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Behaviour and body size: plasticity and genotypic diversity in larval *Ischnura elegans* as a response to predators (Odonata: Coenagrionidae)

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This article is dedicated to Georg Rüppell, who always gave us the opportunity and the freedom to follow up our ideas and conduct experiments within his department.

Phenotypic plasticity represents an adaptive tool in organisms including odonates to cope with heterogeneous environmental conditions. However, while some odonate species can occupy various changing habitats, other species are adapted to a narrow range of environmental conditions. Commonly, behavioural modifications are applied to avoid detection and encounters with predators. But reduced behavioural activity results in decelerated growth and reduced body size, a key fitness attribute in odonates. Using larval *Ischnura elegans* we quantified predator induced plastic behavioural reaction norms in order to manifest variance, and by this evolvability of larval behavioural plasticity. In addition we test for potentially underlying genetic correlations of behavioural traits with body size. Our results show that there is large genotypic variance in plastic reaction norms. Furthermore, no present genetic constraints between behaviour and body size were detected, suggesting potential for independent optimisation of behaviour and body size across environments. Our data indicate that independent phenotypic plasticity in behaviour and body size might enable species to occupy a wide range of environmental conditions.

Keywords: body size; damselfly; dragonfly; inducible defence; genetic correlations; life history; phenotypic plasticity; antipredator behaviour

Introduction

Predators represent a major threat across different habitats, shaping appearance as well as distribution of their prey and structuring communities (Barbosa & Castellanos, 2005; Benard, 2004; Kerfoot & Sih, 1987). Because predators are such a strong selective force, prey species segregate along habitat gradients defined by the presence of different top predators, with each habitat type showing a characteristic prey assemblage (Wellborn, Skelly, & Werner, 1996). An example of an aquatic habitat with such a predator gradient is permanent ponds being either dominated by predatory fish or large invertebrate predators like dragonfly larvae (Johansson &

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Mikolajewski, 2008; McPeek, 1990a; Wittwer, Sahlen, & Suhling, 2010). Within odonates, well-studied examples of species distribution depending on the appearance of top predators are the genera *Enallagma*, *Lestes* and *Leucorrhina*. A certain species assemblage inhabits each pond type, with species expressing characteristic phenotypic adaptations in behaviour, morphology, physiology and life history as an evolutionary response to the top-predation regime (McPeek, 2004; Mikolajewski & Johansson, 2004; Mikolajewski, Johansson, Wohlfahrt, & Stoks, 2006; Mikolajewski et al. 2010; Stoks & McPeek, 2006).

In contrast, some species, e.g. *Ischnura* spp. or *Pachydiplax longipennis*, can occur in both habitat types seemingly unaffected by the difference in predator-induced selection (Gyssels & Stoks, 2005; Hopper, 2001; McPeek, 1998). Phenotypic plasticity, the ability of a genotype to express at least two different phenotypes depending on environmental conditions (Pigliucci, 2001), poses one option to deal with changing predation regimes. If plastic phenotypes meet or are close to a fitness optimum in each environment such adaptive changes would facilitate species to successfully occur in both habitat types. In odonates, adaptive phenotypically plastic behaviour represents an effective way to successfully occupy different predator dominated habitats (e.g. Gyssels & Stoks, 2005; Hopper, 2001; Wohlfahrt, Mikolajewski, Joop, & Suhling, 2006). Further, variation in plasticity among genotypes within a population would indicate evolvability of plasticity per se (Pigliucci, 2005). Few studies investigated variation in plasticity among genotypes in odonate behaviour (but see Brodin & Johansson, 2004).

Plastic behavioural modifications represent the fastest adaptation to new environmental conditions (Sih, 2004). Within the broad diversity of different behavioural features in animals, foraging activity is one of the most well-studied behavioural traits, as it mediates food intake and thus energy gain. The foraging-mediated resource acquisition/mortality trade-off is well established (reviewed in Benard, 2004). Since high foraging activity is directly linked to more encounters with food and by this growth, but also increased predation rates, a reduction in foraging in the presence of predators thus results in reduced mortality but probably also decreases growth and body size (Abrams & Rowe, 1996). Being smaller comes with significant cost, since body size in most insects, including odonates, represents an important fitness component (Anholt, 1991; De Block & Stoks, 2005; Sokolovska, Rowe, & Johansson, 2000). However, while there is proof of a positive correlation between foraging activity and body size in some odonate species (e.g. Brodin & Johansson, 2004), no such link was present in others (e.g. McPeek, 2004; Suhling, Sahlen, Kasperski, & Gaedecke, 2005). This indicates differences in the relationship of foraging and body size in different odonate species. Strong genetic links of foraging behaviour across environments as well as between foraging and body size within environments might substantially complicate the optimisation of both traits. Therefore, we hypothesise that genetic correlations of foraging behaviour across environments and between foraging activity and body size within environments might be particularly uncommon in highly plastic specimens.

In the current study, we investigate larval behavioural reaction norms in the absence/presence of predatory fish and large odonate predators using *Ischnura elegans* (Vander Linden, 1820) as a model system. We use a genotype approach to quantify behavioural reaction norms to be able to manifest variance of the slope and by this evolvability of larval behavioural plasticity. In addition we test for potential underlying genetic correlations of behavioural traits across environments and of foraging behaviour with body size. A lack of strong across-situation genetic correlations of foraging and within-situation correlations of foraging and body size would indicate independent adaptive evolution of foraging behaviour and body size in response to changes in predation regime. In contrast, strong across-situation genetic correlations between behaviour and body size would indicate constraints on the independent optimisation of foraging behaviour and body size, since, for example, a reduction in foraging behaviour would inevitably entail a reduction in body size.

Materials and methods

Study organism and animal collection

Ischnura elegans is an abundant and widespread damselfly species across central, eastern and southern Europe (Dijkstra, 2006). The species occupies a wide range of habitats from running to standing water, while not showing distinct habitat preferences, developing in a wide range of salinity, flow velocity, water size and predation regimes (Sternberg, 1999). In central Germany, *I. elegans* starts emerging from the end of April onwards, and is on the wing until the end of September (Sternberg, 1999). Eggs are pierced into soft plant tissue, larvae hatch ~ 3 weeks later, depending on the temperature, and most individuals emerge the following year (Sternberg, 1999).

On 1 August 2003, we collected 20 egg clutches of *I. elegans* near Braunschweig, Germany (52°19′N, 10°27′E) following the method described by Mikolajewski, Brodin, Johansson, and Joop (2005). Several small fishless ponds, larger lakes containing predatory fish, as well as the River Oker dominate the immediate vicinity, with *I. elegans* breeding in each of those habitats. This way we guaranteed sampling from a population that occurs in a spatial heterogeneous predator environment, containing individuals from different developmental backgrounds (e.g. from ponds and lakes with and without predatory fish). Since spatially heterogeneous environments with smaller scale than the dispersal distance are likely to select for phenotypic plasticity (Baythavong, 2011), we increased chances for plasticity to be present in the studied population. We only caught couples right before completion of copulation, nearly ensuring that we collected full sibling (sib) offspring. Because females can store sperm from previous matings and males do not remove the entire previous sperm during copulation, we cannot fully exclude the possibility that a few offspring of certain females represent paternal half-sibs. But since large proportions of eggs become fertilised with the sperm of the last male, the proportion of paternal half-sibs is expected to be very small (Cooper, Miller, & Holland, 1996). Egg clutches were brought to a controlled temperature room at 21 °C and a day/night light cycle of 17/7 h corresponding to the natural photoperiod of early August in central Europe. Light regime was adjusted weekly to follow progress in the natural light regime (Mikolajewski, De Block, & Stoks, 2015). Egg clutches were kept individually in opaque 20 × 15 cm plastic container (height 10 cm) filled up to 5 cm with de-chlorinated tap water. One egg clutch did not show any sign of development and was discarded from the experiment.

Larvae hatched about three weeks later and were separated in groups of 20 in similar containers as above, each containing eight strips of plastic gauze (2 × 2 cm) as perching substrate. We fed larvae *ad libitum* with laboratory reared *Artemia* nauplii twice daily. Keeping larvae in low-density groups after hatching is known to reduce larval mortality in the critical first days of development (De Block & Stoks, 2003).

Experimental design

On 5 September 2003 larvae of each female were randomly allocated to the experimental treatments. The experiment took place in nine 150 × 50 cm (height: 50 cm) aquaria filled up to 40 cm with dechlorinated tap water. In each of three aquaria we either introduced one perch (*Perca fluviatilis*) of ~ 15 cm body length (treatment fish) or 10 last stadium larval aeshnids (*Aeshna cyanea*) (treatment aeshnid). Both species are known to represent severe predators for larval *Ischnura* (e.g. Stoks, Swillen, & De Block, 2012; Van de Meutter, De Meester, & Stoks, 2005). The last three aquaria served as a control without any predator cues (control treatment). Separated by females, we placed larval *I. elegans* in groups of 10 individuals into opaque plastic vials

(8.5 × 8.5 cm, height: 11 cm) with a volume of 1 l. This density falls within the natural occurring densities of *I. elegans* (Suhling et al., 2000). For perching, each vial contained 10 stripes of 5 × 5 cm plastic gauze. Vials floated at the water surface being stabilised with a ring of Styrofoam. All vials had walls with 5 × 5 cm mesh windows (mesh width: 500 µm) to ensure water exchange between the aquaria and the vials. Each aquarium contained a single vial with larvae of each 19 females. Predators were fed daily *ad libitum* with larval chironomids, and larval *I. elegans* were fed *ad libitum* daily with laboratory reared *Artemia* nauplii.

Response variables

For all behavioural measurements we determined larval head width as a measurement of body size after each trial to correct for size differences in subsequent statistical analyses (see below). Larval head width was measured using a dissection microscope.

Larval activity

In the first week of November 2003 (larval stadium F-2/F-3) and first week of March 2004 (larval stadium F-0) we measured larval activity by monitoring the number of position changes of each larva individually within a fixed time following the protocol of Mikolajewski et al. (2005). In brief, trials were conducted in nine plastic aquaria (9 × 16 cm, height: 10 cm) filled with 600 ml water from tanks containing perch, larval aeshnids or control dechlorinated tap water. This way, the larvae of *I. elegans* could still receive chemical cues by predators but were not disturbed by foraging predators. The bottom substrate consisted of a 0.5 cm layer of fine sand. Larvae did not receive any food during trials. For recording position changes, a transparent film with a 1 × 1 cm grid pattern was placed on top of each aquarium. Larval activity was only tested for their respective treatment and of each vial larval activity of four randomly chosen larvae was recorded. Before trials started larvae were given one hour to acclimatise. Position change was recorded every 10 min for 2.5 h, giving a maximum number of 15 moves. Position change was defined as larvae having moved from one grid to another, at which head location was determined. Note that number of position changes is positively correlated to distance moved in odonate larvae (Johansson, 2000).

Larval escape distance

In the second week of December 2003 (larval stadium F-1/F-2), we measured escape distance of four larval *I. elegans* of each vial following the protocol of Mikolajewski et al. (2006). Larval damselflies usually evade an attacking predator by swimming away fast. Experimental trials took place in an opaque plastic box (30 × 20, height: 5 cm) filled with dechlorinated tap water to a height of 3 cm. Larval were placed individually in the plastic box and we waited until the larvae had settled. A predator attack was simulated by gently touching the end of the larval abdomen with a pair of tweezers. The swim distance was instantly measured using a ruler. Maximum escape distance measured in our experiment was 17 cm.

Larval foraging behaviour

In the first week of February 2004 (larval stadium F-0/F-1) we recorded foraging behaviour in one larva of each vial following the protocol of Mikolajewski, Wohlfahrt, Joop, and Beckerman (2013). Trials took place in the same plastic aquaria and under conditions as described above for larval activity. However, this time 20 daphnids were added as food and each larva was

observed for 30 min. We recorded nine behavioural variables: *time walking*, time spent walking not directly orientated towards prey; *number walking*, number of walks not directly orientated towards prey; *time swimming*, time spent moving in the water column; *number of swims*, number of moves in the water column; *number orient*, number of times larva moved its head towards prey without moving legs; *time advancing*, time spend advancing towards prey moving at least one leg; *number advancing*, number of advances towards prey; *number strike*, number of times a larva struck with its labium towards prey; *number capture*, number of times a larva successfully caught a daphnid.

Larval body size

On 8 March 2004 larvae were collected and final body size was measured. Note that at this point all larvae had reached the final stadium F-0. We monitored head width and dry body mass as estimates for body size, because both can differ in their response to environmental constraints (Strobbe & Stoks, 2004). Head width of each larva was measured using a dissection microscope and dry body mass was determined after placing individuals at 60 °C for 48 hours in a drying oven. Dry body mass was measured to the nearest 0.001 mg using a Sartorius scale.

Data analyses

All analyses were conducted using the open source program R 3.1.1 (R Core Team, 2014). In all behavioural analyses we used current larval head width as a continuous fixed effect in the models to control for body size differences among larvae. For all models (see below) we used type II sum of squares with Wald χ^2 statistic to test for any fixed effects (package car version 2.0-21; Fox and Weisberg, 2013). Presented data in Figures 1–4 show least-squares means from each full mixed effect model (see below) (package lsmeans 2.11; Lenth, 2013).

Larval activity

Repeated measure data on larval activity (position changes) were analysed using a generalised linear mixed effect model (package lme4 version 1.1-7; Bates, Maechler, Bolker, & Walker, 2013) using binomial error distribution due to the fact that maximum number of position changes
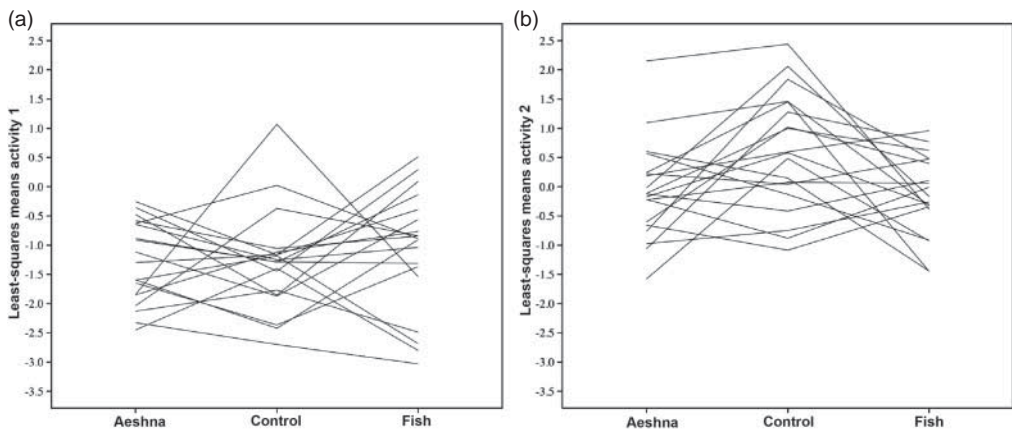

Figure 1. Reaction norms (full sib means) in the presence of predatory fish (*Perca fluviatilis*), aeshnid larvae (*Aeshna cyanea*) and control treatment for early larval (A) and late larvae (B) activity in *Ischnura elegans* (see methods). Least-squares means from a full statistical model are presented (see methods).
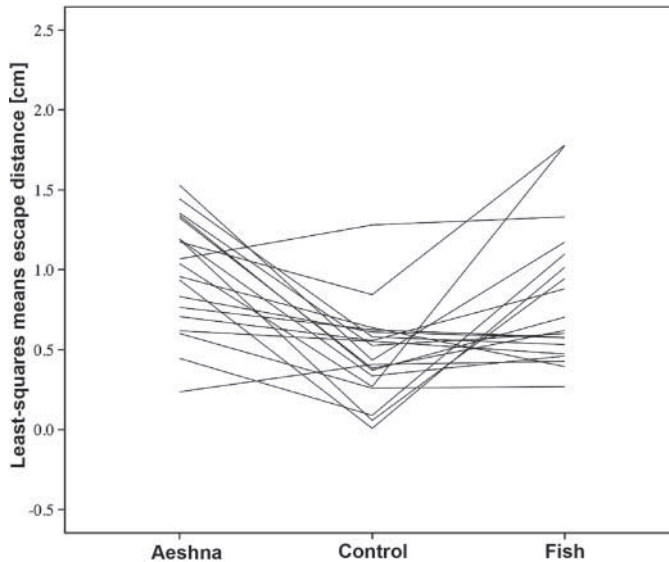
Figure 2.   Reaction norms (full sib means) in the presence of predatory fish (*Perca fluviatilis*), aeshnid larvae (*Aeshna cyanea*) and control treatment for escape distance [cm] in *Ischnura elegans*. Least-squares means from a full statistical model are presented (see methods).

was limited to 15 (see above). As fixed effects we fitted predator treatments (fish/aeshnid/control) and female identity. As random effects we fitted the aquarium the larva came from as well as time of measurement due to the repeated nature of our measurements. Note that we did not fit separate slopes for individual larva due to a lack of power in our data set.

Larval escape distance/larval foraging behaviour/larval body size

The nine measurements on larval behavioural traits were first reduced using principal component analysis (PCA) of the correlation matrix on the $\log(n + 1)$ transformed variables (package psych 1.4.8.11, Revelle, 2014). PCA extracted three principal components with an eigenvalue > 1 explaining 76% of the variance (PC1: 33%, PC2: 22%, PC3: 21%). Behavioural traits associated with activity loaded high on PC1 (from now on PC activity) (time walking $= 0.80$, number walking $= 0.86$, time swimming $= 0.87$, number of swims $= 0.89$). Behavioural traits associated with foraging loaded high on PC2 (from now on PC foraging) (number orient $= 0.73$, number strike $= 0.79$, number capture $= 0.78$), whereas behavioural variables associated with advancing toward prey loaded high on PC 3 (from now on PC advancing) (time advancing $= 0.95$, number advancing $= 0.92$). The scores of PC activity, PC foraging and PC advancing were used in subsequent analyses.

Data on escape distance, PC activity and dry mass were $\log(n + 1)$ transformed to fulfil normal distribution of residual errors. All data on escape distance, PC activity, PC foraging, PC advancing, dry mass, and head width were analysed using linear mixed effect models (package lme4 version 1.1-7, Bates et al., 2013) with predator treatment (fish/aeshnid/control) and female identity as fixed effects and the aquarium larvae were collected from as random effect.

To evaluate the presence of genetic correlations of behavioural variables and body size we calculated Pearson product–moment correlations. Because we used full-sibs, these represent broad-sense genetic correlations (Brodin & Johansson, 2004; Strobbe & Stoks, 2004). We calculated genetic correlations (1) between the trait means of each full-sib family across each of

the three environments for behavioural traits with body size (control/aeshnid/fish); and (2) across treatments for each behavioural trait and body size (control–aeshnid; control–fish; aeshnid–fish). We applied two-sided tests with *p*-values indicating if the genetic correlation differs from zero. The *p*-values were Holm adjusted for multiple testing.

Result

Larval activity

In general, sibs from different females differed in their activity (female: Wald $\chi^2 = 32.265$, df $= 19$, $p = 0.029$) and activity increased with time (time: Wald $\chi^2 = 3.906$, df $= 1$, $p = 0.048$) (Figure 1A, B). Note that the increase in activity with time was most pronounced in the control (ls means $\pm$ SE: activity 1 $= -1.393 \pm 0.191$; activity 2 $= 0.585 \pm 0.222$) and less in the aeshnid (ls means $\pm$ SE: activity 1 $= -1.338 \pm 0.194$; activity 2 $= -0.141 \pm 0.215$) as well as fish treatments (ls means $\pm$ SE: activity 1 $= -1.205 \pm 0.196$; activity 2 $= -0.194 \pm 0.229$) (treatment $\times$ time: Wald $\chi^2 = 6.500$, df $= 2$, $p = 0.039$). This resulted in a pattern of early larval activity being similar among treatments whereas late larval activity was lower in the predator treatments than in the control treatment (Figure 1A, B). In general, sibs responded differently to treatments (treatment $\times$ female: Wald $\chi^2 = 66.787$, df $= 38$, $p = 0.003$) and sibs' response changed over time (treatment $\times$ female $\times$ time: Wald $\chi^2 = 76.004$, df $= 38$, $p = 0.002$). Whereas some sibs reduced activity in the presence of a predator, others increased activity and some showed no plasticity at the first activity measurement (Figure 1A), no sibs increased activity in the presence of predators at the second activity measurement, but either decreased activity or showed no plasticity in larval activity (Figure 1B). No other significant main effects or interactions were detected (all $p > 0.314$).

Larval escape distance

In general, sibs differed in the escape distance (female: Wald $\chi^2 = 45.200$, df $= 18$, $p < 0.001$), and escape distance was larger in individuals from the aeshnid (ls means $\pm$ SE $= 0.987 \pm 0.089$) and fish treatment (ls means $\pm$ SE $= 0.823 \pm 0.092$) than from the control treatment (ls means $\pm$ SE $= 0.465 \pm 0.090$) (Wald $\chi^2 = 11.543$, df $= 2$, $p = 0.003$) (Figure 2). However, this response differed among sibs (female $\times$ treatment: Wald $\chi^2 = 71.786$, df $= 36$, $p < 0.001$) with some sibs expressing a long escape distance to both predator treatments, some just increasing escape distance in the face of predatory aeshnids or fish and others not differing among all three treatments (Figure 2).

Larval foraging behaviour

Individuals did not differ comparing aeshnid (ls means $\pm$ SE: PC activity $= -0.399 \pm 0.175$; PC foraging $= -0.162 \pm 0.122$; PC advancing $= -0.180 \pm 0.120$), fish (ls means $\pm$ SE: PC activity $= -0.089 \pm 0.161$; PC foraging $= 0.010 \pm 0.130$; PC advancing $= 0.138 \pm 0.128$) and control treatment (ls means $\pm$ SE: PC activity $= -0.507 \pm 0.158$; PC foraging $= 0.163 \pm 0.124$; PC advancing $= 0.096 \pm 0.121$) for PC activity (treatment: PC activity $=$ Wald $\chi^2 = 0.045$, df $= 2$, $p = 0.978$), PC foraging (treatment: Wald $\chi^2 = 3.092$, df $= 2$, $p = 0.213$) and PC advancing (treatment: Wald $\chi^2 = 1.942$, df $= 2$, $p = 0.379$). However, sibs differed in their response to predators for PC activity, PC foraging and PC advancing (treatment $\times$ female: Wald $\chi^2_{(PCactivity)} = 56.504$, df $= 36$, $p = 0.016$; Wald $\chi^2_{(PCforaging)} = 56.375$, df $= 36$, $p = 0.017$; Wald $\chi^2_{(PCadvancing)} = 63.239$, df $= 36$, $p = 0.003$). Again, some sibs decreased, some
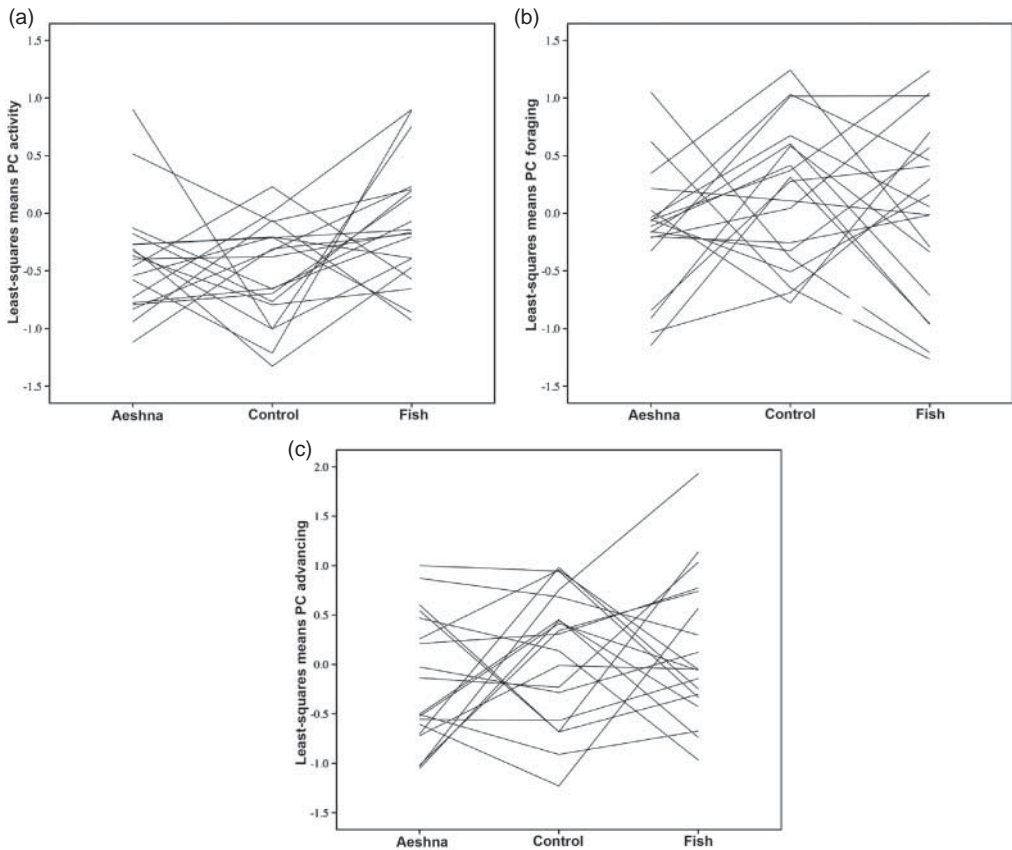
Figure 3.   Reaction norms (full sib means) in the presence of predatory fish (*Perca fluviatilis*), aeshnid larvae (*Aeshna cyanea*) and control treatment for foraging behaviour in *Ischnura elegans*. Note that PCactivity, PCforaging and PCadvancing are scores from a principal component analysis (PCA) (see methods). Least-squares means from a full statistical model are presented (see methods).

increased and others did not change any of their behaviours among the treatments (Figure 3A–C). No other significant effect was detected (all $p > 0.051$).

Larval body size

In general, body mass was lower in individuals from the aeshnid treatment (ls means $\pm$ SE $=$ 1.834 $\pm$ 0.031) compared to fish (ls means $\pm$ SE $=$ 1.876 $\pm$ 0.033) and control treatment (ls means $\pm$ SE $=$ 1.880 $\pm$ 0.032) (treatment: Wald $\chi^2 = 8.826$, df $= 2$, $p = 0.012$) and sibs differed in their body mass (female: Wald $\chi^2 = 44.202$, df $= 18, p < 0.001$) (Figure 4A). However, sibs differed in response to treatments in their larval body mass (female $\times$ treatment: Wald $\chi^2 = 62.164$, df $= 36$, $p = 0.004$). Whereas some sibs had a lower body mass in the presence of predators, others did not show any change or even increased body mass (Figure 4A).

In contrast, head width was kept constant comparing aeshnid (ls means $\pm$ SE $=$ 3.348 $\pm$ 0.024), fish (ls means $\pm$ SE $=$ 3.387 $\pm$ 0.025) and control (ls means $\pm$ SE $=$ 3.373 $\pm$ 0.024) treatments (treatment: Wald $\chi^2 = 5.925$, df $= 2$, $p = 0.052$), but sibs did differ in head width (female: Wald $\chi^2 = 44.202$, df $= 18, p < 0.001$) (Figure 4B). Furthermore, sibs also did not differ in head width across treatments (female $\times$ treatment: Wald $\chi^2 = 37.030$, df $= 36$, $p = 0.421$).
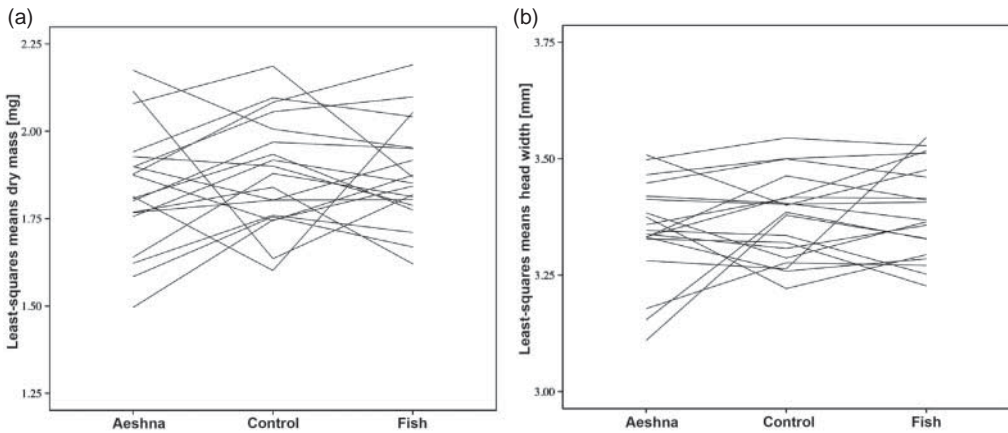
Figure 4.   Reaction norms (full sib means) in the presence of predatory fish (*Perca fluviatilis*), aeshnid larvae (*Aeshna cyanea*) and control treatment for larval body mass (dry mass) and head width in *Ischnura elegans*. Least-squares means from a full statistical model are presented (see methods).

Table 1.   Genetic correlations of behavioural traits with dry mass as well as larval head width within predation (control/aeshnid/fish) treatments in larval *Ischnura elegans*. Sibs mean genetic correlations are presented. None of the coefficients differs from zero (all $p > 0.100$). Note that PC activity, PC foraging and PC approaching are extracted components from a principal component analysis (see the methods section).

	Dry mass			Head width		
	Control	Aeshnid	Fish	Control	Aeshnid	Fish
Activity 1 (control/aeshnid/fish)	0.12	0.28	0.17	−0.16	0.16	0.11
Activity 2 (control/aeshnid/fish)	−0.02	−0.21	−0.31	−0.10	0.11	−0.06
Escape distance (control/aeshnid/fish)	0.36	0.19	0.09	0.17	0.23	0.06
PC activity (control/aeshnid/fish)	0.09	−0.18	0.15	−0.05	−0.31	0.03
PC foraging (control/aeshnid/fish)	0.06	−0.20	0.02	0.13	−0.25	−0.26
PC advancing (control/aeshnid/fish)	−0.17	−0.39	0.14	−0.18	−0.28	0.09

Genetic correlations between behavioural traits and body size

The signs of the estimated genetic correlations between each behavioural trait and body size surrogate differed among treatments (Table 1). There was also no constant positive or negative link between behavioural traits and dry mass or head width within each treatment (Table 1). Further, we did not detect any significant genetic correlations between behavioural traits and body size (Table 1).

Signs of the estimated genetic correlations within behavioural traits and body size across treatments differed between traits and treatment combinations (Table 2). Overall, we did not detect significant genetic correlations across treatments, except for head width between the control and fish treatment and for early larval activity between aeshnid and fish treatment (Table 2).

Discussion

Studying environmentally induced reaction norms represents a compelling approach to understand the potential evolvability of plasticity and adaptation of organisms (Pigliucci, 2005). It may add to our understanding of why some species seem to be able to occupy heterogeneous habitats,

Table 2.   Genetic correlations of behavioural traits, dry mass and larval head width across treatments (control–aeshnids; control–fish; aeshnids–fish). Coefficients that differ from zero are indicated in bold ($p < 0.05$). Note that PC activity, PC foraging and PC approaching are extracted components from a principal component analysis (see the methods section).

	Control–aeshnid	Control–fish	Aeshnid–fish
Activity 1	0.10	0.13	**0.71**
Activity 2	0.36	0.08	–0.23
Escape distance	0.13	0.14	0.23
PC activity	–0.23	–0.23	–0.03
PC foraging	–0.13	0.08	–0.25
PC approaching	0.04	–0.03	–0.14
Dry mass	0.38	0.41	0.35
Head width	0.43	**0.59**	0.49

whereas others are adapted to a narrow range of environmental conditions. This is, to our knowledge, one of the few studies focusing on reaction norms in Odonata (but see Johansson, 2002 for morphology; Brodin & Johansson, 2004 for behaviour; and Shama, Campero-Paz, Wegner, De Block, & Stoks, 2011; Strobbe & Stoks, 2004 for life history). Our results show that there is large phenotypic variance among sibling plasticity. Further, no present genetic constraints of behaviour and body size were detected, suggesting potential for independent optimisation of behaviour and body size across environments.

A common response to the presence of any predator is a decrease in activity and foraging, since this should result in fewer encounters with predators and consequently reduced mortality rate (Lima & Dill, 1990), but also translate into reduced encounters with food and by this growth and size (Gerritsen & Stickler, 1977). Many odonate species indeed reduce activity and foraging in the presence of predators (e.g. Johansson, Stoks, Rowe, & De Block, 2001; Mikolajewski et al. 2005; Mikolajewski, Joop, & Wohlfahrt, 2007; Stoks & Johansson, 2000). Our data reveal that larval *I. elegans* overall reduce activity in later instars in response to predators, whereas in earlier instars larvae did not differ in activity comparing treatments. Schaffner and Anholt (1998) have shown a similar reduction of larval *I. elegans* in activity to avoid aeshnid predators. Interestingly, activity was overall higher in last compared to earlier stadia independent of treatments. Differences in last instar activity among treatments stemmed from stronger increasing activity in the control than the predator treatments. Such differentiation only in later instars has been previously shown in another odonate species (Wohlfahrt, Mikolajewski, Joop, & Vamosi, 2007). Perch and last stadium aeshnid larvae preferentially select large prey. (Corbet, 1999; Mehner, Plewa, Hulsmann, & Worischka, 1998), reducing the risk of mortality in earlier, smaller developmental stadia. Therefore, an adaptive reduction in activity seems only to be essential in larger larvae. However, we do not know why we did not detect a similar overall response to the presence of predators in the foraging behaviour. Larvae were large enough to be preyed upon by the two predators.

In contrast, different sibs express a wide behavioural response to the presence of both predators by either increasing or decreasing activity and foraging behaviour. This may represent two different strategies being present among sibs: avoiding predation by reducing activity and foraging but paying the costs of decreased growth and body size; or increasing activity and foraging with the correlated higher risk of dying, but faster growth and the ability to complete development earlier (Mikolajewski, Johansson, & Brodin 2004; Wohlfahrt et al., 2006). Such deceleration (Schaffner & Anholt, 1998) as well as acceleration (Slos & Stoks, 2006; Stoks et al., 2012) of growth rate in response to predatory invertebrates and fish has been documented.

Larval *I. elegans* also responded to both predators by swimming away a longer distance than in the control treatment. However, escape distance did not differ comparing aeshnid and fish

treatments. Former results showed larval *I. elegans* to be able to distinguish between the two predators in their antipredator response (Gyssels & Stoks, 2005). But escape distance did not differ among *I. elegans* larvae in an experiment comparing predatory aeshnids and fish (Gyssels & Stoks, 2005). Larval *I. elegans* seemingly try to escape from both predators by quickly escaping a shorter distance followed by hiding in submerged vegetation or detritus (Mikolajewski & Rolff, 2004). Similar to activity and foraging behaviour, escape distance showed genetic as well as plastic diversity. Burst swimming away after a predator attack is a common response by odonate larvae (McPeek, 1990b; Mikolajewski et al., 2010). However, in contrast to anisopteran larvae (Mikolajewski et al., 2010), this evasive behaviour in damselfly larvae is most often in response to predatory invertebrates, because larvae cannot outcompete pursuing fish (McPeek, 1990b). Gyssels and Stoks (2005) stated that larval *I. elegans* frequently apply thanatosis in the event of a predatory fish attack rather than escape swimming or rely on crypsis (McPeek, 1990b).

Body size represents an important attribute defining fitness in odonates (Anholt, 1991; De Block & Stoks, 2005; Sokolovska, Rowe, & Johansson, 2000). However, body size surrogates do differ in their response to environmental constraints (Strobbe & Stoks, 2004). Because head width is fixed in insects after metamorphosis, odonates try to keep head width constant and optimise it during larval development (Strobbe & Stoks, 2004). In contrast, body mass can even vary remarkably after emergence due to constant foraging during the adult phase (Anholt, Marden, & Jenkins, 1991; De Block & Stoks, 2005; Stoks, 2001). We found a corresponding pattern in our data, with sibs differing overall in body mass and head width but plasticity and genotype differences in plasticity only being present in body mass.

Ischnura elegans occupies a wide range of different habitats including the presence of different predator species (Sternberg, 1999). Strong plasticity among traits indicates that adaptive plasticity is important for *I. elegans* to successfully settle in a variety of habitats (Hopper, 2001; Gyssels & Stoks, 2005). However, genotypic differences in plasticity suggest the absence of directional selection (Johansson, 2000), as well as support the evolution of plasticity (Pigliucci, 2005). This is obviously beneficial for a species of such diverse environmental range. In *Coenagrion hastulatum*, a species occupying a far narrower range of habitat preferences (Sternberg & Röhn, 1999), genotype differences and plasticity for activity have been described (Brodin & Johansson, 2004). However, no potential for evolution of predator-induced plasticity was present due to the absence of genotypic differences in plasticity (Brodin & Johansson, 2004).

In our data set, the independent optimisation of body size is not constrained by genetic correlation with behavioural traits. The foraging-mediated resource acquisition/mortality trade-off is well established, including in odonates (e.g. Brodin & Johansson, 2004; Johansson et al., 2001). Higher foraging activity leads to more encounters with predators and increased mortality but also increased growth due to more food intake (Stoks, De Block, van de Meutter, & Johansson, 2005). However, in *Ischnura verticalis*, a species also showing high levels of plasticity and wide environmental preferences, no such relationship is present (McPeek, 2004). Comparing activity levels with *Enallagma* species, higher activity in *Ischnura* larvae does induce increased mortality but does not translate into higher feeding rates (Stoks, De Block, & McPeek, 2005). Instead, *Ischnura verticalis* larvae convert more energy from their food into body size than *Enallagma* species, making it unlikely that foraging mediates the growth mortality trade-off in this system (McPeek, 2004). The absence of any genetic correlations between body size and behavioural traits in our study means that other traits, for instance growth efficiency, may be needed to explain body size. Further comparisons of highly plastic and less plastic species could yield insights about the linkage of foraging activity and body size as well as how heterogeneous environments select for changes in the relationship of growth and behaviour.

Acknowledgements

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