Selection on escape performance during ecological speciation driven by predation

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1 Despite the many study systems where predation has played a major role in phenotypic
2 diversification and speciation, the underlying selective regimes imposed by different
3 predator assemblages have rarely been quantified. We did so for the damselfly genus
4 *Enallagma* that strongly diverged in anti-predator traits when the ancestral species
5 occupying lakes containing fish (hereafter fish lakes) repeatedly invaded fishless lakes
6 with dragonfly larvae as top predators (hereafter dragonfly lakes). In two selection
7 experiments in field enclosures we quantified the selection on two key escape traits of
8 two fish-lake *Enallagma* species associated with survival selection by fish in the ancestral
9 fish lakes and by dragonfly predators in the invaded fishless, dragonfly lakes. In
10 accordance with the different hunting modes, fish imposed selection for a decreased
11 swimming propensity while dragonfly larvae imposed selection for increased swimming
12 speed in one of the two species. In two complementary quantitative genetic rearing
13 experiments we found relatively low but significant broad-sense heritabilities for both
14 escape traits. Integrating these estimates for the selection coefficients and the
15 heritabilities, suggests that the evolutionary increase in swimming speed associated with
16 the habitat shift may have occurred rapidly. Our study suggests that the phenotypic
17 evolution of ecologically important traits related to habitat shifts may occur at an
18 ecological timescale.

Keywords: anti-predator behaviour, ecological speciation, *Enallagma* damselflies, habitat shifts, phenotypic diversification.

INTRODUCTION

Predators are a major selective force (reviewed in Endler, 1986; Tollrian & Harvell, 1999) and their role in phenotypic diversification has attracted considerable attention both at the intraspecific level (e.g. Carlson et al., 2009; Marchinko, 2009; Urban, 2010; Harris, et al., 2011; Ghalambor et al., 2015; Stoks et al., 2016) and at the interspecific level, where diversification is expected to have occurred on a much longer timeframe (e.g. McPeck et al., 1996; Langerhans et al., 2007; Nosil & Crespi, 2006; Mikolajewski et al., 2010; Abuckle & Speed, 2015). Predator assemblages that differ in hunting mode can impose different selection pressures on antipredator behaviour. This may lead to phenotypic diversification of prey populations between habitats with different predator assemblages, eventually accumulating into ecological speciation (Wellborn et al., 1996; Vamosi, 2005; Stoks & McPeck, 2006; Schluter, 2009; Nosil, 2012).

In relatively few study systems where different predator assemblages played a major role in phenotypic diversification and speciation, have the underlying selective regimes imposed by different predator assemblages been quantified (Nosil & Crespi, 2006; Carlson et al., 2009; Marchinko, 2009; Svanbäck & Eklöv, 2011; Gordon et al., 2015). Such studies are important to rule out alternative hypotheses underlying the phenotypic diversification such as differential habitat use and differences in other biotic interactions (Irschick et al., 2007; Goodman et al., 2008; Urban & Richardson, 2015). Moreover, links between the phenotype and its adaptive value against certain predators, such as the survival value of a higher escape speed, cannot be taken for granted (Walker et al., 2005; Holmes & McCormick, 2009). For example, Johnson et al. (2008) showed that a higher burst swimming speed in tadpoles of *Rana sphenoscephala* did not influence

survival in the presence of dragonfly predators. Of the studies explicitly quantifying selective regimes imposed by contrasting predator assemblages nearly all focused on intraspecific differentiation (for the only examples of incipient speciation see Nosil & Crespi, 2006; Marchinko, 2009). Moreover, no studies so far have reconstructed the initial stages of predator-driven diversification in a system where the direction of the evolutionary change in predator assemblage is known. Ideally, studies quantifying the predator-mediated selection strength should also estimate the heritability of the traits under selection to evaluate the potential of the selection pressures to drive the evolutionary trajectories when predator regimes changed (Arnold et al., 2001).

An elegant study system to explicitly quantify predator-driven survival selection on escape behaviour is provided by the North American species of the damselfly genus *Enallagma*. Most *Enallagma* species occur in lakes containing fish (hereafter called fish lakes), the ancestral habitat, and three independent invasions have occurred to fishless lakes where large predatory dragonfly larvae are the top predators (hereafter called dragonfly lakes) (Brown et al., 2000; McPeck & Brown, 2000; Turgeon et al., 2005). These invasions into dragonfly lakes were associated with the evolution of higher values for two related escape traits: swimming propensity and escape swimming speed (McPeck, 1999; McPeck et al., 1996). Escape swimming in damselfly larvae occurs by moving the abdomen from side-to-side (Brackenbury, 2002). This includes a behavioural component: larvae swim faster if they beat their abdomens faster (McPeck et al., 1996). The evolution of both traits is linked to the different efficacy of escape swimming as an antipredator strategy against fish and dragonfly predators. Damselfly larvae have little chance in outswimming a fish (McPeck, 2000; Stoks & De Block, 2000), moreover swimming

attracts the attention of the fish predators (Baker et al., 1999). Instead, damselfly larvae have a good chance of avoiding capture when attacked by dragonfly predators (McPeck, 1990b; McPeck et al., 1996; Stoks & De Block, 2000). In line with this, ongoing survival selection for a higher swimming propensity and a higher swimming speed has been documented in a derived dragonfly-lake *Enallagma* species using an enclosure experiment (Strobbe et al., 2009).

Two important pieces of information to assess the role of selection imposed by different predator assemblages in the ecological speciation in this genus (and in most other study systems) are, however, still missing. Firstly, while we quantified ongoing selection on the two key escape traits (swimming propensity and swimming speed) in a derived dragonfly-lake *Enallagma* species (Strobbe et al., 2009), it has not been investigated whether these traits are under different selection between fish lakes and dragonfly lakes in the ancestral fish-lake *Enallagma* species. This is crucial to reconstruct what happened under natural conditions during the initial stages of the habitat shifts when fish-lake *Enallagma* species invaded the fishless dragonfly lakes. For example, when escape speed would be so low that even individuals with the highest speed do not have a better chance of escaping the new top predator, no survival selection on this trait will be detected (Brodie & Brodie, 1999), and directional selection cannot have played its assumed role. Secondly, we need not only estimates of survival selection but also of heritabilities for these key traits to evaluate the potential of directional selection to drive the observed evolutionary trajectories associated with the shift in predator regimes and obtain a tentative time scale of the evolution of these antipredator behaviours during the habitat shift.

89 Here, we present the results of two field enclosure experiments where we
90 quantified selection pressures on both escape traits (swimming propensity and swimming
91 speed) in two fish-lake *Enallagma* species in the ancestral fish lakes and in the derived
92 dragonfly lakes to test for different patterns of directional selection between habitats with
93 different predator assemblages. We studied two fish-lake species, *E. geminatum* and *E.*
94 *hageni* (Brown et al., 2000; McPeck & Brown, 2000; Turgeon et al., 2005), that represent
95 the two clades (and the associated ancestral phenotypes) that independently gave rise to
96 the dragonfly-lake *Enallagma*. We quantified selection by fish on these traits in their
97 natural fish-lake habitat and by dragonfly predators in the derived dragonfly lakes. These
98 coupled experiments thus simulate the initial change in phenotypic selection regime
99 presumably experienced by the ancestral fish-lake species when they invaded dragonfly
100 lakes within the last 20,000 years (McPeck & Brown, 2000; Turgeon et al., 2005). We
101 expected fish predation to select for a lower propensity to swim but not to impose
102 selection on escape swimming speed, while we expected dragonfly predation to select for
103 a higher propensity to swim and a higher escape swimming speed. In a companion paper
104 we quantified selection on foraging activity (number of food items eaten) based on the
105 enclosure experiment in the fish-lake habitat (Strobbe et al., 2011), here we focus on
106 selection on escape traits. In another study (Strobbe et al., 2009) we have documented
107 selection on escape speed by a derived dragonfly-lake *Enallagma* species in a dragonfly
108 lake, allowing a qualitative comparison with ongoing selection pressures after the habitat
109 shift in the evolved dragonfly-lake species. Moreover, by estimating the heritabilities of
110 both escape behaviours for the two species of the lineages sharing most recent ancestry

with current dragonfly-lake species, we were able to assess the scope for evolution by natural selection on these traits.

METHODS

Selection experiments

We quantified selection by fish and by dragonfly larvae on larval escape traits of the two fish-lake *Enallagma* species in field enclosures. We did so by contrasting trait values at the end of the enclosure experiment between two predator treatments: one where predators had been able to consume damselfly larvae ('free-ranging predator') and one where predators had been caged ('caged predator'), hence had not been able to consume damselfly larvae (see also McPeck, 1997; Strobbe et al., 2009, 2011; Siepielski et al., 2014). In the enclosures with a free-ranging predator three drivers of phenotypic change through time may be present: plasticity, non-consumptive predator-driven selection and consumptive predator-driven selection. Indeed, the presence of fish and dragonfly predators may cause plastic changes in *Enallagma* damselfly larvae: for example, a growth reduction and differential allocation of energy (McPeck et al., 2001; Stoks et al., 2005) and a reduction in swimming speed caused by stress-induced oxidative damage (Janssens & Stoks, 2014). Further, predation risk may impose mortality in damselfly larvae not caused by consumptive killing by the predator but caused by physiological stress (Stoks, 2001; Siepielski et al., 2014; see also McCauley et al., 2011). It has been shown in damselfly larvae that this stress-induced mortality may not be random with regard to phenotype, hence may impose non-consumptive predator-driven selection (Siepielski et al., 2014). Given that any plasticity and non-consumptive predator-driven

selection would also be present in a treatment with a caged predator, this is a more appropriate control than a treatment without predators to quantify the selection imposed by consumptive survival selection by the predators. Because all larvae were randomized across the enclosures of the two treatments at the start of the experiment, it is highly unlikely that there were initial differences in the trait distributions between treatments. Any differences in the trait means at the end of the enclosure experiment between the treatments with caged predators and with free-ranging predators therefore likely reflect selection imposed by consumption by the predators. As all three drivers of trait change may be at work through time in the enclosures with a free-ranging predator, also the alternative approach of comparing the phenotypic distribution of the larvae between the start and end of the enclosure experiment is less appropriate.

The experiments were performed in a fish lake and in a dragonfly lake so that the natural selective environments are matched as closely as possible and to enable reconstruction of the change in selection strength experienced by the ancestor species when they switched from fish lakes toward fishless, dragonfly lakes. In the companion paper of the fish-lake experiment (Strobbe et al., 2011) we reported the survival data and focused on selection on foraging activity, here we focus on unpublished data on selection on escape behaviour. In addition, we here present data on a similar unpublished dragonfly-lake experiment with the same two species to quantify selection on escape behaviour when fish-lake *Enallagma* invaded dragonfly lakes. We will describe the common experimental protocols implemented in the two lake types and indicate where differences occurred.

We used semi-permeable enclosures that allowed small prey items to enter the enclosures, but prevented damselfly larvae from escaping. These enclosures were 1.2 m high cylindrical chicken wire frames (mesh size 2cm) with a diameter of 65 cm for the fish-lake experiment, and of 30 cm for the dragonfly-lake experiment (McPeck, 1990a, 1998). Enclosures were placed at a depth of 90 cm, with their open top extending 30 cm out of the water. The enclosures were covered with mosquito netting and had a plastic dish with pebbles at the bottom. To provide natural structure, we added local densities of *Chara vulgaris* stems. Note that we used larger enclosures in the fish lake as fish are larger predators than dragonfly larvae (following McPeck, 1990a; McPeck, 1998, 2004). It is, however, unlikely that the enclosure size would affect the sign of the selection coefficient imposed by the predator on the performance traits. Moreover, mortality rates and growth rates in enclosures of these sizes and where selection can occur (enclosures with predators that were able to kill the damselfly larvae) do not differ from those in natural *Enallagma* populations in both lake types (McPeck, 1990a, 2004). This enclosure setup therefore likely allows obtaining realistic estimates of selection imposed by fish and dragonfly predators.

Predators used were *Lepomis gibbosus* sunfish (standard length ~6 cm) in the fish-lake experiment and antepenultimate instar *Anax junius* larvae (standard length ~4 cm) in the dragonfly-lake experiment. For the caged predator treatment, one predator was placed in a small cage covered with mosquito netting within the enclosure. Damselfly larvae had all means of detecting that a predator was present in the enclosure (e.g. vision and olfaction), but could not be consumed by the predator. In the fish-lake selection experiment this cage was a 20 cm × 20 cm × 20 cm cube made from plastic tubes and

surrounded with a mosquito netting bag, and in the dragonfly-lake selection experiment this cage was a 11 cm × 11 cm × 6 cm coarse-mesh (openings 1.7 cm × 1.0 cm) plastic container similarly covered by a mosquito netting bag. For the free-predator treatment, identical cages without a predator were placed in the enclosures, and one predator was introduced in each enclosure that could freely consume damselflies.

We placed the enclosures in the lakes on 1 September 2003. We placed 12 enclosures (caged-fish treatment: 4, free-ranging-fish treatment: 8) in the fish lake McDaniels Marsh (Enfield, NH, USA) and 17 enclosures (caged-dragonfly treatment: 4; free-ranging-dragonfly treatment: 13) in the dragonfly lake Sylvester Pond (Norwich, VT, USA). Given that we expected lower survival in the enclosures where the predators were able to consume the damselfly larvae, we set up more replicates of the free-ranging predator treatments. In addition, three control enclosures that were not stocked with damselfly larvae were placed in each lake. These enclosures were still without damselfly larvae by the end of the experiment, indicating that no damselfly larvae of the natural lake populations entered the enclosures through the netting. In contrast, all enclosures were colonized in high numbers by prey (mainly midge larvae, mayfly larvae and zooplankton).

At the start of the experiment, on 8 September 2003, we introduced one predator and 30 *E. hageni* and 30 *E. geminatum* larvae in each enclosure. These larval densities reflect densities in natural *Enallagma* populations (McPeck, 1990a). All larvae were collected from Little Salem Lake (Derby, VT, USA). So, in neither experiment larvae were tested in their native lake thereby avoiding potential effects of local adaptation (Siepielski et al., 2016). In the larger enclosures in the fish-lake experiment we also

added 60 damselfly larvae of other genera than *Enallagma* to mimic total densities of damselfly larvae in fish lakes. This also ensured to recover enough larvae of the focal species in the presence of a free-ranging sunfish. After 35 days, the experiment was stopped. Mortality rates were calculated per species and per enclosure as $-\ln(\text{final number of damselfly larvae}) - \ln(\text{initial number of damselfly larvae}) / 35 \text{ days}$.

Recovered larvae with three intact lamellae were kept individually in plastic cups (100 mL) until their escape traits were quantified (within 48h and frozen afterwards). We measured both escape traits (swimming propensity and swimming speed) by videotaping larvae swimming in a 34 cm x 26 cm x 6 cm container in the laboratory. We followed the methodology described in McPeck et al. (1996) and Strobbe et al. (2009). Briefly, a single larva was placed in the container, allowed to settle, and then gently prodded to swim by lightly tapping behind the larva with blunt forceps. A larva was given a swimming propensity score 1 when it swam away, and 0 when it did not swim away. This measure is highly repeatable for a given larva (Strobbe et al., 2009). Three swimming bouts per larva were videotaped and digitized and the fastest swimming speed was retained for analysis. As heavier larvae swim faster we also weighed the larvae to the nearest 0.01 mg on an electronic balance after gently blotting them dry with towel paper. In the fish-lake experiment the final body masses were on average $15.95 \pm 0.32 \text{ mg}$ (mean $\pm 1 \text{ SE}$) for *E. hageni* and 18.94 ± 0.38 for *E. geminatum*. In the dragonfly-lake experiment final body masses were on average 17.50 ± 0.51 for *E. hageni* and 20.13 ± 0.43 for *E. geminatum*.

We quantified swimming propensity of 274 larvae and swimming speed of 221 larvae (not all larvae did swim when prodded, hence the lower number). Specific sample sizes per combination of species and predator enclosure treatment are given in Figure 2.

Quantitative genetic rearing experiments

To estimate the broad-sense heritabilities of both antipredator behaviours for the two study species we carried out common-garden full-sib rearing experiments. Females in copula were collected in June-July at the fish lakes McDaniel's Marsh (*E. geminatum*) and Little Salem Lake (*E. hageni*) in respectively 2003 and 2004. In the laboratory, females were individually placed in glass jars covered with mosquito netting in a temperature-controlled room at 21°C (L:D 15h:9h photoperiod). To provide oviposition substrate, filter paper was provided. After oviposition, the filter papers with eggs were placed in plastic containers (18 × 12 × 7 cm) filled to a height of 1 cm with filtered pond water. Although female damselflies can store sperm from previous partners, a study on *E. hageni* showed that the last male to mate was found to fertilize up to 95% of the eggs of the first clutch laid after mating (Fincke 1984). Therefore, the incidence of paternal half-sibs in a given clutch is expected to be very low. The number of full-sib families included in the experiment was 28 for *E. geminatum* and 30 for *E. hageni*.

Larvae were reared individually in non-transparent plastic cups (diameter 4 cm, height 10 cm, filled to a height of 5 cm with water). Larvae were daily fed *Artemia* nauplii ad libitum. When larvae moulted into the final instar, swimming propensity and swimming speed were scored using the same methodology as used in the selection experiment. Depending on the species, 5-16 larvae per full-sib family were measured for swimming propensity and speed (total of 770 larvae).

Statistical analyses

We tested for effects of predator treatment and species on mortality rate using a mixed model ANOVA. Enclosure nested in predator treatment was added as a random variable to take into account that we had two mortality rates per enclosure, one for each species.

To specifically test for phenotypic selection on the escape behaviours, we directly compared the caged and free predator treatments for each species separately. For swimming propensity, a binary response variable, we defined a binomial error structure and the logit link using the lme package in R 3.3.0. For swimming speed we defined a normal error structure and the identity link and added mass as a covariate. In these models we included enclosure as a random variable to take advantage of the full dataset while overcoming the problem of pseudoreplication within enclosures (Brown & Prescott, 1999; Millar & Anderson, 2004). Given that we had a priori expectations that fish (dragonfly) predation would select for a lower (higher) swimming propensity, and dragonfly predation would select for a higher swimming speed, we provide one-tailed p-values for these tests. Note that we did not expect selection by fish predation on swimming speed. The value of the corresponding selection coefficient was estimated by dividing the difference in swimming traits between the free-ranging predator and caged-predator treatments by the pooled standard deviation (Lande & Arnold, 1983).

To estimate broad-sense heritabilities, we analysed each trait separately per species using animal model analyses (see Alho et al., 2011) in the MCMCglmm package (Hadfield, 2009) with R 2.10.1 (R Development Core Team 2009). In the models the term ‘animal’ (which is referenced back to the pedigree) was included as a random effect.

The heritability estimates are reported as posterior means together with 95% highest posterior density intervals (95% HPDIs). We obtained an estimate of the broad-sense heritability by applying the basic formula $H^2 = V_A/V_P$. Using Bayesian inference, more specifically MCMC (Markov Chain Monte Carlo), we estimated the 95% highest posterior density intervals to see whether it spans zero. For swimming speed, the Markov chains were run for 65,000 iterations. The first 15,000 iterations were discarded as these are less reliable. Iterations were sampled every 50th sample to reduce autocorrelation between successive samples (Wilson et al., 2010). For swimming propensity (a binary trait) we used a probit link. The analysis was run for 1,000,000 iterations where the first 10,000 iterations were discarded and an interval of 100 samples was used (Villemereuill et al., 2012).

RESULTS

Fish-lake selection experiment

Fish imposed strong mortality on both *Enallagma* species (Predator treatment: $F_{1,9} = 12.32$, $P = 0.0067$), and the increase in mortality rate due to direct fish predation was not different for the two *Enallagma* species (Species $\times$ Predator treatment: $F_{1,9} = 0.48$, $P = 0.51$; Fig. 1a).

Swimming propensity was lower for *E. hageni* larvae recovered from the free-ranging predator treatment compared to those recovered from the caged-predator treatment (Fig. 2a: $\chi^2 = 3.73$, $df = 1$, $P_{\text{one-sided}} = 0.026$). This was not the case for *E. geminatum* (Fig. 2a; $\chi^2 = 0.02$, $df = 1$, $P_{\text{one-sided}} = 0.44$). The associated standardized selection coefficient on swimming propensity for *E. hageni* was -2.87 standard deviation

units. Swimming speed did not differ between predator treatments in either species (both $P_{\text{one-sided}} > 0.06$, Fig. 2b).

Dragonfly-lake selection experiment

Dragonfly larvae imposed similar strong mortality on both *Enallagma* species (Predator treatment: $F_{1,30} = 8.88$, $P = 0.0057$, Species $\times$ Predator treatment: $F_{1,30} = 1.04$, $P = 0.32$; Fig. 1b).

Swimming propensity did not differ between larvae recovered from both predator treatments (both $P_{\text{one-sided}} > 0.30$, Fig. 2c). Swimming speed was higher for larvae recovered in the free-Dragonfly treatment compared to those recovered in the caged-Dragonfly treatment in *E. hageni* ($t_{14} = 2.07$, $P_{\text{one-sided}} = 0.028$) but not in *E. geminatum* ($t_{10} = 0.45$, $P_{\text{one-sided}} = 0.331$) (Fig. 2d). The associated selection coefficient on swimming speed for *E. hageni* was +0.74 standard deviation units.

Quantitative genetic rearing experiments

The full-sibling estimated heritabilities of the two escape behaviours were significantly different from zero in each species and varied between 0.14 and 0.28 (Table 1). Given the full-sibling design and hence potential maternal effects, the obtained heritability estimates may be biased upward.

DISCUSSION

In line with the strong mortality imposed by fish and dragonfly predators, the results of this study demonstrate different patterns of phenotypic selection on each type of escape

traits between the two habitats for *E. hageni*. Some mortality may have been caused by cannibalism, yet this would have occurred in both treatments with caged and with free-ranging predators and therefore is unlikely to have affected treatment differences. The observed patterns were expected based on *a priori* knowledge of the role of swimming to survive fish attacks versus dragonfly attacks in *Enallagma* damselflies (McPeck, 1990b, 1997; McPeck, et al. 1996). These results are also consistent with evolutionary reconstructions within this genus showing that species that invaded dragonfly lakes evolved a higher propensity to swim and a higher escape swimming speed (McPeck et al., 1996; McPeck, 2000), and genetic evidence indicates that *E. hageni* is the fish-lake progenitor from which one of the habitat shifts into the dragonfly lake habitat was derived (Turgeon et al., 2005).

Fish predation favoured larvae with a lower propensity to swim in *E. hageni*. Not swimming in the presence of fish is favoured for two reasons. Swimming makes larvae more detectable by fish predators (Baker et al., 1999), and swimming away to escape fish predation is not effective because fish can swim much faster than damselfly larvae (Stoks & De Block, 2000). This also explains why no selection on swimming speed was detected in the fish-lake experiment: if larvae do not express the behaviour to swim, selection cannot operate on swimming speed. Field studies evaluating survival selection on the propensity to escape when attacked are rare. One notable exception is the study by Janzen (1995) who reported similar negative survival selection on the propensity of turtle hatchlings to run. The proposed underlying explanation was similar to our study: motionless animals are cryptic and therefore have increased survivorship over more

active individuals that flee when they are faced with a predator that they have little chance of escaping pursuit.

In contrast, we could detect no selection by dragonfly predation for a higher swimming propensity. Given that larvae of *Enallagma* species native to dragonfly lakes use swimming to escape attacks by dragonfly predators and we documented ongoing selection for a higher swimming propensity in a derived dragonfly-lake *Enallagma* species (Strobbe et al., 2009), such selection pressure was expected in this study. Phenotypic variability among the damselfly larvae used in this experiment may have been too small to detect phenotypic selection on swimming propensity over a month of exposure to dragonfly predators. These results may also suggest that an ordering effect is important to elaborate escape from attacking predators as an effective antipredator adaptation. The dragonfly-lake *Enallagma* larvae of comparable sizes to individuals used in the present studies have swim speeds of 10-25 cm/s, whereas the fish-lake *E. hageni* and *E. geminatum* had maximum speeds of 7-10 cm/s (this study and McPeck et al., 1996; McPeck, 2000). Perhaps the low escape swimming speeds of the fish-lake *Enallagma* species generated a too limited escape probability when attacked by dragonfly predators to give animals with a higher propensity to swim away a detectable survival advantage.

Despite no significant phenotypic selection on swimming propensity, we did detect significant survival selection imposed by the dragonfly predators for a higher swimming speed in *E. hageni*. This is consistent with previous laboratory studies showing that larvae that swim faster have a higher probability of surviving dragonfly attacks (McPeck et al., 1996).

In contrast to these results for *E. hageni*, we could detect no significant phenotypic selection on *E. geminatum* larvae in either habitat. The *E. geminatum* larvae recovered from the fish predation experiment already had a much lower swimming propensity than *E. hageni* (Fig. 2a), suggesting a lack of significant variation on which selection could have acted. The lack of a response for swimming propensity or speed in the dragonfly-lake selection experiment is more puzzling. Possibly, the dragonfly predators may have preferred other prey with less efficient escape traits thereby precluding detecting selection on escape traits in *E. geminatum*.

Our results do not support the hypothesis of divergent survival selection pressures imposed by fish and dragonfly predators on swimming propensity. Our results demonstrate in one species (*E. geminatum*) that selection in the ancestral fish-lake environment operated against the use of swimming as an evasive tactic. Consistent with an overall scenario of opposing selection on swimming propensity across lake types, a previous experiment showed that survival selection imposed by dragonfly predators favoured higher swimming propensities in the derived dragonfly-lake species *E. annexum* (Strobbe et al., 2009). Yet, we could not detect dragonfly-imposed survival selection for an increased swimming propensity in the present study utilizing fish-lake *Enallagma*. This may not be that surprising. When a lineage invades a new ecological environment, individuals in many founder populations likely often are too maladapted for selection to act (McPeck 2000). This is because only a very weak phenotype-fitness covariation will be present when phenotypes are too far from the phenotypic optimum (Brodie & Brodie, 1999; Toju & Sota, 2006). Only the rare invasion will be successful: those were the invaders that probably by chance had the least phenotypic distance to match with the new

adaptive peak (McPeck, 2000), hence those where survival selection will be strong thereby pushing the phenotype to the new optimum rapidly.

Our results provided some support to the hypothesis that fish predation did not impose survival selection on swimming speed in the ancestral fish lakes, while dragonfly predation imposed survival selection for a faster swimming speed. Yet, the latter was only observed in *E. hageni*. Together with the here documented selection for increased swimming speed on the fish-lake *Enallagma* by the new predator in the dragonfly lakes (which is still ongoing in dragonfly-lake *Enallagma*, Strobbe et al., 2009), this may generate differentiation in swimming speed between species in both lake types.

For both escape traits we have shown significant broad-sense heritabilities that are close to measured estimates of heritability of other anti-predator behaviours (0.33 ± 0.038 , Stirling et al., 2002). This indicates that the documented selection pressures imposed by fish and dragonfly predators indeed may result in evolutionary responses contributing to the differentiation in both escape traits when fish-lake *Enallagma* invaded dragonfly lakes (McPeck et al., 1996; McPeck & Brown, 2000). Admittedly, some degree of caution must be exercised when interpreting the estimates of the variance components for swimming propensity as quantitative genetic analyses of binary data are not without caveats (de Villemereuil et al., 2013). Also, the relatively wide 95% HPD intervals indicate low precision of the heritability estimates. Nevertheless, our data suggest minimal heritability for both traits.

By integrating data on selection strength and heritabilities, crude estimates can be made on how rapidly these evolutionary changes during habitat shifts may have occurred. For example, phylogenetic analyses suggest that *E. hageni* is the fish-lake ancestor of the

dragonfly-lake clade including *E. annexum* (Turgeon et al., 2005). Based on swimming speed data obtained from larvae recovered from the caged predator treatments in *E. hageni* (mean $\pm$ STD: 77.99 ± 6.91 mm/s, current study) and *E. annexum* (104.95 ± 5.32 mm/s, Strobbe et al., 2009), swimming speed differs between these species by 5.07 STD units. Given the degree of heritability of swimming speed we have estimated in *E. hageni* (0.14) and the documented selection coefficient (0.74 STD units) it may have taken only ca. 43 years (*Enallagma* have one generation per year) to evolve the higher swimming speed in the derived dragonfly-lake species. A similarly short period has been estimated from similar information on lamellae size (McPeck, 1997). Such rapid evolutionary responses would be critical for a successful habitat shift to turn the founder population from a sink population being driven extinct by dragonfly predation to a source population that is adapted to living with dragonflies. The associated evolutionary rate of 0.10 haldanes indicates rapid evolution (sensu Hendry & Kinnison, 1999). Interestingly, the observed rapid evolution in escape performance is similar to the reported rapid (26-36 generations) evolution of escape ability in guppies when they invaded high-predation habitats (O'Steen et al., 2002). This would suggest that for predator escape traits, phenotypic evolution associated with ecological speciation linked to habitat shifts may at least have the potential to occur as rapidly as evolutionary responses within species, although counteracting gene flow might have slowed down the speciation process. By embedding selection experiments in an evolutionary historical analysis a deeper understanding about the genesis of biological diversity can be gained.

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TABLES

Table 1. Heritabilities (h^2) of swimming propensity and escape swimming speed for two fish-lake *Enallagma* species: *E. geminatum* and *E. hageni* based on a full-sib rearing experiment. Confidence intervals are expressed as 95% highest posterior density intervals (HPDI).

Performance trait	<i>E. geminatum</i>		<i>E. hageni</i>	
	h^2	HPDI	h^2	HPDI
Swimming propensity	0.23	<0.001-0.675	0.28	0.078 - 0.755
Swimming speed	0.24	0.039 - 0.601	0.14	0.052 - 0.547

615 FIGURE LEGENDS

616 **Fig. 1.** Daily mortality rates of *Enallagma geminatum* and *E. hageni* larvae in the caged-
617 and free-predator treatments of the (a) fish-lake and (b) dragonfly-lake selection
618 experiments. Given are least square means $\pm$ 1SE. Means are based on (a) four caged-fish
619 and eight free-fish enclosures and (b) four caged-dragonfly and thirteen free-dragonfly
620 enclosures.

621

622 **Fig. 2.** Swimming propensity and swimming speed of *Enallagma geminatum* and *E.*
623 *hageni* larvae in the caged- and free-predator treatments of the (a) fish-lake and (b)
624 dragonfly-lake selection experiments. Given are least square means $\pm$ 1SE. Numbers with
625 symbols represent sample sizes.

626

Figure 1

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