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Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha*

Abstract—In standardized growth experiments, newly hatched larvae of the zebra mussel *Dreissena polymorpha* were fed diets representing different biochemical compositions. Algae that were rich in (n-3) polyunsaturated fatty acids (PUFAs), except for long-chained (>C18) PUFAs (*Chlorella minutissima* and *Monoraphidium minutum*) were of low food quality. Higher growth than on *C. minutissima* or *M. minutum* was supported by a culture of the cyanobacterium *Aphanothece* sp., which contained traces of a long-chained (n-3) PUFA, docosahexaenoic acid (DHA, 22 : 6n-3). The alga *Isochrysis* aff. *galbana,* which contained high amounts of the long-chained (n-3) PUFAs DHA and eicosapentaenoic acid (EPA, 20 : 5n-3), supported the highest growth. The alga *Nannochloropsis limnetica,* which differed from *I. galbana* by a deficiency in DHA, allowed slightly, but significantly lower, growth. Growth of larvae on *N. limnetica* was increased by enrichment of *N. limnetica* cells with a lipid extract of *I. galbana,* showing that larval growth on *N. limnetica* was limited by the deficiency of a compound that was present in *I. galbana.* Growth was also enhanced by feeding *N. limnetica* cells supplemented with DHA, but not by cells enriched with EPA, indicating that DHA was the limiting factor. We conclude that, on DHA-deficient food, the larvae of *D. polymorpha* were not able to sufficiently convert C18-PUFAs into long-chained (n-3) PUFAs and that the rates for elongation and desaturation of EPA into DHA limited growth.

Larvae of *Dreissena polymorpha* have been raised successfully in only two studies. Wright et al. (1996) reported that the culturing of *D. polymorpha* larvae in the laboratory requires live algal food in very good condition. Vanderploeg et al. (1996) suggested that larvae of *D. polymorpha* require food rich in long-chained (n-3) polyunsaturated fatty acids (PUFAs, >C18), but it remains unclear whether the long-chained (n-3) PUFAs eicosapentaenoic acid (EPA; 20 : 5n-3) and docosahexaenoic acid (DHA; 22 : 6n-3) are of similar importance. A possible demand of *D. polymorpha* for EPA is in agreement with the hypothesis that EPA is of great importance for trophic transfer via the keystone species *Daphnia* in freshwater systems (Müller-Navarra et al. 2000). A requirement for DHA has been demonstrated for marine systems only (e.g., in the successful rearing of marine bivalve larvae in aquaculture, Enright et al. 1986; Volkman et al. 1989; Delaunay et al. 1993). Considering the close phylogenetic relationship between the freshwater species *D. polymorpha* and its marine relatives, it is reasonable to hypothesize that larvae of *D. polymorpha* also require long-chained (n-3) PUFAs, in particular DHA. Consequently, we examined the relative importance of the dietary fatty acids EPA and DHA for the growth and survival of *D. polymorpha* larvae. We used different algal or cyanobacterial diets, which were selected on the basis of the content of particular fatty acids. Although Vanderploeg et al. (1996) and Wright et al. (1996) studied the influence of algae differing in fatty acid composition on larvae, we additionally tested for a putative limitation by the algal food with the lacking compound.

Preparation of food—Freshwater algae were obtained from culture collections of the Institute of Freshwater Ecology and Inland Fisheries (IGB Berlin, Germany) and the University of Göttingen (SAG, Germany). Marine algae were obtained from the culture collection of the University of Texas (UTEX, U.S.A.). The cyanobacterium *Aphanothece* sp. (IGB Berlin) and the algae *Chlorella minutissima* (UTEX 2219), *Monoraphidium minutum* (SAG 243-1), and *Nannochloropsis limnetica* (SAG 18.99) were cultured in modified WC medium with vitamins (Guillard 1975). *Isochrysis* aff. *galbana* (T-Iso, UTEX 2307) was cultivated in artificial seawater (Starr and Zeikus 1993). All algae and the cyanobacterium were grown semicontinuously (20°C, 100 μmol photons m^{-2} s^{-1}) by harvesting 25–50% of the culture every other day and by restoring the volume with freshly prepared medium. Cells were concentrated by centrifugation and resuspended in filtered lake water (0.45-μm pore size membrane filter). Microscopical examination exhibited that cells were not affected by centrifugation. Carbon concentrations of these stock solutions were estimated from photometric light extinction (800 nm) using carbon extinction equations. Aliquots of algal or cyanobacterial stock solutions were added to filtered lake water, and the resulting suspensions were used as food for zebra mussel larvae. Adding marine algae to filtered lake water did not result in osmotic lysis of algal cells (determined as particulate organic carbon after 4 h incubation); therefore, the carbon concentrations remained constant in the food suspensions.

Particulate parameters and fatty acid patterns of the food organisms—Aliquots of algal or bacterial food were filtered onto precombusted glass fiber filters (Whatman GF/F, 25-mm diameter) and acid-rinsed polysulfon filters (HT-200, Pall Gelman Laboratory) for determination of particulate organic carbon (POC) and nitrogen (PON) and phosphorus (P_{part}) and analyzed according to Wacker and Von Elert (2001). For determination of fatty acids, aliquots of food suspensions corresponding to approximately 1.0 mg POC were filtered through a precombusted GF/F filter (25-mm diameter), extracted, transesterified, and analyzed according to Von Elert and Stampfl (2000) using an HP 6890 GC (Agilent Technologies).

The algae and the cyanobacterium represented three different groups of biochemical composition: Group 1 comprised the cyanobacterium *Aphanothece* sp., which should only contain small amounts of PUFAs and no long-chained PUFAs (Kenyon and Stanier 1970; Kenyon 1972; Cobelas

Table 1. Fatty acid content of *Aphanothece* sp. preparations. The cyanobacterial suspensions, with or without previous washings, were filtered through a GF/F filter. Filtrate (not washed) was pelleted. See the text for details. Fatty acid concentrations are given in μg mg^{-1} cyanobacterial biomass (determined as particulate organic carbon, POC). The values are means of $n = 2$ independent cultures. Only detected fatty acids are given. n.d., not detected.

Fatty acid	Cells retained on filter	Washed cells	Pelleted filtrate (26,000 × g)
C14:0	9.17	5.24	0.26
C14:1	0.36	0.20	n.d.
C15:0	1.02	0.33	0.14
C16:0	38.90	17.47	0.93
C16:1	27.85	25.44	0.13
C17:1	0.55	0.49	n.d.
C18:0	3.16	1.60	0.67
C18:1n-12/n-9	6.23	0.69	0.43
C18:1n-7	3.39	4.05	0.05
C18:2n-6	0.09	n.d.	0.13
C18:4n-3	0.43	0.17	n.d.
C20:0	0.14	0.10	0.13
C20:1n-7	0.23	0.13	0.07
C22:0	0.41	n.d.	0.13
C22:2n-6	0.28	n.d.	n.d.
C22:6n-3	0.54	n.d.	0.08
C24:0	0.72	0.25	0.13
C24:1	0.07	n.d.	n.d.
Total	93.52	56.15	3.34

Table 2. Fatty acid content of algae used as food sources for larvae of *D. polymorpha.* Fatty acid concentrations are given in μg mg^{-1} biomass (determined as particulate organic carbon, POC). The values are means of $n = 3$ independent algal cultures. Only detected fatty acids are given. n.d., not detected.

Fatty acid	*I. aff. galbana* (T-Iso)	*N. limnetica*	*C. minutissima*	*M. minutum*
C14:0	23.10	9.06	2.38	2.49
C15:0	2.14	0.89	0.60	0.55
C16:0	32.67	55.09	39.73	51.72
C16:1	13.03	75.01	4.16	1.38
C17:1	n.d.	0.27	0.24	n.d.
C18:0	12.48	2.71	3.80	4.58
C18:1n-12/n-9	46.42	33.59	30.67	80.04
C18:1n-7	14.51	1.28	2.06	2.54
C18:2n-6	10.34	5.95	15.06	17.60
C18:3n-6	0.79	2.37	1.35	1.34
C18:3n-3	31.11	0.92	93.47	54.51
C18:4n-3	26.51	n.d.	10.29	6.02
C20:0	n.d.	0.59	0.59	0.31
C20:1n-9	0.95	0.17	0.38	1.03
C20:2n-6	n.d.	0.81	n.d.	n.d.
C20:3n-6	n.d.	1.24	n.d.	n.d.
C20:4n-6	0.69	14.62	n.d.	n.d.
C20:5n-3	3.71	87.68	n.d.	n.d.
C22:0	n.d.	n.d.	0.93	0.98
C22:6n-3	22.13	n.d.	n.d.	n.d.
C24:0	0.91	n.d.	7.03	0.76
Total	241.51	292.41	212.99	226.37

and Lechardo 1988). Small amounts of PUFAs were detected in *Aphanothece* sp. cells retained on the GF/F filter and in a pellet, which was obtained from centrifugation at 26,000 × g of the filtrate from the GF/F filter (Table 1). Surprisingly, traces of the long-chained (n-3) PUFA, DHA, also were detected (Table 1). In order to identify the origin of DHA, aliquots of *Aphanothece* sp. were centrifuged at 3,000 × g, and the cells were resuspended in sterile WC medium to remove associated bacteria and centrifuged again. The washing procedure was repeated five times. The resulting pellets were resuspended and filtered through GF/F filters, and fatty acids were extracted. Such washed cells contained no PUFAs except for stearidonic acid (SA, 18:4n-3), which shows that the cyanobacterial culture was nonaxenic and that the cells were probably associated with other bacteria (Worm and Sondergaard 1998) that produced long-chained (n-3) PUFAs. Such a situation was reported for bacteria used as food for *Brachionus* (Lewis et al. 1998; Nichols et al. 1996). Because DHA was not detected in *C. minutissima* and *M. minutum,* which were also resuspended in filtered lake water, it is unlikely that DHA was unintentionally introduced to *Aphanothece* sp. with filtered lake water. Algae rich in PUFAs, except for long-chained PUFAs (group 2), were represented by two green algal species, *C. minutissima* and *M. minutum,* which contained considerable amounts of linoleic acid (18:2n-6) and α-linolenic acid (α-LA, 18:3n-3) but were deficient in long-chained fatty acids (Table 2). Group 3 consisted of algae that contained significant amounts of long-chained PUFAs, such as arachidonic acid (ARA, 20:4n-6), EPA, and DHA. Members of this group include *N. limnetica* (high in ARA and EPA, no DHA) and *I. galbana* (high in DHA, low in ARA and EPA; Table 2).

Growth on different diets—Mature zebra mussels 2 or 3 yr old were collected from the littoral zone of Lake Constance and were gradually warmed up over 5 d to the spawning temperature of 17–20°C. Animals were pretreated according to Wright et al. (1996) and induced to spawn according to Vanderploeg et al. (1996). The suspension of fertilized eggs was diluted to 1 or 2 eggs ml^{-1} with filtered lake water and kept at 20°C in autoclaved 1.5-liter vessels. A 0.25-ml aliquot of an aqueous solution of antibiotics (5,000 units penicillin, 5 mg streptomycin, and 10 mg neomycin ml^{-1}, Sigma-Aldrich P3664) was added to each liter of cultured water to minimize bacterial contamination.

On the third day after fertilization when larvae were 107 μm in size, larval suspensions obtained from one female were poured through a 30-μm Nitex screen. Aliquots of larvae retained on the screen were resuspended in several 100-ml beakers filled with filtered lake water. Every other day, larvae were collected on a screen and transferred to autoclaved vessels containing freshly filtered water and were fed live cultures of four different algal species (*C. minutissima, M. minutum, N. limnetica,* and *I. galbana*) and one cyanobacterium (*Aphanothece* sp.) at a nonlimiting food concentration of 3 mg C L^{-1}. Food suspensions were carefully stirred four times a day to prevent sedimentation of algal or bacterial cells. Each feeding treatment consisted of three replicates with approximately 200 animals each and lasted 18

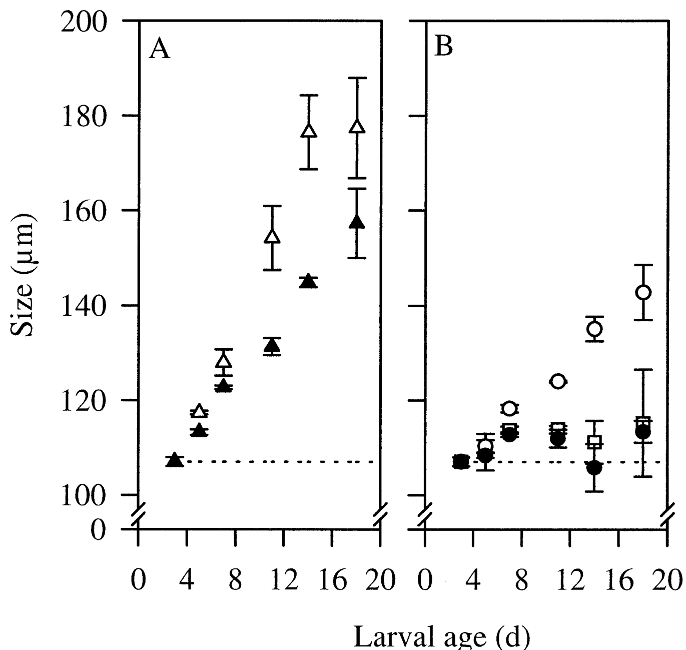

Fig. 1. Mean size (±SD, $n = 3$) of larvae versus age since fertilization of *D. polymorpha* fed (A) *I.* aff. *galbana* (open triangle) and *N. limnetica* (filled triangle) and (B) *C. minutissima* (square), *M. minutum* (filled circle), and *Aphanothece* sp. (open circle). The dashed line marks the larval size of 107 μm at the beginning of the experiment.

d. The larval cultures were maintained at a light intensity of 30 μmol photons m^{-2} s^{-1} and 20°C. At intervals of 3 to 4 d, larvae were sampled from the beakers with a pipette after gentle mixing and immediately inspected under a stereomicroscope (Zeiss; ×100 magnification). With the use of an image analysis system, at least 10 randomly selected animals per replicate were measured in height (here referred to as size), which is the distance from the ventral to the dorsal margin of the shell parallel to the axis of the hinge. Data of larval size were $\log(1 + x)$-transformed, to meet assumptions of normality and homogeneous variance. Data were analyzed using STATISTICA 5.5 (StatSoft).

The larvae grew at substantially different rates on the various food organisms (Fig. 1). Both the food source and time had highly significant effects on the size of the larvae (two-way ANOVA, $F_{5,60} = 150$, $F_{4,60} = 193$, $P < 0.001$). The effect of the interaction ($F_{20,60} = 26.4$, $P < 0.001$) particularly shows that different food sources resulted in significant differences in larval growth over time. The increase in size of the animals was largely linear during the growth experiments (Fig. 1). Therefore, size at a larval age of 18 d was subsequently used as the indicator of food quality.

Algal food quality for larvae of *D. polymorpha* could depend on a variety of factors, including composition of fatty acids and nutrients. The C : P molar ratios found for the food organisms in the present study ranged from 80 to 100, except for *I. galbana* (C : P = 150 : 1). Because *I. galbana,* larvae, fed the food with the highest C : P ratio, showed the best growth (Fig. 1), we assume that factors other than phosphorus were more important in determining growth of *D. polymorpha* larvae.

The size of larvae fed the green algae *C. minutissima* or *M. minutum* did not increase significantly from day 3 to day 18 (Tukey's HSD multiple comparison test, $P = 0.76$ and 0.89). Because of their size (3.56 and 4.48 μm equivalent spherical diameter [ESD], respectively), both algae have to be considered as ingestible (Sprung 1989); their ESD did not differ from those of *I. galbana* and *N. limnetica* (4.48 and 3.56 μm, respectively), which supported somatic growth (Tukey's HSD multiple comparison test, $P < 0.001$). Because the fatty acid compositions (Table 2) of these green algae indicate that they are deficient in long-chained PUFAs, the absence of larval growth suggests that the deficiency in long-chained (n-3) PUFAs limited the growth of the larvae (Fig. 1).

The easily ingestible cyanobacterium *Aphanothece* sp. was deficient in PUFAs but contained traces of DHA (Table 1). Larvae reared on the cyanobacterial cells showed significantly higher growth than on *C. minutissima* and *M. minutum* (Fig. 1, Tukey's HSD multiple comparison test, $P < 0.01$). Other cyanobacteria, which are usually deficient in long-chained PUFAs, have been shown to be inadequate food (Vanderploeg et al. 1996; Wright et al. 1996).

N. limnetica proved to be of very high food quality (Fig. 1A); it differed from the cyanobacterium by a high content of the long-chained (n-3) PUFA, EPA (Tables 1, 2). Animals fed *I. galbana* increased in size to 180 μm, which showed that this alga supported even better growth of the larvae than equivalent food concentrations of *N. limnetica* and the other food organisms tested (Fig. 1, Tukey's HSD multiple comparison test, $P < 0.01$). *I. galbana* differed from *N. limnetica* in having high amounts of the long-chained (n-3) PUFA, DHA (Table 2), and of the (n-3) PUFAs α-LA and -SA. Because *C. minutissima* and *M. minutum* also have abundant α-LA and -SA (Table 2) but are not of high food quality, the larval endogenous synthesis of DHA from these (n-3) C18-PUFAs seems to be of minor importance. The content of DHA therefore appears to be of high relevance for the food quality. This also explains the relatively good food quality of the cyanobacterium *Aphanothece* sp., where DHA was detectable in the culture and the culture medium (Table 1), although at a 41 times lower concentration than in *I. galbana.* Because the growth of larvae fed *Aphanothece* sp. was suboptimal, growth of the animals was limited by a still-persistent deficiency of DHA in the cyanobacterium or by the absence of other components that were not investigated in the present study.

Growth on supplemented diets—A deficiency of *N. limnetica* in DHA present in *I. galbana* could account for a limitation in growth of the larvae of *D. polymorpha* on *N. limnetica* (Fig. 1). A putative limitation can be tested in a straightforward manner by supplementing the lacking compound. We therefore enriched cells of *N. limnetica* with fatty acids; 250 μg EPA or DHA (Sigma-Aldrich Chemie) dissolved in 100 μl ethanol (pro analysis) was incubated with 5 mg bovine serum albumin (Sigma-Aldrich) and 1 mg carbon from algal stock solutions in 10 ml WC medium for 4 h. Thereafter, algal cells were washed repeatedly by centri-

Table 3. EPA and DHA content of *N. limnetica* and of *N. limnetica* enriched with a lipid extract of *I. galbana* or with EPA or DHA, which were used as food sources for larvae of *D. polymorpha.* Fatty acid concentrations are given in μg mg^{-1} biomass (determined as particulate organic carbon, POC). The values are means of $n = 2$ independent algal cultures. Only fatty acids are given that were supplemented to algae. n.d., not detected.

Fatty acid	*N. limnetica*	*N. limnetica* + *I. galbana* extract	*N. limnetica* + DHA	*N. limnetica* + EPA
C20:5n-3 (EPA)	66.83	83.86	75.23	83.19
C22:6n-3 (DHA)	n.d.	2.16	5.82	n.d.
Total	359.07	422.28	338.28	394.26

fugation and resuspended in WC medium. The resulting suspension was diluted with filtered lake water and provided the experimental food for the growth experiment. The content of EPA, which was already abundant in *N. limnetica* cells, increased when cells were incubated in the presence of EPA (Table 3). *N. limnetica* cells, which had been incubated in the presence of DHA, contained DHA, whereas DHA was not detected in *N. limnetica* alone. This indicated that these single compounds were successfully supplemented to *N. limnetica.* Additionally, *N. limnetica* cells were supplemented with lipids of *I. galbana*; *I. galbana* cells were extracted with a mixture of dichloromethane/methanol (2:1, v/v), and the lipid extract was evaporated with nitrogen to dryness and resuspended in ethanol before *N. limnetica* cells were added as described above. This supplementation led to changes in the content of EPA and DHA of *N. limnetica* (Table 3). For statistical analysis, the growth rate of larvae during a 10-d growth experiment with experimental food was calculated. The addition of the lipid extract of *I. galbana* to *N. limnetica* led to significantly higher growth of larvae than on *N. limnetica* alone (Fig. 2; ANCOVA, $F_{3, 90} = 3.2$, $P < 0.05$; contrast analysis, $P < 0.05$), indicating that cells of *I. galbana* contained ingredients that improved the quality of *N. limnetica* and that lower growth of larvae on *N. limnetica* than on *I. galbana* was not caused by differences in ingestion or digestion (e.g., like cell wall properties, Van Donk et al. 1997). A main difference between the content in fatty acids of *N. limnetica* and *I. galbana* was the absence of DHA in *N. limnetica,* whereas it was present in *I. galbana.* We therefore supplemented cells of *N. limnetica* with DHA. The enrichment of *N. limnetica* with DHA led to a significantly higher growth of larvae fed these enriched cells than larvae fed *N. limnetica* alone (Fig. 2; contrast analysis, $P < 0.05$), suggesting that DHA was the limiting factor of larvae fed *N. limnetica. N. limnetica* cells supplemented with EPA did not affect larval growth, indicating that larvae were not limited by the content of EPA in *N. limnetica* (contrast analysis, $P = 0.83$).

Because the effects of pure fatty acids were of greater interest, these experimental treatments were replicated more frequently than the supplementation treatments with the lipid extract of *I. galbana,* leading to a higher statistical power for the fatty acid supplementation treatments than for the

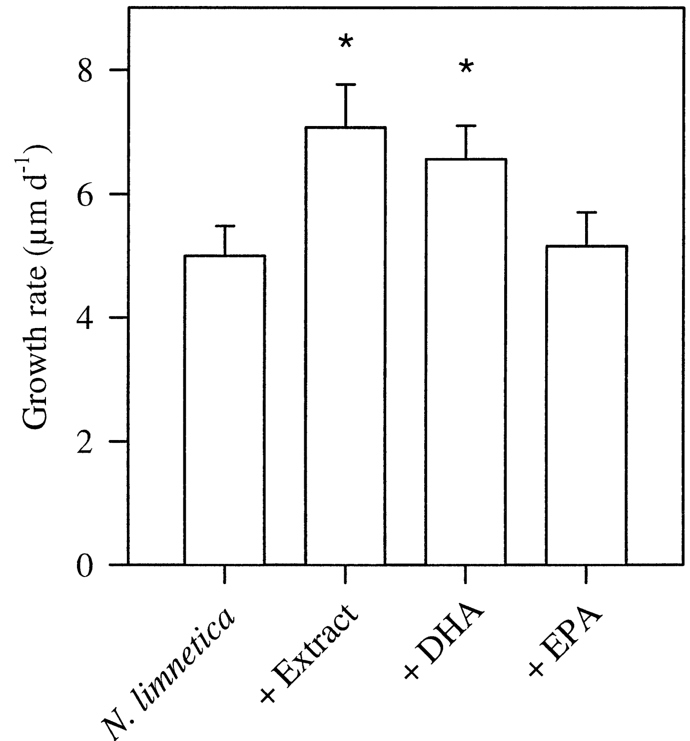

Fig. 2. Larval growth rates (mean ± SD) of planktonic *D. polymorpha* fed *N. limnetica* ($n = 31$) and *N. limnetica* enriched with a lipid extract of *I. galbana* ($n = 15$), with DHA ($n = 25$), or with EPA ($n = 24$). Asterisks indicate significant differences of the treatments to control (*N. limnetica,* contrast analysis following ANCOVA, $P < 0.05$).

lipid extraction treatment. Nevertheless, supplementation with the lipid extract of *I. galbana* significantly improved the growth rate of the larvae, so that different numbers of replication had no effect.

Larvae reared on *N. limnetica* and used for the experiment with supplemented diets showed higher growth rates (5.0 μm d^{-1}, Fig. 2) than larvae on *N. limnetica,* which were used for the feeding experiment with different algal species (Fig. 1). For these larvae, a growth rate of 3.45 μm d^{-1} was calculated for the first 11-d interval (from day 3 until day 14, Fig. 1), which was very close to the 10-d interval of the supplementation experiment (Fig. 2). Because the mussels, and therefore the planktonic larvae on supplemented diets, originated from different cohorts than for the animals used in the feeding experiment with pure algae, differences in growth on *N. limnetica* in different experiments were observed. For marine mussels, maternal effects have been shown to affect larval development and growth (Gabbott 1976; Soudant et al. 1996), suggesting that the differences in performance of *Dreissena* larvae on *N. limnetica* were due to maternal effects, particularly because mussels were collected in different years. Therefore, the growth rates cannot be compared directly between the two growth experiments.

Rearing success—Growth is an important factor in the development of zebra mussel larvae, but further insights on

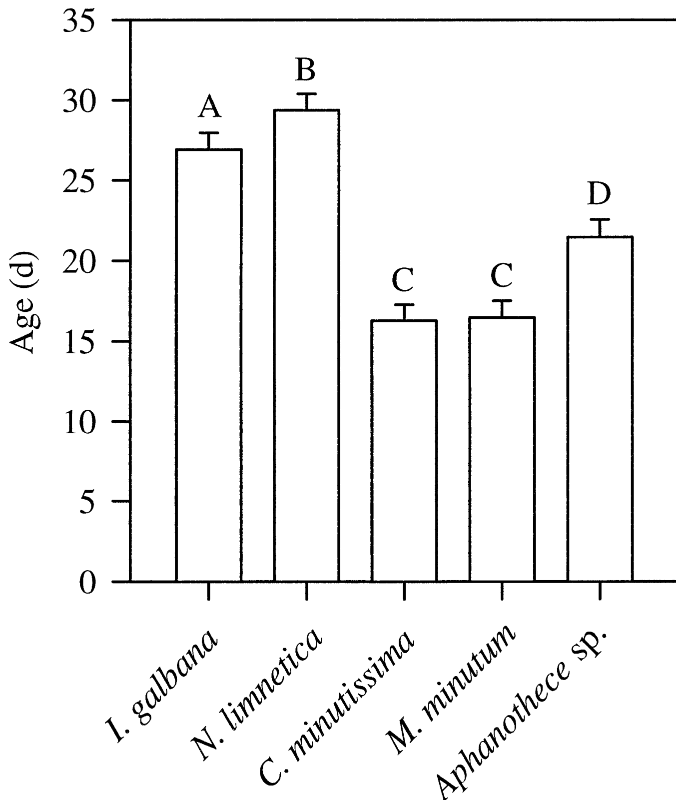

Fig. 3. Larval age (mean ± SD, $n = 3$) when the percentage of dead animals in a sample reached 50% of all initially present individuals (probit regression analysis). Bars showing the same capital letter are not significantly different at $P < 0.05$ (z-test; Natrella 1963) after sequential Bonferroni adjustment (Rice 1989).

the success of larvae reared on a food source can be gained from mortality rates. Therefore, the ability of the food organisms to support survival of the larvae was studied over a 32-d period. In order to investigate which food organism supported the survival of the larvae best, the larval ages and mortality were used for a probit regression model. Age data were log-transformed, and a probit regression model was computed using the maximum likelihood method. Resulting mean ages (50% mortality) were compared (Lozán and Kausch 1998), and the sequential Bonferroni adjustment was carried out for an overall significance level of 0.05 (Rice 1989). Data were analyzed using STATeasy (Wissenschaftliche Anwendungen).

The choice of the food organism had a highly significant effect on the survival of the larvae. Larvae feeding on *C. minutissima* or *M. minutum* suffered the earliest; approximately 2 weeks after fertilization, a mortality of 50% was observed (Fig. 3). The best survival was obtained with *N. limnetica* and *I. galbana* (z-test, $P < 0.05$). Because *I. galbana* supported better growth of larvae than *N. limnetica* (Fig. 1), animals fed *I. galbana* reached the size threshold for settlement (~220 μm, Sprung 1989) sooner, when mortality is high during settlement (Lewandowski 1982). Hence, better growth on *I. galbana* led to significantly less survivorship than larval growth on *N. limnetica* (Fig. 3; z-test, $P < 0.05$).

Our results suggest that the importance of the long-chained (n-3) PUFA, EPA, for the growth of *D. polymorpha* larvae might be due to a requirement for EPA, since higher growth was obtained on *N. limnetica* (rich in EPA) than on algae, deficient in EPA. This result is in agreement with findings of Vanderploeg et al. (1996), who successfully reared larvae on *Rhodomonas minuta* and on strains of *Chlorella minutissima,* which contained significant amounts of EPA. In the present study, we detected no EPA in our culture of *C. minutissima,* whereas Vanderploeg et al. (1996) reported EPA in their culture. We used the same strain of *C. minutissima,* but not the same culture, as Vanderploeg et al. (1996); our culture was obtained from the UTEX culture collection. However, in agreement with differences in the EPA content of the two *C. minutissima* cultures, we found poor growth, whereas Vanderploeg et al. (1996) obtained high growth of larvae. Different origins of cultures, different methods of cultivation, or both could have led to a different EPA content (Cobelas and Lechardo 1988; Harrison et al. 1990).

In general, one reason long-chained (n-3) PUFAs are important for the growth of *Dreissena* larvae might be that EPA (and additionally DHA) are required, since maximum growth was obtained on *I. galbana,* which is rich in both long-chained (n-3) PUFAs. This is consistent with results for marine environments, where EPA and DHA are known to support the growth of marine prawns (Kanazawa et al. 1977), fish (Watanabe et al. 1983*b*; Watanabe et al. 1983*a*), and molluscs (Langdon and Waldock 1981) because these species have very low physiological capabilities to convert dietary C18-PUFAs into long-chained PUFAs (Owen et al. 1975; De Moreno et al. 1976; Kanazawa et al. 1979; Waldock and Holland 1984; Tocher et al. 1989).

Growth was lower on *N. limnetica* than on *I. galbana.* Because *N. limnetica* provided EPA but no DHA, a larval requirement for DHA is indicated. The lower food quality of *N. limnetica* compared to *I. galbana* suggests that the ability of EPA to substitute for DHA was limited, probably because of the underlying enzymatic elongation and desaturation.

Our results clearly indicate that larval growth might become limited by the availability of DHA because (1) a cyanobacterium associated with DHA-producing bacteria allowed growth of the larvae, even though EPA was lacking in the food suspension; (2) growth was lower on *N. limnetica* (EPA, no DHA) than on *I. galbana* (EPA and DHA), indicating a larval requirement for DHA; and (3) the enrichment of *N. limnetica* with DHA improved growth, as in the enrichment with the lipid extract of *I. galbana.* Our finding that DHA is of great importance is in contrast to Vanderploeg et al. (1996), who showed that *C. minutissima* (no DHA) and *Rhodomonas minuta* (DHA present) led to similar growth of larvae. We can not explain the contradictory results at present; however, the improvement of larval growth by supplementation of *N. limnetica* with pure DHA in our experiment clearly shows that DHA is of great importance.

Further evidence is provided from studies of marine mollusc larvae (Volkman et al. 1989) and from reported changes in fatty acid composition during the larval development of scallops (Marty et al. 1992; Delaunay et al. 1993). The pref-

erential incorporation of DHA in well-fed scallop larvae (Delaunay et al. 1993) and the selective retention of DHA in starved scallops, in contrast to a decrease of EPA (Coutteau et al. 1996), clearly indicates that DHA is of major importance. DHA is therefore believed to play a significant role during larval development and metamorphosis of marine molluscs (Marty et al. 1992; Delaunay et al. 1993). If we consider the close taxonomic relationship between marine molluscs and *D. polymorpha,* which retained the archetypal larval form of marine bivalves, our results suggest that long-chained (n-3) PUFAs might be of comparable importance for larvae of *D. polymorpha* and that the importance of DHA reflects the close phylogenetic relationship of *Dreissena* to marine bivalves.

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Avoiding offshore transport of competent larvae during upwelling events: The case of the gastropod *Concholepas concholepas* in Central Chile

Abstract—The coast of central Chile is characterized by the occurrence of coastal upwelling during the austral spring and summer seasons, which probably has important consequences for the cross-shelf transport of larval stages of many species. Three cruises were conducted off the locality of El Quisco during upwelling-favorable wind periods to determine the surface distribution of epineustonic competent larvae of the gastropod *Concholepas concholepas* during such events. Contrary to the predictions of a traditional model, where neustonic-type larvae are transported offshore under such conditions, competent larvae of this species were exclusively found in the area between the shore and the upwelling front. Two additional cruises were conducted during calm periods to determine diel variation in the vertical distribution of *C. concholepas* competent larvae. The absence of competent larvae at the surface during early night hours suggests a reverse vertical migration. Thus, the retention of *C. concholepas* competent larvae in the upwelled waters could be the result of the interaction between their reverse diel vertical migration and the typical two-layer upwelling dynamics.

Over the past two decades, oceanographers and marine ecologists have dedicated intensive efforts to determining the links among physical oceanography, larval distribution, and their dispersal and subsequent recruitment to adult habitats. Most of these studies have demonstrated the relationship between the supply of competent larvae and temporal and spatial variability in settlement of invertebrate species (e.g., Roughgarden et al. 1988; Young 1997). Results from these studies have led to the belief that larval advection mechanisms are key factors explaining the dynamics of nearshore benthic populations of invertebrates with pelagic larval stages (Roughgarden et al. 1988; Botsford et al. 1994). In this context, and perhaps with the exception of some late larval stages of fish and crustaceans (Luckenbach and Orth 1992; Stobutzki and Bellwood 1997), it is generally considered that larval horizontal swimming capability is of minor importance regarding larval transport (Shanks 1995) and that net transport is essentially driven by the interaction of physical oceanic processes and the vertical distribution of larvae in the water column (Roughgarden et al. 1988; Shanks 1995). Several cross-shelf larval transport processes have been identified on different coasts of the world, including wind drifting, onshore propagating tidal waves and bores, and upwelling fronts moving onshore during relaxation. Along eastern ocean boundary conditions, like those found on the Pacific coasts of South and North America and the Atlantic coast of Africa, coastal upwelling forced by equatorward winds is a dominant oceanographic feature (Strub et al. 1998). Thus, it is expected that upwelling conditions will exert a strong influence on the cross-shelf transport of larval stages of many species on these coasts. Indeed, simulation and field studies have shown the importance of Ekman-driven circulation on larval transport and their subsequent settlement (Roughgarden et al. 1988; Shanks 1995; Brubaker and Hooff 2000). The position of larvae in the water column determines the net transport they undergo. Neustonic larvae are first advected offshore by Ekman transport, concentrated by the upwelling front, and then driven back toward the coast during the relaxation phase of the event, causing a settlement pulse (Wing et al. 1995; Shanks et al. 2000).

Many holoplanktonic species undergo daily vertical migrations (Thorson 1964; Mileikovsky 1973; Forward 1988), a pattern also shown by pelagic larval stages of some fish and invertebrate species (e.g., fish, Forward et al. 1996*a,b* and crustacean, Shanks 1986). The most common diel vertical migration type (DVM) corresponds to a deeper distribution of larvae during daytime and surfacing at night (Richards et al. 1996). However, in some cases, planktonic organisms follow a reverse pattern with nocturnal descent (Ohman et al. 1983 and references therein). Besides DVM, other characteristics such as larval buoyancy and sinking or swimming behavior can interact with water mass movements