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Evaluation of Different Phytoplankton for Supporting Development of Zebra Mussel Larvae (*Dreissena polymorpha*): The Importance of Size and Polyunsaturated Fatty Acid Content

Henry A. Vanderploeg, James R. Liebig, and Amy A. Gluck

Great Lakes Environmental Research Laboratory
National Oceanic and Atmospheric Administration
2205 Commonwealth Blvd.
Ann Arbor, Michigan 48105

ABSTRACT. *A marine alga and variety of freshwater algae of known polyunsaturated fatty acid (PUFA) composition were evaluated in the laboratory for their ability to promote development of* Dreissena polymorpha *from egg through settling and metamorphosis. The three species of algae which promoted development—the marine and the freshwater strain of* Chlorella minutissima *and the cryptophyte* Rhodomonas minuta*—were all rich in long-chain (≥ 18 C) n-3 PUFAs, including some 20:5 or 22:6 PUFAs.* Dreissena*'s need for long-chain n-3 PUFAs is consistent with the needs of marine bivalves and freshwater zooplankton. Larval growth rate on the freshwater strain of* C. minutissima *was about the same as that for* R. minuta*, but much faster than that for the marine strain of* C. minutissima*. Mean ages at settling for larvae fed the freshwater* C. minutissima *were 15 d at 26°C, 17 d at 24°C, and 22 d at 22°C. Low survival rates reported for the larvae in nature may be related to low concentrations of long-chain n-3 PUFAs in blue-green and some green algae that dominate eutrophic lakes in summer.*

INDEX WORDS: *Zebra mussel, culture, diet, fatty acids, survival.*

INTRODUCTION

The pelagic phase of the zebra mussel (*Dreissena polymorpha*) may be a weak link in its life cycle. Mortality in nature is variable, and can exceed 99% (Stanczykowska 1977; 1978; Walz 1978; Sprung 1989, 1992). It is not clear to what degree environmental conditions, starvation, food quality, and predation contribute to this high mortality. The ecology of zebra mussel larvae, including nutrition, must be understood before we can understand the trophic consequences of *Dreissena* in an ecosystem, the factors that regulate its populations, and the potential for control.

A major obstacle to understanding the dynamics of the larval period has been the lack of reproducible culture methods to rear larvae (Sprung 1989, 1992). *Dreissena polymorpha* are filter feeders of picoplankton and small particles (~1–5 μm), and their nutritional requirements are not known despite attempts to culture larvae on 50 different species of algae and bacteria (Sprung 1989).

Studies on marine and freshwater zooplankton (bivalve larvae and crustacean zooplankton) have shown that unialgal cultures that support growth are usually rich in n-3 polyunsaturated fatty acids (PUFAs) (e.g., DePauw and Pruder 1986, Ahlgren *et al.* 1990). Many species not only need 18:3 (where x : y = carbon chain length : number of double bonds) n-3 PUFAs, but also 20:5 and 22:6 n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (e.g., Langdon and Waldock 1981, DePauw and Pruder 1986, Ahlgren *et al.* 1990). All algae successfully used in aquaculture of marine bivalve larvae are small (~ 2–5 μm) and rich in EPA or DHA (e.g., DePauw and Pruder 1986, Enright *et al.* 1986, Volkman *et al.* 1989).

We hypothesized that *Dreissena* larvae, which retain the archetypal larval form of marine bivalves, would also require food rich in EPA or DHA. We suspected that many of the species of algae used by Sprung (1989) were not PUFA-rich, especially not in EPA or DHA, or if they were, the cells were too large to be ingested. In this paper we report progress in our ability to culture *D. polymorpha* larvae using different species of algae in pure culture and mixtures of species. The major goal was to define their nutritional requirements. Special empha-

sis was placed on relating suitability of algae for food to known or assumed PUFA concentrations. Development of bivalve larvae or other zooplankton is often faster, with lower mortality, using mixtures of algae than using individual species (Bayne 1965, DePauw and Pruder 1986). Frequently in lakes there are phytoplankton assemblages that do not include small algae rich in EPA or DHA. We considered the possibility that mixtures of algae, composed of species that would not support growth alone, would support larval development.

METHODS AND EXPERIMENTAL DESIGN

To obtain fertilized eggs of *Dreissena polymorpha*, adult mussels maintained in the laboratory were induced to spawn using serotonin or 8-OH-DPAT [(±)-2-dipropylamino-8-hydroxy-1, 2, 3, 4,-tetrahydronaphthalene hydrobromide available from Research Biochemicals Incorporated, Natlick, Massachusetts], a serotonin agonist, after methods modified from Ram *et al.* (1993). Ripe brood-stock mussels were collected from western Lake Erie and Thunder Bay (Lake Huron) during June and July, respectively. These stocks were maintained in ripe condition in aerated 20-L aquaria at 4-6°C (Bayne 1965) with daily feeding of 1 mL of "Algae Diet I" (preserved *Thalassiosira*) or "Algae Diet C" (preserved *Thalassiosira*, *Skeletonema*, *Isochrysis*, and *Chaetoceros*) (Coast Seafoods Co., South Bend, Washington) until needed for production of larvae up 11 mo. later. Mussels were gradually warmed to the spawning temperature of 17 to 22°C over 4–14 d.

Spawning was induced using 10^{-4} M serotonin or 8-OH-DPAT in 0.2-μm filtered water from the Huron River (Ca = 64, Mg = 34, and K = 1.4 mg • L^{-1}) near Ann Arbor, Michigan, and each of the approximately 20 mussels spawned for an experiment was confined to a single 50-mL beaker. After gamete production started, the mussel was rinsed and transferred to a new beaker containing only filtered river water to complete spawning. Males invariably produced sperm before females spawned. Sperm suspension produced by all of the males was collected and about 1 mL was added to each beaker containing a female to further encourage egg production and fertilization. The concentrated suspension of fertilized eggs was diluted to 4-12 eggs • mL^{-1} in autoclaved 1- or 2-L beakers. In about 2 d embryos became D-stage larvae, at which time they were fed.

On the third day, larval suspensions were poured through a 37-μm Nitex screen. Larvae collected on the screen were added to a freshly autoclaved beaker filled with filter-sterilized (0.2 μm) river water containing algae. Larval concentrations at 3 d ranged between 4 and 8 larvae • mL^{-1}. Typically each feeding experiment consisted of three treatments of different species of algae with each treatment in duplicate. Algae were presented to larvae at a concentration of 1–2 mm^3 • L^{-1}. A volume concentration of 1 mm^3 • L^{-1} was near or slightly above that necessary to saturate feeding and support maximum growth rate in marine larval bivalves when *Isochrysis galbana* (1 mm^3 • L^{-1} = 21 cells • μL^{-1}) was used (Sprung 1984 a,b; Gallager 1988; Riisgard 1988). After the first draining and refilling of beakers, beakers were drained and refilled every other day, and a fresh algal suspension was added. As larvae grew, larger and larger screens were used. To minimize the potential for contamination by microorganisms (bacteria, fungi, algae, rotifers, protozoans), all glassware and other materials coming in contact with the cultures or culture water were autoclaved or immersed in hot water.

Larval cultures, maintained at a light intensity of 10–20 μEinst • m^{-2} • s^{-1} on a 14:10 h light:dark cycle, were aerated gently with 5-mm diameter bubbles. Bubbles were introduced through a glass tube placed at the center of each beaker near the bottom at a rate of ~ 1 • s^{-1} until larvae were large enough (> 200 μm) to settle. Bubbling rate was then increased to 3 • s^{-1} to encourage settlement and to insure there was significant water motion near the bottom for settled larvae (e.g., Helm and Spencer 1972). Rate of bubbling did not affect growth or mortality rates before settlement (Vanderploeg, unpublished); however, the gentle mixing provided by aeration kept algae suspended as a homogeneous mixture, which allowed us to get good estimates of algal concentration from random samples with a pipette.

Algal concentrations were sampled daily by counting on a fluorescent microscope at 500 × magnification. If algal concentrations dropped significantly (~20%), algae were added. Larvae were sampled with a pipette from the beakers after gentle mixing. Samples of larval suspension (10 or 25 mL) were taken from each beaker and preserved with formalin for settling down for counting and sizing on the inverted microscope. Lengths were measured at 200 × magnification on about 50 "live" individuals selected at random, and larvae were sampled at intervals of 3 to 4 d until settlement. All larvae with significant tissue within the shell were considered to be alive.

Species and sizes of algae used in this study, along with the culture media used to grow them, are shown in Table 1. The PUFA composition of these or related taxa are shown in Table 2. Algae chosen for this study included a variety of small algae (Table 1) of differing PUFA profiles.

Algae were grown at 20°C at a light intensity of 80–100 μEinst • m^{-2} • s^{-1} in Erlenmeyer flasks without aeration, and were harvested while in exponential-phase growth. Algae grown in BG-11 (Stainer *et al.* 1971) and Mann and Meyers' (1968) medium were centrifuged before adding to larval cultures to avoid introducing high concentrations of NO_3 and Na into experimental containers. Because of the tendency of freshwater *Chlorella minutissima* to form clumps, the algal culture was filtered under low vacuum pressure through a 5-μm mesh Nitex screen.

Survival at settling was a problem in the present study. Settling success appeared to be related to the nature of the container surface film and to the presence of rotifers and ciliates that attacked the larvae. In later experiments freshly autoclaved beakers were used between water changes. As a result, little surface film was available, and larvae tended to settle in groups associated with clumps of algae. Mortality was often high, perhaps because larvae became tangled in algal clumps. The best consistent success at settling was obtained by confining larvae in plexiglass cylinders, 8-cm-diameter × 6-cm-high, with 153-μm Nitex screen on the bottom. These "baskets" were suspended in 2-L beakers, and an air lift pumped water at a rate of ~ 60 mL • min^{-1} into baskets from beakers (Fig. 1). Larvae settled on the screens; water motion may have also encouraged settlement by providing water flow that contained food and removed metabolic wastes. Water flow may also have discouraged growth of ciliates and rotifers. Baskets were removed from beakers and placed in petri dishes for counting and sizing of larvae under a binocular scope at 70 × magnification.

In one experiment, we compared survival and growth of larvae on a basket with survival and growth on a polycarbonate disk coated with a film of the benthic diatom *Navicula* (Carolina Biological

TABLE 1. Algae and culture media used in experiments. Size of the alga is expressed as equivalent spherical diameter (ESD).

Alga	Source	ESD (μm)	Culture medium
Cyanophyta			
Synechococcus sp. (PC)[a]	UTEX ?	1	BG-11[b]
Synechococcus sp. (PE)[c]	Lake Huron[d]	1	BG-11
Chlorophyta			
Chlamydomonas oblonga	UTEX 219	4	WC[e]
Chlorella minutissima (marine)	UTEX 2341	2-3	M&M[f]
Chlorella minutissima (freshwater)	UTEX 2219	3-4	WC
Nannochloris sp.	UTEX 2291	3	WC
Nannochloris ?	Lake Huron[g]	1	WC
Chrysophyta			
Chromulina chinophila	CCAP 909/9	4-5	WC
Cryptophyta			
Cryptomonas ozolini	UTEX 2194	8	WC
Rhodomonas minuta	R. Stemberger[h]	5-6	WC

[a] PC = strain or species rich in phycocyanin pigments
[b] Stainer *et al.* (1971)
[c] PE = strain or species rich in phycoerythrin pigments
[d] Fahnenstiel *et al.* (1991a)
[e] Guillard and Lorenzen (1972)
[f] M&M = medium of Mann and Meyers (1968)
[g] Fahnenstiel *et al.* (1991b)
[h] Dartmouth College, Hanover, New Hampshire, USA

TABLE 2. Fatty acid profiles found in representative freshwater algae or algae used in this study compiled from the literature.[a, b, c, d]

Alga	% Total fatty acids								
	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:6
Cyanophyta									
Synechococcus spp.									
MUFA-rich strains[a]	28.0	42.3	1.7	11.9	0.4	0.0	0.0	0.0	0.0
PUFA-rich strains[b]	24.1	9.8	1.7	15.5	22.5	8.4	0.0	0.0	0.0
Chlorophyta									
Chlamydomonas sp.[c]	9.8	22.9	0.7	6.8	9.0	23.0	0.0	0.0	0.0
Chlorella minutissima									
Marine[d]	12.5	19.4	0.4	4.5	2.1	3.6	5.4	31.8	0.0
Freshwater[d]	21.1	7.4	0.7	7.3	6.1	14.7	3.2	4.6	0.0
Scenedesmus quadricauda[c]	12.7	14.8	0.6	12.0	12.9	23.5	0.0	0.0	0.0
Chrysophyta									
Chromulina chinophila[c]	5.0	2.1	1.5	3.6	7.1	10.0	0.6	0.6	2.5
Cryptophyta									
Cryptomonas sp.[c]	11.1	0.6	1.3	1.7	1.0	9.7	0.0	20.5	7.2
Rhodomonas lacustris[c]	12.8	0.3	0.7	3.1	0.9	21.3	0.0	15.8	4.3

[a] MUFA = monounsaturated fatty acids (Kenyon 1972)
[b] PUFA = polyunsaturated fatty acids (Kenyon 1972)
[c] Ahlgren *et al.* (1992)
[d] Yongmanitchai and Ward (1991)

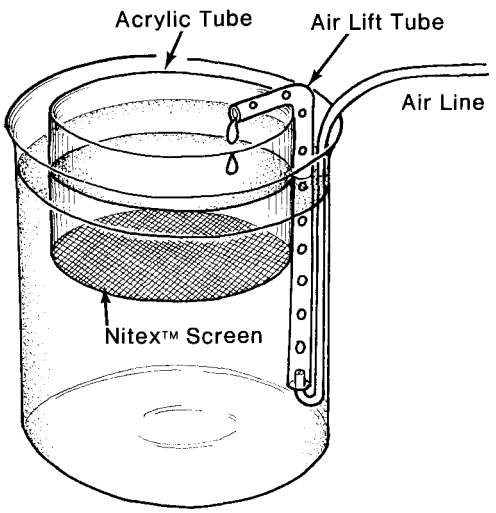

FIG. 1. The air-lift system for larval settling. Air from an aquarium pump was pumped into the glass tube, causing the water to be pumped up as the bubbles rose. Larvae settled out on the 153-μm screen across the bottom of the plexiglas tube.

Supply Co., catalog number 15-3045). The disk with diatom film represented a natural surface colonized by periphyton, but did not have contaminating protozoa that would compete with or attack larvae. A thin polycarbonate disk having the same diameter as the bottom of the 2-L beaker was coated with the film by placing the autoclaved disk in a sterile beaker filled with filter-sterilized Huron River water plus 10% WC medium (Guillard and Lorenzen 1972) inoculated with *Navicula* for 2 weeks.

RESULTS AND DISCUSSION

Ability of Individual Species to Support Development

Evaluation of different unialgal diets is shown in Table 3. Success of a food was defined as its ability to promote survival to the pediveliger stage. Further insights on success of a food were gained from mean lengths to which larvae grew and percent of

TABLE 3. Evaluation of unialgal diets for **Dreissena polymorpha** *larvae on the basis of their ability to promote survival to at least the settling pediveliger stage. N = number of experiments performed; NS = number of experiments in which the larvae survived to at least the pediveliger stage and started to settle. Maximum mean length and maximum survival refer to results for the best experiments. To account for effects due to variable viability of larvae produced by different adult brood stocks, we note whether we had success in rearing larvae with different algae within the same experiment or among different experiments using the same brood stock.*

Alga/condition	Temp (°C)	N	NS	Max mean length (μm)	Broodstock successful: For other algal treatments?	Broodstock successful: For other exps?	Max survival: D-stage to pediveliger (%)
No food	18, 22	4	0	118	yes	yes	0
Cyanophyta							
Synechococcus sp. (PC)	18, 22	4	0	138	no	yes	0
Chlorophyta							
Chlamydomonas oblonga	18	2	0	116	no	yes	0
Chlorella minutissima (marine)	18-26	9	3	231	yes	yes	27
Chlorella minutissima (freshwater)	18-26	16	11	1,120[a]	yes	yes	34
Nannochloris sp.	21, 24	3	0	171	yes	yes	0
Nannochloris ?	18	2	0	112	no	yes	0
Chrysophyta							
Chromulina chinophila	22, 26	2	0	170	yes	yes	0
Cryptophyta							
Cryptomonas ozolini	18	2	0	112	no	yes	0
Rhodomonas minuta	21	2	1	1,506[a]	no	no	9

[a]Experiment terminated before these larvae, now juveniles, died.

D-stage larvae which survived to the pediveliger stage. All measures of success were presented as maximum mean values of separate experiments, i.e., the mean result of the best experiment. For a reference, length attained under conditions of no food is given. Pediveligers varied in length between 200 and 360 μm, metamorphosing individuals ranged between 280 and 360 μm, and individuals ≥ 400 μm were juveniles. Because quality of brood stock for producing viable larvae varied with place and time of collection, and time held in the laboratory, we also note in Table 3 whether the brood stock was successful for other experiments and whether there were treatments with other algae within the same experiment that were successful.

Three species of algae promoted development of larvae: the marine and freshwater strains of the green alga *Chlorella minutissima* and the cryptophyte *Rhodomonas minuta*; best survival from D-stage to pediveliger stage was 27, 34, and 9%, respectively (Table 3). All three algae were small (≤ 6 μm) and they or related species had appreciable quantities of EPA or DHA (Table 2). Both species of *C. minutissima* had previously been examined for fatty acids (Table 2), and all cryptophytes, regardless of species, have been shown to be rich in EPA or DHA (e.g., Ahlgren *et al.* 1992). *Cryptomonas ozolini,* presumably rich in EPA and DHA, probably did not support growth because it was too large too be ingested. Note that lengths of larvae fed this alga were similar to the very low maximum mean length (112 μm) attained by starved larvae (Table 3). Many experiments were performed with the two strains of *C. minutissima* because of our initial success with these species (Table 3). We obtained higher growth rates with the freshwater strain (see below), and for this reason stopped further experimental work with the marine

strain. Experiments with *Rhodomonas minuta* indicate it is a superior food because it promoted growth when other algae, including freshwater *C. minutissima*, did not.

Fatty acid profiles of the algae (Table 2) suggest that many small (≤ 6 μm) species did not support development because of their low long-chain PUFA concentrations. *Synechococcus* sp. (PC) supported a maximum length of only 138 μm. This result is not surprising in that even PUFA-rich strains of *Synechococcus* spp. are relatively low in 18:3 n-3 fatty acids, and contain no EPA or DHA (Table 2). That *Chlamydomonas oblonga* and *Nannochloris* did not support full development is consistent with the observation that most species of greens do not have appreciable quantities of EPA or DHA (Ahlgren *et al.* 1992). Although *Chromulina chinophilia* supported growth almost to the pediveliger stage, it is very low in all the 18:3, 20:5, and 22:6 PUFAs (Table 2).

Ability of Species Mixtures to Support Development

With one exception, only mixtures containing an alga that would support growth individually promoted development to the pediveliger stage. Further, it appeared that the addition of algae that did not support larvae to a species that did support larvae diluted the effect of the desirable species. The only two-species mixtures that promoted development to the pediveliger stage were mixtures that contained the freshwater or the marine strain of *Chlorella minutissima*, which would support growth when offered alone. These mixtures were marine *Chlorella* + *Synechococcus* (PC) and freshwater *Chlorella* + *Cryptomonas ozolini* (Table 4); best respective survivals to pediveliger stage were 16 and 21%. It is possible that *Cryptomonas* was too large to be eaten by the small larvae, but could have been eaten by large larvae.

TABLE 4. Evaluation of multispecies algal diets for* Dreissena polymorpha *on the basis of their ability to promote survival to at least the stage of settling pediveligers. Species abbreviations use the first few letters and other identifying features of the algal species given in Tables 1 and 3. N = number of experiments performed; NS = number of experiments in which the larvae started to settle. Maximum lengths and survival refer to the results for the best experiments. To account for effects due to variable viability of larvae produced by different adult brood stocks, we note whether we had success with different algae within the same experiment or among different experiments with the same broodstock.

Algae	Temp (°C)	N	NS	Max mean length (μm)	Broodstock successful: For other algal treatments?	Broodstock successful: For other exps?	Survival: D-stage to pediveliger (%)
Two species mixtures							
Syn (PC)/ *Syn* (PE)	22	1	0	175	no	yes	0
Syn (PC)/ *Nanno*?	22	2	0	184	no	yes	0
Syn (PC)/ *Chlamy*	22	1	0	160	yes	yes	0
Syn (PC)/ marine *Chlor*	22	1	1	229[a]	yes	yes	16
Chlamy/ *Crypto*	18	1	0	113	no	yes	0
Freshwater *Chlor*/ *Crypto*	20	1	1	441[a]	yes	yes	21
Three species mixtures							
Syn (PC)/ *Syn* (PE)/ *Nanno*?	22	1	1	481[a]	yes	yes	4
Syn (PC)/ *Chlamy*/ *Nanno*?	22	1	0	156	no	yes	0
Syn (PC)/ *Chlamy*/ marine *Chlor*	22	1	1	197[a]	yes	yes	2
Syn (PC)/ *Chlamy*/ freshwater *Chlor*	22	1	1	229[a]	yes	yes	8

[a]Experiment was terminated before these larvae died.

Survival of larvae in three-species mixtures was not outstanding (0–8%). As might be expected, three-species mixtures supporting development included all mixtures that contained the freshwater or marine strain of *C. minutissima*, algae that would support growth on their own. Curiously, the mixture *Synechococcus* (PC) + *Synechococcus* (PE) + *Nannochloris* (?) supported growth with 4% survival (Table 4), when the two-species mixtures *Synechococcus* (PC) + *Synechococcus* (PE) and *Synechococcus* (PC) + *Nannochloris* (?) did not. However, this was not unexpected since each of these two-species mixtures supported development almost to settling size (Table 4).

Larval Growth Rates

Representative length vs. age curves for *Dreissena polymorpha* larvae for those experiments in which they reached pediveliger or more advanced stages are shown in Figures 2 and 3. Growth rates of larvae on the freshwater strain of *Chlorella minutissima* increased with temperature over the range 22 to 26°C. A few larvae in each of the treatments reached settling size (~ 250 μm) and began to settle out at 18 d at 22°C and at 12 d at 24°C and 26°C. Mean settling age, defined by when the mean lengths reached 250 μm, were 22 d at 22°C, 17 d at 24°C, and 15 d at 26°C. These rates are consistent with rates extrapolated from Sprung's (1989) temperature relation (covering the range 14–21°C) for time of development to 220 μm, the length of settling. Sprung's rates were calculated from cohort analysis of natural populations. His predictions and our results (from Fig. 2) were, respectively, 25 vs. 18 d at 22°C, 16 vs. 14 d at 24°C, and 11 vs. 13 d at 26°C. Rates in the present study were generally a little faster than Sprung's except at 26°C, which is probably the upper limit of temperature tolerance of larvae. Sprung (1987) reported that *Dreissena* sperm lost its motility at 26°C.

Growth rate of larvae fed the freshwater strain of *C. minutissima* was about the same as that for *Rhodomonas minuta*, and was much faster than that of the marine strain of *C. minutissima* (Fig. 3). When the experiment with the marine algal strain ended at 34 d, some of the larvae had reached > 250 μm and settled out, at which time the mean population length was 231 μm.

Substrate and Shape of Growth Curves

The relationship between length and age of bivalve larvae from D-stage to metamorphosis has

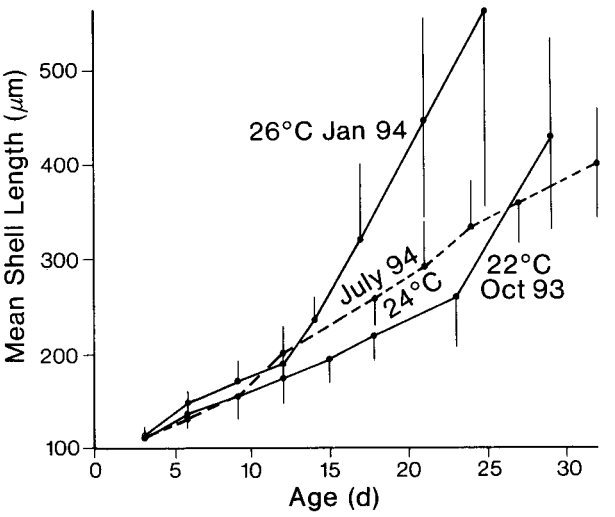

FIG. 2. Mean length (±SD) vs. age since fertilization of* Dreissena polymorpha *larvae fed the freshwater strain of* Chlorella minutissima *as related to temperature and start date of experiment (indicated by month and year). The experiments performed in October 1993 and January 1994 used the same brood stock collected from Thunder Bay in July 1993, and the experiment of July 1994 used brood stock from Thunder Bay collected in that same month. SDs were calculated from pooled estimates of length from the different replicates. Baskets for larval settlement were used in the experiments of January and July 1994.

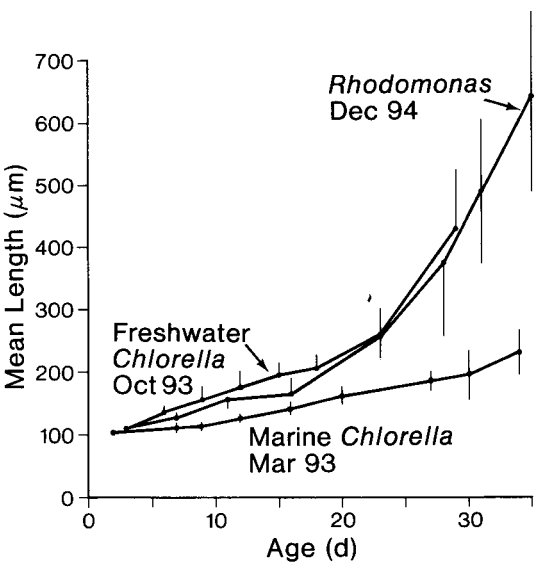

FIG. 3. Mean length (±SD) vs. age since fertilization for different successful foods in experiments run at 21 or 22°C.

been associated with quality of rearing conditions in the laboratory (Bayne 1983). Linear and, especially, exponential growth relationships have been identified with optimal rearing conditions, including water and food quality (Bayne 1983). Asymptotic growth has been associated with delayed metamorphosis due to lack of suitable substrate for settling. Relationships in Figures 2 and 3 appear to be linear or exponential, indicating results are consistent with no lack of suitable substrate for settling. For the curves that exhibit an exponential shape, the dramatic increase in growth occurred after metamorphosis. This may represent increased ability of *Dreissena* to feed and grow after metamorphosis.

Further insight into the importance of substrate came from the comparison of survival and growth of larvae offered a basket or disk to settle on. Both larvae on the disk and basket showed high mortality during settling (Fig. 4A). Once settled, the larvae on the basket exhibited high survival, whereas those on the disk continued to show high mortality. Growth rates were higher on the basket than disk (Fig. 4B). We do not know if better survival and growth on the basket resulted from factors associated with increased turbulence and flow (see methods) or from its being a better surface for attachment than the *Navicula* coated disk. Stanczykowska (1977) reported that in Taltowisko Lake mortality was most intense (> 99%) during settlement and the first few months of life, and she speculated this mortality was due to lack of suitable substrate. Our results emphasize the importance of a suitable substrate.

Ecological Implications of Food Quality

Our results are consistent with the long established findings of marine aquaculture that bivalve larvae require or develop best on food rich in PUFAs, including EPA and DHA (Langdon and Waldock 1981, DePauw and Pruder 1986, Volkman *et al.* 1989). Results are consistent with recent ob-

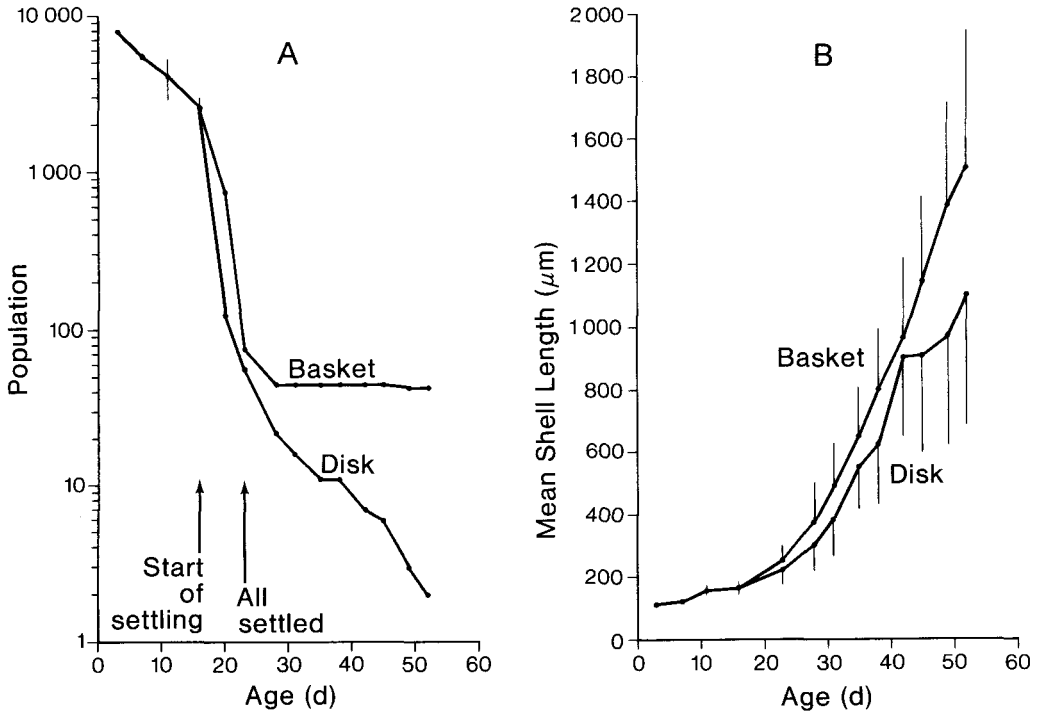

FIG. 4. Population survival (A) and growth (B) of* Dreissena polymorpha *larvae growing in* Rhodomonas minuta *in 2-L beakers with either a basket or a* Navicula*-coated disk as substrate. At the time of start of settling, the two replicates of larvae in* Rhodomonas *were combined and then split between a basket treatment and a disk treatment. Error bars in A are SEs, and those in B are SDs.

servations of Wright *et al.* (1996), who successfully reared *Dreissena polymorpha* on the T-iso clone of *Isochrysis galbana*, a DHA-rich alga (Enright *et al.* 1986) long used as a preferred food in marine bivalve culture.

It is of special interest that results for *Dreissena polymorpha* add to the growing body of evidence that many freshwater zooplankton require or develop better on long-chain n-3 PUFAs. Our results bear striking similarity to results of Ahlgren *et al.* (1990) for the growth rate of three species of cladocerans given different algae of ingestible size and known PUFA content. Ahlgren *et al.* (1990) found that populations of all three species of cladocerans—*Chydorus*, *Daphnia*, and *Eubosmina*—grew fastest on PUFA-rich (Table 2) cryptophytes offered— *Rhodomonas lacustris* and *Cryptomonas* sp. *Daphnia* and *Chydorus* populations were able to grow on some species of green algae. *Eubosmina* grew only on cryptophytes. Only the population of *Chydorus*, which is a neritic or eutrophic-water species, exhibited some growth on blue-green algae or on the crysophyte *Chromulina chinophila*. All green algae in their study (like *Chlamydomonas* and *Scenedesmus* in Table 2) were rich in 18:3 n-3 PUFAs, but had no 20:5 or 22:6 n-3 PUFAs. Thus our results for *Dreissena* are consistent with the need or desirability for not only 18:3 n-3 PUFAs but also longer chain PUFAs, since all unialgal cultures supporting complete development contained some 20:5 or 22:6 PUFAs (Table 2). Our results are in agreement that cryptophytes (*Cryptomonas*, *Rhodomonas*) are good for culture of a variety of freshwater rotifers, cladocerans, calanoid and cyclopoid copepods (Stemberger 1981, Ahlgren *et al.* 1990, Chen and Folt 1993, Hart and Santer 1994, Santer 1994).

Our results imply that food quality, and not size alone, is important to the survival of zebra mussel larvae. This has obvious implications for survival in nature. Because the general pattern of seasonal succession from diatoms and cryptophytes to greens and blue-greens in eutrophic lakes during summer (Sommer 1989) leads to low concentrations of long-chain PUFAs—particularly EPA and DHA, survival of larvae should be low in these systems, particularly in late summer. This situation would be further exacerbated by presence of large inedible (canopy) species that can dominate because of grazing pressure by a variety of grazers that removes edible species (Sterner 1989). Since bacteria, like blue-greens, are not a source of long-chain PUFAs (Kenyon 1972, Volkman *et al.* 1989), bacteria associated with detrital food webs of eutrophic lakes would not be a supplemental source of long-chain PUFAs. Recent work has shown that seasonal patterns of long-chain PUFAs in natural lake seston follow this expected pattern, and that growth of *Daphnia* is highly correlated with EPA concentration (Mullernavarra 1995). The rarity of *Dreissena polymorpha* in hypereutrophic lakes and their declining populations in lakes increasing in eutrophy (Stanczykowska and Lewandowski 1992) are consistent with the role of food quality for larval survival.

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