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Survival selection on escape performance and its underlying phenotypic traits: a case of many-to-one mapping

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Abstract

Selection often operates not directly on phenotypic traits but on performance which is important as several traits may contribute to a single performance measure (many-to-one mapping). Although largely ignored in the context of selection, this asks for studies that link all relevant phenotypes with performance and fitness. In an enclosure experiment, we studied links between phenotypic traits, swimming performance and survival in two *Enallagma* damselflies. Predatory dragonflies imposed survival selection for increased swimming propensity and speed only in *E. annexum*; probably *E. aspersum* was buffered by the former species' presence. Accordingly, more circular caudal lamellae, structures involved in generating thrust while swimming, were selected for only in *E. annexum*. Other phenotypic traits that contributed to swimming speed were apparently not under selection, probably because of many-to-one mapping (functional redundancy). Our results indicate that not only the phenotypic distributions of syntopic prey organisms but also many-to-one mapping should be considered when documenting phenotype–performance–fitness relationships.

Introduction

Predation can be a strong selective force as it can instantaneously reduce the fitness of a prey organism to zero. Many studies have indeed demonstrated phenotypic selection imposed by predators on prey morphology, behaviour and physiology (Endler, 1986; Hoekstra *et al.*, 2001; Kingsolver *et al.*, 2001). However, selection imposed by predators will often act only indirectly on these phenotypic traits, through their link with performance variables (like escape speed) that directly influence the prey's vulnerability to the predator, and that are the more direct targets of selection (Arnold, 1983). Relationships between phenotypic traits and performance are sometimes weak and occasionally nonexistent (Garland & Huey, 1987), underscoring the need for an integrated approach to studying selection (Irschick *et al.*, 2007). Moreover, several phenotypic traits may contribute to a single performance measure, so-called many-to-one mapping (Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005). This phenomenon of functional redundancy has been largely ignored in the context of selection studies but may make identifying selection on individual phenotypic traits challenging by generating unexpected results where phenotypic traits contributing to performance are apparently not under selection. Under the scenario of many-to-one mapping, animals with suboptimal values for one phenotypic trait may still have high performance, hence substantial fitness, given optimal values for other phenotypic traits. Despite this, studies simultaneously linking phenotypes with performance and identifying mortality by predation are rare (Jayne & Bennett, 1990; Downes & Shine, 1999; Miles, 2004; Janzen *et al.*, 2007). Yet, such studies are needed to unambiguously demonstrate the adaptive significance of phenotypic variation with regard to predation and may identify cases of many-to-one mapping (Arnold, 1983; Wainwright, 1994).

One particularly well-studied system in the context of predation is given by the North American *Enallagma* damselfly species. Most *Enallagma* species occur in fish

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lakes, the ancestral habitat, and three independent invasions have occurred to fishless lakes where large predatory dragonflies are the top predators (hereafter called dragonfly lakes) (McPeek, 1990a, 1995, 1996, 1997, 1999, 2000; McPeek *et al.*, 1996; McPeek & Brown, 2000; Turgeon *et al.*, 2005). One set of performance traits crucial for damselflies to survive in dragonfly lakes are swimming propensity and swimming speed. Damselfly larvae swim by moving their abdomens from side to side and using three caudal lamellae to generate thrust (Brackenbury, 2002). Small-scale laboratory experiments showed that larvae that swim away when attacked by a predatory dragonfly have a higher probability of survival (McPeek, 1990b). In another laboratory experiment, larvae with artificially shortened lamellae swam more slowly and had a lower probability of surviving an attack by a dragonfly larva (McPeek *et al.*, 1996). Swimming away is, however, not a good strategy to escape fish predation. In line with this, *Enallagma* species in fish lakes have a low propensity to swim away when attacked and a low swimming speed, whereas dragonfly lake *Enallagma* developed a higher swimming propensity and higher mass-corrected swimming speeds (McPeek, 1999; McPeek *et al.*, 1996). Associated evolutionary changes in morphological (e.g. increased lamella size) and physiological (i.e. increased mass-specific enzyme activity of arginine kinase (Ak), the enzyme that recharges ATP from ADP) swimming-related phenotypic variables were identified through the comparative method (McPeek, 1995, 1999; McPeek *et al.*, 1996).

In this study, we simultaneously study links between phenotypic traits, performance and survival in the presence of predatory dragonflies in two *Enallagma* species occupying dragonfly lakes. By jointly studying phenotype–performance–fitness relationships for all relevant phenotypic traits known to underlie a well-understood performance trait, we will be able to evaluate the role of many-to-one mapping in this study system where so far only the link between phenotypic variation for lamellae size and shape and survival in the presence of predators had been demonstrated (McPeek, 1997). Specifically, we quantify phenotypic selection imposed by dragonfly predation and acting through survival on two performance variables, swimming propensity and swimming speed, under semi-natural conditions using field enclosures. Additionally, we simultaneously evaluate selection on all known morphological, behavioural and physiological traits that are related to swimming speed to identify ongoing selection on phenotypic traits known to have evolved in relation to the habitat shift from fish lakes to dragonfly lakes.

Methods

Set-up selection experiment

We performed the selection experiment in Sylvester Pond (Norwich, VT, USA), a small fishless farm pond where *Anax junius* and various *Aeshna* dragonfly species are the top predators (M.A. McPeek, unpublished data). We evaluated ongoing selection by dragonfly larvae on the larval traits of two *Enallagma* species, *E. annexum* and *E. aspersum*, that are abundant in Sylvester Pond and other dragonfly ponds across eastern North America (McPeek, 1990a, 1998). The selection experiment closely followed the protocol used in previous field enclosure studies of *Enallagma* damselflies (e.g. McPeek, 1990b, 1997, 1998). We installed two predator treatments: one with a caged predator and one with a free-ranging predator. Predator cues may induce morphological changes in prey that give them a higher escape performance. For example, it is well documented that cues from dragonfly predators induce deeper tail fins in tadpoles which as a result swim faster (e.g. Teplitsky *et al.* 2005). As previous experiments showed no such predator-induced plasticity in the traits under study in *Enallagma* (McPeek, 1997; M.A. McPeek, F. Strobbe and R. Stoks, unpublished data) and other damselfly species (Stoks *et al.*, 1999a) we did not include a treatment with no predators.

We identified selection on traits based on differences in trait values between caged predator and free-ranging predator treatments at the end of the experiment. The alternative approach of a longitudinal assessment of phenotypic change was not possible for several reasons. First, enzyme kinetics (i.e. activity if Ak) cannot be measured in a noninvasive way. Moreover, measuring lamellae morphometrics in advance is almost impossible as due to handling these structures lamellae loss (autotomy) will probably occur (e.g. McPeek, 1990a; Stoks, 1999). Finally, to be able to detect selection we ran the experiment long enough (i.e. 35 days); during this period larvae will inevitably moult and grow. As far as we know, no marking method exists for damselfly larvae (or other invertebrates that moult) where the marks remain after moulting, thereby making longitudinal monitoring of individuals not possible.

We used semi-permeable enclosures that allowed small prey items to enter but prevented damselfly larvae from escaping. These enclosures were 1.2-m-high cylindrical cages with a diameter of 30 cm. Enclosures were made of 2-cm mesh size chicken wire covered with mosquito netting (0.6 mm × 1.2 mm mesh size). Cages were sealed at the bottom ends with plastic dishes containing approximately 1 cm of pebbles. The tops of the enclosures extended 30 cm out of the water and were left uncovered. All enclosures were linearly arranged in the pond at a depth of 90 cm. The macrophyte *Chara vulgaris* from the same pond was added to each cage in natural density. When free predators are present, *Enallagma* mortality rates and growth rates in the type of cages used have been shown to be indistinguishable from those in natural populations (McPeek, 1990b, 2004), suggesting that phenotypic selection measured in these enclosures should accurately reflect selection in natural populations.

For the caged dragonfly treatment, one antepenultimate instar *A. junius* larva was placed inside a small cage, which was placed inside the enclosure. The dragonfly cage was 11 cm × 11 cm × 6 cm, and was constructed by placing a small, coarse-mesh (openings 1.7 cm × 1.0 cm) plastic container inside a bag constructed of mosquito netting. This type of container allowed damselflies visual and olfactory cues for detecting that a large dragonfly larva was present in the cage but prevented the dragonfly from eating the damselflies. Identical containers without a dragonfly were placed in all other enclosures. For the free-ranging dragonfly treatment, one antepenultimate instar *A. junius* larva was placed inside the enclosure.

Fourteen enclosures were installed in the pond on 29 August 2005. Four replicates were run for the caged dragonfly treatments and 10 replicates for the free-ranging dragonfly treatment. We performed more replicates for the free-ranging predator treatments, because fewer individuals were expected to survive in this treatment. Enclosures were installed 8 days prior to the addition of odonates to allow colonization by prey for damselflies and dragonflies through the netting. Chironomids, littoral cladocerans, littoral copepods, ephemeropterans, annelids and amphipods were all abundant in cages at the end of the experiment. On 5 September 30, *E. annexum* and 30 *E. aspersum* larvae were added to each enclosure, together with one *Anax* larva. We worked with the natural available distribution of larval instars at the time of the experiment for both damselfly species. Larval densities used were within the natural density ranges as described in McPeek (1990b). Only larvae with three intact lamellae were used. All *E. annexum* and *E. aspersum* larvae were collected from Sylvester Pond. On 29 August 2005, we also placed three control enclosures into which we introduced only *Chara* to check for possible immigration of *Enallagma* larvae into cages. No immigrants were detected in any of these enclosures at the end of the experiment.

At the end of the experiment, after 35 days, the contents of the enclosures were returned to the laboratory and all surviving larvae were immediately removed from the samples and kept individually in 100-mL cups in a room at 20 °C, under the natural photoperiod and fed *Artemia* sp. nauplii *ad libitum* daily, until all swimming trials were conducted (within 48 h).

Response variables

Mortality rate was calculated separately for each species in each enclosure as mortality rate = −[ln(recovered number) − ln(initial number)]/(duration of the experiment). This equation assumes a constant mortality rate throughout the experiment.

For each recovered larva (n = 327), we measured wet mass by weighing it to the nearest 0.01 mg on a Mettler Toledo electrobalance. For subsets of larvae, we also measured two performance variables, swimming propensity and swimming speed, and a set of swimming speed-related variables which we describe in turn below. These included various aspects of the size and shape of the caudal lamellae, the morphological structure used to generate thrust during swimming; the mass-specific activity of Ak, the enzyme that phosphorylates ADP into ATP and thus resupplies ATP pools during the first few seconds of strenuous activity (Morrison, 1973); and various aspects of behaviour during swimming.

Lamellae morphometrics

We quantified various aspects of the morphology of caudal lamellae following McPeek (1995). Larvae with larger and more circular lamellae and that make more beats with their abdomen while swimming have a higher swimming speed (McPeek *et al.*, 1996; Stoks, 1999). Caudal lamellae are routinely damaged and lost under natural conditions; up to 60% of larvae in any given *Enallagma* population may have at least one caudal lamella which has been lost or regenerated (McPeek, 1990a). Moreover, lamellae could be damaged and lost when retrieving larvae from the enclosures. We therefore quantified morphological variables on the median lamella and one of the two lateral lamellae (the two lateral lamellae are identical to one another) for every larva with an unregenerated median lamella and at least one unregenerated lateral lamella (n = 243). We digitized each lamella while viewing its lateral surface. For each lamella, we recorded the area, perimeter, length of the major axis (major axis), the largest width perpendicular to the major axis (width), base width and the distances between the upper and lower ends of the lamella base and the lamella tip (length 1 and length 2 respectively) (see also McPeek, 1995). We calculated an index of lamellar circularity using the formula circularity = $(\text{perimeter})^2/\text{area}$; this index has a minimum value of 4π for a perfect circle and becomes larger as the shape of the object becomes less circular. Images of lamella were recorded with Cam2Disk 2.2 (DVC Machinevisions, Breda, The Netherlands) connected to a Matrox Meteor Framegrabber (Matrox Electronic Systems, Ltd, Dorval, QC, Canada). Images were digitized with Image-Pro Plus 5.0 (Media Cybernetics, Inc., Bethesda, MD, USA).

Arginine kinase activity

We measured the enzymatic activity of Ak on almost all recovered larvae (n = 311, as some samples accidentally thawed before processing). Immediately after being weighed alive, the lamellae of larvae were removed for morphometric studies and the body was placed in a microcentrifuge tube and stored at −80 °C until processing. We quantified Ak activity spectrophotometrically after adapting the protocol of McPeek (1999) for 96-well plates. Briefly, larvae were homogenized in a microcentrifuge tube in ice-cold 50 mM imidazole buffer (pH 7.0).

The volume of imidazole buffer used for grinding varied between 100 and 600 μL depending on larval size. We centrifuged this sample for 10 min at 1310 *g*, and the supernatant was assayed for enzyme activity. Samples were held on ice until assayed, which was completed within 3 h after homogenization. All enzyme assays were run on a Bio-Rad Benchmark Plus microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) and performed at 25 °C in Falcon 96 multiwell flat-bottom culture plates. Ak of each sample was assayed in a 200-μL volume of the following reagents: 50 mM imidazole (pH 7.0), 10 mM glucose, 5 mM magnesium acetate, 5 mM AMP, 1.5 mM ADP, 5 mM phospho-L-arginine, 0.5 mM NADP, 5 U hexokinase (5 U/5 μL; Sigma H-5500) and 0.4 U glucose-6-phosphate dehydrogenase (0.4 U/1 μL; Sigma G-8529). Five microlitres of sample were added to this reagent mixture, and the absolute absorbance at 340 nm was recorded every 11 s for 5 min. This assay measures the rate of production of NADPH in the solution as measured by the change in absorbance at 340 nm. The change in absorbance between 30 and 60 s was used as the estimate of Ak activity in the sample (the reaction was linear over this time interval). The amount of grinding buffer and the individual body mass were then used to calculate mass specific Ak activity in units of μM NADPH produced × (g body mass)$^{-1}$ × min^{-1}.

Swimming propensity and swimming speed

Before weighing larvae, we also quantified swimming propensity, swimming speed, lateral beats of the abdomen and the start angle of the abdomen on a smaller subset (n = 157) of larvae that had all three lamellae intact. The specific numbers of larvae scored per combination of species and predator treatment are given in the figures. Swimming propensity and speed were scored as described in McPeek *et al.* (1996) and McPeek (2000). All swimming trials were videotaped in a 20-cm-long × 30-cm-wide × 3-cm-deep Plexiglass container filled to a height of 1 cm with 21 °C tap water. This container was illuminated from above with indoor fluorescent room lighting. A ruler, graduated in millimetres, was positioned at the bottom of the container for calibration. The magnification of the camera was adjusted so that the container filled the entire field of the video image. Fast starts were filmed at 30 frames per sec with a Hitachi KP-D50 camera (Hitachi Kokusai Electric Canada, Ltd., Scarborough, Ontario, Canada) mounted above the container and connected to a Sony SVO-9500MD VHS video recorder (Sony Electronics Inc., New Jersey, USA). Recorded sequences were converted from analogue (VHS) to digital format at 30 frames per second and saved as AVI files. Swimming bouts were digitized with the image analysis software Image-Pro Plus 5.0 (Media Cybernetics, Inc., Bethesda, MD, USA). During the first prod, we also scored the swimming propensity for that individual (1 if the larva swam away and 0 if not). Pilot trials showed that this behaviour was highly repeatable across prods with > 95% of the larvae showing consistent swimming propensity (F. Strobbe, unpublished data). For later analyses, we included only the swimming speed associated with the fastest swim performed by each individual.

Based on the video-taped sequences, we also quantified the number of abdominal beats and the start angle of the swimming burst. For each digitized swim bout we measured the number of complete beat cycles of the tip of the abdomen. One complete cycle is defined as the tip of the abdomen swinging to one side, then to the other, then returning to the initial position. The rate at which the larva swings its abdomen is a measure of the effort of the larva during the swimming event. We calculated the rate of abdomen beats (hereafter swimming beats) as the number of cycles per duration of swim, which has units of beats per second (McPeek *et al.*, 1996). We also measured the smallest start angle in the bent abdomen (C-start, Brackenbury, 2002) before starting their fastest swim bout.

Data analyses

Prior to statistical testing, a principal components analysis was performed on the 16 lamellae morphometric variables. The first two principal components (PC1 and PC2) were extracted from the covariance matrix and the analysis was run on the PC scores. Extracting separate principal components per species gave loadings that were highly correlated between species for both axes (PC1: r = 0.99; P < 0.0001; and PC2: r = 1.00; P < 0.0001). Therefore, we extracted PCs from the combined data of both species.

We separately tested for an effect of predator treatment and species on mortality rate, swimming propensity and swimming speed with mixed model AN(C)OVAS using PROC MIXED in SAS 9.1 (SAS Institute Inc., Carry, NC, USA). For swimming propensity and speed, enclosure was added as a random variable to take advantage of the full data set while overcoming the problem with pseudoreplication within enclosures (Brown & Prescott, 1999; Millar & Anderson, 2004). This way we also took into account that larvae of two species were within the same enclosure. Mass was included as a covariate when analysing the effects on swimming propensity and swimming speed. Initially, we also included interactions with mass in the model, but as none were significant, they were removed from the final models. For swimming propensity, a binary response variable, we used a binomial error structure and the logit link and used the GLIMMIX macro of SAS 9.1.

To test whether swimming-related variables contributed to swimming speed we ran a mixed model ANCOVA with predator treatment and species as categorical predictors and all swimming-related variables and mass as continuous predictor variables (covariates) and swim-

ming speed as dependent variable. Initially, we also included interactions between predator treatment and species with the swimming-related variables; but as none were significant, they were removed from the final model. Next, we tested for effects of predator treatment and species on the swimming-related variables in a mixed model MANCOVA with mass as a covariate and enclosure as a random factor. Additionally, we ran separate univariate ANCOVAS per swimming-related variable. Again, interactions with mass were not significant, and were removed from the final model. Across all analyses, the intraclass correlation coefficients associated with enclosure ranged from 0% to 12% except for swimming beats were it was 23%.

To specifically test for phenotypic selection on the traits identified in previous analyses, we contrasted both predator treatments for each species using the contrast procedure in the previous analyses. As mass was included as a covariate, the value of the corresponding selection coefficient was estimated by dividing the difference in intercepts between the free-ranging predator and caged dragonfly treatments by the standard deviation in residuals around the caged predator regression; this metric measures the displacement of the free-ranging predator regression from the caged predator regression in phenotypic SD units (see McPeek, 1997).

Results

Survival and performance

Free-ranging dragonfly larvae considerably increased mortality of both *Enallagma* species (Table 1, Fig. 1a). Swimming propensity was higher for *E. annexum* larvae recovered in the free predator treatment compared with those recovered in the caged predator treatment, but not for *E. aspersum* (predator treatment × species; Table 1, Fig. 1b). The associated selection coefficient on swimming propensity for *E. annexum* was +0.58 SD units (planned contrast, $F_{1,167} = 5.12$, $P = 0.025$), for *E. aspersum* this was −0.17 SD units (planned contrast, $F_{1,167} = 0.00$, $P = 0.96$).

Swimming speed was higher for larvae recovered in the free predator treatment compared with those recovered in the caged predator treatment (predator treatment, Table 1, Fig. 1c). Although there was no significant species × predator treatment interaction, the associated selection coefficient for *E. annexum* was significantly different from zero (+0.95 SD units; planned contrast, $F_{1,151} = 5.10$, $P = 0.025$), whereas the associated selection coefficient for *E. aspersum* was not (+0.13 SD units; planned contrast, $F_{1,151} = 1.28$, $P = 0.26$).

Phenotypic correlates of swimming speed

The first two PCs extracted from the set of 16 lamellae variables summarized 94% of the variation (Table 2). PC1 was interpreted as a size metric having large positive loadings for the area and perimeter of both lamellae. Larvae with more positive scores for PC1 had larger lamellae. PC2 quantified shape with large, negative loadings for the circularity of both lamellae. Larvae with more positive scores for PC2 had more circular lamellae.

The above-mentioned effect of predator treatment on swimming speed disappeared when correcting for the swimming-related variables (ANCOVA, Table 3). As also species and the predator treatment × species interaction were not significant, we directly evaluated the contribution of the swimming-related variables using multiple regressions. For *E. annexum*, lamella shape (PC2, partial correlation coefficient +0.47) and beats (+0.45) and to a lesser extent lamellae size (PC1, +0.36) had a positive contribution to swimming speed. For *E. aspersum*, only beats (+0.23) had a positive contribution to swimming speed, although this was marginally nonsignificant.

A MANCOVA with mass as a covariate showed effects of species ($F_{5,123} = 36.52$, $P < 0.0001$) but neither of predator treatment ($F_{5,123} = 0.80$, $P = 0.55$) nor of predator treatment × species interaction ($F_{5,123} = 0.97$, $P = 0.44$) on the set of swimming-related variables. Separate AN(C)OVAS showed that the latter two factors had no significant effect on mass or any of the mass-corrected swimming-related variables (all $P > 0.11$), except for lamellae shape (PC2, Table 4). Larvae recovered in the free predator treatment had more circular lamellae compared with those recovered in the caged predator treatment, but this was only true for *E. annexum*

Table 1 Results of mixed model AN(C)OVAS testing for effects of species and predator treatment on daily mortality rate, swimming propensity and swimming speed of *Enallagma aspersum* and *Enallagma annexum* larvae.

	Mortality rate			Swimming propensity			Swimming speed		
	d.f.	*F*	*P*-value	d.f.	*F*	*P*-value	d.f.	*F*	*P*-value
Predator treatment (P)	1	16.11	0.0017	1	2.34	0.13	1	6.04	0.015
Species (S)	1	0.28	0.61	1	25.89	< 0.0001	1	0.69	0.41
P × S	1	3.54	0.084	1	4.49	0.036	1	1.00	0.32
Mass	–	–	–	1	22.55	< 0.0001	1	86.28	< 0.0001
Error	12			167			151		

Mass was included as a covariate when analysing effects on swimming propensity and swimming speed.

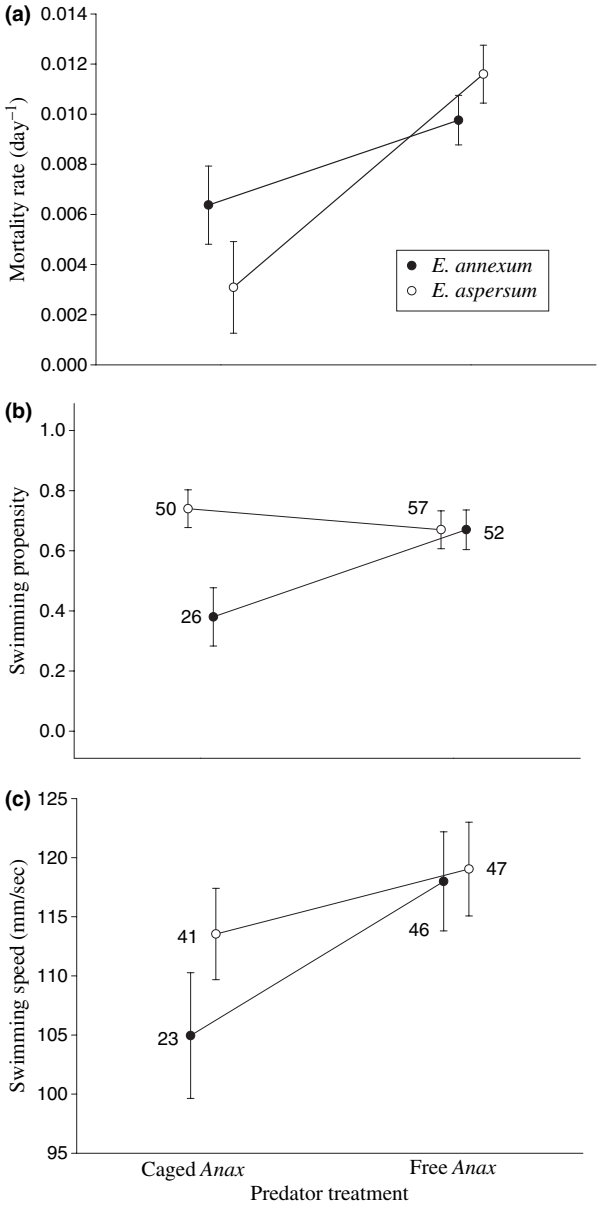

Fig. 1 Daily mortality rates (a), swimming propensity (b) and swimming speed (c) of *Enallagma aspersum* and *Enallagma annexum* larvae in the caged and free *Anax* predator treatments. Given are least square mean values (±1 SE); swimming propensity and swimming speed were corrected for mass. Numbers with symbols in plots b and c denote total number of larvae measured; in plot means are based on four enclosures for the Caged *Anax* treatment and on 10 enclosures for the Free *Anax* treatment.

(predator Treatment × species, Table 4, Fig. 2c). The associated selection coefficient was +0.73 SD units (planned contrast, $F_{1,225} = 5.33$, $P = 0.022$) for *E. annexum* and +0.055 SD units (planned contrast, $F_{1,225} = 0.00$, $P = 0.99$) for *E. aspersum*. This selection on lamellae shape in *E. annexum* disappeared when correcting for swimming speed (planned contrast, $F_{1,118} = 2.77$, $P = 0.10$). Species differences in the MANCOVA were due to a larger mass, larger lamellae (PC1), less circular lamellae (PC2), fewer swimming beats and lower Ak activity in *E. annexum* compared with *E. aspersum* (Table 4, Fig. 2).

Table 2 Loadings of the 16 original morphometric variables of the median and lateral lamellae on the first two principal components (PC1 and PC2) for *Enallagma annexum* and *Enallagma aspersum*.

	PC1	PC2
Median lamella		
Area	0.83	0.53
Width	0.71	0.69
Major axis	0.94	0.29
Perimeter	0.95	0.25
Circularity	−0.16	−0.96
Base width	0.73	0.55
Length 1	0.95	0.21
Length 2	0.95	0.22
Lateral lamella		
Area	0.83	0.53
Width	0.71	0.69
Major axis	0.91	0.35
Perimeter	0.94	0.31
Circularity	−0.14	−0.95
Base width	0.56	0.61
Length 1	0.95	0.26
Length 2	0.95	0.26
Percentage of variation explained	65%	29%

See the Methods section for explanation of raw variables.

Discussion

In line with previous studies (McPeek, 1990b, 1997, 1998; Stoks *et al.*, 2005), *Anax* predatory dragonflies imposed strong mortality on both studied *Enallagma* species typical of dragonfly lakes. Linked to this, we could detect ongoing selection on both studied swimming-related performance variables to avoid predation and on one phenotypic trait underlying swimming speed, lamellae shape. We consider it unlikely that our experimental approach for detecting selection, i.e. comparing phenotypic distributions between caged predator and free-ranging predator treatments at the end of the exposure period, could have biased our results. The observed patterns were all expected based on *a priori* knowledge of the role of swimming to survive dragonfly attacks and the role of phenotypic traits in shaping swimming speed based on short-term laboratory trials (e.g. McPeek, 1990b, 1997; McPeek *et al.*, 1996) and consistent with macro-evolutionary reconstructions showing that larvae of species that invaded lakes with a new top predator (i.e. dragonfly larvae) developed a higher propensity to swim, higher swimming speeds and more circular lamellae (McPeek, 1995, 1999; McPeek *et al.*, 1996).

Table 3 (a) Results of a mixed model ANCOVA (mass was included as a covariate) testing for effects of predator treatment, species and swimming-related variables and (b) results of separate multiple regressions of swimming-related variables on swimming speed of *Enallagma annexum* and *Enallagma aspersum*.

	(a)		(b)							
			E. annexum				*E. aspersum*			
	$F_{1,110}$	*P*-value	$F_{1,29}$	*P*-value	Slope (SE)	Partial *R*	$F_{1,64}$	*P*-value	Slope (SE)	Partial *R*
Predator treatment (P)	0.15	0.70								
Species (S)	2.82	0.096								
P × S	0.15	0.70								
Mass	0.06	0.80	0.49	0.49	−0.23 (0.31)	−0.11	2.85	0.096	0.27 (0.29)	0.093
Lamella size (PC1)	13.11	0.0004	6.21	0.019	0.48 (0.20)	0.36	0.96	0.33	0.21 (0.18)	0.13
Lamella shape (PC2)	12.27	0.0007	11.85	0.0026	0.67 (0.20)	0.47	0.00	0.97	0.035 (0.22)	0.018
Angle	0.00	0.99	0.02	0.88	0.014 (0.10)	0.021	0.09	0.77	−0.028 (0.10)	−0.031
Beats	12.19	< 0.0007	9.86	0.0039	0.35 (0.11)	0.45	3.91	0.052	0.23 (0.10)	0.24
Arginine kinase activity	0.01	0.91	2.01	0.17	−0.16 (0.10)	−0.23	1.30	0.26	0.080 (0.10)	0.091

Table 4 Results of AN(C)OVAS testing for effects of predator treatment and species on mass and all mass-corrected swimming-related variables of *Enallagma annexum* and *Enallagma aspersum*.

	d.f.	*F*	*P*-value	d.f.	*F*	*P*-value	d.f.	*F*	*P*-value
	Mass			Lamella size (PC1)			Lamella shape (PC2)		
Predator treatment (P)	1	0.65	0.42	1	2.36	0.13	1	2.63	0.11
Species (S)	1	284.06	< 0.0001	1	73.87	< 0.0001	1	295.72	< 0.0001
P × S	1	0.67	0.41	1	1.77	0.18	1	4.26	0.040
Mass	–	–	–	1	286.56	< 0.0001	1	306.46	< 0.0001
Error	311			225			225		
	Angle			Beats			Ak		
Predator treatment (P)	1	0.12	0.73	1	0.13	0.72	1	0.10	0.75
Species (S)	1	0.049	0.49	1	6.37	0.013	1	12.89	0.0004
P × S	1	1.56	0.21	1	0.03	0.86	1	2.32	0.13
Mass	1	7.58	0.0067	1	0.77	0.38	1	0.82	0.37
Error	139			139			294		

Mass was included as a covariate for the swimming-related variables.

The alternative explanation that the observed differences in these variables are confounded with predator-induced plasticity in these traits, potentially linked with training where damselflies in the free-ranging predator enclosures that managed to avoid these attacks learned to improve their escapes via faster swimming or quicker starts (training effects), seems unlikely. In previous experiments designed to evaluate the degree of phenotypic plasticity in these traits absolutely no hint of plasticity was found at all (McPeek, 1997; M.A. McPeek, F. Strobbe and R. Stoks, unpublished data; for *Lestes* larvae see Stoks *et al.*, 1999a, b). Moreover, after having video-taped thousands of larvae of these and other damselfly species and where we simulated a predator attack by coaxing larvae to swim multiple times (across a wide range of time intervals, from minutes to days), we never found a pattern consistent with training (i.e. increased swimming speed after repeated simulated attacks) (M.A. McPeek, F. Strobbe and R. Stoks, unpublished data).

Moreover, if learning played a significant role, one would also expect those larvae that learned to deal with the predator to have higher survival and thus learn to coexist with the predator. However, our experience with predator introductions to new habitats (i.e. introducing fish into previously fishless ponds or removing fish from fish ponds [e.g. via winter kills]) shows that the native *Enallagma* species are rapidly driven locally extinct by the new predator (M.A. McPeek, personal observation). If larvae did learn to some degree to deal with the new predator, this amount of learning was not enough to prevent extinction. Given that the phenotypic differences between species that inhabit the two lake types are completely consistent with selection pressures we infer from this and other experiments, and laboratory performance experiments have demonstrated that the inferred mechanisms of these selection pressures are also operating exactly as expected, we feel that any learning effect must be substantially subordinate to the phenotypic selection imposed by the direct mortality from predators.

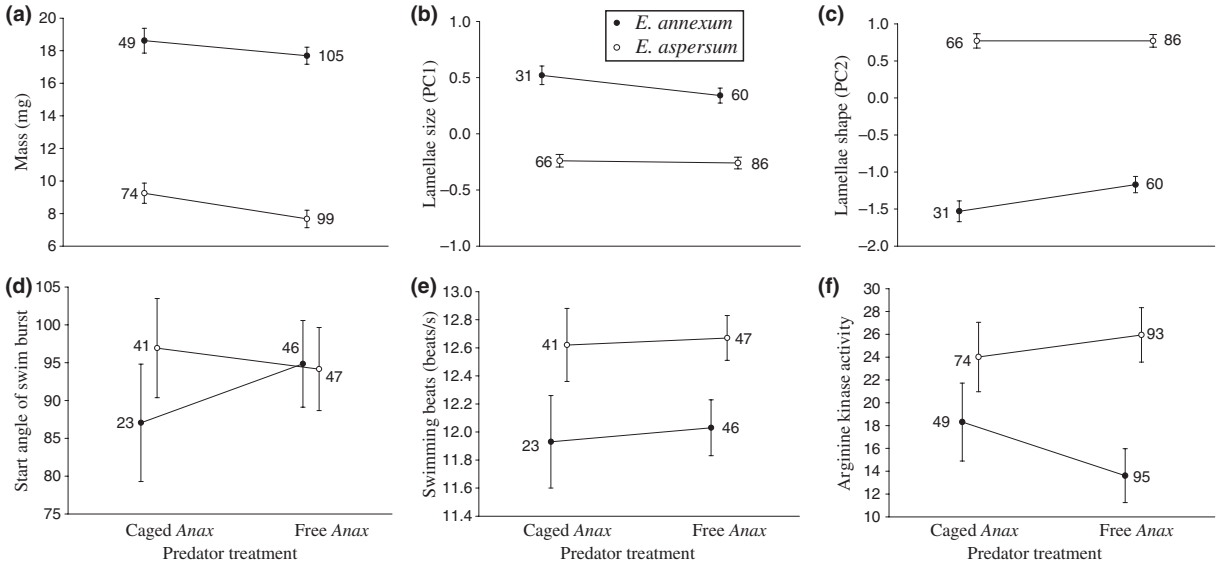

Fig. 2 Swimming-related variables of *Enallagma aspersum* and *Enallagma annexum* larvae in the caged and free *Anax* predator treatments. Except for mass, least square mean values (±1 SE) are given corrected for mass. Arginine kinase activity is expressed as μM NADPH produced × (g body mass)$^{-1}$ × min^{-1}. Numbers with symbols denote total number of larvae measured.

We identified positive survival selection for faster swimming propensity and for a higher swimming speed in *E. annexum*. This finding confirms under semi-natural field conditions previous observations in the laboratory of a lower survival probability when attacked by large dragonfly larvae in *Enallagma* larvae that do not swim away when attacked (McPeek, 1990b), and that swim slower due to an artificial shortening of the lamellae (McPeek *et al.*, 1996). The general pattern emerging from the few studies directly showing survival selection by predators on escape performance under semi-natural conditions is, as expected, positive selection for escape speed (overview in Irschick *et al.*, 2007; Janzen *et al.*, 2007; this study). Field studies on propensity to escape are very scarce. In contrast to our study, Janzen (1995) reported negative survival selection on the propensity of turtle hatchlings to run. He suggested that snapping turtles that remain motionless are cryptic and may have increased survivorship over more active individuals that flee.

Despite being syntopic with *E. annexum*, selection on both performance variables could not be shown in *E. aspersum*. Because both species had the same mortality rates in the presence of a free-ranging *Anax* predator, the absence of selection in *E. aspersum* was not because the predator did not impose a less strong mortality by predation on this species. The failure to detect an increase in swimming propensity and swimming speed due to survival selection in this species may be explained by the already high values for both variables in the caged *Anax* treatment when compared with *E. annexum* (Fig. 1). The detection of selection by predation on a prey trait may be critically dependent on the phenotypic distribution of that trait in other syntopic prey species. The presence of alternative prey species with less optimal values for that trait may to some extent have buffered selection on that trait in *E. aspersum*. This is an important largely neglected aspect that may prove important in the recent paradigm shift toward considering natural selection in a community context (Irschick *et al.*, 2007). Interestingly, this situation may cause convergence in performance traits among species sharing the same selective environment. Further experiments are needed to explore this possibility.

At the phenotypic level, we could demonstrate survival selection for more circular lamellae in *E. annexum*. This confirms the results of previous studies under similar semi-natural conditions in the morphologically similar sister species *E. boreale* (McPeek, 1997). In line with this, larvae with more circular lamellae swim faster (McPeek *et al.*, 1996; this study) and predatory dragonflies select for faster swimming speed in *E. annexum* (this study). Alternatively, survival selection on lamellae morphology may have not worked through swimming speed but through a lure effect where dragonfly larvae would have deflected their attacks toward the lamellae and this increasingly so in larvae with a certain morphology. Such mechanism has been invoked in the interactions between tadpoles and *Anax* predators (Van Buskirk *et al.*, 2003; Johnson *et al.*, 2008). This lure effect generated selection for increased tailfin depth in tadpoles (fig. 3 in Johnson *et al.*, 2008), this metric corresponds to lamella width in our study. Yet, it is unlikely to have played a role in our study as lamellae width had equal loadings on

both lamellae PCs (Table 2), whereas we only detected selection on PC2. Further, in case of a lure effect we would expect larvae with more circular lamellae to be more attacked at the lamellae, hence having a higher chance of autotomizing lamellae. Yet, lamellae shape (PC2) in *E. annexum* did not differ between larvae with both and those with only one remaining lateral lamella ($t_{58} = -1.39$; $P = 0.17$). Additional proof for survival selection on lamellae shape operating through swimming speed itself comes from the observation that when adding swimming speed as a covariate to the analysis selection on lamellae shape was no longer significant.

Our study adds to the few others that demonstrate survival selection by predation on a performance trait and the underlying phenotypic trait (Jayne & Bennett, 1990; Downes & Shine, 1999; Miles, 2004; Janzen *et al.*, 2007) and is the first demonstration for an invertebrate. The associated selection coefficient for lamellae shape (+0.73) was somewhat higher than the one reported by McPeek (1997) (+0.24) but within the range of other studies of natural selection which predators are thought or known to be the agents of selection (Kingsolver *et al.*, 2001). Comparative methods may give insight into the links between changes in selection environments and changes in trait values (Pagel & Harvey, 1988). However, they are not sufficient to demonstrate adaptation, and need to be complemented with experiments showing ongoing selection (Doughty, 1995). Together with the evolutionary change toward more circular lamellae when *Enallagma* larvae invaded dragonfly ponds as identified by the comparative method (McPeek, 1995), our current experimental results strongly indicate that more circular lamellae are an adaptation to live with large predatory dragonflies.

Although several phenotypic traits contributed to swimming speed, survival selection could only be detected on lamellae shape (PC2). As in McPeek *et al.* (1996), lamellae size and number of swimming beats positively contributed to swimming speed, yet we did not find that they were higher in larvae that survived the free *Anax* treatment. One reason for this outcome may be that these traits were less important in contributing to swimming speed, because past selection may have shifted their trait mean to a fitness optimum (Arnold *et al.*, 2001) and reduced their phenotypic variation making current selection harder to quantify (Arnold & Wade, 1984). Although the partial correlation of lamellae shape (PC2) on swimming speed was fast (+0.47) in *E. annexum,* this was, however, also the case for swimming beats (+0.45) and to a lesser extent for lamellae size (PC1, +0.35). Two of these variables are orthogonal (PC1 and PC2), whereas swimming beats was not related to lamellae size (PC1) or lamellae shape (PC2) neither in *E. annexum* larvae recovered from caged predator enclosures as in those recovered from free-ranging predator enclosures ($-0.13 < r < 0.14$, all $P > 0.44$). A more likely explanation is therefore that swimming speed can be achieved through various combinations of underlying independent phenotypic variables. Larvae may differ in the combinations of the phenotypic traits they use to generate a swimming speed high enough to survive attacks by dragonfly larvae. Swimming speed may thereby represent a case of redundant many-to-one mapping of phenotypic traits on performance (Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005). Under such scenario of many-to-one mapping, different traits can perfectly not be correlated and still larvae with different values for the traits may all end up with the same fitness because they all have optimal values for different traits despite having suboptimal values for other traits. For example, a larva with small lamellae may still swim fast because it has very circular lamellae, another larva with small lamellae may swim fast because it makes many abdominal beats, and so forth. The traits just add up to give a certain swimming performance and many trait combinations may give the same swimming performance (functional redundancy). A related, yet fundamentally different, scenario that may explain why larvae with suboptimal values for a given trait still have high fitness is one where suboptimal values for a given trait are consistently associated, hence correlated, with more optimal values for another trait. Such alternative scenario would not necessarily imply a mechanistic explanation where a set of traits shape one performance trait, because the necessary correlations may just exist because of linkage on the chromosomes (Lynch & Walsh, 1998). This alternative scenario does not fit the pattern we observed as it would need traits shaping performance and fitness to be strongly correlated among each other. Many-to-one mapping may increase the difficulty in detecting selection on the individual phenotypic traits underlying swimming performance. Intriguingly, historic reconstructions reveal increases in such traits, lamellae size and number of swimming beats, in response to the habitat shift toward dragonfly lakes (McPeek, 1995). One reason for this apparent discrepancy may be that the covariation structure between the swimming-related variables and swimming speed also changed through evolution (Begin & Roff, 2001).

Many-to-one mapping may be a general phenomenon with regard to escape performance. For example, Vanhooydonck *et al.* (2006) reported at the between-species level that similar sprint speeds in *Anolis* lizards could be achieved through various combinations of different limb segment lengths and knee extensor muscle mass. Obviously, many-to-one mapping may relate to other performance variables and linked to both natural and sexual selection. For example, many phenotypic traits may independently contribute to flight ability (e.g. Berwaerts *et al.*, 2002) which may determine mating success. We therefore hypothesize that besides the phenotypic distributions of syntopic prey organisms, hence the community context, many-to-one mapping should also be taken

into account when documenting phenotype–performance–fitness relationships and hence when trying to document adaptation in performance-related phenotypic traits.

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