

Environmental stress and local adaptation in *Daphnia magna*

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Abstract

The effects of fish kairomones, crowding chemicals, and day length on the life-history traits of a set of 16 *Daphnia magna* clones, derived from four populations that differ in fish-predation pressure, were studied. Significant among-population differences were observed, the differences being in concordance with the hypothesis of local adaptation. The among-population genetic differences were not mediated through a change in response to fish kairomones, but through an overall smaller body size, smaller eggs, and a higher number of eggs in clones derived from habitats in which fish are present. Using a model, we show that the observed changes in life-history characteristics may lead to differences in fitness under different predation regimes, such that populations from habitats with fish have highest fitness under fish-predation regimes and populations without a fish background have higher fitness values under invertebrate predation regimes.

Local genetic differentiation and local adaptation have been observed in a variety of species and habitats (Endler 1986) and have led to the introduction of the ecotype concept as far back as 1922, when Turesson (1922) realized that differences between organisms from different locations must have a genetic basis and must have resulted from evolutionary differentiation within species according to habitat. Several studies have shown that also for aquatic organisms (mainly *Daphnia* species), differences in genetic structure between habitats exist (e.g., Hebert 1974; Boileau and Taylor 1994). If this genetic differentiation results from an adaptive response to local selective forces rather than stochastic events, organisms should have highest relative fitness in their site of origin, compared to other sites. The straightforward way to test this would be by means of reciprocal transplantation experiments (Ayre 1985; van Tienderen and van der Toorn 1991; Via 1994). As this is not always possible, organisms of different populations could be cultured in common environments (mimicking environmental conditions of different backgrounds), and among-population differences can be quantified. From these experiments, one can infer that local adaptation has occurred if there are significant among-population genetic differences in the traits and if there is within-population genetic variability allowing for a micro-evolutionary response of the trait value. This allows for the

exclusion of the alternative hypothesis that among-population differences are the result of chance effects such as a founder event (De Meester 1996a,b). In zooplankton populations, not many studies have gone beyond detecting among-population differences in genetic structure to actually investigate local adaptation. Of the ones that have, most focus on the response of *Daphnia* clones from different habitats to the presence of predator kairomones (Parejko and Dodson 1991; Pijanowska et al. 1993; Weider and Pijanowska 1993; De Meester 1996a), while others investigate the correlation between predation pressure of local habitats and body size of *Daphnia* populations (Leibold and Tessier 1991).

Several studies have convincingly shown that predation has a major impact on freshwater communities in general (Gliwicz and Pijanowska 1989; Carpenter and Kitchell 1993) and also on the genetic composition of *Daphnia* species (Pijanowska et al. 1993; De Meester et al. 1995). These predators can structure zooplankton populations directly through size-selective predation, with fish usually preferring larger individuals and a variety of invertebrate predators selecting for smaller sized individuals. Moreover, they can structure populations indirectly through the induction of defense mechanisms in their prey species (reviewed by Larsson and Dodson 1993; Tollrian and Harvell 1998). As a consequence, given the importance of predation and the islandlike nature of many limnetic habitats with limited gene flow between them, it is to be expected that populations of *Daphnia* are adapted to the predation pressure in their local habitat (Parejko and Dodson 1991).

In this study, we set out to contrast life-history responses in the absence and presence of fish kairomones in clones derived from locations with and without fish. As the presence or absence of fish in a certain habitat is likely to also cause other differences between these habitats, such as differences in population densities of daphnids, we additionally tested the effect of crowding on *Daphnia* life histories (e.g., Burns 1995). In temperate regions, fish-predation pressure is only important during summer. To test for a photoperiod-dependent expression of induced changes in life-history characteristics in different clones or populations studied, we also included photoperiod as a factor in our experiments.

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We tested the following hypotheses: (1) As fish mostly show positively size-selective feeding behavior, the presence of fish chemicals should lead to a decrease in size and a decrease in egg size. (2) Clones are adapted to their habitat. Populations from locations with fish should either react more strongly to the presence of fish kairomones than ones isolated from habitats without fish or should on average remain smaller both in the absence and presence of fish kairomones (constitutively defended). (3) As crowding (high densities of competitors) usually results in food stress, and assuming that daphnids use cues associated with crowding to adjust their life histories to food stress, we expect crowding chemicals to lead to an increase in egg size, which will cause an increase in starvation resistance of these neonates. Clones from fishless environments are expected to react more strongly than clones isolated from habitats containing fish, since crowding is likely to be more important in fishless habitats. Finally, (4) we expect no differences in the reaction of different clones to differences in photoperiod. However, if a short-day photoperiod is used as a cue indicating the onset of winter conditions, we expect that short-day photoperiods will result in a decreased response to the presence of fish kairomones.

Materials and methods

Clones—Clonal lineages were established from *D. magna* populations from four habitats in Germany and Belgium: two lakes with fish present and two ponds that contained no fish. From each population, we worked with four clones. The Belgium clones originated from Lake Blankaart, a shallow hypertrophic lake in which fish, including bream (*Abramis brama* L.), roach (*Rutilus rutilus* L.), rudd (*Scardinius erythrophthalmus* L.), and perch (*Perca fluviatilis* L.) are present in relatively high densities, and from Citadelpark, a fishless pond in the city of Gent. Both sets of clones have been characterized for their phototactic behavior in a previous study (De Meester 1996a). The German clones were collected from the Großer Binnensee, a shallow slightly brackish and hypertrophic coastal lake, which contains unknown densities of mainly bream, roach, and perch (Lampert 1993), and from the Rixdorfer Pferdetränke, a small eutrophic fishless pond. These clones were used previously in life-table experiments involving different levels of salinity (Teschner 1995) and fish kairomones (Teschner unpubl. data). All experimental clones originated from ephippia to ensure that all clones were genetically distinct and represented a random sample of the gene pool at the start of the growing season. Ephippia collected from a given habitat were hatched in the laboratory, except for the clones from Citadelpark, which hatched in situ. Four clones per location were randomly chosen and cultured in 1-liter jars in the laboratory for several years before the experiment was started. Although in this period, mutations will have occurred (Toline and Lynch 1994), these mutations are not expected to show any direction, especially since all clones were treated identically. Hence, potential mutations will only lead to an increase in the among-clone variability, but it is highly unlikely that the direction of the "mutational load" will be different for different populations.

Clonal diversity of the four populations under study, expressed as the Shannon-Wiener index (e^H) (Peet 1974), was variable. Values ranged from 1.65, for the Citadelpark population, through 9.11 for the Großer Binnensee and 15.21 for Lake Blankaart to 25.53 for the Rixdorfer Pferdetränke (Mitchell 1997; Vanoverbeke and De Meester 1997). These values originated mostly from one sampling date, with only three to four enzyme loci analyzed, and hence are an underestimation of the within-population genetic variability. Except for the Citadelpark population, the values are relatively high, which means that the probability that these populations have been established by small founder populations is low and hence, that the patterns observed are likely to be a result of selective processes rather than drift.

Life-table experiment—Neonates were collected from stock cultures, and 6–10 individuals were placed into 200-ml jars. These individuals, grandmothers of the experimental animals, were kept at 20°C, in a long-day photoperiod (16:8 light:dark [LD]). All animals were fed 0.8 mg C liter⁻¹ of the green alga *Scenedesmus acutus* Meyen, cultured in 3-liter chemostats on a Chu-12 medium, and medium was refreshed daily. Third brood neonates of the grandmothers were isolated and subjected to the same conditions, and they served as mothers for the experimental animals; synchronized neonates from their second brood were used in the life-table experiment.

The experimental vessels (200 ml) were stocked with seven neonates per vessel within 24 h of birth and were subjected to the following treatments in a full factorial design: fish water/nonfish water, crowded water/noncrowded water, short day/long day. Fish water was obtained from a 40-liter aquarium that contained two ide (*Leuciscus idus* L.) of approximately 5 cm in length. Water was collected daily from this aquarium, filtered through a 0.45- μ m filter, and aerated for 10 min. The aquarium was refilled with 10- μ m filtered lake water from nearby lake Schöhsee, and the fish were fed daphnids after water for the day had been taken out, to ensure that fish-conditioned water contained only fish kairomones and no other cues such as alarm substances of *Daphnia* (e.g., Pijanowska and Kowalczewski 1997). Crowded water was obtained by filtering water (0.45- μ m filter) previously inhabited by a very dense (>300 individuals liter⁻¹) population of a *Daphnia pulex* clone originating from Bergen, Norway. This density represents a density of individuals occasionally observed in nature (Barker and Hebert 1990; Kvam and Kleiven 1995; Young and Watt 1995). *D. pulex* was used for generating crowded water to avoid interference of a closer relationship of one of the experimental clones with animals that conditioned the crowded medium. Moreover, the *D. pulex* clone used has been shown to affect life histories of *D. magna* (Hobæk and Larsson 1990). Crowded cultures were resupplied daily with 0.45- μ m filtered Schöhsee water and were fed three to four times daily with *Scenedesmus*, at a concentration $>1 \times 10^6$ cells ml⁻¹. Fish media and crowded media consisted of half conditioned water and half 0.45- μ m filtered Schöhsee water; fish plus crowded water consisted of half fish and half crowded water. Half of the animals were subjected to long-day photoperiod (16:8 LD), and the other half were kept at a short-day photoperiod

(8:16 LD). Experimental animals were transferred to clean vessels and fed 0.8 mg C liter⁻¹ of algae daily.

For the first five adult instars of the animals in the experimental vessels, we measured body size on one randomly selected adult from each culture vessel, which was measured from the top of the eye to the base of the tail spine, and one individual for the first, third, and fifth adult instars from each vessel was culled to establish weight of their eggs. Only young eggs (stage 1, following Threlkeld 1979) were analyzed. The weight of eggs in the brood pouch was established by dissecting mothers and transferring the total clutch to preweighed small silver weighing boats. These were dried for 24 h at 60°C, stored in a desiccator, and weighed to the nearest 0.1 µg using a Sartorius electronic microbalance. Instar duration was estimated using developmental rates of all animals in the vessels, and the combination of development rates with clutch sizes of the different instars was used to compute the intrinsic rate of population increase, r , using the Euler-Lotka equation. In this equation, survival rates were set to one, as mortality in "well-kept" cultures is normally very low and mainly a result of handling (Vijverberg 1989). Male neonates were observed (*see also* Boersma et al. 1998), but differences between populations were significant only after the third brood and hence, not important for the computation of r .

To test whether the results observed in this study were not an effect of differences in food level as a result of different treatments, we measured dissolved organic carbon (DOC; Beckman organic carbon analyzer) content of fish-, crowded-, and fresh lake water after filtering through a 0.45-µm filter and established algal densities in vessels in which animals had been present for 24 h, using an electronic particle counter (Casy; Schärfe Systems).

This experimental setup resulted in a total of two (day length) × two (fish factor) × two (crowding factor) × four (populations) × four (clones) × two (replicates per clone) = 256 experimental vessels. Within-population interclonal differences were not the main interest of this study. Therefore, we averaged replicate values of the clones and entered these values as the lowest level of replication in the analyses of variance (ANOVAs), yielding a total of 128 observations. This averaging yields a more accurate estimator of clonal values and avoids complications of a cross-nested analysis (compare, for example, Milliken and Johnson 1984 and Neter et al. 1990 with Carvalho 1987; Parejko and Dodson 1991; Weider and Wolf 1991; Weider 1993; Young and Watt 1994; and Repka 1996). For a detailed analysis of clonal reaction norms, we refer to Boersma et al. (1998). The ANOVAs were carried out with background (fish and nonfish), country of origin (Belgium and Germany), and three treatment effects: fish factor, crowded factor, and day length as fixed main effects. Measurements on three adult instars (first, third, and fifth) were incorporated as repeated measures. One of the major differences between animals originating from different backgrounds is body size (Leibold and Tessier 1991), and our treatments were expected to affect body size as well. To correct for these differences in size when investigating other traits that are known to correlate with body size, we also analyzed the data with body length of the an-

imals as a changing covariable. The assumption of homogeneity of slopes was met in all tests.

Local adaptation describes the evolutionary change of a population to local natural selection. Selection acts on fitness. The life-history traits calculated above are most likely important components of fitness, but they do not represent fitness completely. Even the intrinsic rate of increase, r , which is often used as a synonym for fitness (e.g., Roff 1992), is not a proper fitness estimate when established from laboratory experiments, as it is calculated without natural mortality rates. Therefore, we computed intrinsic rates of population increase of the animals in the life-history experiment under different mortality schemes. If clones are locally adapted to their habitats, then animals taken from fish habitats should have highest values of r under a fish type mortality regime, and clones isolated from ponds without fish should have highest fitness when adult mortality is low. We used the simple survival-rate model of Taylor and Gabriel (1992), where survival rate, p , over an instar of duration D days is a function of length L in millimeters:

$$p = (aL + b)^{D/DAD},$$

where DAD is adult instar duration. Two mortality regimes were tested, one mimicking fish selectivity, with a decrease in survival probability with length ($a = -0.22$; $b = 1.03$), and one mimicking invertebrate selectivity, with an increasing survival probability with length ($a = 0.22$; $b = 0.22$). This was done since the absence of fish often causes an increase in densities of (negatively size-selective) invertebrate predators (Brooks and Dodson 1965; Post and McQueen 1987). These survival rates were incorporated in the Euler-Lotka equation, and r values for all clones under different predation regimes were computed. Taylor and Gabriel (1992) presented their parameters ($a = -0.18$; $b = 1.03$ for fish predation; $a = 0.18$; $b = 0.271$ for invertebrate predation) without any information on source of the parameters. We found that these parameters yielded much lower r values for the invertebrate mortality regimes. As there is no a priori reason to expect this to be the case, we modified the original parameters slightly to ensure similar average r values for both predation regimes.

Results

DOC values for fish media and crowded media were 3.05 mg liter⁻¹ and 4.25 mg liter⁻¹, respectively, whereas freshly filtered lake water contained only 1.00 mg C liter⁻¹. DOC is taken up by bacteria (Azam et al. 1983), which may in turn be taken up by daphnids (Brendelberger 1991), but it is unlikely that the observed differences in DOC content affected the quality of the food for the *Daphnia* in our experiment. Filtering through a 0.45-µm filter reduces the number of bacteria that are large enough to be taken up by daphnids to low levels (Brendelberger 1991), and bacterial densities are unlikely to recover in 24 h, the time interval between daily changes of experimental media. Therefore, although DOC contents were different between treatments, it is not likely that these directly affected food levels.

Neither fish factor nor photoperiod showed an effect on the amount of algae that disappeared in 24 h. In crowded

cultures, however, clearance rates of animals were significantly lower (about 75% of the noncrowded cultures). This observation suggests that daphnids consumed fewer algae when cultured under crowded conditions. Moreover, algal growth in crowded cultures was less: in cultures without animals, algal densities increased 50% in 24 h in cultures without crowded medium, whereas only a 10% increase was found under crowded conditions. This implies that clearance rates of daphnids under crowded conditions were even lower.

Body size—The size of the first, third, and fifth mature instars was found to be significantly affected by all main effects, except by day length (Table 1). Populations with fish (circles in Fig. 1) were smaller than populations isolated from fishless locations (triangles in Fig. 1), and animals taken from German lakes were larger than those isolated in Belgium. All populations showed a significant response to the presence of fish kairomones, resulting in smaller sizes (Fig. 1 top). No significant interaction between fish factor and background or country of origin was observed, meaning that different populations reacted in a similar manner to the presence of fish chemicals. The significant interaction between background and instar is caused by the smaller difference in body size for populations from habitats with fish and from fishless locations for the fifth adult instar.

The general reaction to the presence of crowded chemicals was a decrease in size. However, this reaction was stronger for populations from the two German locations (Fig. 1 bottom), causing a significant interaction between crowding factor and lake origin (Table 1). In fact, Tukey's honest significance test showed that the response to crowding was significant for the Rixdorf population only ($P = 0.02$). Of all the multiway interactions, only one was significant (Table 1). In all of the traits measured, all but one significant multiway interaction involved instar as an effect. This implies that the observed patterns change as an animal gets older, and in most cases, this simply means that differences between treatments became larger in older animals.

Reproduction—Egg number was significantly influenced by the presence of fish factor and crowded factor, with the presence of fish kairomones leading to a higher number of eggs and generally crowded chemicals causing a reduction in egg numbers. The presence of a significant interaction term between fish factor and instar reflects the fact that the effect of the presence of fish chemicals is stronger for older animals (Fig. 2). Again, as was the case for body size, a significant interaction between country of origin and crowded factor was observed, with no differences in egg number between crowded and noncrowded conditions for the Belgian populations, and a difference of around two eggs per brood for the German ones. To correct for differences in size of animals in different treatments, we reanalyzed the data with body length of animals as a changing covariable (Table 1). This caused the highly significant instar effect to become insignificant; i.e., differences between instars are mainly a result of differences in size. In addition, the crowding effect could be explained by differences in size only. Background and origin significantly affected egg number after correction for body size, with significantly more eggs produced by Ger-

man animals and (length adjusted) 2.5 eggs more for populations from fish backgrounds. The effect of presence of fish chemicals became even more significant, whereas the interaction between origin and crowded factors was no longer significant, indicating that for egg number, this effect is caused mainly by differences in size.

Average egg weights were significantly influenced by two main effects, background of the populations and the presence of fish factor (Table 1), with heavier eggs produced by animals taken from fish-free backgrounds (significant only for the Belgian populations) and presence of fish chemicals causing a decrease in egg size for all populations (with a stronger reaction for older females) (Fig. 3). The presence of crowded chemicals did not influence all of the populations in the same way. Hence, no significant main effect was observed. However, a significant interaction was found between lake origin and crowding, with populations originating from Citadelpark and Lake Blankaart producing smaller eggs when growing under crowded conditions, whereas populations isolated from the two other locations produced larger eggs when exposed to crowded media. This phenomenon was most pronounced in first and third adult instars and was less in the fifth instar; this resulted in a significant interaction between instar, lake, and crowded factor. Tukey's honest significance test showed that the difference in individual egg weight between crowded and noncrowded conditions was significant for the population isolated from Citadelpark only. These animals significantly decreased their egg weight when cultured in medium conditioned by a crowded *Daphnia* population (Fig. 3). All significant effects remained significant when length of animals was incorporated as a covariable, and hence, changes in body size alone could not account for the observed differences in egg weight. No significant two-way interaction between significant main effects (background and fish factor) was found (Table 1), indicating that, irrespective of background of the populations, they reacted in a similar manner to the presence of fish kairomones.

Reproductive investment is usually defined as a percentage of total energy reserve or biomass available. As we did not establish total body weight of animals at different instars, it is not possible to compute this quantity. However, under the assumption that growth in daphnids is allometric, reproductive effort can be inferred by the product of average egg weight and number of eggs, divided by length of the animals to the power 3. The reproductive effort was affected significantly by main effects lake background and fish factor. We observed higher reproductive effort for *D. magna* originating from environments with fish. Additionally, when exposed to fish factors, reproductive effort was also higher (Fig. 4). Crowding and day length had no significant effect on reproductive investment.

Age at maturity and intrinsic rate of population increase—Age at maturity was significantly affected by clonal background ($F_{1,96} = 14.2$; $P < 0.001$) and by the interaction between background and country ($F_{1,96} = 9.4$; $P < 0.01$), with fish-adapted populations showing a significantly later age at maturity than nonfish populations (Fig. 5). The effect of crowding on age at maturity approached significance ($F_{1,96} = 3.6$; $P = 0.07$), with a tendency for animals under crowd-

Table 1. Summary table of the ANOVAs, with body size, egg number, egg weight, and reproductive effort (Rep. eff.) of the first, third, and fifth instar as the dependent variables in a repeated measurement design, and origin of the lake (OR), background (BA), and fish factor (FF), crowded factor (CF), and day length (DL) as the independent variables. For the sake of clarity, only the mean squares (MSs) of the main effects and the two-way interactions are shown, as well as the significant (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) multiway interactions. Egg number and egg weight were analyzed without (no cov) and with (cov) body length as a covariable, to infer how many of the responses were primarily attributable to changes in body size. All MSs including instar effects were tested over the error (instar term (MS = 0 indicates MS < 0.5).

Effect	df	Size	Egg number		Egg weight		Rep. eff.
			no cov	cov	no cov	cov	
Background (BA)	1	15,530***	58	371***	44***	19**	20***
Lake origin (OR)	1	14,236***	39	52*	0	0	2
Fish factor (FF)	1	10,427***	70*	279***	42***	26***	19***
Crowded factor (CF)	1	4,497**	80*	5	1	1	1
Day length (DL)	1	1,113	16	2	0	0	0
Instar	2	224,446***	636***	1	157***	0	4***
BA × OR	1	12	1	0	38***	36***	0
BA × FF	1	16	5	1	1	1	0
OR × FF	1	888	40	16	1	1	0
BA × CF	1	867	0	25	1	0	2
OR × CF	1	2,086*	103*	14	20**	20**	0
FF × CF	1	42	43	38	4	3	2
BA × DL	1	11	2	9	1	1	0
OR × DL	1	33	1	1	0	0	0
FF × DL	1	4	43	27	1	1	6*
CF × DL	1	160	0	0	0	0	0
BA × instar	2	549*	5	3	2	1	0
CO × instar	2	30	5	4	2	2	2**
FF × instar	2	245	15*	13*	5*	5*	0
CF × instar	2	114	7	6	0	0	1
DL × instar	2	446*	9	3	3	1	0
BA × OR × instar	2		30**	20**	4*	4*	
OR × CF × instar	2	660**	25**	19**			
FF × CF × instar	2						2*
FF × DL × instar	2		18*	13*			
BA × FF × CF × instar	2			20**			2*
OR × FF × CF × DL	1						4*
OR × FF × CF × instar	2		16*	12*			
Error	95	409	16	10	2	2	0.9
Error (instar)	190	112	4	3	1	1	0.5

ed conditions to mature later. All other effects were nonsignificant.

Although fish-adapted populations needed a longer time to mature, this is counterbalanced by the production of more eggs. Consequently, the intrinsic rate of population increase is not significantly affected by background or by the presence of fish factor. Only the presence of crowded chemicals ($F_{1,95} = 4.3$; $P = 0.04$) caused a significant decrease in r (Fig. 5). The only other significant term in the ANOVA table was the interaction between country of origin and presence of crowded substance ($F_{1,96} = 4.2$; $P = 0.04$); all other terms were not significant.

Fitness—To test whether all of the above changes in life history could indeed be a result of local adaptation, we re-computed the values for intrinsic rate of increase under the two mortality regimes described in “Materials and methods.” For the fish-predation regime, we used life-history characteristics of animals under conditions with fish kairomones present, whereas for the invertebrate predation sim-

ulation, life histories of nonfish treatments were taken. Figure 6 shows the outcome of this simulation experiment. In an ANOVA with lake background and predation regime as main effects, only the interaction between these two was significant ($F_{1,123} = 5.33$; $P = 0.02$), indicating that populations from different backgrounds reacted differently to different mortality regimes. Indeed, under the invertebrate predator regime, r values for animals from fishless ponds were higher than those for fish-adapted populations, whereas the opposite was the case when a fishlike-predation regime was applied (Fig. 6).

Discussion

The food uptake of daphnids under crowded conditions was lower than under noncrowded conditions. The average response to the presence of crowding chemicals was to remain smaller and produce fewer eggs. No significant influence of crowding on egg size could be observed. The former two observations suggest that daphnids experience crowded

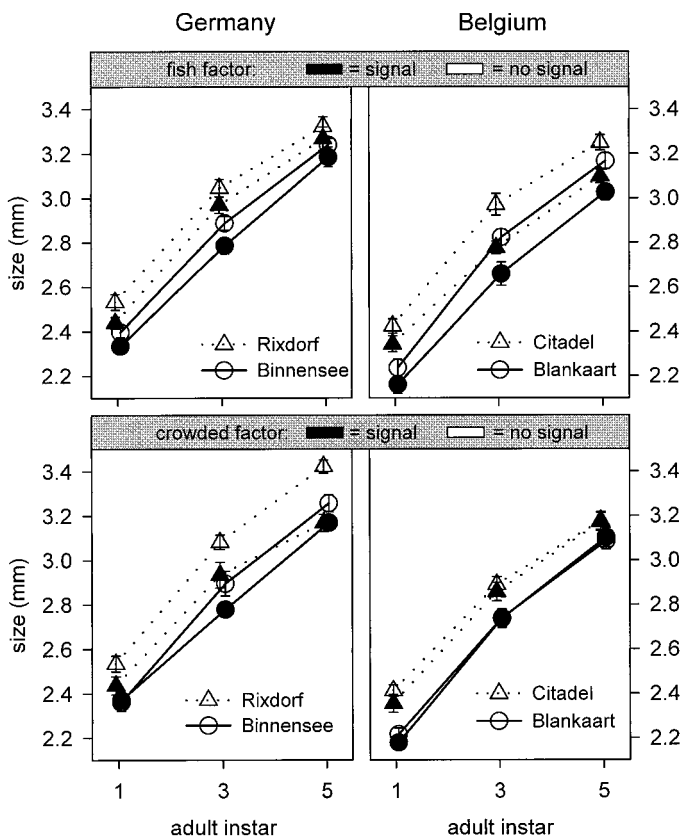


Fig. 1. Size of the first, third, and fifth adult instars of clones taken from different populations, under different conditions. Top panels: treatment with (filled symbols) and without (open symbols) fish factor; bottom panels: treatment with (filled symbols) and without (open symbols) crowded factor. Populations with a fish background (circles) and without a fish background (triangles) are shown. Error bars indicate standard errors.

conditions as conditions of food stress, although they were fed the same amount of algae. Only a few studies have tried to disentangle the direct effect of crowding from food concentration effects in the laboratory (Seitz 1984; Guisande 1993; Matveev 1993; Gosler and Ratte 1994; Burns 1995). With the exception of Seitz (1984), all of these authors reported the same pattern as found in our study: although food was abundant, animals reacted as if they were food limited. Of these authors, only Matveev (1993) measured food uptake by crowded daphnids, and similar to our observations, he reported that animals consume less when crowded than when not crowded.

Variation existed in response to crowding chemicals. Only the two German populations significantly decreased their size and the number of eggs they produced, whereas only animals originating from Citadelpark significantly changed size of their eggs. The effect of allelopathic substances released by daphnids may be different for different populations. Mitchell (1997) indeed observed that *D. magna* collected from one population (Lebrader Fischteich) were suppressed more by a crowded culture of another *D. magna* population (Rixdorfer Pferdetränke) than by a crowded culture of the same population. It remains difficult to explain,

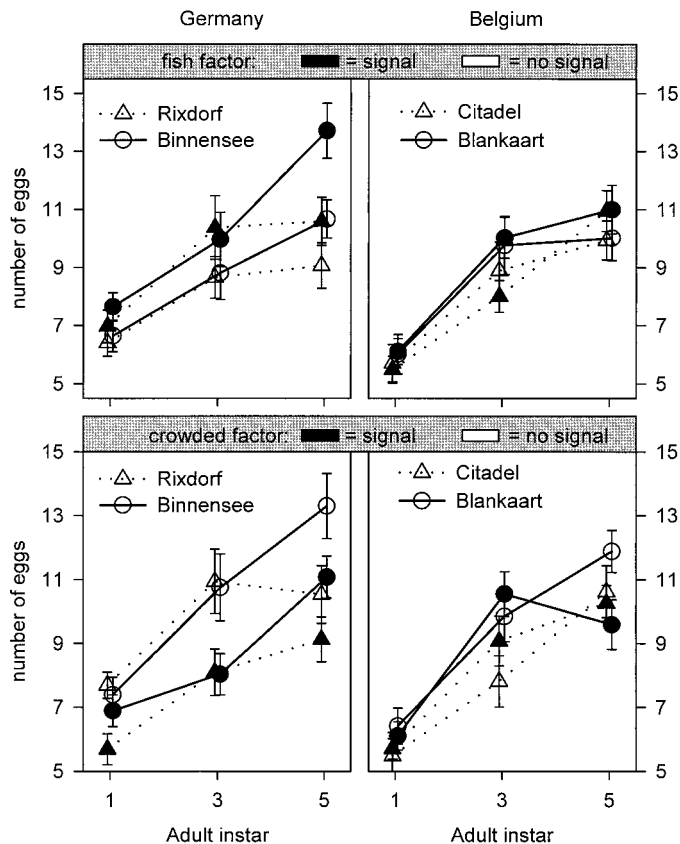


Fig. 2. Number of eggs of the first, third, and fifth adult instars of clones taken from different populations, under different conditions. Top panels: treatment with (filled symbols) and without (open symbols) fish factor; bottom panels: treatment with (filled symbols) and without (open symbols) crowded factor. Populations with a fish background (circles) and without a fish background (triangles) are shown. Error bars indicate standard errors.

however, why the Citadelpark population decreased egg weight of individual eggs when cultured in crowded medium, a response normally observed when food conditions increase. The Citadelpark pond population cooccurs with other *Daphnia* species, including *D. pulex*, during a large part of the year. This may explain why this population reacts differently to the crowding factor produced by *D. pulex* than the other populations. More importantly, however, the link between crowding and subsequent food stress is likely to be different in different habitats. The Citadelpark pond functions as a passage pond for water from a larger pond, as part of an artificial waterfall system, and hence, conditions in this pond resemble to some extent a flow-through culture, in which the continuous supply of fresh food results in an uncoupling of crowding effects and food stress. Because of the hypertrophic nature of both Blankaart and Großer Binnensee, phytoplankton densities are always high. Consequently, the link between high *Daphnia* densities and low food availability is also not strong in these lakes. In contrast to this, the Rixdorfer Pferdetränke can contain high densities of *D. magna*, leading to severe food stress in this habitat (Mitchell 1997) and hence, a tight coupling between crowding and subsequent food stress in this population. The reaction to the

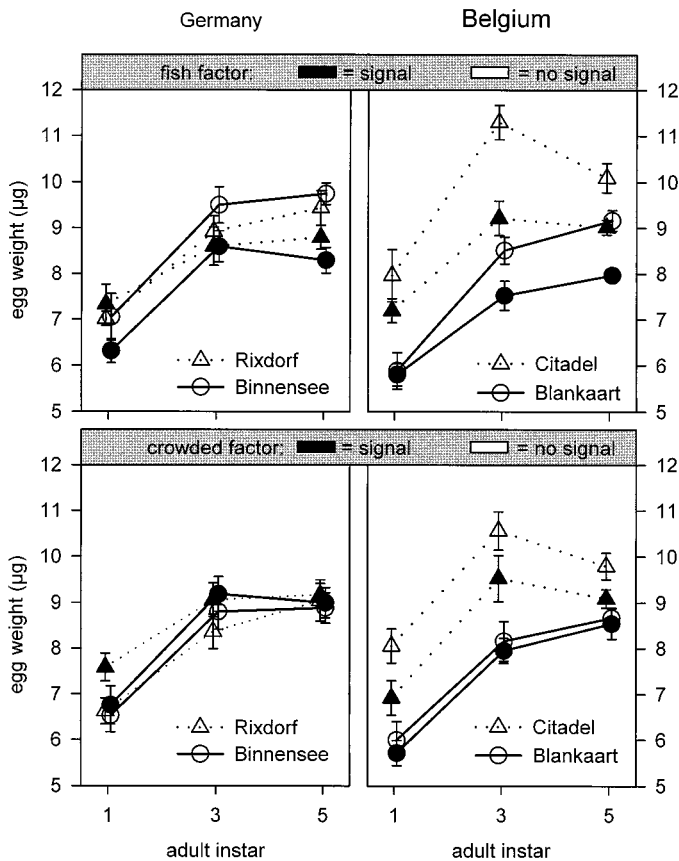


Fig. 3. Egg weight of eggs produced by the first, third, and fifth adult instars of clones taken from different populations, under different conditions. Top panels: treatment with (filled symbols) and without (open symbols) fish factor; bottom panels: treatment with (filled symbols) and without (open symbols) crowded factor. Populations with a fish background (circles) and without a fish background (triangles) are shown. Error bars indicate standard errors.

crowding factor of the Rixdorf population was indeed strong, and the direction of the response is in accordance with most other published reports. Hence, we conclude that our hypothesis, which states that populations collected from fishless habitats should respond more to crowding chemicals than populations isolated from habitats with fish, should be rejected. The interaction effect between lake origin and crowding factor might reflect differences in specific characteristics of different habitats rather than the presence or absence of fish. However, more data are needed to clarify this.

Overall, the addition of fish kairomones caused a decrease in average size, an increase in egg number, and a decrease in average weight of eggs, irrespective of the type of habitat daphnids were derived from (*see also* Weider and Pijanowska 1993). Published data on the influence of fish kairomones on intrinsic rate of increase vary between no effect (Stibor 1995) and an increase in r caused by fish chemicals (Reede and Ringelberg 1995). In this study, we observed no effect of presence of fish kairomones on intrinsic rate of population increase. However, populations taken from different backgrounds significantly differed in their r values,

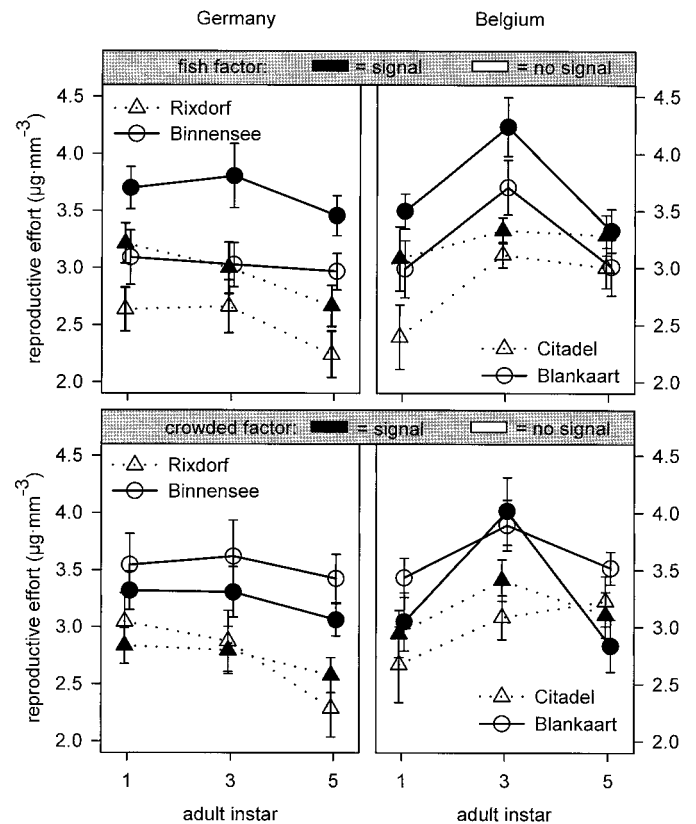


Fig. 4. Reproductive effort of the first, third, and fifth adult instars of clones taken from different populations, under different conditions. Top panels: treatment with (filled symbols) and without (open symbols) fish factor; bottom panels: treatment with (filled symbols) and without (open symbols) crowded factor. Populations with a fish background (circles) and without a fish background (triangles) are shown. Error bars indicate standard errors.

with populations taken from locations with fish showing a higher value of r . As these values were established without any mortality present, it is difficult to use them to determine whether populations are locally adapted. The results of the simulation with different mortality regimes indicate, however, that observed differences in life-history traits lead to “real” fitness differences under the two predation regimes, and hence, we conclude that populations are indeed adapted to their local habitat. Obviously, the selectivity functions we applied are an oversimplification of what happens in reality, as selectivity is not likely to depend linearly on size (Taylor and Gabriel 1992) but might also depend on availability of alternative prey (e.g., Chesson 1983). However, as it is not possible to make specific statements on field situations, we chose the simplest generalization for selective predation for this analysis.

Our results indicate that populations derived from different habitats are adapted to their local predation pressure. This adaptation consists of “doing the right thing” at all times (*see also* Leibold and Tessier 1991). The populations under study apparently do not differ in their reaction to fish kairomones. Since clones were randomly selected, originating from the ephippial bank of different populations studied,

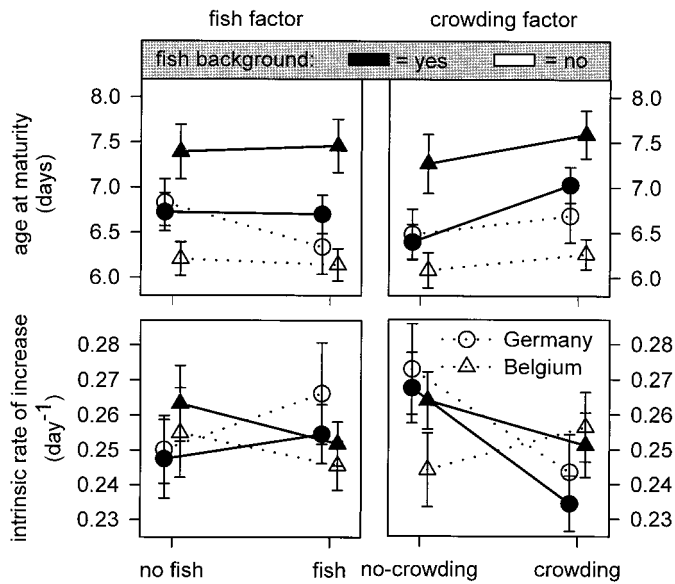


Fig. 5. Age at maturity (top panels) and intrinsic rate of population increase (bottom panels) of clones taken from different populations, under different conditions. Filled symbols indicate populations with a fish background, and open symbols indicate populations without fish present in the original location. German (circles) and Belgian (triangles) populations are shown. Error bars indicate standard errors.

our findings can indeed be used to make inferences about the degree of local adaptation of these field populations. We stress that the results obtained in our study underestimate the degree of local adaptation, since the clones are new recombination products, with a potentially lower adaptation to specific conditions of different habitats than populations that would be sampled during the growing season (e.g., Lynch and Deng 1994).

Our observation that populations derived from a given habitat tend to be adapted with respect to their life histories to the presence or absence of fish-predation pressure in the local habitat is in concordance with observations made by De Meester (1996a), who studied phototactic behavior of *D. magna* clones isolated from different populations. However, whereas De Meester observed that for phototactic behavior, clones collected from Lake Blankaart were more inducible by fish kairomones than clones originating from fishless Citadelpark pond, we observed that there were no differences in inducibility with respect to size, egg weight, and egg number between these populations but that overall size was smaller, egg weight was lower, and numbers of eggs produced were higher in populations derived from habitats containing fish. Although our results are consistent with the hypothesis of local microevolution, local adaptation is expressed as differences in overall size of animals rather than as differences in response to presence of fish kairomones. Indeed, fishless populations reacted to presence of fish-specific chemicals by becoming smaller in a way similar to that of populations isolated from habitats in which fish are present. A possible explanation for this might be that small body size has a large ecological cost (explaining why some plasticity is still retained in populations coexisting with

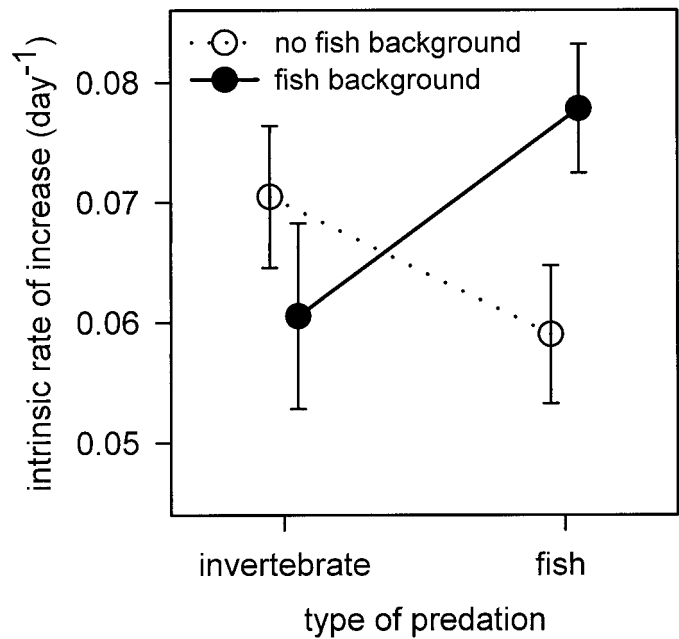


Fig. 6. Intrinsic rate of population increase of populations with a fish background (filled symbols) and without a fish background (open symbols) under two predation regimes. Analysis of the different fitnesses was carried out with the original data and hence, involved clonal reactions and not population averages. Error bars indicate standard errors.

fish), combined with the fact that the response time for life-history traits, in contrast to behavioral traits, is relatively long (explaining why fish populations have an overall smaller body size than populations from fishless ponds). Time lags may indeed be an important cost with respect to evolution of inducible defenses (Padilla and Adolph 1996). Whereas a change in behavioral traits can be induced within a few hours (De Meester and Cousyn 1997), most changes in life-history traits take at least as long as one egg development time interval (about 3 d at 20°C in *Daphnia*) (Macháček 1995) and often as long as one egg-to-egg generation time (e.g., for size at maturity). Therefore, behavioral traits would be expected to be more plastic. Important for this, however, is the time scale with which predators (fish) come and go in a given environment. If this time scale is in the order of days, then a response that takes several days to take effect would be too slow, and hence, plasticity in this trait would be pointless. As it indeed has been shown that juvenile fish can appear in the open-water zone of lakes suddenly (e.g., Ringelberg et al. 1991), the observed differences in inducibility between traits seem sensible.

As local adaptation is a trait of a population, we opted to contrast populations in the present analysis. One of our main conclusions is that all populations showed similar changes in life-history traits in response to the presence of kairomones. Earlier, we (Boersma et al. 1998) showed that the clones used in the present study react to presence of fish chemicals with different traits and that even within populations, large differences exist in type of responses exhibited by specific clones. However, this is not in contradiction with

the results presented here: when we look at the level of the population, these differences between clones are averaged out, and average response for life-history traits is similar for all populations. In his study on local adaptation in phototactic behavior in *Daphnia*, De Meester (1996a) came to the same conclusion: although the population on average exhibited differences in behavioral responses that were concordant with differences in fish-predation pressure among habitats, different populations harbored clones with widely different phototactic behavior and different responses to presence of fish kairomones.

Conclusions

The main conclusion of our experiment is that there are significant among-population differences in body size and number and weight of eggs produced by *D. magna* populations and that these differences are in concordance with the hypothesis of local adaptation. All populations respond to presence of fish kairomones by remaining smaller and producing smaller eggs. Thus, among-population genetic differences are not mediated through a change in response to fish kairomones but through an overall smaller body size and egg size in populations derived from habitats in which fish are present.

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