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# Food quality controls egg quality of the zebra mussel *Dreissena polymorpha*: The role of fatty acids

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*Abstract*

We investigated the investment of adult *Dreissena polymorpha* to the eggs by sampling mussels monthly from 4- and 15-m water depth. The fatty acid composition of eggs differed significantly between depths and over time. To assess whether temperature and food conditions led to the differences observed for mussels sampled from the two depths, mussels were reared in the laboratory under two different 3-month temperature regimes, simulating the temperature of the lake at 4- and 15-m depth. Possible effects of food quality were tested in each simulation using four diets differing in fatty acid composition: *Cryptomonas erosa, Nannochloropsis limnetica* [rich in polyunsaturated fatty acids (PUFAs) and long-chained PUFAs (>C18)], *Scenedesmus obliquus,* and the cyanobacterium *Aphanothece* sp. (deficient in long-chained PUFAs). In newly released eggs, specific (n–3) and (n–6) long-chained PUFAs increased when these fatty acids were available in the natural seston or in the laboratory diets. Mussels fed organisms deficient in long-chained PUFAs were still able to allocate arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid into eggs, which suggests that minimum levels of particular fatty acids were maintained in eggs by transfer from internal reserves of the female mussels to oocytes. In contrast to the diet, there were no effects of the temperature on the fatty acid composition of eggs.

Studies of food requirements are important because food is an unpredictable, fluctuating resource within the immediate surroundings of individuals in their natural environment and might inhibit successful survival and reproduction. This is particularly important for sessile animals, which cannot move to more favorable environments, such as the zebra mussel *Dreissena polymorpha,* which has a great impact on the structure of the benthic community (Mörtl and Rothhaupt 2003) and contributes substantially in the transfer of primary to secondary production (Mörtl and Rothhaupt 2003). Among the freshwater bivalves, *D. polymorpha* is one of the rare species that reproduces via a pelagic larva. Its reproduction is characterized by high fecundity, which is probably one reason why the zebra mussel has been able to spread rapidly in lakes and rivers with favorable conditions (Sprung 1989). In Europe, the zebra mussel has colonized waters of different trophic status and with various temperature conditions (e.g., Stanczykowska 1977) and is often studied because it plays an important role in aquatic ecosystems (MacIsaac 1996).

For species that produce eggs and provide no parental care, the entire maternal nutritive contribution to the subsequent generation is provided in the egg. Organisms invest considerable energy to ensure that their eggs will survive. The source of material for early larval development is provided by gametogenesis of adult mussels. This reproductive investment is characterized by the internal reserves of the eggs. Female mussels store high amounts of lipids in eggs (Sprung 1995); therefore, high fecundity and successful reproduction are probably limited by the amount that can be transferred from the female to the egg. The allocation of lipids to eggs is important for the further reproductive success because internal stores of the eggs affect development and growth of larvae during the first days after liberation; the growth of newly released marine mussel larvae is positively correlated with larval lipid contents (Gabbott 1976). Hence, nutritional stress of the female affects subsequent larval development, which is evident for marine mussels (Gabbott 1976; Bayne 1976).

Since polyunsaturated fatty acids (PUFAs) play an important role for a successful development of zebra mussel larvae (Wacker et al. 2002; Wacker and Von Elert 2002), adult mussels may transfer PUFAs in sufficient amounts to their eggs to ensure a good egg quality. Egg quality can be described by determining the fatty acid content of eggs (Cahu et al. 1995; Soudant et al. 1996) and is positively correlated with successful development (Soudant et al. 1996) and growth of marine mussel larvae during the first few days of life (Bayne 1976). Effects of temperature on lipid composition and egg quality were demonstrated on commercially used fish only (Webb et al. 2001). To our knowledge, information on egg quality in terms of fatty acids in freshwater invertebrate species comes only from commercially used freshwater prawns (Cavalli et al. 1999); however, there is a lack of studies for freshwater mussels. We considered the factor temperature to be important because in spring when the temperature in the littoral zone increases, a thermocline divides the water column into two parts. In the warmer surface layer, phytoplankton species different from those in the lower layer may develop (Stewart and Wetzel 1986), thereby providing mussels with a different composition of seston at different water depths.

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When we consider the spawning interval of *D. polymorpha* in Lake Constance from May until August, egg quantity did not differ between two water depths: mussels collected at 4-m depth released on average 1.3 mg dry weight eggs per mussel (0.4–2.3 mg) and mussels at 15-m depth released 1.2 mg dry weight eggs per mussel (0–2.8 mg) (Wacker and Von Elert 2003). However, egg quality in terms of fatty acids may play an important role, as was shown for freshwater prawns, which are used commercially (Cavalli et al. 1999). Although mussels from different depths produced the same egg quantity, egg quality might differ. Low food quality could lead mussels to produce high egg quantity with low quality or to produce low egg quantity with high quality. In the laboratory part of our previous study it was shown that food quality affected egg quantity, and mussels in the 15-m temperature simulation spawned later than mussels in the 4-m temperature simulation (Wacker and Von Elert 2003). However, since no significant interaction between the factors temperature regime and diet quality was found, these two factors should operate independently. Temperature played an important role as a trigger for spawning. The type of diet clearly affected the reproductive investment. Mussels fed the alga *Nannochloropsis limnetica* and *Cryptomonas erosa* [both of which are rich in arachidonic acid (ARA) and eicosapentaenoic acid (EPA)] released on average 1.5 and 0.9 mg dry weight eggs per mussel. Mussels fed these algae produced a threefold higher egg quantity than mussels fed the cyanobacterium *Aphanothece* sp. or the green alga *Scenedesmus obliquus* (both of which are deficient in ARA and EPA) (0.4 and 0.5 mg dry weight eggs per mussel). Since there is evidence for differences in egg production, it might be useful to examine the quality of eggs produced in order to understand the energy allocation of zebra mussels. This way, it will be possible to predict reproductive success of *D. polymorpha* with a given information of diet quality (fatty acid composition) and water temperature.

The aim of this study was to investigate to what extent the egg quality of zebra mussels in the field is affected by environmental factors, such as temperature and food. The reproductive investment of adult *D. polymorpha* sampled monthly at two water depths (4 and 15 m) was determined qualitatively (fatty acid composition of eggs). While Borcherding (1995) studied the influence of food quantity in laboratory experiments on gametogenesis, we examined possible impacts of food quality on reproductive investment. Additionally, we investigated the natural food source and its possible influence on the reproductive investment in the littoral zone. Since field analyses would not measure how much the observed differences in the reproductive investment of the mussels in the lake are attributable to different temperature or food conditions, the importance of temperature and food regimes for the reproductive investment of adult mussels was also studied in controlled laboratory experiments. Mussels in the laboratory were fed with food organisms, which differed in their fatty acid composition. By determining the fatty acid content of eggs as a parameter for egg quality (Soudant et al. 1996), we extended the study made by Stoeckmann and Garton (2001). Mussels were exposed to two different 3-month temperature regimes simulating the temperature of the lake at the two depths and were fed with food organisms that differed in their fatty acid composition.

## Materials and methods

*Monthly field sampling*—Lake Constance is a mesotrophic lake of warm monomictic character at the northern border of the Alps in Central Europe. The study was carried out from March until October 2000 at the littoral zone close the Limnological Institute. Three rocks each at 4- and 15-m depth were collected monthly by scuba diving, and adult zebra mussels (18–25 mm in length) were isolated from the each of the three rocks of each depth. Mussels were scrubbed free of debris and transported to the laboratory in filtered lake water (0.45-$\mu$m pore-sized membrane filter). The mussels were maintained at 8°C until they arrived at the laboratory. Directly above the zebra mussel banks, close to the sampling sites at each depth, seston was collected 0.5 m above the lake bottom either from a boat equipped with a sonic depth finder using a Ruttner sampler (10 liters) or by self-contained underwater breathing apparatus (SCUBA) diving. The water was immediately filtered through 55-$\mu$m pore-size filters, which represents the preferred size fraction of food for adult zebra mussels (Ten Winkel and Davids 1982). The water samples were used for analysis of particulate parameters.

*Preparation of food*—Freshwater algae were obtained from culture collections of the Max-Planck-Institute for Limnology (MPIL), the Institute of Freshwater Ecology and Inland Fisheries (IGB), and the University of Göttingen (SAG). Algae were grown semicontinuously in aerated 5-liter vessels by harvesting 25–50% of the culture every other day and by restoring the volume with freshly prepared medium. The small and unicellular cyanobacterium *Aphanothece* sp. (IGB), which was successfully used to rear zebra mussel larvae (Wacker and Von Elert 2002), the cryptomonad *Cryptomonas erosa* (MPIL), the heterokont chromophyte alga *Nannochloropsis limnetica* (SAG 18.99), and the green alga *Scenedesmus obliquus* (SAG 276-3a) were cultured in modified WC medium with vitamins at 20°C with a photosynthetic photon flux density of 100 $\mu$mol m$^{-2}$ s$^{-1}$. To remove growth media, cells were concentrated by centrifugation and resuspended in filtered lake water (0.45-$\mu$m pore-sized membrane filter). Carbon concentrations of these stock solutions were estimated from photometric light extinction (800 nm) using carbon-extinction equations previously determined. Equations were determined separately for each species and were consistent over time because food organisms were cultured under standardized conditions and were harvested in the same growth phase. Aliquots of algal or cyanobacterial solutions of estimated carbon contents were added to filtered lake water, and the resulting standardized suspensions in terms of carbon were used as food for the adult zebra mussels.

The food organisms represented three different groups of biochemical composition: (1) containing few or no PUFAs, (2) rich in PUFAs except for long-chained PUFAs (>C18), and (3) rich in almost all PUFAs. Group 1 comprised the cyanobacterium *Aphanothece* sp., which contained only

Table 1. Fatty acid composition of algae or cyanobacteria used as food sources for adult *Dreissena polymorpha.* Contents of polyunsaturated fatty acids are given in micrograms of fatty acid per milligram of POC. The values are means of $n$ = 3–4 independent cultures; the standard error is given in parentheses (n.d. = not detected).

| | Group 1 | Group 2 | Group 3 | |
|---|---|---|---|---|
| Fatty acid | *Aphanothece* sp. ($n$ = 4) | *Scenedesmus obliquus* ($n$ = 4) | *Nannochloropsis limnetica* ($n$ = 4) | *Cryptomonas erosa* ($n$ = 3) |
| C18:2n-6 | n.d. | 27.95 (4.94) | 6.14 (0.33) | 6.40 (0.48) |
| C18:3n-6 | n.d. | 1.66 (0.12) | 2.20 (0.32) | 0.42 (0.21) |
| C18:3n-3 | n.d. | 59.21 (9.79) | 1.12 (0.23) | 50.95 (2.03) |
| C18:4n-3 | 0.09 (0.09) | 7.24 (1.64) | n.d. | 32.92 (14.02) |
| C20:4n-6 | n.d. | n.d. | 15.94 (1.56) | 0.44 (0.22) |
| C20:5n-3 | n.d. | n.d. | 103.32 (17.32) | 37.49 (3.94) |
| C22:6n-3 | n.d. | n.d. | n.d. | 3.95 (1.25) |

small amounts of PUFAs and no long-chained PUFAs (Table 1). Group 2 comprised the green alga *S. obliquus,* which contained considerable amounts of linoleic acid (18:2n–6) and α-linolenic acid (α-LA, 18:3n–3), but was deficient in long-chained PUFAs (Table 1). Group 3 consisted of algae that contained significant amounts of long-chained PUFAs, such as ARA, EPA, and docosahexaenoic acid (DHA, 22: 6n–3). Members of this group included *N. limnetica* (high in ARA and EPA, no DHA) and *C. erosa* (moderate in EPA, low in ARA, and traces of DHA) (Table 1).

*Broodstock conditioning*—Zebra mussels (18–25 mm in length) were collected on 14 March 2000 from the littoral zone (close to our institute) of Lake Constance at 10-m depth by SCUBA diving. The intermediate depth of 10-m between 4- and 15-m was chosen in order to integrate between random effects. A difference of temperature and food composition between the depths can be neglected because the temperature at 4- and 15-m depth was identical in March when mussels were collected. Usually this time of the year high water exchange rates between the depths occur (warm monomictic lake). Mussels were cleaned and immediately brought to the laboratory. Zebra mussels were then randomly selected, and 24 vessels (2-liter volume) were stocked with 20 animals each. The water in the vessels was aerated with an airstone and recirculated at a flow rate of 300 ml $h^{-1}$ to prevent food particles from settling.

Food suspensions were prepared from aliquots of the algal or cyanobacterial stock suspensions added to filtered lake water in separate flasks (1 liter) and kept homogeneous by constant stirring. Food suspensions in flasks were renewed daily and pumped continuously into the rearing vessels to minimize fluctuations in food quantity. Each mussel was provided with gradually increasing food levels, from 0.57 to 0.71 mg algal carbon $mussel^{-1}$ $d^{-1}$ within 3 months to simulate increasing food levels in the littoral zone in spring. This food quantity exceeded the metabolic demands estimated from data given in Walz (1978) and Borcherding (1995). Every other day, the animals were checked for vitality and transferred to new vessels with freshly filtered lake water to remove pseudofeces and debris. The mussels were kept at dim light, with a light : dark cycle of 12 : 12 h.

In addition to various food, the mussels were also exposed to different temperature regimes. Two different 3-month temperature regimes, each with four different food regimes, were set up with three replicate experimental 2-liter vessels each. The first temperature regime consisted of mussels kept at 6°C, mimicking the temperature of the lake at 15 m (15-m temperature simulation). In the second regime, mussels were kept at 6°C and after 1 month were exposed to 11°C when the littoral temperature at 4-m depth rose (4-m temperature simulation). In order to study the effect of food quality on the reproductive investment, the mussels within each temperature regime were fed either the cyanobacterium *Aphanothece* sp., the cryptomonad *C. erosa,* the heterokont chromophyte alga *N. limnetica,* or the green alga *S. obliquus.*

*Composition of eggs*—The quality of eggs was tested with mussels of each temperature/food quality regime sampled monthly, and with mussels from the field at 4- and 15-m depth. Animals were scrubbed free of debris under running water and transferred to filtered lake water, which was aerated and replaced twice a day. The mussels were gradually warmed up to the spawning temperature of 20°C over 2 d. The mussels were starved during the warming period. Mussels of each replicate sampling were then exposed to 0.15 mmol $L^{-1}$ serotonin-creatinine-sulfate complex (Sigma-Aldrich Chemie) in 300-ml filtered lake water to induce spawning (Vanderploeg et al. 1996; Wacker et al. 2002). Aliquots of released eggs (0.2–1 mg dry weight) were collected on preweighed and precombusted glass-fiber filters (Whatman GF/F, 25-mm diameter) to determine dry weight and fatty acids. Samples for determination of dry weight were dried overnight at 50°C and weighed after cooling in a desiccator. Dry weights were determined on an electronic balance (Mettler UMT 2) to the nearest 0.1 $\mu$g.

*Particulate parameter and fatty acid analysis*—Aliquots of algal or cyanobacterial food suspensions, seston, or mussel eggs were filtered through precombusted glass-fiber filters (Whatman GF/F, 25-mm diameter), dried, and analyzed for particulate organic carbon (POC) by oxidation in an NCS-2500 analyzer (ThermoQuest GmbH) (Wacker and Von Elert 2001). For fatty acid analysis, aliquots of algal or cyanobacterial food suspensions or mussel eggs corresponding to approximately 1.0 mg POC were filtered through a precombusted Whatman GF/F filter (25-mm diameter). For natural lake water, filters 47 mm in diameter were used. The

loaded filters were either extracted immediately for analysis of fatty acids or stored at −80°C for later analysis. For extraction of lipids, the loaded filters were extracted twice with 7 ml of dichloromethane/methanol (2 : 1, v/v). Particles were removed by centrifugation (3,500 × g, 2 min) and the supernatant evaporated to dryness under nitrogen. The dried sample was resuspended in 3 ml of 3 mol $L^{-1}$ methanolic HCl (Sigma-Aldrich Chemie) and subsequently incubated 15 min at 60°C in a sealed vial in order to transesterify fatty acids into methyl esters. After the sample had cooled down, fatty acid methyl esters (FAMEs) were extracted twice with 3 ml of iso-hexan. The fraction of iso-hexan was evaporated to dryness under nitrogen and resuspended in a volume of 10 to 50 $\mu$l iso-hexan. Prior to extraction of the loaded filter, 2 $\mu$g of heptadecanoic acid methyl ester and 2.5 $\mu$g of tricosanoic acid methyl ester were added as internal standards. FAMEs were analyzed by gas chromatography using an HP 6890 GC (Agilent Technologies) with the following configuration: column, DB 225 (30 m × 0.25 mm inner diameter × 0.25 $\mu$m film; J&W Scientific); oven, 60°C (1 min) to 150°C at 30°C $min^{-1}$, then to 170°C at 3°C $min^{-1}$, then to 220°C at 2°C $min^{-1}$, and held for 6 min; carrier, helium, 35 cm $s^{-1}$; flame-ionization detector (FID), 250°C; injector, 250°C; total run time, 42 min $sample^{-1}$. The sample (1 $\mu$l) was injected splitlessly. FAMEs were identified by comparison of retention times with those of reference compounds (Sigma-Aldrich) and by gas chromatography–mass spectrometry (Finnigan GCQ, ThermoQuest). The fatty acids were quantified by comparison to internal standards and to response factors determined for each FAME from mixtures of known composition. The detection limit was 40 ng FAME $mg^{-1}$ POC; it was not possible to distinguish between petroselinic acid (C18:1n–12) and oleic acid (C18:1n–9). The absolute amount of each FAME was normalized to the independently determined POC content or dry weight of the sample.

*Data analysis*—If the concentrations of fatty acids in the seston differed between 4- and 15-m water depth, analyses of covariance were undertaken using the time as covariate. Data of the fatty acid contents from field mussel eggs were log ($x$ + 1) transformed to meet assumptions for analysis of variance (ANOVA). For fatty acid compositions of broodstock conditioning, raw data met assumptions. The experimental factors were either depth and date (field sampling) or food category and temperature regime (broodstock conditioning). Whether the experimental factors accounted for significant variation in the fatty acid composition of the eggs was tested using a repeated-measurement ANOVA involving seven polyunsaturated fatty acids (18:2n–6, 18:3n–6, 18:3n–3, 18:4n–3, 20:4n–6, 20:5n–3, and 22:6n–3) as within dependent variable (fatty acid composition [FAC]).

According to Underwood (1981), those factors can be excluded from the ANOVA, in which the significance of the $F$ values is $p > 0.25$. Therefore, these factors ($p > 0.25$) were excluded in a second ANOVA and the following tests. When an experimental factor (main effects or interactions) showed a significant interaction with the within-factor that represents the fatty acid composition (FAC), Tukey Honest Significant Differences (HSD) multiple-comparison tests were carried out in order to distinguish between effects of different depths (field sampling) or different diet types (broodstock conditioning). All analyses were carried out using the general linear model module of Statistica 6.0 (StatSoft Inc.).

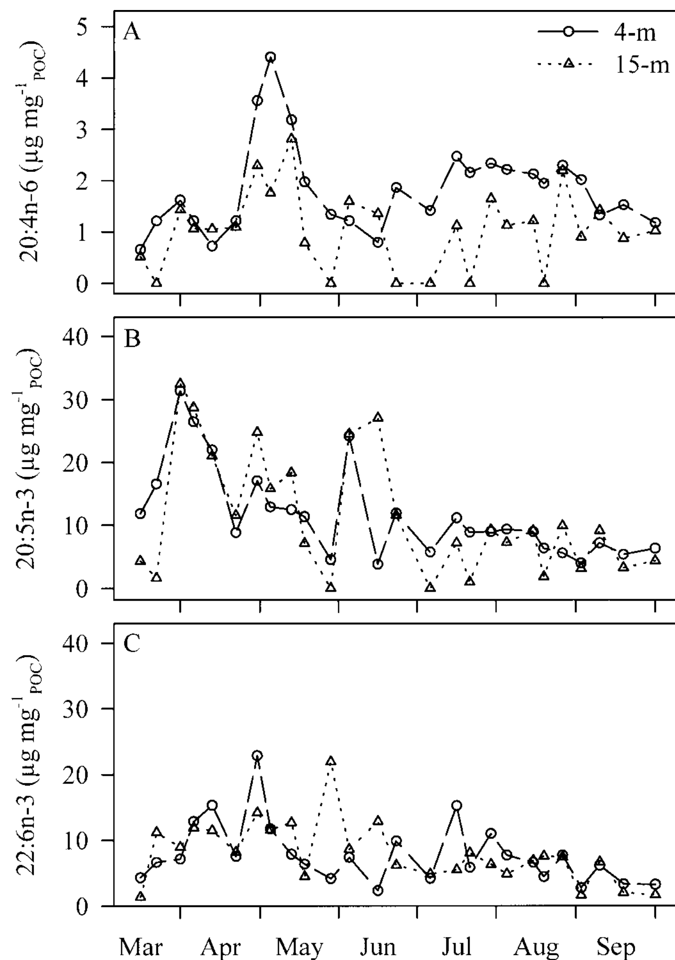


Fig. 1. Seasonal pattern of the sestonic long-chained PUFA content: (A) arachidonic acid (ARA, 20:4n–6), (B) eicosapentaenoic acid (EPA, 20:5n–3), and (C) docosahexaenoic acid (DHA, 22:6n–3). Data refer to the edible (<55 $\mu$m) fraction of natural seston at 4- and 15-m depth.

## Results

*Environmental factors*—The content of polyunsaturated fatty acids (PUFAs) in the natural seston, which is often used as a measure for food quality, in particular eicosapentaenoic acid (EPA, 20:5n–3), was high in spring and substantially lower in summer (ANCOVA, $F_{1,35} = 7.97$, $p < 0.01$, Fig. 1). The content of arachidonic acid (ARA, 20:4n–6) was usually lower at 15-m depth than at 4-m depth (ANCOVA, $F_{1,35} = 7.87$, $p < 0.01$). The mean daily temperature at the 15-m depth rose very slowly from 5°C in March to 12°C in August, while the temperature at the 4-m depth reached 12°C already in May.

Table 2. Results of statistical analysis of the content of polyunsaturated fatty acids of eggs released by *Dreissena polymorpha* sampled from two different depths. Data of fatty acid contents from field mussel eggs were log ($x + 1$) transformed to meet assumptions for ANOVA. The factor of the composition of seven different polyunsaturated fatty acids is shown as FAC. According to Underwood (1981), factors ($p > 0.25$) were excluded in a second ANOVA and the following tests. When an experimental factor showed a significant interaction with the factor that represents the FAC, a Tukey HSD multiple-comparison test was carried out to distinguish between effects of different depths. SS = sum of squares. MS = mean squares.

| | SS | *df* | MS | *F* | *P* |
|---|---|---|---|---|---|
| Full factorial | | | | | |
| FAC × depth | 0.0591 | 6 | 0.0099 | 2.16 | 0.06 |
| FAC × date | 0.0275 | 6 | 0.0046 | 1.01 | 0.42 |
| FAC × depth × date | 0.0841 | 6 | 0.0140 | 3.08 | <0.01 |
| Error | 0.3281 | 72 | 0.0046 | | |
| Factors with $p > 0.25$ excluded in analysis | | | | | |
| FAC × depth | 0.4067 | 6 | 0.0678 | 14.87 | <0.001 |
| FAC × depth × date | 0.4318 | 6 | 0.0720 | 15.78 | <0.001 |
| Error | 0.3556 | 78 | 0.0046 | | |
| Tukey HSD for depth | | | | | |
| | 18:2n-6 | 78 | 0.0047 | | 0.32 |
| | 18:3n-6 | 78 | 0.0047 | | 0.73 |
| | 18:3n-3 | 78 | 0.0047 | | 0.51 |
| | 18:4n-3 | 78 | 0.0047 | | 0.56 |
| | 20:4n-6 | 78 | 0.0047 | | <0.001 |
| | 20:5n-3 | 78 | 0.0047 | | <0.01 |
| | 22:6n-3 | 78 | 0.0047 | | <0.05 |

*Fatty acid composition of eggs of mussels feeding in the field*—According to Underwood (1981), we excluded those factors in which the significance of the *F* values was $p > 0.25$ (Table 2). The factor depth exhibited significant differences of the PUFA composition in eggs released by mussels from the two different depths (ANOVA, $F_{6,78} = 14.9$, $p < 0.001$, Table 2); the interaction depth × time showed that 4- and 15-m mussels changed their PUFA composition of eggs differently over time (ANOVA, $F_{6,78} = 15.8$, $p < 0.001$, Table 2). The eggs from mussels collected at different depths and dates significantly differed in the content of the single fatty acids ARA, EPA, and DHA (Fig. 2, Table 2). No other PUFA in the eggs was significantly different (Table 2). The different conditions at the two depths led on average to a content of ARA in the eggs of mussels collected from 4-m depth significantly higher than that from 15-m depth (Fig. 2, Table 2), which is consistent with the finding that the natural food source at 4-m depth usually contained more ARA than that at 15-m depth (Fig. 1). This suggests that the mussels transferred abundantly available ARA of the seston to the eggs. Eggs of mussels collected in July at 15-m depth had contents of EPA and DHA higher than those collected at 4-m depth (Fig. 2, Table 2).

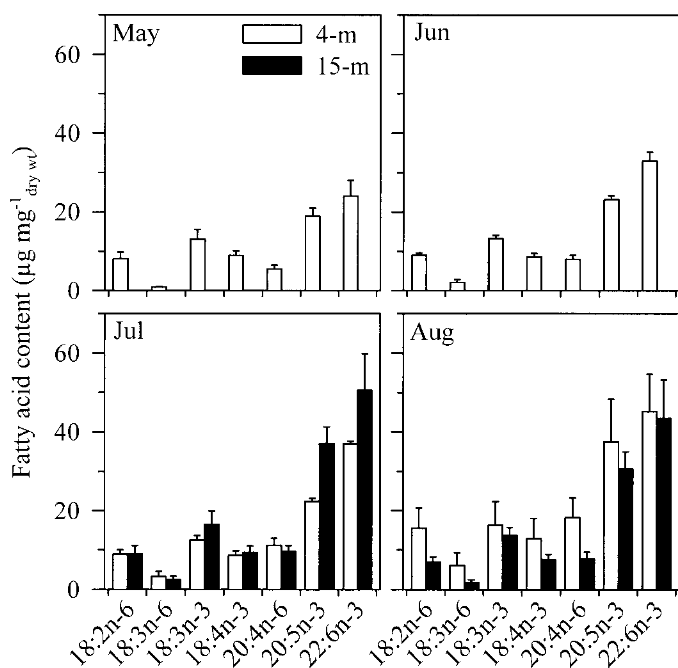


Fig. 2. Fatty acid content of eggs (mean ±SE, $n = 3$) released by females collected on different dates at 4- and 15-m depth. Mussels were sampled from March until October, and mussels spawned from May to August (4-m) and July to August (15-m).

*Fatty acid composition of eggs of mussels conditioned differently*—The released mussel eggs did not show significant differences in the fatty acid composition between the two temperature simulations (ANOVA, $F_{6,144} = 0.32$, $p = 0.92$, Table 3). However, the factor food category (*Aphanothece* sp., *S. obliquus, N. limnetica,* or *C. erosa*) showed that there were significant differences in the fatty acid composition in the eggs of adult mussels that were reared on different food sources (ANOVA, $F_{18,144} = 6.21$, $p < 0.001$, Table 3). There was no other significant factor or two-way interaction (Table 3). For further tests we excluded those factors in which the significance of the *F* values was $p > 0.25$ (Underwood 1981). The different food regimes resulted in significant differences in single PUFAs. Mussels fed *C. erosa* released

Table 3. Results of statistical analysis of the content of polyunsaturated fatty acids of eggs released by differently conditioned *D. polymorpha.* Fatty acid data met assumptions for ANOVA. The factor of the composition of seven different polyunsaturated fatty acids is shown as FAC. According to Underwood (1981), factors ($p > 0.25$) were excluded in a second ANOVA and the following tests. When an experimental factor showed a significant interaction with the factor that represents the FAC, a Tukey HSD multiple-comparison test was carried out to distinguish between effects of different diet types.

| | SS | *df* | MS | *F* | *p* |
|---|---|---|---|---|---|
| Full factorial | | | | | |
| FAC × diet | 1,862,650,498 | 18 | 103,480,583 | 6.21 | <0.001 |
| FAC × temperature | 32,476,042 | 6 | 5,412,674 | 0.32 | 0.92 |
| FAC × diet × temperature | 147,982,801 | 12 | 12,331,900 | 0.74 | 0.71 |
| Error | 2,399,950,051 | 144 | 16,666,320 | | |
| Factors with $p > 0.25$ excluded in analysis | | | | | |
| FAC × diet | 2,095,898,391 | 18 | 116,438,799 | 7.32 | <0.001 |
| Error | 2,578,313,695 | 162 | 15,915,517 | | |
| Tukey HSD for diet | | | | | |
| | 18 : 2n-6 | 162 | 15,915,517 | | 0.85 |
| | 18 : 3n-6 | 162 | 15,915,517 | | 0.14 |
| | 18 : 3n-3 | 162 | 15,915,517 | | <0.05 |
| | 18 : 4n-3 | 162 | 15,915,517 | | <0.05 |
| | 20 : 4n-6 | 162 | 15,915,517 | | <0.05 |
| | 20 : 5n-3 | 162 | 15,915,517 | | <0.05 |
| | 22 : 6n-3 | 162 | 15,915,517 | | 0.23 |

eggs with significantly higher mean contents of C18:3n–3 (α-LA) and C18:4n–3 (stearidonic acid, SA) than mussels fed *N. limnetica* (Fig. 3) (HSD multiple-comparison test, $p < 0.05$, Table 3). Mussels fed *N. limnetica* (rich in ARA and EPA) allocated a higher content of 20:4n–6 (ARA) and an almost twofold higher mean content of 20:5n–3 (EPA) to eggs than mussels fed *Aphanothece* sp., *C. erosa,* or *S. obliquus* (HSD multiple-comparison test, $p < 0.05$). Therefore, the contents of C18:3n–3 (α-LA), C18:4n–3 (SA), 20:4n–6 (ARA), and 20:5n–3 (EPA) of released eggs reflected the composition of the corresponding diets of the broodstock. The other PUFAs in the eggs did not significantly differ (Table 3).

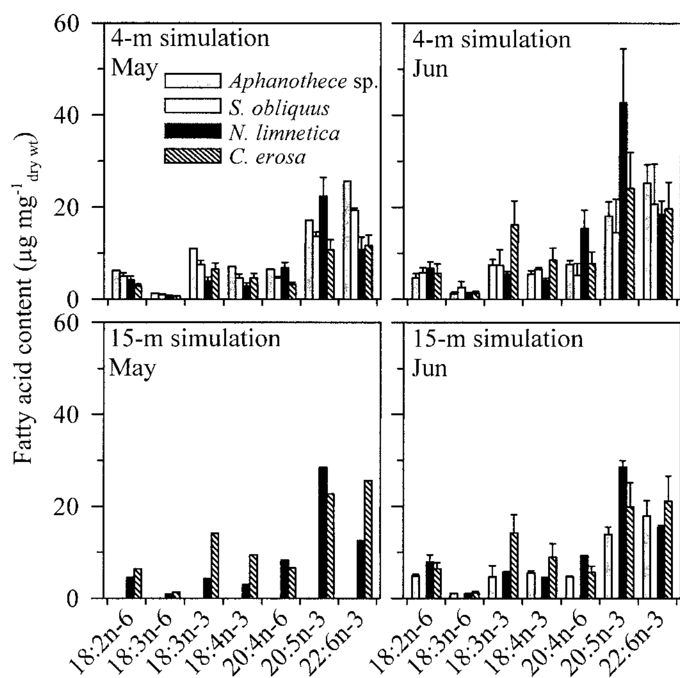


Fig. 3. Effect of broodstock conditioning diet and temperature on the content of single fatty acids of eggs. Values are means (±SE) of eggs released by mussels previously fed *Aphanothece* sp., *Scenedesmus obliquus, Nannochloropsis limnetica,* or *Cryptomonas erosa.* Bars without an error bar are single observations; others are means of $n = 3$. Not all food treatments in the 15-m simulation resulted in release of eggs (no vertical bars shown).

## Discussion

The extent to which a species can spread, as well as its success, in a given environment is related mainly to those factors that can limit reproduction. Gametogenesis creates the source of material for larval development, and this reproductive investment is reflected by qualitative parameters (e.g., biochemical composition of eggs). In this study, the fatty acid composition of the eggs of adult mussels from two different depths in a lake was investigated. Food quality has been reported to affect reproduction of marine mussels (Andersen and Ringvold 2000) and might similarly influence the proliferation of *D. polymorpha.* In the warmer surface layer, phytoplankton species different from those in the lower layer might have developed (Stewart and Wetzel 1986), thereby providing mussels with a different composition of seston at 4-m and at 15-m depth (Fig. 1). This different composition was reflected in a significantly different content of ARA in the two water depths and led to differences in the availability of this fatty acid for the mussels (Fig. 1). Since some fatty acids, which are used as constituents of biological membranes and serve as precursors for signal molecules, are essential and cannot be synthesized de novo by most invertebrates (Stanley-Samuelson et al. 1988), the availability of

PUFAs (Fig. 1, Table 1) might have affected the quality of eggs produced (Fig. 2, Fig. 3).

The spawning of mussels from 15-m depth later in the season than those from 4-m depth (Fig. 2) is consistent with findings of Borcherding (1991), who observed a delayed onset of spawning at 9-m depth than at 2-m depth. Borcherding (1991) found a temperature threshold of 12°C for spawning. Also, Gist et al. (1997) suggested that temperature regulates the onset of major reproductive events. A temperature difference may explain the delayed onset of spawning at 15-m depth compared to 4-m depth. However, a temperature threshold can not explain the difference between the 4- and the 15-m depth with respect to egg quality. Egg quality could have been influenced by spatial and temporal differences in environmental factors, such as differences in food composition between the two water depths.

In summer, the temperature at 15-m depth increases above the threshold for successful spawning (Borcherding 1991), so that mussels are likely to spawn. Therefore it is evident that temperature regimes at 4- and 15-m depth, which show significant differences in food quality (e.g. ARA), are warm enough for natural spawning. In conclusion, quality of spawned eggs may differ within sites of a lake, as well as between lake types with different seston composition.

The mussels were starved during the warming period, yet differences in egg PUFA content corresponded to diet differences during the previous cold-water period (<12°C). This implies that PUFA allocation occurred even under cold temperature regimes in the laboratory (this study), even though eggs are relatively small and immature (Ram et al. 1996). The results at 15-m depth also seem to indicate that food quality can affect PUFA content of eggs at low temperature, unless allocation primarily occurred after mussels were brought back to the lab and warmed for spawning. Since mussels were starved during the warming up, it is likely that only fatty acids of internal reserves were allocated to the eggs. This is supported further by findings of Mantecca et al. (2003), who found that zebra mussels had reproductive active ovaries in depths were the temperature is below the threshold of 12°C.

The multitude of environmental factors in the field may mask the true relationship between limiting factors and physiological processes such as reproduction. Hence, the causal relationship must be evaluated in controlled laboratory experiments, as done in our study in which mussels were reared on different temperature and food quality regimes. The lower temperature in the 15-m depth temperature simulations resulted in a delayed spawning of these animals in comparison with animals in the 4-m depth (Fig. 3). This indicates that temperature plays an important role as a trigger for egg development and spawning, consistent with the findings of Borcherding (1995). In laboratory experiments, food quality clearly affected egg quantity (Wacker and Von Elert 2003). This result is in accordance with data from Stoeckmann and Garton (2001) who showed that the energy allocated for the reproduction of mussels fed a dried green alga was lower than that of mussels fed a mixed diet of live algae. In the present study, we extended the study of egg quantity and determined the egg quality by measuring the fatty acid composition of the eggs.

In our study, the content of single PUFAs in newly released eggs (Fig. 3) was dependent on their proportion in the diet (Table 1). This is consistent with results of Soudant et al. (1996), who have shown that PUFA contents of marine mussel eggs reflect those of the corresponding diets. The present study presents evidence for the importance of long-chained PUFAs in reproduction of *D. polymorpha* because mussels fed *N. limnetica* (rich in ARA and EPA) invested two times more ARA and EPA in eggs than mussels fed food deficient in ARA and EPA. Additionally the mussels released three times more eggs than mussels fed PUFA-deficient food organisms (Wacker and Von Elert 2003). In consequence, the effect of egg quantity is slightly higher than the effect of fatty acids transferred to the eggs. However, these two effects have the same order of magnitude, so that differences in the transfer of fatty acids to eggs should be of similar importance for the recruitment of the zebra mussel. One can expect that low food quality conditions will lead mussels to produce low number of eggs with high quality maintained: *D. polymorpha* fed a diet totally deficient in long-chained PUFAs invested C18-PUFAs in oocytes and were still able to allocate 20:4n–6 (ARA), 20:5n–3 (EPA), and 22:6n–3 (DHA) to oocytes, which has also been reported for marine mussels (Soudant et al. 1996). That mussels fed cyanobacteria were still able to spawn (Fig. 3) may result from the fact that these mussels reabsorbed oocytes (Borcherding 1995) in order to support a remaining, smaller number of oocytes (Wacker and Von Elert 2003). Female *D. polymorpha* may transfer 20:4n–6 (ARA), 20:5n–3 (EPA), and 22:6n–3 (DHA) from internal reserves to oocytes, maintaining a minimum content of these PUFAs in the eggs, which would point to the importance of these PUFAs. EPA and DHA are of importance in marine aquaculture (Enright et al. 1986; Delaunay et al. 1993), and the importance of ARA can be mainly attributed to its function as a precursor of prostaglandins (Cook 1996), which influence reproduction of mollusks (Osada et al. 1989; Martinez et al. 2000).

In conclusion, our results indicate that the biochemical composition of the eggs of the zebra mussel *D. polymorpha* is strongly influenced by food quality. These food quality effects vary seasonally and differ in different water depths in large temperate lakes and may therefore restrict successful reproduction to mussels living at particular water depths. Storage of fatty acids allows reproduction to occur for limited periods of poor food quality. Since hypertrophic lakes are dominated by cyanobacteria, poor in long-chained PUFAs, the ability of this invasive bivalve to spread throughout different types of lakes might be limited by food quality.

## References

Andersen, S., and H. Ringvold. 2000. Seasonal differences in effect of broodstock diet on spawning success in the great scallop. Aquacult. Int. **8:** 259–265.

Bayne, B. L. 1976. The biology of mussel larvae, p. 293–356. *In* B. L. Bayne [ed.], Marine mussels: Their ecology and physiology. Cambridge Univ. Press.

Borcherding, J. 1991. The annual reproductive cycle of the freshwater mussel *Dreissena polymorpha* Pallas in lakes. Oecologia **87:** 208–218.

———. 1995. Laboratory experiments on the influence of food availability, temperature and photoperiod on gonad development in the freshwater mussel *Dreissena polymorpha.* Malacologia **36:** 15–27.

CAHU, C. L., G. CUZON, AND P. QUAZUGUEL. 1995. Effect of highly unsaturated fatty acids, alpha-tocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus.* Comp. Biochem. Physiol. **112:** 417–424.

CAVALLI, R. O., P. LAVENS, AND P. SORGELOOS. 1999. Performance of *Macrobrachium rosenbergii* broodstock fed diets with different fatty acid composition. Aquaculture **179:** 387–402.

COOK, H. W. 1996. Fatty acid desaturation and chain elongation in eukaryotes, p. 129–152. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of lipids, lipoproteins and membranes. Elsevier Science, Amsterdam.

DELAUNAY, F., Y. MARTY, J. MOAL, AND J. F. SAMAIN. 1993. The effect of monospecific algal diets on growth and fatty-acid composition of *Pecten maximus* (L.) larvae. J. Exp. Mar. Biol. Ecol. **173:** 163–179.

ENRIGHT, C. T., G. F. NEWKIRK, J. S. CRAIGIE, AND J. D. CASTELL. 1986. Evaluation of phytoplankton as diets for juvenile *Ostrea Edulis* L. J. Exp. Mar. Biol. Ecol. **96:** 1–14.

GABBOTT, P. A. 1976. Energy metabolism, p. 293–356. *In* B. L. Bayne [ed.], Marine mussels: Their ecology and physiology. Cambridge Univ. Press.

GIST, D. H., M. C. MILLER, AND W. A. BRENCE. 1997. Annual reproductive cycle of the zebra mussel in the Ohio River: A comparison with Lake Erie. Arch. Hydrobiol. **138:** 365–379.

MACISAAC, H. J. 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of North America. Am. Zool. **36:** 287–299.

MANTECCA, P., G. VAILATI, L. GARIBALDI, AND R. BACCHETTA. 2003. Depth effects on zebra mussel reproduction. Malacologia **45:** 109–120.

MARTINEZ, G., A. Z. OLIVARES, AND L. METTIFOGO. 2000. In vitro effects of monoamines and prostaglandins on meiosis reinitiation and oocyte release in *Argopecten purpuratus* Lamarck. Invertebr. Reprod. Dev. **38:** 61–69.

MÖRTL, M., AND K. O. ROTHHAUPT. 2003. Effects of adult *Dreissena polymorpha* on settling juveniles and associated macroinvertebrates. Int. Rev. Hydrobiol. **88:** 561–569.

OSADA, M., T. NISHIKAWA, AND T. NOMURA. 1989. Involvement of prostaglandins in the spawning of the scallop, *Patinopecten yessoensis.* Comp. Biochem. Physiol. C **94:** 595–601.

RAM, J. L., P. P. FONG, AND D. W. GARTON. 1996. Physiological aspects of zebra mussel reproduction: Maturation, spawning, and fertilization. Am. Zool. **36:** 326–338.

SOUDANT, P., Y. MARTY, J. MOAL, AND J. SAMAIN. 1996. Fatty acids and egg quality in great scallop. Aquac. Int. **4:** 191–200.

SPRUNG, M. 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: Abundance, growth, mortality and food demands. Arch. Hydrobiol. **115:** 537–561.

———. 1995. Physiological energetics of the mussel *Dreissena polymorpha* in lakes: I. Growth and reproductive effort. Hydrobiologia **304:** 117–132.

STANCZYKOWSKA, A. 1977. Ecology of *Dreissena polymorpha* (Pall.) (Bivalvia) in lakes. Pol. Arch. Hydrobiol. **24:** 481–530.

STANLEY-SAMUELSON, D. W., R. A. JURENKA, C. CRIPPS, G. J. BLOMQUIST, AND M. DERENOBALES. 1988. Fatty acids in insects: Composition, metabolism, and biological significance. Arch. Insect Biochem. Physiol. **9:** 1–33.

STEWART, A. J., AND R. G. WETZEL. 1986. Cryptophytes and other microflagellates as couplers in planktonic community dynamics. Arch. Hydrobiol. **106:** 1–19.

STOECKMANN, A. M., AND D. W. GARTON. 2001. Flexible energy allocation in zebra mussels *(Dreissena polymorpha)* in response to different environmental conditions. J. N. Am. Benthol. Soc. **20:** 486–500.

TEN WINKEL, M. E. H., AND C. DAVIDS. 1982. Food selection by *Dreissena polymorpha* Pallas (Mollusca: Bivalvia). Freshw. Biol. **12:** 553–558.

UNDERWOOD, A. J. 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Annu. Rev. **19:** 513–605.

VANDERPLOEG, H. A., J. R. LIEBIG, AND A. A. GLUCK. 1996. Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): The importance of size and polyunsaturated fatty acid content. J. Gt. Lakes Res. **22:** 36–45.

WACKER, A., P. BECHER, AND E. VON ELERT. 2002. Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha.* Limnol. Oceanogr. **47:** 1242–1248.

———, AND E. VON ELERT. 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata.* Ecology **82:** 2507–2520.

———, AND ———. 2002. Strong influences of larval diet history on subsequent post-settlement growth in the freshwater mollusc *Dreissena polymorpha.* Proc. R. Soc. Lond., B **269:** 2113–2119.

———, AND ———. 2003. Food quality controls reproduction of the zebra mussel *(Dreissena polymorpha).* Oecologia **135:** 332–338.

WALZ, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. 1–4. Arch. Hydrobiol. Suppl. **55:** 83–156.

WEBB, M. A. H., J. P. VAN EENENNAAM, G. W. FEIST, J. LINARES-CASENAVE, M. S. FITZPATRICK, C. B. SCHRECK, AND S. I. DOROSHOV. 2001. Effects of thermal regime on ovarian maturation and plasma sex steroids in farmed white sturgeon, *Acipenser transmontanus.* Aquaculture **201:** 137–151.

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