JOURNAL OF PLANKTON RESEARCH | VOLUME 29 | NUMBER 5 | PAGES 463–470 | 2007

Effects of essential fatty acids on the reproduction of a generalist herbivore

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Received August 7, 2006; accepted in principle January 11, 2007; accepted for publication March 28, 2007; published online April 16, 2007

Communicating editor: K.J. Flynn

We studied direct and indirect effects of essential fatty acids (EFA) on Daphnia magna reproduction. Daphnia females that received EFA enriched algae over their entire lifespan produced larger broods than females fed control algae. However, EFA-enriched females were also 91% heavier than control females, which potentially explain the larger investment in reproduction. Thus, with such a large difference in female mass, it is difficult to differentiate the direct effect of the EFA addition from the indirect (maternal size) effects on reproduction. To assess the direct effects on reproduction, we performed two experiments in which we enriched female diets with a range of fatty acids. To minimize maternal size differences, we applied the EFA enrichments only to mature daphniids and studied the effects on reproduction during and after two time intervals (15–16 and 7 days). Limiting the enrichment phase until after maturity decreased the maternal size differences over the enrichment phase among the fatty acid treatments from an average of 91% (for life time enrichment) to 29% after 15–16 days maternal enrichment interval, to 18% after the 7 day interval. Minimizing size differences between differently enriched females decreased the differences in brood size and offspring size. Neonates from control females were more severely affected by starvation than offspring from females that received saturated and polyunsaturated fatty acid enrichments. Under low food concentrations, only neonates that had access to polyunsaturated fatty acids had positive growth rates, thus showing that although dietary fatty acids can be used for energy purposes, specific fatty acids are required to build new biomass. One consequence of our findings is that offspring size does not serve as a good estimate of offspring quality when feeding on different resource qualities.

INTRODUCTION

Offspring size is an important trait in life history, since larger offspring typically have higher fitness than smaller offspring (Stearns, 1992). However, allocating more resources to single offspring should result in a lower total brood size, provided the resources available for reproduction are constant. Hence, Smith and Fretwell (Smith and Fretwell, 1974) suggested that in any given environment parents should trade-off offspring size against brood size in order to maximize offspring fitness per unit effort. However, for this to work, the parents must have an indication of the future resource levels. *Daphnia* neonates and females generally utilize the same pool of resources, and feeding conditions for the offspring may broadly be similar to what the reproducing female encounter at the time of egg production (Gliwicz and Guisande, 1992). Several zooplankton studies have indeed found evidence that with changing resource quantity, females trade brood size against offspring mass (Tessier and Consolatti, 1991; Guisande and Gliwicz, 1992; Ebert, 1993; Boersma, 1997a). However, the direction of the adjustment has

doi:10.1093/plankt/fbm030, available online at www.plankt.oxfordjournals.org

not always been consistent across studies. In concordance with Smith and Fretwell (Smith and Fretwell, 1974), some researchers found that increasing food concentration resulted in smaller offspring (Gliwicz and Guisande, 1992; Ebert, 1993; Boersma, 1997a, 1997b). Other researchers described contradictory effects with increasing resource concentrations: no correlation with offspring size (Burns, 1995), heavier offspring (Lynch, 1989) or heavier offspring at intermediate food concentrations (Tessier and Consolatti, 1991; Boersma, 1995; Trubetskova and Lampert, 1995). One explanation for these inconsistencies could be that some individuals command more resources than others, which would result in a positive correlation between offspring size and brood size over different animals (van Noordwijk and de Jong, 1986).

Although the importance of resource quality (mineral and biochemical composition of the resources) on somatic growth is well established, only fairly recently have studies also included food quality effects on reproduction and the next generation (Brett, 1993; Sterner and Schulz, 1998; Urabe and Sterner, 2001). For example, females cultured on high-quality algae (i.e. *Rhodomonas*) produced offspring of higher quality, having positive effects on offspring population growth and life history (Brett, 1993). Further, several studies investigated the effects of phosphorus (P) or nitrogen (N) on *Daphnia* reproduction (Urabe and Sterner, 2001; Faerovig and Hessen, 2003; Becker and Boersma, 2005). P-limitation was associated with an increased proportion of eggs that failed to develop (Urabe and Sterner, 2001). Under P-limitation egg volumes decreased, but egg yolk volumes increased (Urabe and Sterner, 2001). The same pattern was also found by Boersma and Kreutzer (Boersma and Kreutzer, 2002) who also observed that P-limited neonates had much higher areas of lipid droplets, probably obtained from the higher lipid concentration of P-limited *Scenedesmus* (Boersma, 2000). Boersma and Kreutzer also found that $<$24 h old neonates from females feeding on P-sufficient algae had a higher P content than neonates from females fed P-limited algae. However, this higher availability of P did not improve the offspring's ability to survive on P-limited resources; in fact, on low concentrations of P-limited algae offspring from P-limited mothers had higher growth rates than offspring from P-sufficient mothers. In contrast to Boersma and Kreutzer (Boersma and Kreutzer, 2002), other studies reported that neither egg weight nor P content per egg was significantly affected by the P concentration in the maternal diet (Becker and Boersma, 2003, 2005; Faerovig and Hessen, 2003).

Different resources may be important during different stages of *Daphnia* life (Urabe and Sterner, 2001; Becker and Boersma, 2003). Phosphorus could be more important during the juvenile phase whereas other resources could become quality determining after maturity. For example, *Daphnia* egg production is coupled to a cyclic process of lipid accumulation from their diet (Tessier and Goulden, 1982; Goulden and Place, 1993), and available polyunsaturated fatty acids (PUFA) are preferably allocated to offspring (Becker and Boersma, 2005), and can improve offspring fitness (Becker and Boersma, 2003). Thus, lipids are most likely especially important for reproduction. In an earlier study, we studied the effects of an essential fatty acid, eicosapentaenoic acid (EPA), on *Daphnia magna* throughout its life history, wherein EPA enrichments resulted in higher (maternal) somatic growth, and earlier and larger investment in reproduction (Becker and Boersma, 2003). Moreover, offspring from dietary enriched females achieved higher growth rates than offspring from control females. However, from that study, it is difficult to assess the direct effect of EFA on reproduction, since increased maternal size typically corresponds to larger clutch sizes as well as larger egg sizes (Lampert, 1993). Hence, here, we investigated how EFA affect *D. magna* investments in reproduction, contrasting the effects of enrichments over the entire lifespan (direct and indirect effects) with the direct effects of fatty acid enrichments, when maternal size differences were minimized. We studied the effects of fatty acids on clutch size, offspring mass and offspring fitness.

METHODS

Animals and algae

We used a clone of *D. magna* previously isolated from a pond in Frankfurt, Germany, and kept at the Max Planck Institute for Limnology for many years. Experimental animals were obtained by cultivating *D. magna* on P-sufficient *Scenedesmus obliquus* at 1 mg C L^{-1}. All daphniids were cultivated in "Aachener Daphnien Medium" (ADaM) (Klüttgen *et al.*, 1994), at 18–20°C under constant dimmed light. The experiments were started with less than 24 h old neonates from third brood females. To study the robustness of fatty acid limitation between algae species, we selected *Chlamydomonas* sp. and *S. obliquus* that both have low concentrations of PUFA. These algae were cultured in semi-continuous set-ups: *Chlamydomonas* in WC-medium (Guillard and Lorenzen, 1972) and *S. obliquus* in P-sufficient (1.39 mg P L^{-1}) conditions in Z/4 medium (Zehnder and Gorham, 1960; Becker and Boersma,

2003, 2005). All algae were cultured at 20°C, in 10 L flask with a flow rate of 1.5 L day^{-1}.

Fatty acid additions and analyses

Algae were enriched with three fatty acids: eicosanoic acid (20:0, ESA), EPA (20:5ω3) and eicosatetraenoic acid (20:4ω6, arachidonic acid, ARA) (Williams *et al.*, 1990; von Elert and Stampfl, 2000; von Elert, 2002). In short, algae were incubated in a suspension of single fatty acids and bovine serum albumin overnight. The incubation was stopped by centrifugation and the algae were washed repeatedly with ADaM and thereafter diluted to acquire food concentrations of 1 mg C L^{-1}. Algal samples were filtered onto GF/C filters and extracted and esterified according to Wiltshire *et al.* (Wiltshire *et al.*, 2000). Fatty acid profiles were analyzed by gas chromatography with the same configurations as von Elert (von Elert, 2002). The FAMEs were identified by comparison of retention times of known reference compounds; heptadecanoic acid methyl-ester (17:0) and tricosanoic acid methyl-ester (23:0) were used as internal standards.

Experimental procedures

Experiment 1—To investigate how EFA additions affect reproduction directly, and via interactions with female size, we reanalyzed data from Becker and Boersma (Becker and Boersma, 2003). In our previous study, life history characteristics were determined for *D. magna* feeding on a range of P-limited algae, enriched with or without EPA. In this reanalysis of our previous data, we use the brood size and maternal mass at the time for the first reproduction for the three highest P additions (i.e. 5, 10 and 20 μg P L^{-1}), where there was no significant effect between these three P additions, and contrast animals with and without EPA addition. In our previous study, we observed that EPA additions increased maternal growth and enhanced the investment in reproduction, but we did not investigate the contribution of female body size on reproduction.

Experiments 2 and 3—In order to isolate effects of various fatty acids on reproduction, we aimed to minimize differences in maternal body mass. This was done by delaying the enrichment of fatty acids until maturity, when somatic growth rates are generally lower (DeMott, 2003). Twenty <24 h old *D. magna* neonates were placed in 1 L glass beakers and were fed P-sufficient *S. obliquus* at 1 mg C L^{-1} daily (e.g. *ad libitum*). Every second day, animals were transferred to fresh food suspensions. After 10 days, most females had matured and egg-carrying females were selected and randomly assigned to the various fatty acid treatments in a batch set-up, each with 12 replicates. From this point, daphniids were transferred to new food suspensions daily. In the second experiment, the *D. magna* food was *Chlamydomonas* sp. incubated with EPA or ARA (von Elert, 2002). The control treatment was treated identically as the EPA and ARA treatments, apart from the fatty acid addition. The experiment on enriched *Chlamydomonas* was stopped after the fourth brood of offspring was released (i.e. 15–16 days of EFA enrichment); brood size was recorded for all four instars, offspring mass was recorded for the third and the forth brood. Maternal growth rates were calculated from weight increments for the 10-day-old females until the end of the experiment (i.e. for the 25–26-day-old females). Further, we investigated if maternal fatty acid enrichments affected the fitness of the fourth brood offspring under starving conditions using growth assays as a proxy. The result from the experiment with enriched *Chlamydomonas* indicated that maternal fatty acid enrichments resulted in offspring weight differences. Such differences can have had implications on the offspring fitness where larger individuals better sustained starvation (Tessier and Consolatti, 1989). Hence, we set up a third similar experiment, where 10-day individuals received EFA enrichments for 7 days. In this experiment, we cultivated daphniids with P-sufficient *S. obliquus*, which similarly to *Chlamydomonas*, has low concentrations of PUFA (Table I) (von Elert, 2002; Becker and Boersma, 2005). Further, since both ARA and EPA enhanced maternal growth rates in the previous experiment, we added a saturated fatty acid (20:0, ESA) to rule out that positive effects were only the consequence of higher energy availability. Control *S. obliquus* was enriched with ESA, EPA or ARA, female and neonate mass was recorded as above, and growth assays were performed on the offspring on two algae concentrations: starvation (only ADaM) and 0.1 mg C L^{-1} *S. obliquus*.

Growth assays

Less than 24 h old offspring were pooled, and from this pool, five individuals were placed randomly in a 120 mL flow-through vessel with a flow rate of 1 L day^{-1}. Food suspensions were replaced daily and each treatment was replicated five times. Neonate dry mass was determined from a sub-sample of the pooled animals and the final mass was established from the experimental animals after 3 days. At sampling, five neonates were transferred to pre-weighed aluminum boats; dried at 60°C overnight and weighed to the nearest 0.1 μg on a sartorius

Table I: Mean fatty acid concentrations (mg FA g C^{-1}) in Chlamydomonas sp. after enrichment with EPA, ARA or unenriched control

Parameters	Control		EPA		ARA	
14:0	0.38	(0.04)[a]	0.73	(0.04)[b]	0.73	(0.07)[b]
16:0	23.98	(2.35)	21.07	(1.34)	22.63	(2.22)
16:1ω7	1.69	(0.47)	1.43	(0.31)	1.51	(0.30)
18:0	5.55	(0.90)	4.27	(0.16)	4.17	(0.32)
18:1ω12/ω9	10.31	(2.20)	8.52	(0.89)	9.12	(0.81)
18:1 ω7	8.07	(0.72)	7.60	(0.18)	6.63	(0.54)
18:2ω6	9.89	(0.89)	10.08	(1.28)	9.19	(2.38)
18:3ω6	1.54	(1.28)	0.17	(0.03)	0.30	(0.02)
18:3ω4	1.33	(0.31)	1.25	(0.22)	1.27	(0.25)
18:3ω3	14.66	(1.08)	19.69	(2.75)	16.28	(2.51)
18:4ω3	0.24	(0.07)	0.23	(0.05)	0.23	(0.06)
20:0	0.26	(0.10)	0.12	(0.06)	0.19	(0.09)
20:2ω6	0.00	(0.00)	0.00	(0.00)	0.03	(0.03)
20:3ω6	0.03	(0.01)[a]	0.17	(0.12)[a]	0.97	(0.29)[b]
20:4ω6	0.03	(0.03)[a]	0.15	(0.08)[a]	25.10	(2.24)[b]
20:3ω3	0.02	(0.02)	0.06	(0.01)	0.00	(0.00)
20:4ω3	0.00	(0.00)[a]	1.07	(0.40)[b]	0.00	(0.00)[a]
20:5ω3	0.00	(0.00)[a]	18.81	(4.70)[b]	0.07	(0.01)[a]
22:0	0.02	(0.02)	0.06	(0.03)	0.07	(0.07)
22:5ω3	0.79	(0.05)[a]	0.37	(0.03)[b]	0.49	(0.08)[b]
22:6ω3	0.06	(0.00)	0.03	(0.01)	0.03	(0.01)
SAFA	30.18	(3.34)	26.24	(1.52)	27.79	(2.68)
MUFA	20.07	(3.01)	17.54	(1.01)	17.26	(1.50)
PUFA	28.58	(0.98)[a]	52.07	(6.89)[b]	53.97	(2.07)[b]

SE from three replicate samples is given in parenthesis. Identical letters in superscript denote non-significant differences among the three fatty acid additions (Tukey's HSD test).

microbalance. From the mass increments, growth rates were computed.

RESULTS

Experiment 1—The effect of EPA addition and maternal mass on reproduction was studied over the first adult instar for the three highest P additions (5, 10 and 20 μg P L^{-1}) using data obtained from Becker and Boersma (Becker and Boersma, 2003). For these animals, the addition of EPA resulted in 91% heavier females than control females at the time for the first reproduction. Maternal mass had a positive effect on brood size (Fig. 1, general linear model (GLM): $F_{1,26} = 59.00$, $P < 0.001$). However, mean brood size was not significantly affected by neither EPA addition nor EPA × maternal size interaction (Fig. 1, GLM: $F_{1,26} < 0.12$, $P > 0.72$). We did not find a significant effect of female mass on offspring mass (GLM: $F_{1,26} = 3.41$, $P < 0.093$) nor an effect of the fatty acid treatments on offspring mass (GLM: $F_{1,26} = 0.076$, $P = 0.78$).

Experiments 2 and 3—*Chlamydomonas* fatty acid composition was altered with the different additions: *Chlamydomonas* enriched with ARA resulted in higher

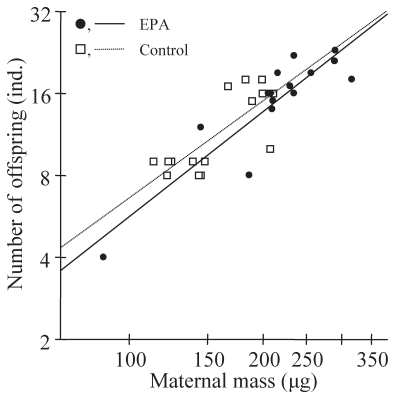

Fig. 1. Relationship between *D. magna* maternal mass and brood size for the first adult instar (log–log). Daphniids were fed P-limited *S. obliquus* supplemented with 5, 10 and 15 μg P L^{-1} and enriched with (EPA) or without EPA (control). Data obtained from Becker and Boersma (Becker and Boersma, 2003).

concentrations of this specific fatty acid and also in a slight increase in the content of 20:3ω6 (Table I); additions of EPA enhanced the content of EPA and 20:4ω3 (Table I). Further, the content of 22:5ω3 was higher in the control treatment than in the enriched treatments (Table I). We studied how fatty acid enrichments affected *D. magna* reproduction when body size differences between females were minimized, by delaying the enrichment phase until maturity. After 15–16 days on the three fatty acid treatments, all animals had produced four clutches of offspring and the experiment was stopped. EPA and ARA enriched females had grown to a heavier mean mass than control females (Fig. 2A, ANOVA: $F_{2,33} = 18.21$, $P < 0.001$), this relative increase was only 29% which was considerably lower than the 91% increase seen when the diet was enriched over the whole juvenile phase (Fig. 1). Both brood size and offspring mass increased with consecutive broods (Fig. 2B and C; repeated measures analysis, brood size: $F_{3,99} = 503.06$, $P < 0.001$; offspring mass: $F_{1,32} = 5.907$, $P = 0.021$). Over all adult instars, fatty acid enrichments did not affect brood size significantly (adult instar, $F_{2,33} = 1.23$; $p = 0.305$), but there was a weak negative effect of fatty acid enrichments on mean offspring mass (adult instar: $F_{2,32} = 3.37$, $P = 0.047$). Conversely, the effect of fatty acid enrichments on brood size differed over time (adult instar × fatty acid treatment: $F_{6,99} = 2.87$, $P < 0.013$). However, this effect was mostly due to increased brood size of the forth-adult instar. The difference in offspring mass between fatty acid treatments did not increase over consecutive broods (adult instar × fat treatment: $F_{2,32} = 0.53$, $P < 0.59$). In order to assess the effects of fatty acid enrichments on the offspring, we determined offspring growth

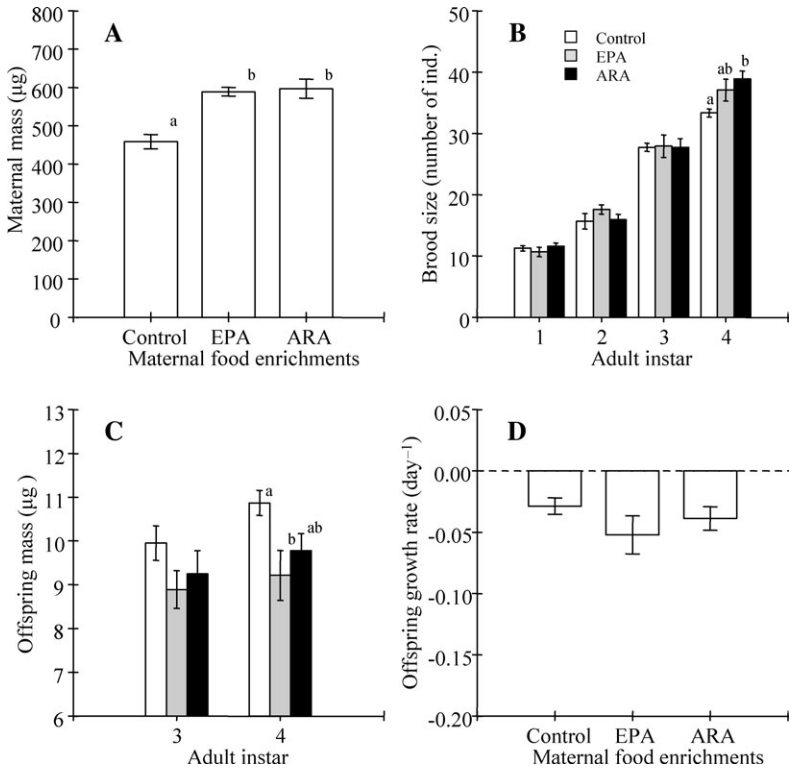

Fig. 2. Effects on *D. magna* grown on unenriched *S. obliquus* for 10 days and thereafter cultivated on *Chlamydomonas* enriched with two fatty acids and an unenriched control: (**A**) maternal mass after 15–16 days growth; (**B**) brood size in four consecutive broods; (**C**) offspring mass; (**D**) offspring growth rate for the forth instar offspring over 3 days starvation. Error bars denote ±SE for 5 (A, D) and 12 replicate samples (B, C). Identical letters denote non-significant differences among treatments (Tukey's HSD test).

rates under starvation over 3 days. All neonates had negative growth rates and there were no significant differences in growth rates between fatty acid treatments (Fig. 3; ANOVA: $F_{2,33} = 1.206$, $P < 0.312$). These results were contrary to our expectations, since forth instar control females produced offspring of a larger mass than EPA and ARA females. Since larger offspring are expected to better sustain harsh conditions (Tessier and Consolatti, 1991), the lack of significant difference in growth response during starvation may thus indicate a hidden positive effect of EFA addition (Fig. 2C and D).To assess if such a hidden effect existed, we performed a third experiment further minimizing the maternal and offspring body size differences by decreasing the enrichment period to 7 days. Over the shorter time interval, we still found that maternal mass differed across the four fatty acid treatments (Fig. 3A, ANOVA: $F_{3,12} = 15.84$, $P < 0.001$). A Tukey *post hoc* test identified that females enriched with EPA and ARA were heavier than control and ESA enriched females. On average, ARA and EPA females were 18% heavier than Control and ESA females. On the other hand, there were no significant effects of the fatty acid treatments on offspring mass (Fig. 3A, ANOVA, offspring mass: $F_{3,4} = 4.364$, $P = 0.094$). These offspring were collected for growth assays and divided over two food treatments, starved and semi-starved (0.1 mg C L^{-1}). Juvenile growth rates were affected by food concentration and maternal fatty acid enrichments (Fig. 3B, two-way ANOVA, maternal fatty acids: $F_{3,29} = 34.36$, $P < 0.001$; food concentration: $F_{1,29} = 292.43$; $P < 0.001$). The effect of maternal fatty acids enrichments on juvenile growth rates differed with food concentration (Fig. 3B, two-way ANOVA, maternal fatty acids × food concentration: $F_{3,29} = 10.70$; $P < 0.001$).

DISCUSSION

Different resources may be limiting during different life stages (Urabe and Sterner, 2001), and during times of reproduction lipids and fatty acids may be especially important (Tessier and Goulden, 1982; Goulden and Place, 1993). Especially, EFA are preferably allocated to offspring, resulting in a drain of these fatty acids from mothers into offspring (Becker and Boersma, 2005). This transport of fatty acids into the offspring had a positive effect on the ability for the offspring to sustain

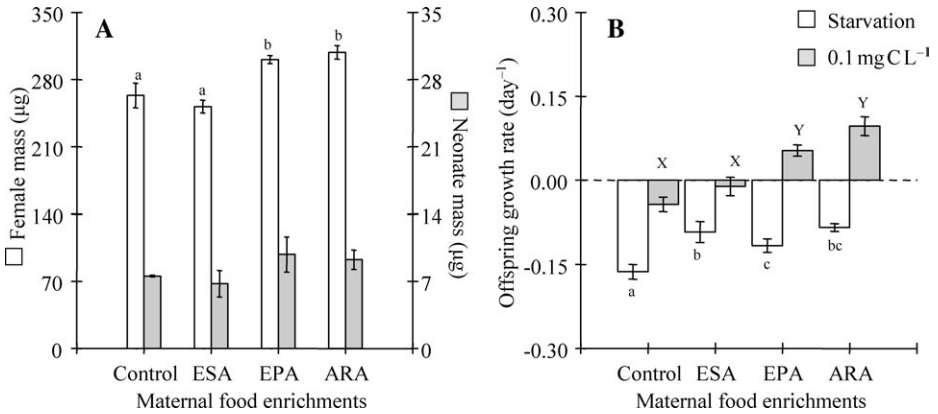

Fig. 3. Effects of maternal fatty acid enrichments on offspring: (**A**) female and offspring mass and (**B**) growth rates. Ten-day-old mature *D. magna* females were fed *S. obliquus* either enriched with ESA, EPA, ARA or Control for 7 days. Error bars denote ±SE for four and five replicate samples, respectively. Identical letters denote non-significant differences between treatments within each food source treatment (i.e. starvation and 0.1 mg C L^{-1}) (Tukey's HSD test).

poor resource quality environments (Plath and Boersma, 2001; Becker and Boersma, 2003). In the current study, we aimed to separate indirect effects of EFA enrichment (e.g. maternal size) from the direct effects on *D. magna* reproduction by minimizing differences in maternal size. Using the data from Becker and Boersma (Becker and Boersma, 2003), it was clear that there were positive relationships between female body size and brood size (Fig. 1). EPA enriched females were heavier at maturity and as expected they produced more offspring (Lampert, 1993). However, when statistically adjusted for maternal size differences, EPA addition did not have a significant effect on mean brood size (Fig. 1). Thus, body size differences, caused by EPA additions, had a considerable effect on *D. magna* reproduction, but we could not identify any direct effects of EPA on reproduction.

To minimize body size differences caused by fatty acid additions, we delayed the EFA enrichment phase until maturity in experiments two and three. Compared to our previous study (Becker and Boersma, 2003), delaying the EFA enrichment did indeed cause a decrease in the differences between the biomass of the females, but even in the third experiment, we observed that maternal mass was still significantly higher in those animals that received EFA additions compared with control animals. Obviously, the fatty acids were not primarily used as a source of energy, as in the third experiment only ARA and EPA additions resulted in heavier females. These smaller differences in maternal size between treatments yielded differences in the number of offspring produced in the second, but not in the third experiment.

The effect of the fatty acid additions on juvenile biomass, and resulting growth and survival was slightly different between the experiments. Where in the second experiment, EFA enriched offspring had a lower mass than control offspring (Fig. 2C), this was not the case when the mothers were supplied with fatty acid additions for a shorter time (Fig. 3A). To determine the consequence of these offspring quality differences, we isolated neonates from each of the treatments from the forth brood in the second experiment, and estimated their growth response during starvation. All treatments had negative growth rates and there were no significant differences between the treatments (Fig. 2D). This result was contrary to our expectations, since control neonates were on average 10–15% heavier than neonates from each of the EFA treatments (Fig. 2C), and larger offspring should sustain poor food conditions better than smaller offspring (Tessier and Consolatti, 1989). Hence, this lack of difference indicates that smaller offspring enriched with EFA sustained starvation equally well as larger offspring from control females (Fig. 2B; Fig. 2C). Adding, a non-EFA (ESA) as an energy control in the third experiment further clarified the role of fatty acids in determining the fitness of juvenile *Daphnia*. Both the essential as well as the ESA enrichments decreased the negative effects of starvation over 3 days (Fig. 3B). This, thus, indeed suggests that under starvation conditions, the fatty acids were used as an energy source and explains the results of the second experiment where the smaller total mass of the offspring was counterbalanced by the higher amounts of fatty acids in the offspring, thus making all offspring equally starvation resistant. On the other hand, for the semi-starved neonates in experiment 3, only the two PUFA supported positive growth rates whereas offspring from control and ESA females had negative growth rates (Fig. 3B). This suggests that under those conditions the fatty acids were not used solely as an energy source, but rather in their

essential capacity to support the build-up of membranes and hormones. Interestingly, the addition of the ω-6 (ARA) and the ω-3 fatty (EPA) fatty acid induced similar results which is somewhat unexpected, since these compounds cannot normally be bio-converted and thus are not substitutable resources. A possible explanation for this finding is that during our experimental conditions these two fatty acids had similar functions, for example, as structural compounds in cell membranes where they are essential to maintain membrane functions (Vance and Vance, 1985).

It is by now a generally accepted fact that EFA are a major factor determining the quality of the food for zooplankton (Brett and Müller-Navarra, 1997; Müller-Navarra *et al.*, 2000; Park *et al.*, 2003), and zooplankters that receive enough fatty acids in their food grow to larger size and produce more offspring (Becker and Boersma, 2003). This study shows that the observation that animals supplied with fatty acids produce more offspring can be contributed almost completely to the larger size of the mothers. The direct effect of fatty acid supplements seems to be mainly in the quality of the offspring. Under starvation conditions, the energy content of the fatty acids allows a higher resistance to starvation, whereas when some food is available, the PUFA regain their role as essential components used for structural growth. One consequence of our findings is that offspring size does not serve as a good estimate of offspring quality when feeding on different resource qualities, as offspring of similar size may differ considerably in quality.

So, if increased concentrations of fatty acids in the food lead to larger daphniids with offspring that are better acclimated to low food situations, we would expect that in lakes with a dominance of high-quality food both *Daphnia* densities as well as their average body size should be larger. And indeed, *Daphnia* growth is often closely linked to the availability of EFA (Müller-Navarra, 1995; Müller-Navarra *et al.*, 2000; Wacker and von Elert, 2001). However, in the field, the potential effects on *Daphnia* density and mean body size are most likely obscured by other factors such as size-selective predation that zooplankton encounter, the presence of infochemicals or simply the size and age structure of natural populations. Nevertheless, it is clear that highly unsaturated fatty acids are of vital importance for the success of herbivorous zooplankton, both as essential components of the food as well as sources for energy.

ACKNOWLEDGEMENTS

Winfried Lampert, Lena Sivars Becker, Meghan Duffy, Spencer Hall and Alan Tessier are thanked for discussions and comments. This research was partly supported by DFG Grant BO 1488/3 and 1488/5. This is contribution 1286 of the W. K. Kellogg Biological Station.

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