ADAPTATION TO PREDATORS IN A NEW COMMUNITY: SWIMMING PERFORMANCE AND PREDATOR AVOIDANCE IN DAMSELFIES

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Abstract. Species evolve in response to the abiotic and biotic interactions they experience in a community, and so studying the adaptation of species in a community context is a necessary component for understanding the development of community structure. Previous studies have suggested that Enallagma damselflies (Odonata: Coenagrionidae) invaded aquatic habitats with large dragonflies as the top predators at least twice, and large changes in morphological structures (e.g., caudal lamellae and abdomen), which should increase swimming speed, are associated with these invasions. This is significant because these species swim away from attacking predators, whereas species in the ancestral habitat (habitats with fish as the top predators) do not swim from attacking predators. We quantified swimming speed over a large ontogenetic size range for larvae of seven Enallagma species, two in dragonfly lakes and five in fish lakes. Results of analysis of covariance and a modification of Felsenstein’s method of evolutionary contrasts indicate that a large evolutionary increase in the slope of the ontogenetic relationship between swimming speed and $\log_{10}$ (body length) is associated with invasions into dragonfly lakes, and this shift results in larvae of dragonfly-lake species being faster swimmers, especially in larger size classes, than larvae of fish-lake species. We also present experimental results demonstrating that damselflies with larger lamellae are faster swimmers and have higher survival when exposed to dragonfly predation. The results of this study indicate that the change in selective environment associated with habitat shifts into dragonfly lakes promoted adaptations for increased swimming speed in Enallagma lineages. The potential ramifications of this adaptation for community structure are discussed.

Key words: adaptation; Enallagma; Odonata; phylogenetic reconstruction; predation; predator avoidance; swimming performance.

INTRODUCTION

Species interactions influence community structure on both ecological and evolutionary time scales. On ecological time scales, species interactions influence which species can coexist and what population dynamics coexisting species display; these are the usual lines of inquiry in investigations of community structure. On evolutionary time scales, species interactions act as agents of natural selection that shape the phenotypic distributions of populations and species and that influence diversification rates of component taxa and ultimately community diversity.

Interacting species are not static entities, but rather entities whose properties change in response to evolutionary forces. Changes in phenotypic properties can profoundly influence the strengths and mechanisms of interactions with other species. Studying how species adapt to interactions with other species in a community context addresses fundamental questions about the mechanisms by which community structure develops over time. Adaptation of coexisting species to interspecific competition has been a major topic of inquiry and debate in community ecology for many years (e.g., Lack 1947, Brown and Wilson 1956, Fenchel 1975, Roughgarden 1976, Davidson 1977, 1978, Connell 1980, 1983, Slatkin 1980, Price and Brown 1983, Schoener 1983, Rummell and Roughgarden 1985, Schluter et al. 1985, Taper and Case 1985, Abrams 1987, Schluter 1988, 1994, Losos 1990, Schluter and McPhail 1992). However, the ramifications to community structure of adaptation to other types of species interactions, most notably predation, until recently have been largely ignored.

Interactions with predators influence prey diversity and abundances in many communities (Sih et al. 1985), and predation is a potent agent of natural selection for many species (e.g., Brodie 1992, Weis et al. 1992; see Endler 1986, Vermeij 1987 for reviews). Theoretical studies suggest that changes in prey species diversity (Holt 1977, Brown 1992), prey abundances and population dynamics (Abrams 1986, 1990, 1991, Abrams and Matsuda 1993), and the nature and strengths of interactions with other species (Matsuda et al. 1993)
may all occur as prey adapt to their predators. Therefore, studies of prey adaptation to their predators, and adaptation in a community context in general, may provide key insights into how mechanisms of multispecies interactions have been elaborated in nature and how present-day communities were assembled.

The study of adaptation is the study of adaptedness in an historical context (Gould and Vrba 1982, Coddington 1988, Baum and Larson 1991, Leroi et al. 1994). Adaptedness is a statement about the relationship between fitness and the phenotype (Gould and Vrba 1982, Coddington 1988, Baum and Larson 1991, Lauder et al. 1993, Reeve and Sherman 1993, Leroi et al. 1994); phenotype A makes an individual more “adapted” to its environment than phenotype B, if phenotype A confers higher fitness on its possessor via its effects on some performance measure than does phenotype B. In a community context, studying the adaptedness of a particular species to its environment then requires that one (1) identify the interactions affecting fitness (i.e., survival and reproduction), (2) distinguish the phenotypic characters influencing performance in these interactions, and (3) demonstrate that altering the phenotype would lead to a decrease in fitness (see also recent critiques of adaptation studies by Lauder et al. 1993, Reeve and Sherman 1993, Leroi et al. 1994). These studies of adaptedness should then be placed in an historical context to show that changes in the phenotype have occurred when the selective environment has changed (Coddington 1988, Baum and Larson 1991). The study of adaptation in a community context must therefore meld mechanistic studies of species interactions, functional studies of the phenotypes of interacting taxa, evolutionary studies of natural selection, and phylogenetic studies of component taxa.

In this paper we examine how Enallagma damselflies (Odonata: Coenagrionidae) adapted, following habitat shifts, to the predators found in the new community. Enallagma species display a striking distribution pattern among the bogs, marshes, ponds, and lakes of eastern North America; one group of species is found as larvae only in bodies of water that lack fish, whereas the remaining species are found as larvae only in bodies of water that support fish (Johnson and Crowley 1980, McPeek 1989, 1990a). The results of field experiments have shown that fish predation in fish lakes and dragonfly predation in fishless lakes (hereafter referred to as dragonfly lakes) maintain the segregation of species between the two lake types, are primary sources of larval mortality, and influence larval growth rates in their respective communities (Pierce et al. 1985, Blois-Heulin et al. 1990, M. A. McPeek 1990a, and unpublished data). Enallagma species in the two community types are differentially susceptible to these predators because they differ in overall levels of activity and in their responses to attacking predators; species in fish lakes are inactive and do not attempt to evade attacking predators, whereas species from dragonfly lakes are more active and swim away from attacking dragonflies (Pierce et al. 1985, McPeek 1990b). Whether these and other phenotypic differences among species in the two lake types are adaptations for coexisting with fish vs. dragonfly predators is one topic of our ongoing inquiry into the ecological and evolutionary mechanisms structuring these communities (McPeek 1995a, b).

Phylogenetic analyses indicate that the fish-lake community is ancestral and that at least two independent invasions of the dragonfly-lake community have occurred (M. A. McPeek 1995a, and unpublished data, M. L. May, personal communication). The results of character reconstruction analyses indicate that changes in morphological structures that should increase swimming speed are associated with these habitat shifts into dragonfly lakes (McPeek 1995a, b). Damselfly larvae swim by moving their abdomen from side to side and using the three caudal lamellae at the end of their abdomen to generate thrust. If a larva loses all three caudal lamellae, its swimming speed is greatly reduced (Robinson et al. 1991a). Reconstructions of evolutionary rates of character change indicate that large increases in the surface areas of caudal lamellae were associated with habitat shifts into dragonfly lakes (McPeek 1995a, b). These results are consistent with the hypothesis that increasing swimming speed via increasing caudal lamellae area is an adaptation to coexisting with dragonflies in dragonfly lakes, but they do not constitute a complete test of this hypothesis.

In this paper we present data that completes the test of increasing swimming speed via increasing lamellae area being an adaptation to dragonfly lakes. First, we measure swimming speed for seven Enallagma species and reconstruct its evolutionary history to test whether significant increases in swimming speed are associated with habitat shifts into dragonfly lakes. Second, we experimentally alter caudal lamellae size to determine whether lamellae size is a functional component that influences swimming speed. Finally, we experimentally test whether caudal lamellae size influences susceptibility to dragonfly predation.

**Materials and Methods**

*Ontogeny of swimming performance among species*

To examine interspecific differences in swimming performance among Enallagma species in the two community types, we videotaped larvae of seven species swimming in a laboratory arena. The seven species were *E. aspersum* and *E. boreale* from dragonfly lakes and *E. hageni, E. geminatum, E. exsulans, E. signatum,* and *E. vesperum* from fish lakes. These species were chosen for a number of reasons: (1) all have large geographic ranges extending across much of northeastern North America (e.g., most range from Maine and the maritime provinces of eastern Canada west to Michigan and western Ontario, and south to North Carolina; M.
Table 1. Source populations and size ranges for the seven *Enallagma* species that were videotaped for swimming performance. The last three columns characterize the size range of larvae used for each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source population</th>
<th>N*</th>
<th>Latitude and longitude</th>
<th>Minimum body length (mm)</th>
<th>Mean body length (mm)</th>
<th>Maximum body length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dragonfly lakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. aspersum</em></td>
<td>Johnson's Pond, Norwich, Vermont</td>
<td>39</td>
<td>43°45'00&quot;N, 72°17'30&quot;W</td>
<td>8.06</td>
<td>11.96</td>
<td>15.36</td>
</tr>
<tr>
<td><em>E. boreale</em></td>
<td>Ferson Road Marsh, Lyme, New Hampshire</td>
<td>40</td>
<td>43°46'05&quot;N, 72°08'49&quot;W</td>
<td>8.31</td>
<td>13.71</td>
<td>20.63</td>
</tr>
<tr>
<td>Fish lakes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. hageni</em></td>
<td>Palmatier Lake, Barry County, Michigan</td>
<td>5</td>
<td>42°34'19&quot;N, 85°26'16&quot;W</td>
<td>7.85</td>
<td>12.16</td>
<td>15.40</td>
</tr>
<tr>
<td><em>E. geminatum</em></td>
<td>Palmatier Lake, Barry County, Michigan</td>
<td>44</td>
<td>42°21'02&quot;N, 85°25'58&quot;W</td>
<td>5.81</td>
<td>9.02</td>
<td>14.85</td>
</tr>
<tr>
<td><em>E. exsulans</em></td>
<td>Connecticut River, Hanover, New Hampshire</td>
<td>27</td>
<td>43°44'20&quot;N, 72°15'05&quot;W</td>
<td>7.45</td>
<td>11.11</td>
<td>14.06</td>
</tr>
<tr>
<td><em>E. signatum</em></td>
<td>Palmatier Lake, Barry County, Michigan</td>
<td>18</td>
<td>42°34'19&quot;N, 85°26'16&quot;W</td>
<td>6.64</td>
<td>9.41</td>
<td>13.92</td>
</tr>
<tr>
<td><em>E. vesperum</em></td>
<td>Three Lakes II, Richland, Michigan</td>
<td>14</td>
<td>42°21'02&quot;N, 85°25'58&quot;W</td>
<td>6.10</td>
<td>10.00</td>
<td>12.39</td>
</tr>
<tr>
<td></td>
<td>McDaniels Marsh, Enfield, New Hampshire</td>
<td>26</td>
<td>43°31'38&quot;N, 72°04'43&quot;W</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Palmatier Lake, Barry County, Michigan</td>
<td>19</td>
<td>42°34'19&quot;N, 85°26'16&quot;W</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N* = the total number of individuals included from that population in the study.

A. McPeek 1989, 1990a, unpublished data); (2) within a lake type they usually all co-occur (e.g., *E. aspersum* and *E. boreale* are usually found co-occurring in lakes where their ranges overlap; McPeek 1990a); (3) they are representative of the breadth of taxonomic diversity within the North American monophyletic group of *Enallagma*; and (4) all are common and readily obtained from the field.

Table 1 gives the locations of source populations, sample size, and the size range of each species. We included as large a size range of larvae as possible in the study. Final instars of all species are included, and for most species the smallest individuals are instars F-6 or F-5 (damselflies have 11 instars, and they are generally identified as F for the final instar, F-1 for the penultimate instar, F-2 for the antepenultimate, etc.). Larvae were collected between 0800 and 1000 and run through swimming trials between 1200 and 1530 of the same day. Only larvae with completely intact and un-regenerated lamellae were used.

All swimming trials were videotaped in a 35.5 cm long × 25.5 cm wide × 6.0 cm deep Plexiglas container filled with 23°C tap water and illuminated with indoor fluorescent room lighting. The temperature of 23°C is well within the range of temperatures experienced by larvae of species in both lake type habitats (M. A. McPeek, unpublished data). A 15-cm clear ruler, graduated in millimetres, was attached to the bottom of the container for calibration during digitizing. Swimming events were recorded with a Cohu Model 4815 camera mounted above the container and a Sony MVD9500MD Super-VHS videorecorder. The magnification was adjusted so that the container filled the entire field of the video image.

For each trial one larva was gently released in the center of the container at the surface of the water. Many larvae swam immediately. Others would float to the bottom, orient themselves, and come to rest. Larvae were coaxied to perform multiple swims by gently tapping them on the dorsal surface of the thorax with a thin metal spatula. Each larva was prodded in this way until it performed four to six swims or clearly would not swim. Swims near the walls of the container were not included in the study because of potential optical distortion effects on the videotape.

Videotapes were digitized using BioScan OPTIMAS software. We digitized the position of the middle point on the thorax of a larva (i.e., the point around which the body pivots as a larva swims) in every third frame of a swim, which translates into recording the position of the larva every 0.05 s. We did not digitize swims if they lasted for <10 frames. The distance (in centimetres) swim by a larva in a swimming event was calculated by summing all the distances between locations in successive digitized frames. The duration of the swim (in seconds) was calculated by multiplying the number of successive digitized frames after the initial frame by 0.05 s(successive digitized frame).

Swimming speed (in centimetres per second) was then calculated as this distance divided by this duration. For each swim we also measured the number of complete beat cycles the tip of the abdomen moved through during the digitized frames of the swim. 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 should be a measure of the intensity of effort of the larva during the swimming event. We calculated the rate of abdomen beats (hereafter beat rate) as the number of cycles per duration of swim, which has units of beats per second.

We also measured the body length of each larva (distance from the tip of the snout to the end of the abdomen) after it had been preserved in 70% ethanol. Body length was measured by magnifying the larva
with a dissecting microscope and importing the image into OPTIMAS.

For analysis we included only data associated with the fastest swim performed by an individual. We used analysis of covariance (ANCOVA) with planned contrasts to test for specific differences among species in abdomen beat rate and swimming speed, with the natural logarithm of body length as the covariate. First, for each variable we tested for differences in slopes among the species. If no significant differences were found among the slopes, we tested for differences in midpoints of the relationships (Sokal and Rohlf 1981). All analyses were performed in the GLM procedure of SAS (SAS 1990).

We also applied phylogenetic analyses to reconstruct the evolutionary history of the slope of the regressions between swimming speed and log$_e$(body length). This metric quantifies the ontogenetic change in swimming speed with body size for each species. To reconstruct the evolutionary history of this character, we utilized the methods developed by McPeek (1995a, b), which are extensions of Felsenstein’s method of evolutionary contrasts (Felsenstein 1973, 1985, 1988). Evolutionary contrasts partition evolutionary change in a character into independent components associated with different parts of a phylogeny (Felsenstein 1973, 1985, 1988). The extended methods allow evolutionary change along some sets of single branches on a phylogeny to be isolated (McPeek 1995a).

To apply these methods, a phylogeny of the species, estimates of branch lengths on the phylogeny, and the variables to be reconstructed are needed. We use a phylogeny that is developed from a maximum parsimony analysis of 41 morphological, structural and pigment characters (McPeek 1995a). A previous analysis of this data set included all the species used here except E. exsulans, and two additional species, E. antennatum and E. traviatum (McPeek 1995a). For the present study an analysis using the exhaustive search algorithm of PAUP (Swofford 1993) found only one tree of shortest length (length 50) (Fig. 1). The phylogenetic relationships among the other species implied by this tree (Fig. 1) do not differ from previous analyses (cf. McPeek 1995a, b), and are consistent with a much larger analysis of 31 species using the same data set (M. A. McPeek, unpublished data). We used MacClade (Maddison and Maddison 1992) to identify the number of unambiguous character state changes in this cladistic data set (Fig. 1), and these numbers were used as estimates of branch lengths for the phylogeny (Garland et al. 1992).

The phylogenetic relationships among the seven species indicate that the two dragonfly-lake species must have arisen via independent evolutionary events leading to successful habitat shifts from fish lakes to dragonfly lakes (Fig. 1). Therefore, if increases in the ontogenetic relationship of swimming speed are critical to adapting to dragonfly lakes, large and directed changes in this character should be evident along the branches leading directly to E. aspersum and E. boreale. We therefore constructed evolutionary contrasts to isolate change along these two branches of the phylogeny (McPeek 1995a, b). The evolutionary contrast set is (1) boreale–immediate ancestor; (2) hageni–geminatum; (3) aspersum–immediate ancestor; (4) vespum–signatum; (5) exsulans–immediate ancestor of vespum; (6) immediate ancestor of geminatum–immediate ancestor of exsulans. Contrasts were standardized by the square roots of their branch lengths for comparison (Felsenstein 1985, McPeek 1995a, b).

Swimming performance of larvae with experimentally reduced lamellae

We experimentally reduced the sizes of caudal lamellae on larvae to examine whether larvae with smaller lamellae are poorer swimmers. Final-instar larvae of E. vernale collected from McDaniels Marsh, Enfield, New Hampshire, were used in this experiment. Larvae were collected between 0800 and 1000 and used in the experiment between 1200 and 1530 of the same day. Only larvae with completely intact and unregenerated lamellae were used.

We applied one of three lamellae tissue removal treatments to larvae and then videotaped them swimming. The three treatments were (1) leaving all three lamellae completely intact (“whole” lamellae), (2) “nipping” off the ends of the three lamellae by making vertical cuts with a single-edged razor to remove approximately the distal 1/10 of each lamella, and (3)
“cutting” off the distal half of all three lamellae by making a vertical cut with a razor. The Nipped treatment is a control for potential detrimental effects of wounding on swimming performance. Larvae were assigned randomly to treatments, with 11 larvae in the Whole Lamellae treatment, 17 in the Nipped treatment, and 15 in the Cut treatment. All larvae were only used once. The treatment was applied to a larva (i.e., the lamellae were cut to the specified level if necessary), and the larva was then immediately placed in the swimming arena and prodded to swim as described in the previous section. All larvae readily swam when prodded. Four to eight swims were recorded for each individual. Trials were performed under fluorescent room lighting at 23°C. Methods for digitizing and analyzing the videotapes are as described in the previous section. We also digitized the lateral surface areas of the three lamellae and body length for each larva after they were preserved in 70% ethanol. The Nipped and Cut treatments are not unusual for *Enallagma* larvae, since caudal lamellae are routinely damaged and lost in nature; up to 60% of larvae in any given population may have at least one caudal lamella which has been lost and regenerated (McPeek 1990a). Although lamellae are primary sites of gas exchange, only \( \approx 20\% \) of the oxygen taken up by larvae enters through the lamellae over a wide range of oxygen concentrations and temperatures, including those of the experimental setup we used (Eriksen 1986). Since all larvae completed the swimming trials within 30–60 s after having lamellae tissue removed, oxygen stress caused by lamellae tissue removal was probably minimal or nonexistent.

Only data associated with the fastest swim by each individual were included in the analyses. We applied analyses of variance with planned orthogonal contrasts to test for significant treatment effects (Sokal and Rohlf 1981). In addition to swimming speed, we analyzed the rate at which larvae swung their abdomens (i.e., abdomen beat rate) during swimming to examine whether the surgical removal of lamellae tissue had any detrimental effect on the intensity of activity during swimming. We also used regression analyses to examine the relationship between swimming speed and average lamella area. First, swimming speed was regressed on abdomen beat rate, and the residuals around this line were calculated. These residuals were then regressed on the average lamella area of individuals. This second regression presents the relationship between swimming speed and mean lamella area, after removing the effects of different intensities of activity during swimming from the analysis.

**Dragonfly selection experiment**

We tested whether *Enallagma* larvae with smaller caudal lamellae are more susceptible to dragonfly predation by experimentally producing larvae that differed in lamellae size and quantifying their relative susceptibilities to dragonfly predators. Two *Enallagma* species were used in this experiment: *E. aspersum*, a dragonfly-lake species, and *E. vespertinum*, a species from lakes with fish. *E. aspersum* larvae were collected from Johnson’s Pond, Norwich, Vermont, and *E. vespertinum* were collected from McDaniels Marsh, Enfield, New Hampshire (see Table 1). These species were chosen because they have been shown to differ in whether they swim away from attacking dragonflies: *E. aspersum* does, and *E. vespertinum* does not (McPeek 1990b). The dragonfly predator used was *Aeshna canadensis*, a large aeshnid common to dragonfly ponds and lakes in New England; dragonflies were collected from Johnson’s Pond. All damselflies and dragonflies were final instars.

A stock of *Enallagma* larvae of one species was collected between 0800 and 1000 on days when trials were performed. Trials were done between 1300 and 1600, and were conducted in 20 cm diameter glass dishes filled to a depth of 5 cm with tap water. The bottoms of dishes were covered with fiberglass window screening to provide footing for the odonates. All trials were run under fluorescent room lighting at 23°C.

Immediately before a trial, two similar-sized *Enallagma* larvae of the same species were selected from the collected stock. Each larva then had a portion of its caudal lamellae removed by making a quick vertical cut with a single-edged razor; one larva had approximately the distal one-fourth of all three lamellae removed, and the other had approximately the distal two-thirds of all three lamellae removed. Treatments were assigned to individuals at random. These treatments resulted in individuals that differed greatly in lamellae surface area. We reduced lamellae size on both larvae so that they would not differ in ways that may attract predators (e.g., hemolymph leaking from new wounds). The two larvae were then introduced into a dish. After 5 min a single *Aeshna* larva, which had been held without food for 2 d, was introduced. A trial ended when the *Aeshna* ate one of the *Enallagma*. Trials generally lasted for \(<5\) min. We did not allow wounds to heal or a longer acclimation period because damselflies autotomize injured lamellae within 20 min (M. A. McPeek, personal observation). Each individual damselfly and dragonfly was used in only one trial. Thirty-one replicate trials were performed for *E. aspersum*, and 16 trials for *E. vespertinum*.

For analysis a 2 \( \times \) 2 contingency table was constructed with *Enallagma* species and the number of trials in which the first larva eaten had larger or smaller lamellae as the rows and columns, respectively. \( G \) tests were used to test whether the frequency of trials in which larvae with larger or smaller lamellae were eaten first differed between the species, and for each species whether the number of trials with each outcome was equal. If larger lamellae result in a greater ability to avoid attacking dragonflies by increasing swimming performance, the larva with smaller lamellae should be eaten first in significantly more trials for *E. aspersum*, because this species attempts to avoid attacking drag-
Fig. 2. The relationship between abdomen beat rate (beats/s) and body length (mm) for the seven species of Enallagma; note that the abscissa is a natural logarithmic scale. For clarity of presentation, species in the two major clades are given in different panels: the left panel presents data for E. aspersum (symbolized by a), E. boreale (b), E. geminatum (g), and E. hageni (h); the right panel presents data for E. exsulans (x), E. sigvatum (s), and E. vesperum (v). Each symbol represents a datum for one individual. Linear least squares regressions for species give the following relationships: E. aspersum Abdomen Beat Rate = \(-22.19 + 15.25 \log_{10}(\text{Body Length})\); E. boreale Abdomen Beat Rate = \(3.17 + 3.38 \log_{10}(\text{Body Length})\); E. geminatum Abdomen Beat Rate = \(-9.72 + 9.52 \log_{10}(\text{Body Length})\); E. hageni Abdomen Beat Rate = \(-12.87 + 8.53 \log_{10}(\text{Body Length})\); E. exsulans Abdomen Beat Rate = \(24.81 - 8.15 \log_{10}(\text{Body Length})\); E. sigvatum Abdomen Beat Rate = \(7.36 + 0.18 \log_{10}(\text{Body Length})\); and E. vesperum Abdomen Beat Rate = \(-7.30 + 6.13 \log_{10}(\text{Body Length})\). The regressions for the two dragonfly-lake species are given by the dashed lines, and solid lines present the regressions for the five fish-lake species.

flies by swimming; but the first larva eaten should be equally apportioned between the two types for E. vesperum, because this species does not swim from attacking dragonflies.

Results

The ontogeny of swimming performance among species

The slopes of the relationships between abdomen beat rates and \(\log_{10}(\text{body length})\) differed significantly among the seven species (Fig. 2; \(F_{6,204} = 2.19, P < 0.05\)). This difference was primarily due to differences between species in the two clades (contrast testing for differences in slopes between species in the two clades [i.e., aspersum, boreale, geminatum, hageni vs. exsulans, sigvatum, vesperum], \(F_{1,204} = 7.20, P < 0.008\)). Therefore, we analyzed species in the two clades separately. For the relationship between abdomen beat rate and \(\log_{10}(\text{body length})\), E. exsulans, E. sigvatum, and E. vesperum did not differ in slopes (\(F_{2,38} = 1.71, P > 0.19\)) or intercepts (\(F_{2,38} = 1.06, P > 0.35\); individual tests for slopes different from zero were nonsignificant for all three species (Fig. 2; all \(P > 0.10\)). Species in the other clade also did not differ in slope among species (\(F_{3,168} = 1.94, P > 0.12\), but they did differ significantly in intercept (Fig. 2: \(F_{3,171} = 20.98, P < 0.0001\)). Although the average intercept of the two species in dragonfly lakes was significantly higher than the average intercept of the two fish-lake species (linear contrast testing for differences aspersum, boreale vs. geminatum, hageni: \(F_{1,171} = 12.95, P < 0.0004\), inspection of the least square means (SAS 1992) for the relationships ordered the species from highest to lowest relationship as aspersum, geminatum, boreale, and hageni (Fig. 2).

The slopes of the relationships between swimming speed and \(\log_{10}(\text{body length})\) also differed significantly among the seven species (Fig. 3: \(F_{6,204} = 4.31, P < 0.0004\)). Although the two clades differed in average slope (\(F_{1,204} = 8.25, P < 0.005\)), this difference was due to the differences in slopes between species in the two community types. Fish-lake species in the two clades did not differ in slope (Fig. 3: contrast testing for difference in slopes between geminatum, hageni vs. exsulans, sigvatum, vesperum, \(F_{1,204} = 1.43, P > 0.23\)). However, slopes of swimming rate against \(\log_{10}(\text{body length})\) were significantly steeper for E. aspersum and E. boreale than for the five fish-lake species (\(F_{1,204} = 16.88, P < 0.0001\)). These results indicate that swimming speed increases more than twice as fast with increasing body size in dragonfly-lake species as in fish-lake species, and that the allometry between swimming speed and body size is similar among species within each habitat (Fig. 3). Consequently, average swimming speed of dragonfly-lake species is greater than that of fish-lake species over much of the size range studied, and this difference increases with increasing size (Fig. 3).

The results of the evolutionary contrasts analysis indicate that a large increase in the allometry of swim-
Fig. 3. The relationship between swimming speed (cm/s) and body length (mm) for the seven species of Enallagma; note that the abscissa is a natural logarithmic scale. As in Fig. 2, species in the two major clades are given in different panels. Symbols for species are as in Fig. 2. Each symbol represents a datum for one individual. Linear least squares regressions for species give the following relationships: *E. aspersum* Swimming Speed = -36.05 + 20.00 log(Body Length); *E. boreale* Swimming Speed = -46.41 + 24.14 log(Body Length); *E. geminatum* Swimming Speed = -16.63 + 10.41 log(Body Length); *E. hageni* Swimming Speed = -15.21 + 7.98 log(Body Length); *E. exsulans* Swimming Speed = 11.48 + -2.97 log(Body Length); *E. signatum* Swimming Speed = -11.73 + 7.41 log(Body Length); and *E. vesperum* Swimming Speed = -9.54 + 6.57 log(Body Length). The regressions for the two dragonfly-lake species are given by the dashed lines, and solid lines present the regressions for the five fish-lake species.

Swimming speed and log(body length) is associated with habitat shifts into dragonfly lakes (Fig. 4). The standardized contrasts associated with the external branches leading to *E. aspersum* and *E. boreale* are significantly larger than the other four standardized contrasts (t<sub>n</sub> = 3.99, P < 0.05). Because habitat shifts from fish lakes to dragonfly lakes are hypothesized to have occurred along these two branches, this result suggests that large increases in the allometric slope of swimming speed against log(body length) occur when lineages shift from occupying fish lakes to occupying dragonfly lakes.

*E. vernal* larvae did not differ in body length among the three treatments (Table 2: F<sub>2,40</sub> = 0.71, P > 0.49). Larvae in the Cut treatment had significantly smaller lamellae than larvae in the other two treatments (Table 2: F<sub>2,40</sub> = 213.26, P < 0.0001). The difference in mean lamellae size between the Nipped and Whole Lamellae treatments was much smaller but also significant (Table 2: F<sub>2,40</sub> = 16.79, P < 0.0002). Larvae did not differ among the treatments in the intensity with which they attempted to swim (abdomen beat rate, Table 2: F<sub>2,40</sub> = 1.10, P > 0.34), suggesting that larvae were not oxygen stressed by the treatments. Also, although not directly quantified, no discernible treatment effects were evident on swimming performance characteristics such as steering, pitch, roll, or yaw control.

The swimming speed of larvae in the Cut treatment was significantly slower than larvae in the other two treatments (Table 2: F<sub>1,40</sub> = 30.47, P < 0.0001). Although the swimming speed of larvae in the Nipped treatment was on average less than in the Whole Lamellae treatment (Table 2), this difference was not significant (F<sub>1,40</sub> = 1.62, P > 0.21).

The results of the regression analysis of these data...
also support the conclusion that lamellae size has a direct functional relationship to swimming speed. The regression of swimming speed on abdomen beat rate produced a significant positive relationship (Fig. 5A), indicating that the intensity with which larvae swim their abdomens during swimming affects speed. The relationship between the residuals from that regression and mean lamellae area had a positive slope that was highly significant (Fig. 5B). In other words, after accounting for variation among individuals in the intensity of swimming effort, swimming speed strongly increases as average lamellae size increases.

**Dragonfly selection experiment**

The lamellae size of the first larva eaten in trials differed significantly between the two *Enallagma* species (Fig. 6; \( G_i = 4.02, \ P < 0.05 \)). In trials with *E. aspersum*, a species that uses swimming to evade attacking dragonflies (McPeek 1990a), larvae with shorter lamellae were eaten first significantly more frequently (Fig. 6; \( G_i = 7.45, \ P < 0.01 \)). In contrast, lamellae size did not affect which larva was eaten first in trials involving *E. vesperum*, a species that does not swim from attacking dragonflies (Fig. 6; \( G_i = 0.24, \ P > 0.70 \)). These results indicate that having larger lamellae increases a damselfly larva’s chances of successfully evading an attacking dragonfly, if the larva uses swimming as an antipredator defense.

**DISCUSSION**

The results of this study indicate that the change in selective environment associated with habitat shifts into dragonfly lakes promotes adaptations for increased swimming speed in larvae of *Enallagma* species. The selection pressures experienced by *Enallagma* larvae in these two communities should be very different. In fishless ponds and lakes, a set of large, active dragonfly species (i.e., *Anax, Aeshna, and Tramea* species), which are also restricted to fishless lakes by fish predation (Crowder and Cooper 1982), are primary sources of mortality for coexisting *Enallagma* species (Blois-Heulin et al. 1990, McPeek 1990a, Wissinger and McGrady 1993). In fact, dragonfly predation can account for > 70% of larval *Enallagma* mortality in

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**Table 2.** Results from the experiment in which the sizes of lamellae for *Enallagma vernale* larvae were reduced by surgically removing various amounts of tissue.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cut lamellae</th>
<th>Nipped lamellae</th>
<th>Whole lamellae</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{X} )</td>
<td>3.57</td>
<td>7.03</td>
<td>8.43</td>
</tr>
<tr>
<td>SE</td>
<td>0.17</td>
<td>0.24</td>
<td>0.30</td>
</tr>
<tr>
<td>Body length (mm)</td>
<td>15.97</td>
<td>15.54</td>
<td>15.70</td>
</tr>
<tr>
<td>SE</td>
<td>0.30</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td>Abdomen beat frequency (beats/s)</td>
<td>10.83</td>
<td>11.98</td>
<td>11.20</td>
</tr>
<tr>
<td>SE</td>
<td>0.51</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>Swimming speed (cm/s)</td>
<td>10.68</td>
<td>15.39</td>
<td>16.91</td>
</tr>
<tr>
<td>SE</td>
<td>0.83</td>
<td>0.81</td>
<td>0.72</td>
</tr>
</tbody>
</table>

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**Fig. 5.** The relationships among swimming speed, abdomen beat rate, and mean lamellae size from the experiment in which *E. vernale* larvae had their caudal lamellae surgically reduced to varying degrees. (A) presents the relationship between swimming speed and abdomen beat rate. The slope of the regression line is significantly positive (1.08 ± 0.22 [slope ± 1 se], \( t_i = 4.91, \ P < 0.0001 \)). In (B) the residuals around the relationship in (A) are regressed on mean lamellae area. This regression quantifies the relationship between swimming speed and mean lamellae area, after removing the effects of differences among individuals in abdomen beat rate. The slope of this relationship is significantly positive (1.08 ± 0.15 [slope ± 1 se], \( t_i = 7.28, \ P < 0.0001 \)). W = larvae in the Whole Lamellae treatment, N = larvae in the Nipped treatment, and C = larvae in the Cut treatment.
dragonfly lakes (McPeek 1990a). In lakes with fish, fish impose substantial mortality on Enallagma larvae (Pierce et al. 1985, 1987, Blois-Heulin et al. 1990, M. A. McPeek 1990a and unpublished data). Although another set of dragonfly species coexist with fish (e.g., Epitheca, Biaiaschma, and Celithemis species), these dragonfly species do not demonstratively affect Enallagma mortality rates in field experiments (M. A. McPeek, unpublished data). These ecological results suggest that dragonfly predation may be a potentially strong agent of natural selection in dragonfly lakes, and likewise fish predation in fish lakes.

One way Enallagma species appear to have adapted to living in dragonfly lakes is by evolutionarily increasing lamellae area to increase swimming speed. Because fish are capable of swimming much faster than damselflies, swimming does not appear to be an effective evasive tactic against attacking fish. Consistent with this, Enallagma species that coexist with fish do not swim away from attacking predators (Pierce et al. 1985, McPeek 1990b). In contrast, swimming is an effective evasive tactic against attacking dragonflies, and Enallagma species in dragonfly lakes employ it (McPeek 1990b). This shift in behavior to swim away from attacking predators should generate direct selection for increased swimming speed, and this should in turn impose selection indirectly on other phenotypic characters that affect swimming speed, such as lamellae area (McPeek 1995b).

Similar changes affecting swimming performance appear to have occurred in at least two separate lineages in the North American Enallagma as these lineages adapted to the selective environment of dragonfly lakes following habitat shifts (Fig. 1, see also McPeek 1995a, b). Large increases in the sizes of lamellae and in the widths of abdominal segments are associated with habitat shifts into dragonfly lakes (McPeek 1995a, b). Along with these changes in morphology, the allometric relationship between swimming speed and body size becomes steeper so that larvae of dragonfly-lake species are on average much faster swimmers than fish-lake species over larger body sizes (Fig. 3), and rapid evolutionary changes in the allometry of swimming speed have occurred in lineages leading to extant dragonfly-lake species as compared to changes occurring within lineages in the fish-lake habitat (Fig. 4). These changes in morphology and swimming speed associated with changes in selective environment are consistent with the hypothesis that they are adaptations for coexisting with dragonflies (Coddington 1988, Baum and Larson 1991, McPeek 1995b).

The results of the present study also demonstrate that the consequences of having larger lamellae for swimming speed make Enallagma larvae more adapted to coexisting with dragonflies in dragonfly lakes. Lamellae size has a direct functional relationship to swimming speed; the lamellae act in similar fashion to a fish’s caudal fin to generate thrust for swimming. Swimming speed is diminished when lamellae are experimentally reduced in size and other variables affecting swimming speed (e.g., body size and swimming effort as characterized by abdomen beat rate) are controlled (Fig. 5). Also, swimming speed is diminished when lamellae are completely removed (Robinson et al. 1990a). Furthermore, having experimentally reduced lamellae make larvae of species which swim from attacking dragonflies more vulnerable to dragonfly predation (Figure 6). Therefore, the larger lamellae of Enallagma species in dragonfly lakes fulfill both the adaptedness (e.g., Lauder et al. 1993, Reeve and Sherman 1993, Leroi et al. 1994) and historical context criteria (Coddington 1988, Baum and Larson 1991) for being an adaptation to coexist with dragonflies in dragonfly lakes.

Predation regime need not have been the only change in the selective environment affecting lamellae size that lineages undergoing habitat shifts experienced. Besides their importance for swimming, caudal lamellae are primary sites of gas exchange for respiration in damselflies (Pennak and McColl 1944, Eriksen 1986, Robinson et al. 1990b). Because dragonfly ponds and lakes tend to be shallower than ponds and lakes with fish (Tonn and Magnuson 1982, Rahel 1984, Robinson and Tonn 1989), daily and seasonal fluctuations in O₂ concentrations may be greater in dragonfly lakes, which should also select for larger caudal lamellae. Whether consistent differences in O₂ regimes exist between fish and dragonfly lakes is unexplored. However, over the normal range of oxygen concentrations experienced in the field, only ~20% of total oxygen uptake diffuses across the lamellae surfaces in other damselflies (Eriksen 1986). Moreover, the potential action of multiple selective agents on caudal lamellae size does not diminish its importance to determining swimming speed and predator avoidance. Multiple selective agents prob-
ably operate on most if not all phenotypic characters, which necessitates the application of careful functional studies to unravel their relationships to performance variables and ultimately fitness components.

Additionally, adaptational responses to changes in the selective environment may generally cause change in multiple phenotypic characters. For example, Schlueter and McPhead (1992) documented changes in diet, the number and length of gill rakers, gape width, body depth, and overall body size in stickleback populations that had apparently undergone character displacement. In *Enallagma* the widths of abdominal segments, lamellae size, and swimming speed have all been shown to increase dramatically in association with habitat shifts in to dragonfly lakes (McPhead 1995a, b, present study), and most likely other characters (e.g., activity [McPhead 1999]) have also changed as species adapted to predation imposed by large dragonflies in dragonfly lakes. Adaptation to this new environment may have involved not only adaptation to a new predator, but also adaptive changes in foraging strategy and metabolic physiological processes to counterbalance changes in predation risk. Studies of adaptation should examine the functional suite of characters that could potentially influence fitness in a new selective environment and not simply single traits responding to single selection pressures.

### Habitat shifts and character change

A shift from one habitat to another will be successful when a founder population is established in the wrong habitat and subsequently adapts to the new ecological niche before going extinct. This implies that founder populations must quickly adapt to their new environment; most founder populations are probably quickly extirpated, but rarely one may survive long enough to adapt (Gomulkiewicz and Holt 1995) and speciate (Bush 1969, 1975, Barton and Charlesworth 1984, Carson and Templeton 1984, Rice 1987, Tauber and Tauber 1989). Dispersal rates among lakes are very low for *Enallagma* species in general (McPhead 1989, Anholt 1990), but females do sometimes oviposit in the wrong lake type (M. A. McPhead, personal observation). Because *Enallagma* adults apparently cannot distinguish between a lake with and without fish (McPhead 1989), founder populations probably primarily result from females simply ovipositing in the wrong lake type and not from any change in active habitat choice (cf. Futuyma and Moreno 1989).

Because character reconstructions are the only available data, we can only postulate the evolutionary changes that occurred during habitat shifts in *Enallagma*. Presumably, larvae in the founder populations for both of these habitat shifts had traits distributionly similar to those of present-day fish-lake species in the clade containing *E. hageni* and *E. geminatum*, given the relatively low rates of evolutionary change for most characters within the fish-lake habitat suggested by the evolutionary contrasts analysis (McPhead 1995a, b, and this study). Once a founder population was established, strong selection would have acted directly on behavioral characters to increase the propensity of swimming from attacking predators and indirectly on morphological structures such as caudal lamellae to increase swimming speed. Previously, we proposed a simple model that shows how these characters may evolve synergistically in response to dragonfly predation to accelerate rates of change in both sets of characters (McPhead 1995b). Initially when a founder population is created, the more important character to determining survival is probably a larva’s propensity to swim from an attacking predator. As swimming propensity increases in response to selection, greater selection pressures would be mediated onto swimming structures, because swimming is used more frequently, and therefore greater selection differentials can be generated on these morphological structures (McPhead 1995b). Simultaneously, increased swimming speed would increase the selective advantage (i.e., selection differential) of swimming away from attacking predators. These considerations suggest that rapid evolutionary responses in both the propensity to swim from attacking predators and morphological characters affecting swimming speed probably occurred in founder populations because of the synergistic interactions between these two sets of characters in determining the probability of escape from predators (McPhead 1995b).

### Phylogenetics and community structure

Phylogenetic methods when combined with mechanistic studies of extant species provide the means to generate and test hypotheses about how species’ abilities to engage in ecological interactions might have changed over evolutionary time. In addition to being adapted to avoiding dragonfly predators, larvae of *Enallagma* species in dragonfly lakes also impose strong per capita competitive effects on both conspecifics and other damselfly species, whereas fish-lake *Enallagma* larvae are weak competitors (Pierce et al. 1985, M. A. McPhead 1990 and unpublished data). This difference in competitive ability between species in the two community types appears to result from differences in activity. Dragonfly-lake species are more aggressive than fish-lake species (Pierce et al. 1985), and they are more active than fish-lake species (moving more frequently and at higher rates of speed), which presumably affords them higher encounter rates with their own prey (Pierce et al. 1985, McPhead 1990b). Increased activity resulting in increased competitive ability is also seen among anuran larvae (Morin 1983, Lawler 1989, Werner 1992, 1994).

Although rigorous phylogenetic analyses have not been completed to date, these differences in activity may also be a consequence of adapting to the dragonfly-lake community and may be functionally linked to adapting to dragonfly predators. Adapting to dragonfly predation by increasing swimming propensity and
speed would have increased the probability of escaping predators once detected and attacked (see above discussion). As this occurred, the balance of selection pressures on characters such as activity that affect detection rates by predators may have shifted to permit higher predator detection rates but more importantly higher feeding rates, because larvae were now successful at avoiding predators even after being detected. In other words, adaptation by increasing swimming speed to avoid attacking dragonflies may have resulted indirectly in a shift in the optimal balance of the foraging rate/predation risk trade-off for Enallagma in dragonfly lakes. Moreover, the resulting shift towards increased activity may explain the increased competitive ability of dragonfly-lake species as an indirect consequence of adapting to dragonfly predation. The phylogenetic relationships among Enallagma species in the two community types provide an evolutionary framework for testing these hypotheses about the coordinated evolution of activity, competitive ability, and predator avoidance ability resulting from their functional linkages.

Incorporating phylogenetic methodologies into studies of community ecology may also allow us to reconstruct how real communities were assembled over time (see also Losos 1992, in press). When considering the development of structure among communities, ecologists generally assume a paradigm in which a defined pool of species exists, and each member of the pool can potentially colonize every site under consideration (e.g., Neil 1975, Drake 1991, Drake et al. 1993, Lawler and Morin 1993, Grover 1994). The abiotic environment and species interactions determine which species can and cannot coexist at each site; some species in the pool can coexist at a given site, and the rest are driven locally extinct. This has been a very useful paradigm for studying dynamical constraints on community structure, but it ignores the important evolutionary question of how the species pool was generated. Species evolve and taxa diversify in a community context, and so the structure of present-day communities result from the evolutionary histories of component taxa. Properties of species in the past can be inferred from plesiomorphic character states and character reconstructions (as in this study). When combined with functional studies of the phenotype and mechanistic studies of species interactions, these character reconstructions will allow formulation of hypotheses about change in community structure over time. Also, paleoecological data and calibrated molecular clocks may permit dating of these changes. Phylogenetic methods are also beginning to be used to examine the role of species interactions in regulating species diversity (Cornell 1985, 1993, Mitter et al. 1988, Farrell et al. 1991, Cornell and Lawton 1992, Cadle and Green 1993). Phylogenetic methodologies provide valuable tools for investigating how the evolutionary histories of component taxa may have shaped community structure.

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