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Nutritional quality of a freshwater heterotrophic flagellate: trophic upgrading of its microalgal diet for *Daphnia hyalina***Alexandre Bec^{1,2,*}, Christian Desvillettes¹, Aurélie Véra¹, Charles Lemarchand¹,
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ABSTRACT: Bacterivorous freshwater heterotrophic flagellates seem to be too poor in (n-3) series polyunsaturated fatty acids (PUFA) to be able to sustain major zooplankton growth by themselves. This study shows that when the heterotrophic flagellate *Aulacomonas submarina* fed on microalgae, either *Rhodomonas lacustris* (*Aulacomonas/R.*) or *Chlorogonium elongatum* (*Aulacomonas/C.*), this species exhibited (n-3) series PUFA in great quantities. Therefore, *A. submarina* PUFA composition seems to depend mainly on its algal diet. However, the biosynthetic capacities of *A. submarina* seem to have endowed it with greater amounts of 20 PUFA and a more diversified PUFA composition. Moreover, this study also shows that *Daphnia hyalina* exhibits a higher fecundity when fed on *Aulacomonas/C.* (or *Aulacomonas/R.*) than is the case when fed on *C. elongatum* (or respectively *R. lacustris*). This suggests that the ability of *A. submarina* to convert dietary lipids results in the biochemical value of its food source being upgraded.

KEY WORDS: Heterotrophic nanoflagellates · Nutritional quality · Fatty acids · PUFA

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Numerous studies devoted to the functioning of the microbial loop in marine or lacustrine environments have shown the key role of protozoans in transferring organic matter from small size particules to higher trophic levels (Sherr & Sherr 1988, Gifford 1991).

Among these protozoans, heterotrophic nanoflagellates (HNF) exhibit sizes and at times relatively great abundance, which render them important prey for zooplanktonic microcrustacea (Sanders & Wickham 1993, Laybourn Parry 2000). Nonetheless, it has been shown that although zooplankton predation rates on HNF are often high, this does not assure that HNF can be a high quality food (Sanders et al. 1996). Indeed, morphology and movement influence the grazing efficiency of zooplankton whereas the biochemical composition of the

food determines its nutritive value (Ahlgren et al. 1990, Mayzaud et al. 1998).

In natural environments, sestonic phosphorus (Elser et al. 2001, Makino et al. 2002) and specific lipid compounds (Arts 1998, Müller-Navarra et al. 2000) can be dependable predictors of food quality for zooplankton. In the same way, ciliates and HNF have a high incorporation of P (Caron & Goldman 1990) and thus may be a particularly important source of phosphorus-containing compounds for zooplankton. Nevertheless, mineral content alone is insufficient to predict food quality (Anderson & Hessen 1995, Müller-Navarra 1995) and it has been suggested that essential food compounds, especially (n-3) series polyunsaturated fatty acids (PUFA), play a significant role (Wacker & Von Elert 2001).

Compared with microalgae, lipid and fatty acid composition of freshwater HNF have attracted less attention (Desvillettes et al. 1997). However, recent work by Véra et al. (2001) showed that typical aquatic HNF, such as *Spumella* and *Paraphysomonas*, have little capacity for synthesizing (n-3) series PUFA and exhibit fatty acid profiles strongly influenced by their food. Furthermore, when fed on bacteria, these HNF species lack (n-3) PUFA as bacteria generally do not produce PUFA (Napolitano 1998). Thus, it is possible that freshwater algivorous flagellates exhibit (n-3) series PUFA in much greater quantities than the bacterivorous ones do. Indeed, in marine environments, it has been suggested that algivorous protozoans contain (n-3) series PUFA in great amounts (Claustre et al. 1989). Moreover, Klein Brettele et al. (1999) suggested that, as protozoans may produce new compounds, they upgrade the biochemical composition of food rather than merely repackage it. In this context, we therefore initially compared the PUFA compositions of the

algivorous flagellate *Aulacomonas submarina* to PUFA compositions of 2 types of algal food, the Cryptophyceae *Rhodomonas lacustris* and the Chlorophyceae *Chlorogonium elongatum*. We then investigated whether the addition of *A. submarina* as a trophic intermediate in the simplified nanoplanktonic microalga-zooplankton food chain affected cladoceran development and reproduction. With a view to this, we conducted growth experiments on *Daphnia hyalina* fed 4 different diets: (1) *A. submarina* fed on *R. lacustris*; (2) *A. submarina* fed on *C. elongatum*; (3) *R. lacustris*; or (4) *C. elongatum* in order to compare their nutritional value for the cladoceran.

Material and methods. Protists cultures: The different protists were obtained from our own laboratory collection. The autotrophic flagellates, *Rhodomonas lacustris* (12 μm long) and *Chlorogonium elongatum* (18 μm long) were mass cultivated in a modified Synura medium (Véra et al. 2001). These cultures were grown semi-continuously at 20°C under a 12:12 h light:dark photoperiod. In order to maintain an exponential growth rate, 20 to 40% of the medium was renewed every other day. The algivorous flagellate *Aulacomonas submarina* (10 to 30 μm long) was grown in 800 ml of Synura medium to which 100 ml of *R. lacustris* culture or *C. elongatum* culture was added daily. Each culture was conducted in triplicate.

Zooplankton culture: *Daphnia hyalina* was isolated from zooplankton samples collected in Lake Annecy,

France, and cultured in spring water, and fed every other day with a 50/50 mixture of freeze-dried fish foods (Tetramin® + Tetraphyll®) broken down into fine particles by ultrasound.

Growth experiment: Twelve 600 ml glass tanks were filled with spring water. Each tank received 50 newly hatched *Daphnia hyalina* (<12 h old). For 10 d, triplicates of 50 *D. hyalina* each were fed daily with *Rhodomonas lacustris* cells, *Aulacomonas submarina* cells grown on *R. lacustris* (*Aulacomonas/R.*), *Chlorogonium elongatum* cells and *A. submarina* cells grown on *C. elongatum* (*Aulacomonas/C.*). This experiment was conducted at 20°C under a 12:12 h light:dark cycle and food concentration was 1.5×10^4 cells ml^{-1} . Thus, the cladocerans in each treatment were not limited by food quantity. At the end of the survey, *D. hyalina* survival, *D. hyalina* length and egg number per female were recorded.

Statistical analysis: The survival rate, the length and the fecundity of the cladocerans at the end of each experiment were compared 2-by-2 with a 1-way ANOVA (Scheffé test) ($p < 0.01$).

Sampling and fatty acid analysis: Microalgae and *Aulacomonas submarina* were recovered by filtration on precombusted GF/F glass-fiber filters. Before filtration or *Daphnia hyalina* feeding, *A. submarina* cells in culture were starved for 36 h in the dark. This treatment reduced the size of the cell to around 10 to 15 μm in length, caused the digestion vacuoles to disappear (see Fig. 1) and allowed us to check that no residual *Rhodomonas lacustris* or *Chlorogonium elongatum* were present in the culture. Fatty acid analyses were performed on samples (3 aliquots of different flasks) of food algae and *A. submarina*. After extraction and derivatization, fatty acids were identified by gas chromatography. A full description of the methodology used was given previously by Véra et al. (2001).

Results and discussion. Changes in PUFA composition of *Aulacomonas submarina* in response to diets (Figs. 2 & 3): *Rhodomonas lacustris*, like other autotrophic cryptomonads, was characterized by high percentages of 18:4(n-3) and 20:5(n-3), whereas *Chlorogonium elongatum* exhibited a different PUFA composition dominated by 16:4(n-3) and 18:3(n-3), both typical of Chlorophyceae PUFA (Sargent et al. 1995). As shown in Figs. 2 & 3, these microalgae had a marked influence on the PUFA composition of *A. submarina*. The PUFA content of starved *A. submarina* was strongly dominated by the (n-3) series, which is not the case for bacterivorous HNF, for which (n-6) fatty acid series constitute the main PUFAs (Desvillettes et al. 1997, Véra et al. 2001). As shown in Figs. 2 & 3, the PUFA composition of *Aulacomonas/C.* differs from that of *Aulacomonas/R.* as *Aulacomonas/C.* had fewer 20 (n-3) series PUFA characteristic of cryptomonads

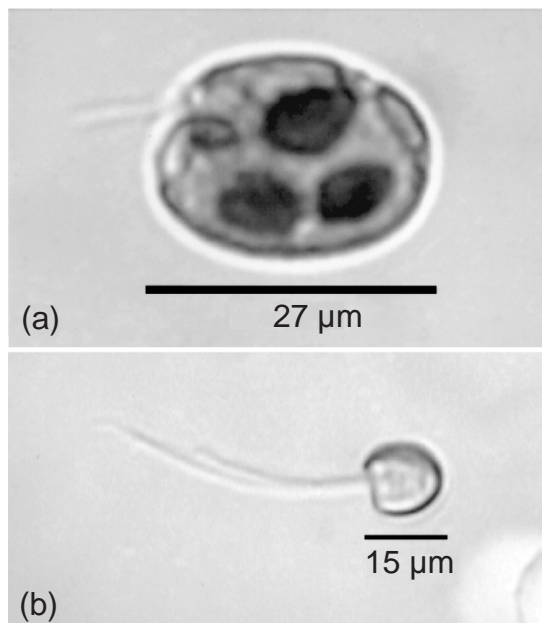


Fig. 1. (a) Live cell of *Aulacomonas submarina* fed on *Rhodomonas lacustris* before starvation. (b) Live cell of *A. submarina* after starvation. Note that the cell biovolume is reduced and that digestion vacuoles have disappeared

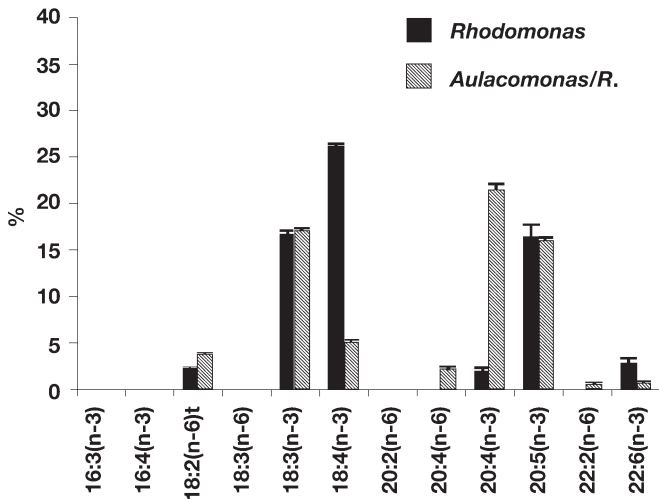


Fig. 2. Polyunsaturated fatty acid compositions (wt % of total fatty acids) of *Rhodomonas lacustris* and *Aulacomonas submarina* fed on *R. lacustris* (*Aulacomonas/R.*)

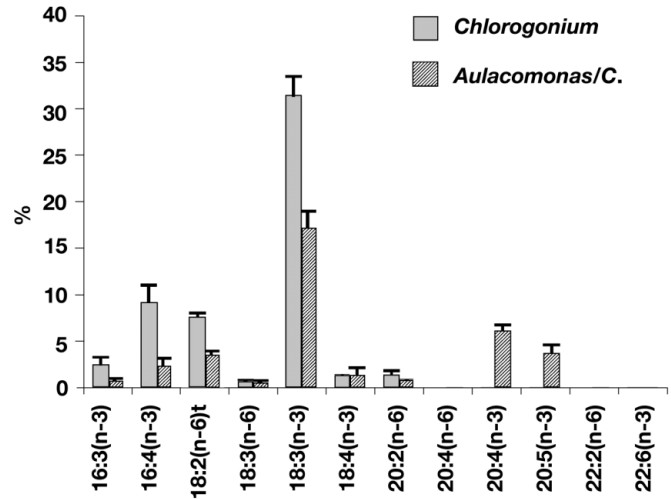


Fig. 3. Polyunsaturated fatty acid compositions (wt % of total fatty acids) of *Chlorogonium elongatum* and *Aulacomonas submarina* fed on *C. elongatum* (*Aulacomonas/C.*)

and more 16 (n-3) series PUFA characteristic of Chlorophyceae. Likewise, Klein Breteler et al. (1999) showed that the heterotrophic dinoflagellate *Oxyrrhis marina* fed on the Chlorophyceae *Dunaliella* sp. (*Oxyrrhis/D.*) also contained 16 (n-3) series PUFA. However, it should be noted that *A. submarina* lipid analyses were performed after a period of starvation, which caused the digestion vacuoles to disappear. This allowed us to hypothesize that these typical algal PUFA had become part of *A. submarina* lipids.

Comparison of the PUFA composition of *Aulacomonas submarina* and its food sources seems to suggest that it is able to bioconvert fatty acid. Indeed, *Aulacomonas/R.*, in contrast to *Rhodomonas lacustris*, contains inverse proportions of 20:4(n-3) and 18:4(n-3), and *Aulacomonas/C.*, in contrast to *Chlorogonium elongatum*, has 20:4(n-3) and 20:5(n-3) in significant quantities. Similarly, Klein Breteler et al. (1999)

reported that, unlike *Dunaliella* sp., *Oxyrrhis/D.* exhibited long chain PUFA like 22:6(n-3). *A. submarina*, therefore, seems to convert PUFA from its food into long carbon chain PUFA through a process of elongation and desaturation. In *Aulacomonas/R.*, dietary 18:4(n-3) was probably elongated into 20:4(n-3) and in *Aulacomonas/C.*, dietary 18:3(n-3) seemed to be partially bioconverted into 20:4(n-3) and finally into 20:5(n-3) by means of Δ6 and Δ5 desaturases. As this algivorous flagellate is a protista incertae sedis distantly related to heterokont flagellates (Brugerolle & Patterson 1990) and does not contain any residual plast, unlike a number of freshwater genera (*Spumella*, *Paraphysomonas*, *Polytoma*; Arndt et al. 2000), these desaturases are probably located in endoplasmic reticulum, as observed for ciliates.

Finally, even if dietary PUFA have a marked influence on *Aulacomonas submarina* lipid composition,

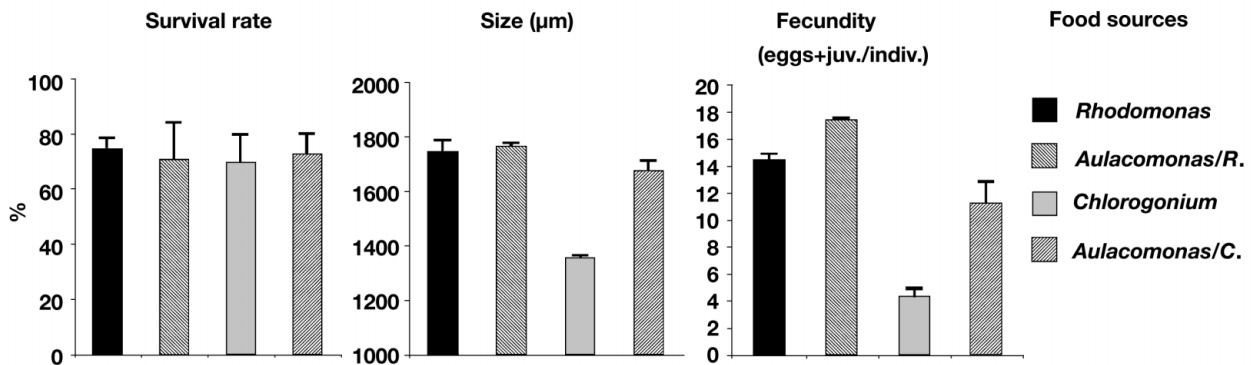


Fig. 4. Survival rate, size and fecundity of *Daphnia hyalina* fed on *Rhodomonas lacustris*, *Aulacomonas/R.*, *Chlorogonium elongatum* and *Aulacomonas/C.*

this PUFA bioconversion results in *Aulacomonas/R.* and *Aulacomonas/C.* having high amounts of both 20:4(n-3) and 20:5(n-3). It is worth noting that the ratios of the percentages of 18:4(n-3):20:4(n-3) and 18:4(n-3):20:5(n-3) calculated for *Aulacomonas/R.* (i.e. 0.22 and 0.30 respectively) are equivalent to those calculated in *Aulacomonas/C.* (i.e. 0.21 and 0.35 respectively). Thus, this PUFA bioconversion seems to play an important role (changes in cellular membrane conformation?) which needs further investigation.

Nutritional quality of *Aulacomonas submarina* and the different food sources: The different food sources all allowed the development and reproduction of *Daphnia hyalina* (Fig. 4). The survival rates measured for the 4 treatments were not significantly different ($p < 0.01$). However, the size and fecundity of the cladocerans fed on *Chlorogonium elongatum* were distinctly low. It is also interesting to note that the presence of neonates or juveniles were not recorded in the *C. elongatum* treatment, which shows that cladocerans fed on *C. elongatum* develop more slowly. Moreover, the fecundity recorded in the *Aulacomonas/R.* treatment was significantly the highest ($p < 0.01$). Thus, these results show that *A. submarina* is of better nutritional quality for *D. hyalina* than the microalga it feeds on. Similarly, recent studies have also suggested that protozoans may improve the food quality for marine copepods by acting as intermediate prey (Klein Breteler et al. 1999, Tang et al. 2001).

In view of the similar morphological characteristics (size, mobility) of the microalgae and the heterotrophic flagellate tested, their biochemical composition may be the main factor influencing their food quality. Thus, the low nutritional value of *Chlorogonium elongatum*, like that of Chlorophyceae in general, seems to be most often connected with a lack of 20:5(n-3) (Ahlgren et al. 1990, Brett & Müller-Navarra 1997). In contrast, *Aulacomonas/C.* could have supplied the cladocerans with important quantities of 20 PUFA notably of 20:5(n-3). The elongation and desaturation capacities of the heterotrophic flagellate therefore seem to explain why *Aulacomonas/C.* is of better nutritional value than *C. elongatum* alone. The addition of *A. submarina* as an intermediate in the *Rhodomonas lacustris-Daphnia hyalina* simplified trophic chain had less influence on the development of the cladocerans than had been previously assumed (Fig. 4). Nevertheless it should be recalled that Cryptophyceae are recognised as already being of high nutritional value due to their high 20:5(n-3) contents (Ahlgren et al. 1990). However, *Aulacomonas/R.* could provide more diversified specific compounds than *R. lacustris* alone, notably specific sterols (Klein Breteler et al. 1999). These latter compounds must be taken into account for determining the quality of zooplankton food (Von Elert & Wolffrom 2001).

Conclusion. Generally speaking, the essential compound contents of protozoans (here fatty acids) depend on the synthetic capacities peculiar to each species but above all on the biochemical composition of the food available. The considerable variations in the biochemical composition of heterotrophic flagellates which may ensue largely explain the extreme variability of their own nutritional value, as observed by Sanders & Wickham (1993). Strictly from the point of view of (n-3) series PUFA contents, free HNF fed on bacteria do not seem to constitute a high quality food (Véra et al. 2001); it seems on the other hand that flagellates able to ingest microalgae are of much better quality. This study shows that an algivorous flagellate, such as *Aulacomonas submarina*, accumulates and also modifies the dietary PUFA and by doing so can upgrade the nutritional value of its algal food source.

Finally, as heterotrophic flagellates are generally considered as the major consumers of autotrophic picoplanktonic species (Weisse 1993, Dolan & Šimek 1998) mainly composed of picocyanobacteria and picochlorophyceae poor in 20 PUFA, trophic upgrading of autotrophic picoplankton food quality by heterotrophic flagellates can become an important source of essential compounds for zooplankton. This may prove to be a key process in the functioning of aquatic ecosystems whose importance remains to be specified.

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