to six replicates by high-performance liquid chromatography (HPLC) using an Alliance system, a 474 scanning fluorescence detector, and a 15 \times 3.9 Nova-Pak C₁₈ column (all from Waters) following the method described by Van Wandelen and Cohen (1997). Amino acid Standard H NCI0180 Pierce was used for the identification and quantification of amino acids.

The method described by Lowry et al. (1951) and modified by Markwell et al. (1978) was used to analyze total proteins.

Similarity index—Average Euclidean distance (D_{jk}) was used to estimate similarity between the EAA of females and the EAA of food and eggs. $D_{jk} = \sqrt{\sum_{i=1}^n (X_{ij} - X_{ik})^2/n}$, where X_{ij} and X_{ik} are the percentages of the amino acid i of the females (j) and the food or eggs (k), and n is the number of amino acids. A higher similarity is obtained as Euclidean distance becomes smaller.

Results

Figure 1 shows daily naupliar production per female of the *E. acutifrons* fed different algal species at various food concentrations. For all the phytoplankton species used as food in this study, there is a concentration range at which a

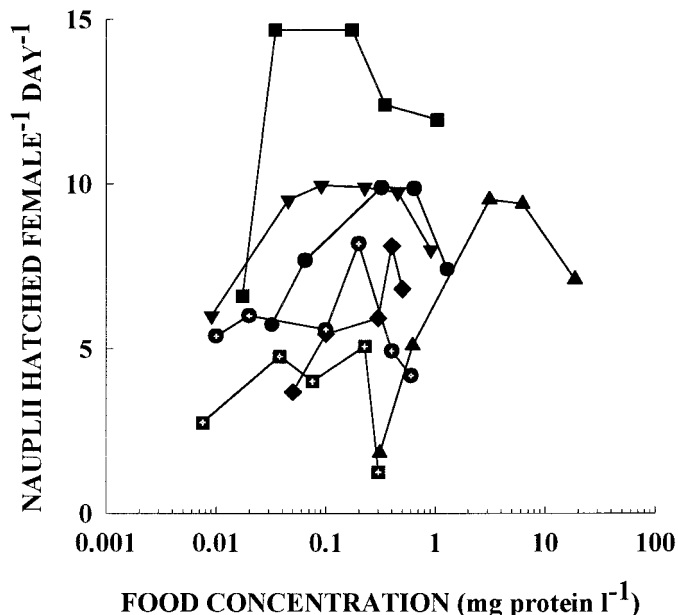


Fig. 1. Mean daily naupliar production per female in the copepod *E. acutifrons* fed different algal species at different food concentrations. *I. galbana* (solid square), *P. lutheri* (small bullet), *T. suecica* (solid triangle up), *Ch. calcitrans* (solid triangle down), *N. gaditana* (large closed diamond), *Ch. autotrofica* (solid circle with plus), and *Ellipsoidion* sp. (solid square with plus). All SEs for the means of naupliar production were <2.1.

maximum daily naupliar production is reached. For each algal species, the mean value of the maximum daily naupliar production obtained in this concentration range was used to compare copepod reproductive success under the different nutritional food supplies. Maximum naupliar production is a good indicator of reproductive success because it gives information about egg production and hatching success and avoids the effect of food concentration.

The reduction in naupliar production at high food concentrations obtained in this study in nearly all treatments (Fig. 1) may be due to a low hatching of eggs exposed to high concentrations of phytoplankton produced by the depletion of oxygen in the incubation water (Jónasdóttir and Kjørboe 1996).

Estimation of daily naupliar production from animals collected from the field was obtained during the spring bloom. In the field during this period, there is no food limitation, and maximum egg production per sac is obtained for this copepod species (Guisande et al. 1996; mean \pm SD; 35.4 \pm 7.0; $n = 79$). In fact, protein concentration (mean \pm SE) of the water column in the 3–20- μ m seston size fraction integrated from seawater samples collected at 2-, 5-, 10-, 15-, and 20-m depths at the sampling station in March 1998 was 0.188 \pm 0.008 mg liter⁻¹. This value is within the range of food concentration at which maximum naupliar production is obtained with the different algal species (Fig. 1).

Table 1 shows the EAA of the microalgae used in this study and of the food available for the copepods collected from the field (seston fraction = 3–20 μ m). The differences observed in the EAA of the diet, as will be shown later, are

Table 1. EAA (mean \pm SD weight percentage of total amino acid yield) of *Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros calcitrans*, *Nannochloropsis gaditana*, *Chlorella autotrofica*, *Ellipsoidion* sp., and the size fraction 3–20 μ m at the station sampled in March 1998. Amino acid abbreviations: ASP, aspartic acid; SER, serine; GLU, glutamic acid; GLY, glycine; HIS, histidine, ARG, arginine, THR, threonine; ALA, alanine; PRO, proline; TYR, tyrosine; VAL, valine; LYS, lysine; ILE, isoleucine; LEU, leucine; PHE, phenylalanine.

	<i>I. galbana</i>	<i>P. lutheri</i>	<i>T. suecica</i>	<i>Ch. calcitrans</i>	<i>N. gaditana</i>	<i>Ch. autotrofica</i>	<i>Ellipsoidion</i> sp.	Field sample (March 1998)
ASP	9.9 \pm 0.4	9.5 \pm 0.4	8.6 \pm 0.8	10.8 \pm 0.3	7.1 \pm 1.0	7.9 \pm 0.6	7.4 \pm 0.5	10.2 \pm 0.2
SER	6.8 \pm 0.0	5.7 \pm 0.2	6.7 \pm 0.3	6.7 \pm 0.4	5.5 \pm 1.2	4.9 \pm 0.5	4.6 \pm 0.2	8.9 \pm 0.2
GLU	12.3 \pm 0.2	12.5 \pm 0.4	12.2 \pm 0.8	12.6 \pm 0.7	13.2 \pm 1.1	12.7 \pm 1.2	12.8 \pm 2.0	14.5 \pm 0.3
GLY	6.3 \pm 0.1	6.9 \pm 0.2	7.7 \pm 0.3	6.6 \pm 0.3	6.7 \pm 0.9	5.6 \pm 0.3	5.5 \pm 0.2	10.1 \pm 0.3
HIS	2.6 \pm 0.0	3.0 \pm 0.1	2.8 \pm 0.2	2.5 \pm 0.1	2.8 \pm 0.3	2.5 \pm 0.4	2.5 \pm 0.4	2.0 \pm 0.1
ARG	6.6 \pm 0.2	5.7 \pm 0.2	6.0 \pm 0.6	4.9 \pm 0.2	6.5 \pm 0.7	6.8 \pm 1.0	6.4 \pm 1.1	5.7 \pm 0.2
THR	7.6 \pm 0.6	5.9 \pm 0.2	7.7 \pm 0.4	5.6 \pm 0.2	5.5 \pm 0.5	5.0 \pm 0.3	5.1 \pm 0.3	5.3 \pm 0.1
ALA	6.1 \pm 0.7	8.5 \pm 0.3	7.0 \pm 0.5	6.3 \pm 0.1	6.0 \pm 1.4	7.0 \pm 0.7	6.2 \pm 0.8	5.1 \pm 0.1
PRO	4.6 \pm 0.1	4.8 \pm 0.2	4.8 \pm 0.1	4.8 \pm 0.3	11.6 \pm 1.8	13.9 \pm 4.2	15.5 \pm 4.5	3.7 \pm 0.1
TYR	4.4 \pm 0.1	3.5 \pm 0.1	3.7 \pm 0.4	3.1 \pm 0.8	3.1 \pm 0.7	5.2 \pm 2.6	5.1 \pm 1.3	3.2 \pm 0.8
VAL	6.0 \pm 0.0	6.5 \pm 0.1	6.5 \pm 0.0	6.2 \pm 0.1	5.8 \pm 0.3	5.4 \pm 0.3	5.3 \pm 0.2	5.1 \pm 0.1
LYS	6.2 \pm 0.5	6.3 \pm 0.6	5.3 \pm 0.6	7.2 \pm 0.4	6.8 \pm 1.2	6.8 \pm 0.6	6.7 \pm 1.3	8.2 \pm 0.4
ILE	5.0 \pm 0.0	5.0 \pm 0.2	4.9 \pm 0.1	6.6 \pm 0.5	5.8 \pm 0.5	4.5 \pm 0.6	4.7 \pm 0.6	5.4 \pm 0.6
LEU	9.7 \pm 0.0	10.2 \pm 0.1	9.2 \pm 0.1	9.3 \pm 0.2	8.3 \pm 0.6	7.5 \pm 0.3	7.7 \pm 0.2	8.1 \pm 0.1
PHE	5.6 \pm 0.4	6.2 \pm 0.3	7.1 \pm 0.6	6.7 \pm 0.2	5.4 \pm 0.7	4.2 \pm 0.3	4.2 \pm 0.6	4.6 \pm 0.1

of importance in terms of the nutritional value of the proteins available for the copepods.

Despite different nutritional supplies, there were no significant differences in the EAA of females (Table 2) (analysis of variance [ANOVA], $F_{7,30} < 1.8$, MS between 0.291 and 1.662, $P > 0.14$ for all amino acids). However, the EAA of eggs (Table 3) varied significantly according to the food source (ANOVA, $F_{7,29} > 2.6$, $P < 0.05$ for all the amino acids). Therefore, it seems that the proportion of amino acids in eggs is not as rigid as in the biomass of adults.

These differences in the range of variation in the EAA of microalgae, females, and eggs are shown in Fig. 2 and Table 4, which give the results of a principal component analysis on data of the EAA of algae, females, and eggs using the covariance matrix. The EAA of algae shows a greater range

of variation than does the EAA of females (as mentioned above, no significant variation), and the EAA of eggs shows an intermediate level of variation.

A higher naupliar production was observed as the EAA of females became more similar to the EAA of food (Fig. 3a) and to the EAA of eggs (Fig. 3b).

Discussion

The results of this study show that, besides the homeostasis observed in zooplankton elemental composition (C:N:P) (Andersen and Hessen 1991), there is also—at least for this copepod species—homeostasis at a biochemical level because the proportion of EAA is constant in adults of this copepod species. However, it should be noted that, as EAA

Table 2. EAA (mean \pm SD weight percentage of total amino acid yield) of females of *E. acutifrons* collected from the field and fed with *I. galbana*, *P. lutheri*, *T. suecica*, *Ch. calcitrans*, *N. gaditana*, *Ch. autotrofica*, and *Ellipsoidion* sp. Amino acid abbreviations as mentioned in Table 1.

	<i>I. galbana</i>	<i>P. lutheri</i>	<i>T. suecica</i>	<i>Ch. calcitrans</i>	<i>N. gaditana</i>	<i>Ch. autotrofica</i>	<i>Ellipsoidion</i> sp.	Field sample (March 1998)	Pooled mean
ASP	8.3 \pm 0.1	8.9 \pm 0.4	8.5 \pm 0.2	8.2 \pm 0.5	8.2 \pm 0.3	8.4 \pm 0.5	8.7 \pm 0.3	8.1 \pm 0.2	8.4 \pm 0.3
SER	5.2 \pm 0.1	5.3 \pm 0.4	5.1 \pm 0.1	5.2 \pm 0.1	5.2 \pm 0.1	5.6 \pm 0.9	5.0 \pm 0.3	4.7 \pm 0.3	5.1 \pm 0.2
GLU	14.8 \pm 0.1	13.0 \pm 0.4	14.5 \pm 0.2	14.6 \pm 0.5	14.4 \pm 0.7	14.1 \pm 0.4	14.3 \pm 0.1	13.7 \pm 0.4	14.2 \pm 0.6
GLY	6.7 \pm 0.4	6.9 \pm 0.3	6.4 \pm 0.1	6.9 \pm 0.4	6.9 \pm 0.5	7.0 \pm 0.4	6.7 \pm 0.1	6.3 \pm 0.4	6.7 \pm 0.3
HIS	2.7 \pm 0.1	2.7 \pm 0.0	2.6 \pm 0.1	2.7 \pm 0.1	2.7 \pm 0.1	2.9 \pm 0.4	2.8 \pm 0.1	2.7 \pm 0.0	2.7 \pm 0.1
ARG	7.9 \pm 0.9	7.7 \pm 0.3	8.4 \pm 0.6	8.5 \pm 0.8	8.9 \pm 0.4	7.0 \pm 0.1	8.6 \pm 0.8	9.1 \pm 0.6	8.3 \pm 0.6
THR	5.2 \pm 0.1	5.1 \pm 0.1	5.1 \pm 0.1	4.9 \pm 0.1	4.9 \pm 0.2	5.0 \pm 0.4	4.9 \pm 0.1	4.9 \pm 0.2	5.0 \pm 0.1
ALA	7.1 \pm 0.2	7.8 \pm 0.3	7.0 \pm 0.3	6.9 \pm 0.3	6.8 \pm 0.2	7.9 \pm 0.1	7.7 \pm 0.2	7.6 \pm 0.4	7.3 \pm 0.4
PRO	4.8 \pm 0.1	5.2 \pm 0.3	5.1 \pm 0.2	4.9 \pm 0.1	5.0 \pm 0.1	5.2 \pm 0.6	5.2 \pm 0.4	6.5 \pm 0.4	5.2 \pm 0.5
TYR	8.2 \pm 0.5	7.9 \pm 0.4	8.2 \pm 0.4	8.3 \pm 0.3	7.9 \pm 0.6	7.6 \pm 0.2	8.2 \pm 0.2	7.8 \pm 0.5	8.0 \pm 0.2
VAL	5.4 \pm 0.2	5.6 \pm 0.1	5.5 \pm 0.1	5.4 \pm 0.1	5.4 \pm 0.1	5.7 \pm 0.5	5.5 \pm 0.1	5.5 \pm 0.1	5.5 \pm 0.1
LYS	7.7 \pm 0.5	7.6 \pm 0.7	7.3 \pm 0.5	7.4 \pm 0.4	7.6 \pm 0.7	7.4 \pm 0.2	6.7 \pm 0.4	7.4 \pm 0.5	7.4 \pm 0.3
ILE	5.1 \pm 0.1	5.0 \pm 0.1	5.1 \pm 0.1	5.0 \pm 0.0	5.1 \pm 0.1	5.0 \pm 0.5	5.0 \pm 0.1	5.0 \pm 0.1	5.0 \pm 0.1
LEU	7.2 \pm 0.2	7.6 \pm 0.2	7.3 \pm 0.1	7.1 \pm 0.2	7.1 \pm 0.1	7.4 \pm 0.2	7.2 \pm 0.1	7.2 \pm 0.3	7.3 \pm 0.2
PHE	3.7 \pm 0.0	3.7 \pm 0.1	3.8 \pm 0.2	3.9 \pm 0.4	3.8 \pm 0.2	3.2 \pm 0.2	3.2 \pm 0.1	3.6 \pm 0.3	3.6 \pm 0.2

Table 3. EAA (mean \pm SD weight percentage of total amino acid yield) of eggs of *E. acutifrons* collected from the field and fed with *I. galbana*, *P. lutheri*, *T. suecica*, *Ch. calcitrans*, *N. gaditana*, *Ch. autotrofica*, and *Ellipsoidion* sp. Amino acid abbreviations as mentioned in Table 1.

	<i>I. galbana</i>	<i>P. lutheri</i>	<i>T. suecica</i>	<i>Ch. calcitrans</i>	<i>N. gaditana</i>	<i>Ch. autotrofica</i>	<i>Ellipsoidion</i> sp.	Field sample (March 1998)
ASP	8.6 \pm 0.1	9.1 \pm 1.3	8.3 \pm 1.1	8.7 \pm 0.5	8.1 \pm 0.2	8.9 \pm 0.7	7.9 \pm 2.1	5.7 \pm 1.7
SER	4.7 \pm 0.0	4.8 \pm 1.1	5.4 \pm 0.9	5.0 \pm 0.4	7.0 \pm 1.9	6.4 \pm 0.9	6.5 \pm 1.0	7.5 \pm 1.6
GLU	14.4 \pm 0.6	14.6 \pm 1.0	15.6 \pm 1.8	15.4 \pm 1.4	14.5 \pm 1.3	16.7 \pm 2.3	18.8 \pm 1.5	14.7 \pm 2.8
GLY	4.9 \pm 0.1	5.0 \pm 0.4	5.4 \pm 1.8	5.7 \pm 1.4	7.8 \pm 2.1	5.9 \pm 0.8	5.8 \pm 1.3	5.4 \pm 0.9
HIS	2.5 \pm 0.0	2.9 \pm 0.1	2.1 \pm 0.1	2.5 \pm 0.1	2.9 \pm 0.3	3.8 \pm 1.0	3.5 \pm 0.5	3.3 \pm 0.3
ARG	11.1 \pm 0.7	9.7 \pm 0.3	10.5 \pm 1.8	10.4 \pm 0.8	10.7 \pm 1.6	9.0 \pm 1.1	9.2 \pm 2.4	11.2 \pm 1.7
THR	5.6 \pm 0.4	4.8 \pm 0.2	4.5 \pm 0.4	4.4 \pm 0.0	4.1 \pm 0.4	4.6 \pm 0.4	3.9 \pm 0.1	5.2 \pm 0.5
ALA	5.1 \pm 0.1	7.1 \pm 0.6	5.7 \pm 1.0	4.9 \pm 0.3	4.5 \pm 0.7	4.7 \pm 0.7	4.7 \pm 0.7	5.0 \pm 0.6
PRO	7.6 \pm 0.1	6.3 \pm 0.6	6.2 \pm 1.5	7.3 \pm 1.1	6.3 \pm 1.3	5.8 \pm 0.5	3.8 \pm 0.6	6.5 \pm 2.0
TYR	5.6 \pm 0.9	5.9 \pm 0.4	6.3 \pm 0.9	6.1 \pm 0.6	5.9 \pm 0.7	5.5 \pm 0.8	6.7 \pm 0.8	5.4 \pm 0.8
VAL	5.3 \pm 0.2	5.1 \pm 0.3	5.4 \pm 0.6	5.2 \pm 0.2	4.9 \pm 0.4	4.9 \pm 0.1	4.8 \pm 0.3	5.2 \pm 0.3
LYS	8.1 \pm 0.0	9.3 \pm 0.7	7.8 \pm 1.2	8.5 \pm 1.1	7.4 \pm 1.3	8.7 \pm 1.0	10.1 \pm 0.9	9.1 \pm 0.9
ILE	5.5 \pm 0.2	4.9 \pm 0.3	4.9 \pm 0.4	4.7 \pm 0.3	5.0 \pm 0.4	4.7 \pm 0.2	4.7 \pm 0.2	5.2 \pm 0.4
LEU	6.4 \pm 0.1	6.5 \pm 0.3	7.2 \pm 0.4	6.6 \pm 0.3	6.4 \pm 0.3	6.4 \pm 0.3	6.3 \pm 0.4	6.6 \pm 0.7
PHE	4.7 \pm 0.1	4.0 \pm 0.1	4.7 \pm 0.7	4.6 \pm 0.2	4.5 \pm 0.1	3.6 \pm 0.7	3.0 \pm 0.4	3.9 \pm 0.6

analyses of laboratory-reared *E. acutifrons* females were performed on individuals cultured for only 7 d at the experimental concentration for each microalgae, it is not possible to reject the possibility that the relative lack of variance in the EAA of females may simply reflect a longer turnover time for EAA.

A higher reproductive success is obtained as the EAA of food becomes closer to the EAA of females (Fig. 3a), which is in agreement with one prediction of the homeostasis theory (Sterner 1990). The importance of the proportion of amino acids in the food on reproductive success could explain why conversion efficiencies of ingested nitrogen may vary

according to the food source (Støttrup and Jensen 1990), and it also should lead to a selective retention of amino acids from the food ingested. This is in agreement with the results obtained by Cowie and Hedges (1996), which showed that the copepod *Calanus pacificus* digested individual amino acids to different degrees.

The relatively high naupliar production observed in copepods fed *I. galbana* (Fig. 3b) shows that other attributes of the food, e.g., particle size and fatty acid composition, could also be important factors affecting copepod production.

One reason a diet balanced in an optimal proportion is an important factor affecting copepod reproductive success could be the short gut residence of ingested food observed in copepods (<30 min), which gives rise to material more highly assimilated and is most abundant in the cytoplasm

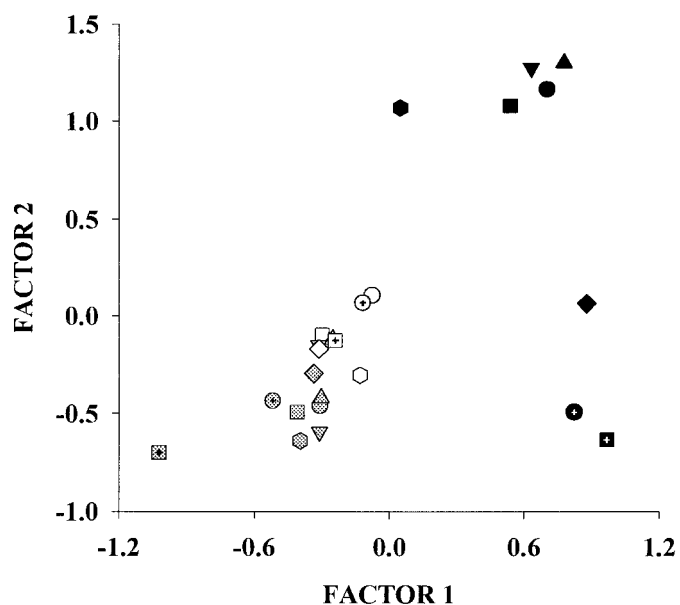


Fig. 2. Plots of the first two principal component analysis scores for the EAA of algae (filled symbols), females (open symbols), and eggs (shaded symbols). Symbols for each algal species are as given in Fig. 1; field sample (●).

Table 4. Factor loadings for the two principal components of a principal component analysis on EAA shown in Tables 1–3. First component explained 38.9% and second component 32.5% of the variance, respectively.

	PC1	PC2
ASP	-0.041	0.733
SER	-0.117	0.588
GLU	-1.100	-0.565
GLY	0.012	0.706
HIS	-0.114	-0.057
ARG	-1.203	-1.144
THR	0.363	0.565
ALA	0.264	0.310
PRO	2.325	-1.868
TYR	-1.120	-0.788
VAL	0.2130	0.303
LYS	-0.678	-0.435
ILE	0.045	0.190
LEU	0.628	0.833
PHE	0.548	0.671

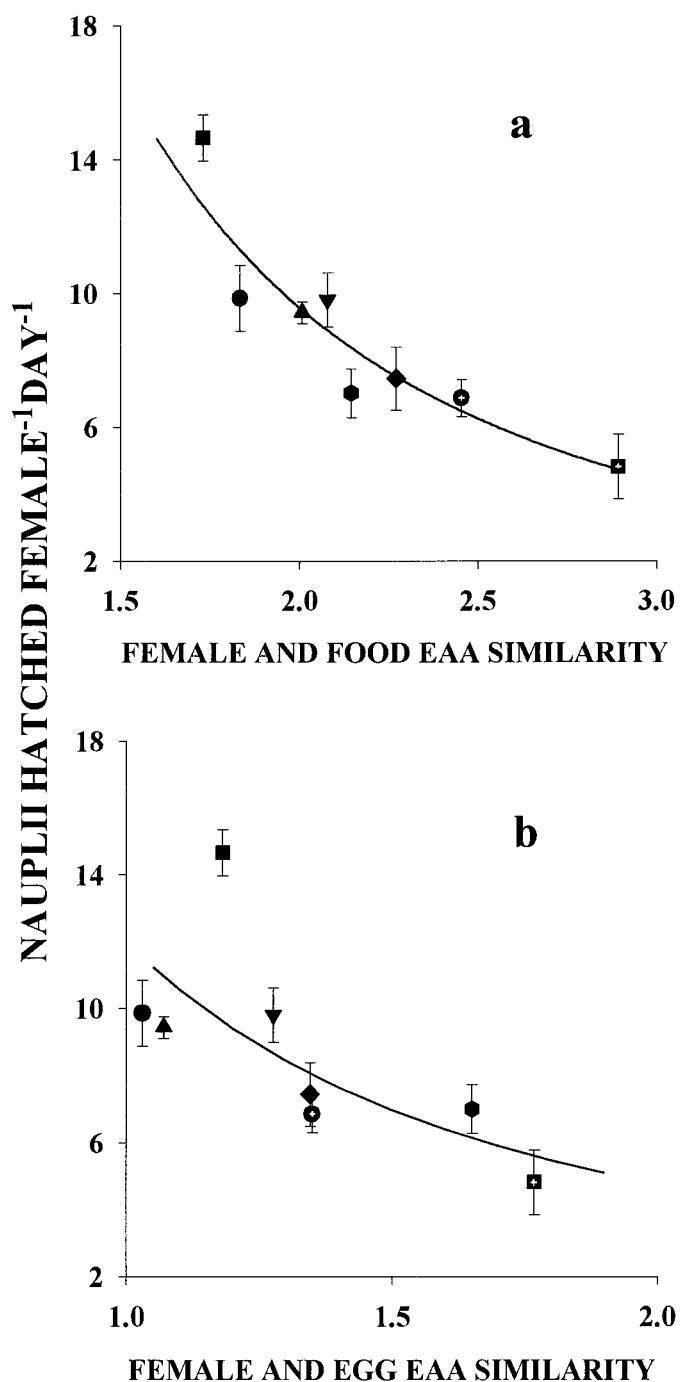


Fig. 3. (a) Relationship between the daily naupliar production per female in *E. acutifrons* and the similarity between the EAA of females and food. Slope is different from zero with a $P < 0.001$. (b) Relationships between the daily naupliar production per female in *E. acutifrons* and the similarity between the EAA of females and eggs. Slope is different from zero with a $P = 0.025$. Symbols are as given in Figs. 1, 2. As there were no significant differences in the EAA of females, the pooled mean of the EAA of females shown in Table 2 was used to estimate the similarities. Similarity was measured by Euclidean distance.

(Reinfelder and Fisher 1991). If the EAA of food is too different from the EAA of females, the lag time between food being ingested and being converted into production of eggs is probably too short to produce eggs with this optimal proportion of amino acids. This is probably why the EAA of eggs varied according to food source, and, as the EAA of eggs became more similar to the EAA of females, a higher naupliar production was obtained (Fig. 3b).

The results of this study may represent only a certain state in the field because of the experimental design. All phytoplankton cultures were given at stationary stage, and at this stage, the chemical composition of the phytoplankton is often not as optimal for copepod growth as it is in actively growing phytoplankton. Moreover, as all the experiments were performed on cultures with unialgal diets, the possibility of food selection by the copepods was not taken into account. Therefore, if *E. acutifrons* is also able to discriminate on the basis of food quality, as has been observed in other copepod species (Cowles et al. 1988; DeMott 1989), a higher naupliar production per female should be expected in the field than in a culture with only one algal species, when considering equal similarity between the EAA of females and the EAA of food.

Copepod reproduction has been shown to be influenced by the availability of food (see Mauchline 1998). However, apart from studies of the effect of fatty acids composition of food on egg production (Støttrup and Jensen 1990; Jónasdóttir 1994; Jónasdóttir et al. 1995; Jónasdóttir and Kjørboe 1996; Pond et al. 1996), the role of the chemical composition of food has not been investigated relative to copepod reproduction (Mauchline 1998). This is probably due to the difficulty of quantifying food quality.

The results obtained in this study show that amino acid composition of food can also play an important role in copepod reproductive success. If other copepod species show a rigid EAA, as has been observed in *E. acutifrons*, comparisons between the EAA of copepods and the EAA of food available for them could be used to quantify the effect of food quality on copepod reproduction.

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