




# The evolution of developmental thresholds and reaction norms for age and size at maturity

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**Developing organisms typically mature earlier and at larger sizes in favorable growth conditions, while in rarer cases, maturity is delayed. The rarer reaction norm is easily accommodated by general life history models, whereas the common pattern is not. Theory suggests that a solution to this paradox lies in the existence of critical size thresholds at which maturation or metamorphosis can commence, and in the evolution of these threshold sizes in response to environmental variation. For example, ephemeral environments might favor the evolution of smaller thresholds, enabling earlier maturation. The threshold model makes two unique and untested predictions. First, reaction norms for age and size should steepen, and even change sign, with decreases in threshold size; second, food reductions at sizes below the threshold should delay maturation, while those occurring after the threshold should accelerate maturation. We test these predictions through food manipulations in five damselfly species that theory suggests should differ in threshold size. The results provide strong support for the threshold model's predictions. In all species, early food reductions delayed maturation, while late reductions accelerated maturation. Reaction norms were steeper, and the effect of food reductions changed from decelerating to accelerating at a much smaller size in species from ephemeral habitats. These results support the view that developmental thresholds can account for the widespread observation of negative correlations between age and size at maturity. Moreover, evolution of the threshold appears to be both predictable and central to the observed diversity of reaction norms for age and size at maturity.**

developmental thresholds | life history | reaction norms | growth conditions

The age and size at which organisms mature or metamorphose are key life history traits that are critical to individual fitness, demographic rates, and population biomass (1, 2). Understanding plastic and evolutionary changes in age and size at life history transitions in response to environmental change is thus both a fundamental question in ecology and evolution and one with important consequences for conservation and resource management. For example, life history models that investigate the effects of size-selective mortality have been crucial for understanding the rapid evolutionary changes in age and size at maturity that have preceded the collapse in exploited fish stocks (3, 4). However, it is remarkable that one of the best-documented patterns in age and size still lacks a well-supported explanation: that most organisms tend to mature or metamorphose both earlier and at larger sizes in environments that favor rapid growth, leading to a reaction norm of decreasing size with age (2, 5–7). Intriguingly, a small number of exceptions to this rule are known, with some amphibians, insects, and fish showing delayed maturity or metamorphosis with improved growth conditions (5, 8–10). It is relatively easy to explain these rarer reaction norms of increasing size with age. If growth is nonasymptotic and lifetime reproductive output increases with size at the transition, theory predicts that it is optimal to delay maturity or metamorphosis to take advantage of favorable growth conditions (1, 5, 11, 12). Models that predict the commonly observed negative relationship, on the other hand,

have often relied on taxon-specific assumptions that are unlikely to be met for the diverse set of taxa where they are observed (2, 11, 13).

General theoretical models that have attempted to explain the negative correlation between age and size at life history transitions such as maturity or metamorphosis have relied on developmental thresholds (12, 14–17). These thresholds describe a minimum size, stage, or state that organisms must reach before the life history transition is possible. The existence of developmental thresholds was first proposed by Wilbur and Collins (14) in an influential verbal model of amphibian metamorphosis and later formalized by Day and Rowe (12). The Day and Rowe model showed that, below the threshold, environmental conditions that reduce growth will delay the transition at least until the threshold is reached. However, above the threshold, conditions that reduce growth will decrease the time to maturation or metamorphosis, as predicted by general life history theory models without a threshold (12). When the threshold size is large and there is variation in growth conditions, the below-threshold delaying effect will dominate, resulting in reaction norms for age and size with shallow negative slopes (Fig. 1A). On the other hand, when the threshold size is small and there is variation in growth conditions, the above-threshold accelerating effect will dominate, resulting in reaction norms with vertical or steep positive segments, often resulting in an L-shaped function (Fig. 1B). Provided that lifetime reproductive output increases with size at the transition, these predictions are qualitatively valid for any type of life history transition (e.g., maturity or metamorphosis) as well as for organisms with both determinate or indeterminate growth

## Significance

**Why most—but not all—organisms mature earlier in better growth conditions remains a mystery. Theory suggest that a solution may lie in the existence and evolution of critical size thresholds during development. The threshold model makes two unique predictions on the evolution of reaction norms between species and the effect of food reductions within species. We test this model experimentally using five damselfly species aligned along an ephemeral–permanent gradient, finding strong support for each prediction. Permanent habitats favor large thresholds and early maturation in better conditions, whereas ephemeral habitats favor small thresholds producing the rarer pattern with delayed maturation. The evolution of developmental thresholds is critical for understanding natural and human-induced variation in age and size at maturity.**

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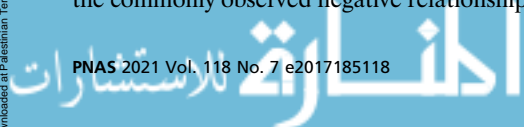
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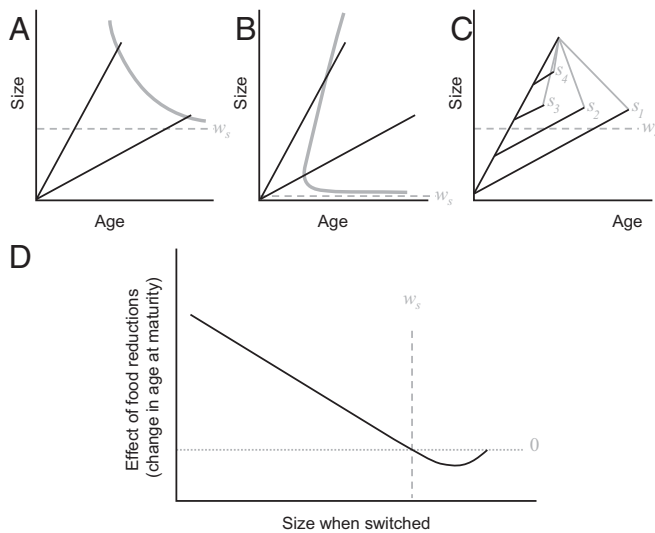
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**Fig. 1.** General life history models predict that it is optimal to delay maturity or metamorphosis when growth conditions are good, contrary to most empirical evidence. The Day and Rowe model (12) shows that the existence of developmental thresholds may resolve this paradox. (A) When a substantial threshold  $w_s$  is present (dashed line), age at maturity decreases under better growth conditions because it takes less time to reach the threshold. This results in shallowly negative reaction norms (gray line), as seen in most species studied. Black lines show linear growth trajectories under favorable and unfavorable growth conditions. (B) When the threshold size is small, age at maturity instead increases under better growth conditions. This results in steeply L-shaped or forward-leaning reaction norms, similar to those predicted by general life history models without a threshold but rarely observed empirically. (C) A unique prediction from this theory is that a switch from favorable to unfavorable growth conditions will cause increased age at maturity or metamorphosis before the threshold ( $s_1, s_2$ ) but decreased age at maturity or metamorphosis above the threshold ( $s_3, s_4$ ). (D) In an experiment where individuals are switched from high to low food over a range of sizes, the effect of food reductions on age is predicted to be nonmonotonic with respect to size when switched if developmental thresholds are present. The effect will be large and positive when switched at small sizes, decline in magnitude with size and shift from positive to negative at the threshold size ( $w_s$ ), reach a minimum slightly above the threshold, and increase to zero again late in ontogeny. A–C data from ref. 12.

(12). Thus, the presence of substantial developmental thresholds can account for the commonly observed decline in age with size at maturity or metamorphosis, and their absence can account for the rare increase in size with age at the transition (12). Furthermore, it raises the possibility that much of the variation in the slope and shape of reaction norms for age and size at maturity or metamorphosis across taxa may reflect variation in the magnitude of the threshold. For example, developmental size thresholds may be much reduced in species from ephemeral habitats where there is intense selection for rapid development and early transitions compared with those in permanent habitats (10, 14).

A number of observational studies have interpreted features of reaction norms, such as reduced variance in size for late-developing individuals, as supporting the developmental threshold model (18–21). However, experimental tests of the predictions that are unique to the theory of developmental thresholds have not been conducted. The first distinguishing prediction concerns the evolution of the threshold: reaction norms will have a shallow negative slope in species where the threshold size is large (Fig. 1A) and become steeply L shaped or have a positive slope in species where the threshold size is small (Fig. 1B). The second prediction of the theory relates to the opposing effects of reductions in the quality of the growth environment (for example, food availability)

at pre- and postthreshold sizes. According to the Day and Rowe model, food reductions prior to the threshold increase the time it takes to reach the threshold, resulting in delayed age at maturity, whereas food reductions after the threshold is reached reduce the time before individuals mature out of the declining growth environment, resulting in decreased age at maturity (Fig. 1C). Testing these predictions, therefore, requires a combination of targeted food manipulation experiments within species and comparative tests for divergence among species that vary in predicted threshold sizes.

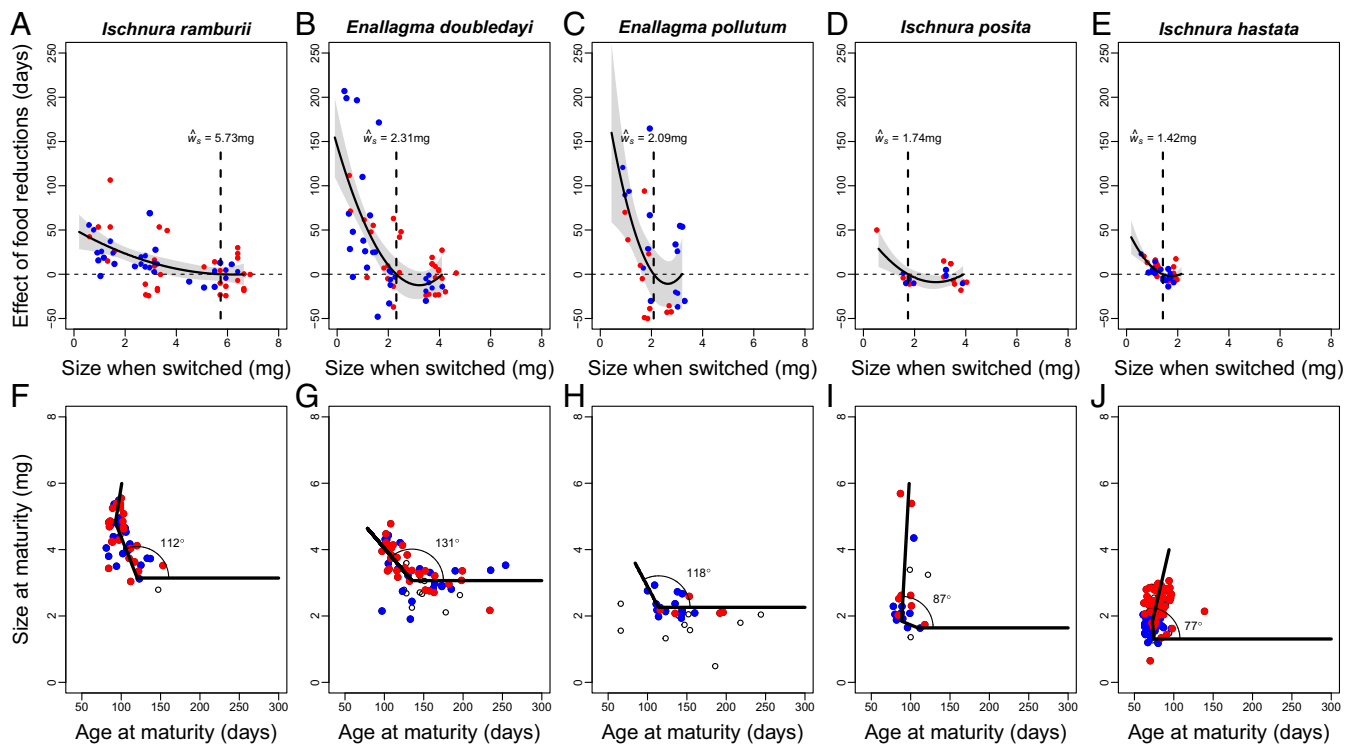
We tested the predictions from the developmental threshold model by performing a comparative food manipulation experiment using five species of North American damselflies (Odonata: Coenagrionidae) that show a range of habitat preferences from permanent lakes to ephemeral ponds, making them strong candidates for predictable evolution of developmental thresholds. We reared multiple family groups of each species from egg to adult in the laboratory and switched random subsets of individuals from high to low food levels at different sizes. Our food reduction treatment thus represents a proxy for a reduction in the quality of growth conditions. If developmental thresholds are present and evolve predictably, we predicted that 1) the food reductions will have a nonmonotonic effect on age at maturity with respect to the size when initiated, with earlier reductions having the strongest delaying effect on age at maturity, switching to an acceleration of age at the threshold size ( $w_s$ ) (Fig. 1D); 2) reaction norms will vary in shape from shallowly negative to steeply L shaped (Fig. 1A and B), and they will be steeply L shaped when the threshold size identified in 1) is small and have a shallow negative slope when the threshold size is large; and 3) species from ephemeral habitats will have smaller thresholds and more steeply L-shaped or positive-sloped reaction norms, whereas species from permanent habitats will have larger thresholds and shallower negative-sloped reaction norms.

## Results

**Effect of Food Reductions.** We quantified the effect of food reductions on age at maturity by calculating the age at maturity (completion of metamorphosis) of individuals switched to low food levels minus the mean age at maturity of high-food individuals from the same family and sex. We also incorporated a species-specific offset to account for the fact that food reductions tended to increase the time from initiation to completion of metamorphosis in low-food larvae (*Analysis*).

In the five species studied, food reductions overwhelmingly had a nonmonotonic effect on age at maturity with respect to the size when switched and changed sign from positive to negative within the observed range of body sizes in all five species (Fig. 2A–E), as predicted by the developmental threshold model (Fig. 1D). The nonmonotonic polynomial model was statistically supported over other candidate models based on Akaike's Information Criterion (AIC) scores in three species (*Ischnura ramburii*, *Ischnura hastata*, and *Enallagma doubledayi*) (SI Appendix, Table S1) and provided a better fit than linear and monotonic models in *Enallagma pollutum*, although a model with sex-specific linear effects had the lowest AIC scores overall in this species (SI Appendix, Table S1). In *Ischnura posita*, the effect of food reductions was significantly curvilinear with respect to size when switched ( $P = 0.019$ ) but was best described with a monotonic polynomial model (SI Appendix, Table S1). Taken together, these results provide strong empirical support for the existence of developmental thresholds in all the species studies. The presence of such thresholds can explain the observed size-specific changes in how the larvae respond to reductions in the quality of the growth environment (Fig. 1D).

**Threshold Size.** We estimated the developmental threshold size of each species as the size where the effect of food reductions switched from positive to negative when modeled as a second-



**Fig. 2.** Evidence for the evolution of developmental thresholds from comparative food manipulation experiments on five species of damselflies. Species are ordered based on habitat permanence preference (permanent on the left; ephemeral on the right). (A–E) In the five species studied, food reductions overwhelmingly had a nonmonotonic effect on age at maturity depending on the size when individuals were switched, with a local minimum within the observed range of body sizes. We estimated the threshold size  $w_s$  as the size where the effect of the food reduction treatment changed sign from positive to negative (dashed lines). (F–J) The shape of reaction norms for age and size at maturity varied from shallowly negative to steeply L shaped with a positive slope, as predicted by developmental threshold theory. We quantified reaction norm shape ( $R_z$ ) as the angle (in degrees) formed between the horizontal and upright portions of each reaction norm. Data for males are shown in blue, and data for females are shown in red.

degree polynomial function ( $\hat{w}_s$ ) (Fig. 2 A–E, dashed vertical lines). This represents a direct estimate of the true physiological threshold size  $w_s$  of each species. The magnitude of developmental thresholds differed considerably across species, with  $\hat{w}_s$  ranging from 1.42 mg in *I. hastata* to 5.73 mg in *I. ramburii*. These results provide strong support for the hypothesis that the magnitude of developmental thresholds can and does evolve.

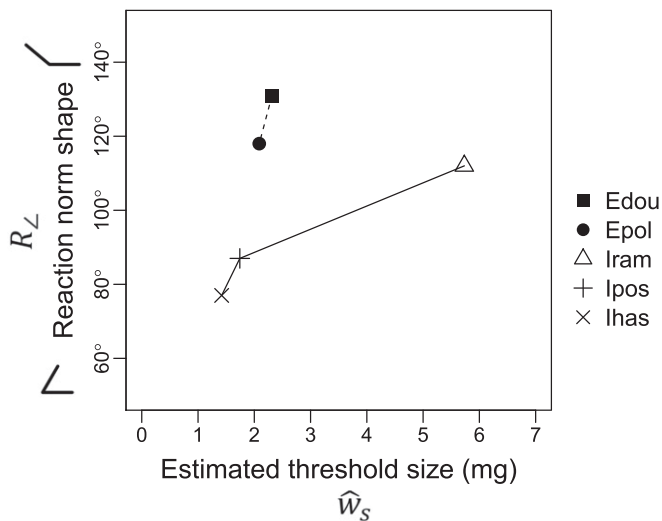
**Reaction Norm Shape.** The shape of reaction norms ranged from shallowly negative to steeply L shaped with a positive slope (Fig. 2 F–J). We quantified reaction norm shape as the angle formed between the horizontal and upright portions of reaction norms after fitting piecewise regressions ( $R_z$ , in degrees). As predicted by theory, the shape of reaction norms was positively correlated with the magnitude of estimated developmental thresholds among species (Fig. 3). Reaction norms tended to be shallower in species with large developmental thresholds and more steeply L shaped in species with small thresholds within each genus, but they were steeper overall in *Ischnura* than *Enallagma* (Fig. 3) (Spearman's partial rank correlation = 0.94,  $P = 0.059$ ; genus as covariate). These results provide strong empirical support for the among-species prediction from theory on developmental thresholds that the evolution of developmental thresholds results in predictable changes in the shape of reaction norms for age and size at maturity.

**Habitat Permanence.** Developmental threshold sizes ( $\hat{w}_s$ ) and reaction norm shapes ( $R_z$ ) were strongly correlated with species' preferences for ephemeral and permanent habitats (Fig. 4). Based on published information, species ranked in habitat permanence preference as follows (from permanent to ephemeral):

*I. ramburii*, *E. doubledayi*, *E. pollutum*, *I. posita*, and *I. hastata* (Materials and Methods). We predicted that permanent habitats would favor larger thresholds and shallower negative reaction norms and that ephemeral habitats would favor smaller thresholds and L-shaped positive reaction norms. The results support both of these predictions. Developmental threshold sizes increased with increasing habitat permanence (Fig. 4A) (Spearman's rank order correlation = 1,  $P = 0.017$ ). Reaction norms tended to be shallower in species from permanent habitat and more steeply L shaped in species from ephemeral habitats within each genus, but they were steeper overall in *Ischnura* than in *Enallagma* (Fig. 4B) (Spearman's partial rank correlation = 0.94,  $P = 0.059$ ; genus as covariate). These results support the view that developmental thresholds and reaction norm shapes evolve predictably during adaption to permanent and ephemeral habits.

## Discussion

Developmental thresholds offer a simple and general explanation for why most, but not all, organisms mature or metamorphose both earlier and at a larger size when faced with better growth opportunities. However, predictions unique to this theory have not been formally tested. Here, we experimentally test the key predictions with food manipulation experiments in five species of damselflies that range in habitat preference from permanent lakes to ephemeral ponds. As predicted by theory, food reductions had a nonmonotonic effect on age at maturity depending on the size when they were initiated: early reductions had the strongest delaying effect and reductions just above the threshold the strongest accelerating effect on maturation. Among species, our estimates of the threshold sizes were related to the shape of the reaction norm for age and size, with steeper L-shaped reactions



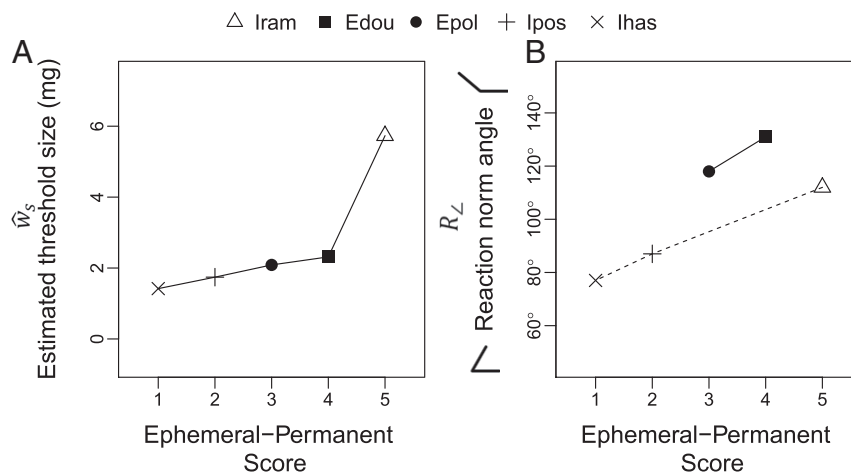
**Fig. 3.** Evolution of developmental threshold sizes ( $\hat{w}_s$ ) predictably alters the shape of reaction norms for age and size at maturity ( $R_L$ ). Within each genus, reaction norms were more steeply  $\perp$  shaped in species with smaller developmental thresholds and shallowly negative in species with larger thresholds. Fast-growing *Ischnura* had steeper reaction norms than *Enallagma* for a given threshold size. Edou, *E. doubledayi*; Epol, *E. pollutum*; Ihas, *I. hastata*; Ipos, *I. posita*; Iram, *I. ramburii*.

norms in species with small thresholds and shallower negative-sloped reaction norms in species with larger thresholds. Finally, as predicted, species from more ephemeral time-constrained habitats tended to have smaller thresholds and steeper reaction norms. These experimental results provide strong support for the ideas that developmental thresholds are central to the evolution of reaction norms for age and size at maturity and that their evolution is both adaptive and predictable.

That reductions in the quality of the growth environment differ in their effect above and below a certain size is a prediction unique to theory on developmental thresholds. This idea traces back to the influential verbal model by Wilbur and Collins (14), who postulated the existence of a minimal body size at which metamorphosis is possible and that metamorphosis should be delayed beyond the threshold when growth conditions are favorable. This

verbal model inspired a number of food manipulation experiments to determine if development rates were accelerated by food reductions late in ontogeny, as predicted by Wilbur and Collins. Two of these are particularly notable as they performed food manipulations in species from ephemeral and permanent habitats (10, 16). The results of these studies were mixed, with some observing slightly accelerated development late in development (10, 22, 23) but most seeing no effect (15, 16, 21, 23–28). However, the formalization by Day and Rowe (12) of the model of Wilbur and Collins (14) derived quantitative predictions for the effect of food reductions above and below the threshold (Fig. 1 C and D), which demonstrated that for many growth functions and threshold sizes, late food reductions will have a relatively small negative effect on age that is difficult to distinguish from a scenario where development rate is fixed at some earlier stage (29). This is further complicated by the fact that food-reduced individuals are likely in poorer condition, which can cause elongation of the maturation process and thus, diminish or even reverse any acceleration caused by food reductions (9), as seen in our experiment and earlier studies (25, 27). With this in mind, the moderate to null observed effects of late food reductions observed previously are consistent with both the threshold model and models with a fixed development rate (16, 29). Our study overcomes these difficulties by employing food reductions across a range of sizes, accounting for the elongation of the maturation process, and analyzing the impact of food reductions on age at maturity as a continuous function of size to infer the presence of developmental thresholds from the shape of this relationship. We suggest that a wider adoption of the approach employed here could resolve many of the apparent inconsistencies observed in previous studies.

Based on theory (12), we hypothesized that ephemeral habitats would favor smaller thresholds and steeper reaction norms, and these predictions were experimentally supported in these damselflies. Habitat permanence represents a key factor structuring aquatic ecosystems (30, 31), and has attracted considerable interest with respect to developmental thresholds in amphibians (10, 14, 32). Our results provide strong support for the hypothesis that a significant portion of variation observed in reaction norm shape—and in the effect of food reductions—arises because ephemeral habitats favor the reduction of developmental thresholds, relative to more permanent habitats. Notably, two of the rare exceptions of favorable growth leading to delayed maturity come from time-constrained anurans who breed in tree holes and



**Fig. 4.** The magnitude of developmental thresholds ( $\hat{w}_s$ ) and the shape of reaction norms for age and size at maturity ( $R_L$ ) evolve predictably during adaptation to ephemeral and permanent habitats. Developmental thresholds were smaller (A) and reaction norms were steeper (B) in species from ephemeral ponds that dry out each year (score = 1) than in species from permanent lakes (score = 5). Reaction norms were steeper overall in *Ischnura* than *Enallagma*. Edou, *E. doubledayi*; Epol, *E. pollutum*; Ihas, *I. hastata*; Ipos, *I. posita*; Iram, *I. ramburii*.



monsoonal pools, which face an extremely high risk of desiccation (8, 10): exactly the conditions we expect would favor the evolution of small thresholds and steep positive reaction norms.

Our observation that the two studied genera differed in the shape of reaction norms for a given threshold size is an intriguing finding (Fig. 3). In the Day and Rowe model, the shape of reaction norms for age and size depends on an organism's initial size, growth, and mortality rates, as well as the threshold size. The species studied here vary strikingly little in initial size (*SI Appendix*, Fig. S1), typical of temperate coenagrionid damselflies, and were reared under identical conditions. This suggests that differences in reaction norms likely reflect differences between genera in intrinsic rates of growth and/or mortality. It is known that *Enallagma* and *Ischnura* have evolved different solutions to the growth–predation trade-off and that these differences are crucial for their coexistence (33). In a common environment, *Ischnura* damselflies grow faster than *Enallagma* but experience higher predator-induced mortality. This suggests that evolution along the growth–predation risk trade-off axis further contributes to variation in the shape of reaction norms for age and size at maturity in this group.

A limitation of the developmental threshold model is that it assumes that growth rates are directly determined by the growth environment and that optimal age at maturity is determined by a single state variable, namely size (12). While these are common assumptions in optimality models, they are not in accord with the facts that organisms can adaptively optimize their growth rate in a given environment to balance their energetic supply, demand, and mortality risk (34) and that other state variables, such as condition and developmental state, can strongly influence reaction norms for age and size (17). The vast majority of studies on developmental thresholds, ours included, have focused on changes in food supply as a proxy for growth conditions (10, 12, 14, 16). Many other environmental variables, however, also influence growth rates, including temperature, salinity, predation risk, and time constraints, but may differ in their effect on age and size. High temperatures, for example, typically result in faster growth and earlier maturity at a smaller size, producing positive reaction norms for age and size that contrast with reaction norms produced by changes in food supply (35). This may reflect the fact that temperature not only has strong effects on energetic supply, through changes in food intake and conversion rates, but also on demand, through changes in metabolic rates, and possibly also on mortality. In addition, temperature tends to have a stronger effect on differentiation rate than on growth (35, 36). A broader exploration of the role of bioenergetic and developmental mechanisms in shaping reaction norms in light of developmental thresholds will be fruitful.

The results presented here provide insights into the forces that generate variation in age and size at maturity or metamorphosis in natural populations. Accordingly, they will be important in identifying whether age or size is more likely to respond to natural or human-induced changes in the environment. Our results suggest that a reduction in habitat quality (e.g., resource density) will result in substantially increased age at maturity in damselflies from permanent lakes but drastic declines in size at maturity in damselflies from temporary ponds. Although we focused on permanent–ephemeral hydrological dynamics that are especially relevant for freshwater ecosystems, the results are likely also applicable to other factors that display ephemeral–permanent dynamics such as resource availability and predation pressure (5, 34, 37, 38).

Our results also provide insight into the mechanisms underlying fisheries-induced life history evolution. In many commercial fish stocks, mean age and size at maturity have declined drastically during periods of increased fishing pressures (3, 39). General life history models can readily explain reductions in mean age at maturity as an adaptive response to increased mortality

(40). However, in several populations of cod and herring, reductions in mean age have been followed by increases in the slope of reaction norms for age and size at maturity, from shallowly to more steeply negative (3, 39, 41). These changes could potentially be explained as an adaptive response to size- or stage-selective harvesting (42) or a plastic response to increases in per-capita resource availability, and hence, growth rates, at low population densities (43). Our results, however, raise another possibility: that high fishing pressures led to the evolution of smaller developmental thresholds, with increases in reaction norm slope evolving as a correlated response. Because most fish continue to grow after reaching sexual maturity, as do reptiles and amphibians but not the insects studied here, the shape of reaction norms predicted by the threshold model will also depend on adult mortality. However, except when adult mortality is very strongly negatively size dependent, we expect evolution of smaller thresholds to produce qualitatively similar changes in reaction norms in fish as those shown here (12). Plastic changes in growth rate, due to the increased resource availability at low densities, could further exacerbate this effect by shifting populations toward the steepest part of the reaction norm. If this is the case, evolution of the threshold could be contributing to the slow recovery of several populations where fishing pressures have been reduced (44). Although it is clear that high mortality in older age classes will lead to strong selection against individuals with large developmental thresholds that take a long time to reach maturity, it is much less clear if or why selection would favor individuals with large developmental thresholds in the absence of such selection pressures. Understanding the adaptive benefit of larger thresholds thus represents a major unresolved issue with important implications for resource management and conservation.

## Materials and Methods

**Study System.** We conducted a common garden experiment using five species of damselflies from eastern North America: *E. doubledayi*, *E. pollutum*, *I. ramburii*, *I. hastata*, and *I. posita* (Odonata: Coenagrionidae). All species studied mate during the warmer months of the year, oviposit in submerged plants, and have aquatic larvae that are generalist predators on smaller invertebrates in the littoral zone. Important for this study, they differ markedly in body size and segregate along the ephemeral–permanent freshwater habitat gradient (30, 31, 33, 45). We therefore expect them to have diverged with respect to their life history and developmental thresholds. We classified habitat permanence types according to criteria in Stoks and McPeck (31). *I. ramburii* is the largest (by mass) species of *Ischnura* in eastern United States and occurs primarily in large permanent ponds and lakes with fish as top predator that rarely or never dry out (Type 4) (31). *E. doubledayi* is a medium-sized species restricted to the Atlantic coastal plain of eastern and southeastern United States, where it is locally common in sparsely vegetated sand-bottomed lakes with dragonflies as top predator that tend to dry out only in the most severe drought years (Type 3) (31). *E. pollutum* is smaller than *E. doubledayi* and almost entirely restricted to Florida, where it is abundant and rapidly colonizes a wide range of aquatic habitats from small ponds to slow streams, many of which likely dry out every few to tens of years (Type 2) (31). The two remaining species, *I. hastata* and *I. posita*, are typical of small ephemeral habitats that dry out every year (Type 1) (31) and represent the smallest species of odonates in eastern North America. Both species can reach extremely high population densities in newly created aquatic habitats and wet, grassy areas. Their population dynamics appear dominated by long-range dispersal, frequent colonization and extinction, and rapid population growth. Based on available information, we scored species' permanence preferences as follows: *I. ramburii* (5), *E. doubledayi* (4), *E. pollutum* (3), *I. posita* (2), and *I. hastata* (1), with permanent = 5 and ephemeral = 1.

**Common Garden Experiment.** We reared multiple family groups of each species from egg to adult in the laboratory to test for the effect of food reductions within species and for divergence in developmental thresholds and reaction norms among species. One subset of larvae from each family experienced high food levels during the entire experiment, whereas random subsets of individuals from each family were switched from high to low food levels at different body sizes. By comparing the age at maturity of high- and

low-food larvae, we estimated the shape of the relationship between the effect of food reductions on age at maturity and the size when switched from high to low food levels. With high-food larvae, we estimated the shape of reaction norms for age and size at maturity to test the prediction that reaction norms become more L shaped when the threshold size is small.

Larvae used in the experiment were hatched from eggs laid by field-collected females sampled at sites in North Carolina, Georgia, and Florida, United States, in June and July 2014 (SI Appendix, Table S2). Mated pairs were collected by sweep netting, and females were kept individually in 500-mL plastic jars lined with wet filter paper as an oviposition substrate until they had laid eggs. Filter papers with eggs were placed in 50-mL polycarbonate tubes filled with 25 mL of bottled water and transported by car or air to the laboratory at the University of Toronto, Canada. Eggs were kept in 500-mL containers in the laboratory at a water temperature of  $21.1\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$  (mean  $\pm 1$  SD,  $n = 27$  randomly measured containers) and natural photoperiod until hatching. Hatching was highly synchronous within families. The first day when more than one individual was observed was used as the hatching date for all individuals of a family. Soon after hatching, 50 first-instar larvae per family were transferred to individual rearing containers. Each species was represented in the experiment by larvae from between 3 and 11 family groups (SI Appendix, Table S2). Four families included in the experiment produced less than 50 (29 to 42) individuals. Total sample sizes at the beginning of the experiment were 550 (*E. doubledayi*), 250 (*E. pollutum*), 250 (*I. ramburii*), 273 (*I. hastata*), and 120 (*I. posita*). Rearing containers were grouped into blocks of 25 that were placed haphazardly inside two walk-in climate chambers set to produce a water temperature of  $21.1\text{ }^{\circ}\text{C} \pm 0.6\text{ }^{\circ}\text{C}$  (mean  $\pm 1$  SD,  $n = 80$  randomly measured rearing containers) and a photoperiod of 16-h:8-h light:dark. The rearing containers consisted of 100-mL plastic cups filled with  $\sim 50$  mL of synthetic freshwater medium (46). The water was changed every 6 wk or more frequently when necessary to prevent excessive algal growth. When larvae reached the final instar, a 3- $\times$  7-cm piece of plastic mesh was added inside each cup as an emergence substrate, and the cups were covered with mesh to prevent adults from escaping. Cups were checked 6 d/wk for emerged adults. Emerged adults were allowed to harden for 24 h before being killed in 70% ethanol and dried (Age and Size at Maturity has measurements).

**Food Reductions and Larval Size Measurements.** From the beginning of the experiment, larvae were fed *Artemia* nauplii ad libitum 6 d/wk ( $174 \pm 34$  nauplii per feeding, mean  $\pm 1$  SD,  $n = 43$  random food doses), which represents our high food-level treatment. At set intervals, random subsets of individuals from each family were switched from high to low food levels. After the switch, low-food individuals were fed 3 d/wk (Monday, Wednesday, Friday) for the remainder of the experiment using the same amount of nauplii as high-food larvae. Because *Artemia salina* nauplii die within hours in the freshwater medium, the amount of food available to low food-level larvae after the switch was effectively half that of larvae at the high food level.

We varied the timing of the switch to the low-food treatment depending on the growth rate of each family group to ensure that the switch was initiated over a suitable size range. To do this, we measured the head width (maximum distance between the distal parts of the eyes) of 10 randomly selected larvae from each family at the age of 42 d. All head width measurements were taken using a Wild M3C stereo microscope fitted with an ocular micrometer. We measured head widths as this trait displays less allometric variation during ontogeny than other measurements that can be reliably taken on living larvae (47). We ranked families based on their average head width at this age and divided them into four groups, with group one containing families with the largest average head width, and group four containing families with the smallest average head width, at this age. For families in the first (fastest-growing) group, individuals were switched from high to low food at the ages of 48, 65, and 79 d; in the second at the ages of 68, 81, and 91 d; in the third at the ages of 84, 100, and 120 d; and in the fourth at the ages of 110, 132, and 158 d. We adjusted the number of individuals switched at each time point depending on the number of individuals alive on that day.

We measured the head width of each individual when it was switched to the low-food treatment. We used these data to analyze how the effect of food reductions changed depending on the size when switched. To facilitate comparisons, we converted larval head widths to dry weights after establishing allometric scaling relationships for each species. To estimate these allometries, we killed approximately four individuals per week and species, measured their head widths when alive, placed them in aluminum weighting trays in a drying oven at  $60\text{ }^{\circ}\text{C}$  for 48 h, and weighted them to the nearest 0.001 mg with a Mettler Toledo X5105 scale. We modeled the relationship between head width and dry weight as a power function and

used these equations to estimate the dry weight of individuals when switched to the low-food treatment (SI Appendix, Fig. S1).

**Age and Size at Maturity.** When high- and low-food individuals emerged, we collected data on their 1) age at maturity, 2) emergence success, and 3) adult dry weight. We define age at maturity as the day when an individual was first observed emerging as an adult from its exuvia (completion of metamorphosis). Emergence success was scored on a scale from zero to four by counting the groups of body part (legs, head, thorax, and abdomen) that remained inside the exuvium. A score of zero thus indicated a successful emergence, and a score of four indicated a complete failure to emerge. Preliminary analyses identified some failed emergences (score = 4) as statistical outliers, suggesting that these may have represented failed attempts to escape containers rather than true decisions to emerge. Subsequent analyses were thus only performed on individuals that exhibited clear morphological evidence of having triggered the physiological process to emerge (i.e., score < 4). Individuals excluded from the analyses are shown as open circles in Fig. 2 F–J.

We measured adult dry weights to the nearest 0.001 mg as described above for killed larvae. For individuals that did not fully emerge, we weighed adults together with their exuvia. To enable direct comparisons, we weighed the exuviae of successfully emerged individuals and estimated the relationship between the total dry weight (adult + exuvium) and adult dry weight (excluding the exuvium) for each species. With these relationships, we converted total dry weights into adult dry weights for individuals that were measured with their exuviae.

**Analysis.** We define the effect of the food reduction treatment as the observed age of maturity of low-food individuals minus the expected age at maturity of high-food individuals from the same family and sex. We first modeled age at maturity as a function of family, sex, and their interaction using high-food individuals and removed nonsignificant terms using backward elimination based on AIC scores. We used the resulting models to predict the expected age at maturity of low-food individuals had they not been subjected to food reductions. According to theory, we expect individuals that are switched to low food levels very late in ontogeny to mature at the same age as individuals that remain on high food levels (c.f. Fig. 1D). However, in our experiment, larvae switched near the upper size range matured later than high-food individuals from the same family and sex (SI Appendix, Fig. S2). The most likely explanation for this is that the imposed low-food treatment increased the time from initiation to completion of metamorphosis, as seen in earlier studies on amphibians (25, 27). In the developmental threshold model, however, maturation is assumed to occur instantaneously. To ensure conformity with predictions from the developmental threshold model, we estimated and corrected for the magnitude of the lengthening of metamorphosis in each species. We first modeled the effect of food reductions (observed minus expected age at maturity of low-food larvae) as a second-degree polynomial function of size when switched and predicted the effect of food reductions if switched at the 99th percentile of observed larval sizes for each species (SI Appendix, Fig. S2, dashed lines). We subtracted this value from the age at maturity of low-food individuals to account for the lengthening of metamorphosis. Using the average value across all species did not qualitatively affect the results.

We used a model selection approach to test the prediction that the effect of food reductions ( $\Delta\text{Age}$ ) changes nonmonotonically with size when switched ( $w_{HL}$ ) and changes sign from positive to negative at the threshold size (Fig. 1C). We compiled a set of candidate models that estimated the relationship between  $\Delta\text{Age}$  and  $w_{HL}$  as linear (model 1), nonmonotonic polynomial (model 2), or monotonic polynomial (model 3). Two further models incorporated sex-specific linear or polynomial size effects (model 4, model 5). The best-fitting model for each species was assessed based on AIC scores (SI Appendix, Table S1). We present  $P$  values for the linear and nonlinear effects of  $w_{HL}$  from models M1 and M2, respectively. We estimated the threshold size of each species ( $\hat{w}_s$ ) as the size where the effect of food reductions changes sign based on the nonmonotonic polynomial.

To quantify reaction norm shape, one approach is to model size as a function of age using hyperbolic or exponential decay functions (18, 19). However, these functions cannot be used for steeply L-shaped reaction norms, as shown by two species here (Fig. 2 I and J). We are not aware of any functions that can accommodate such different reaction norms. Instead, we define an operational measure of reaction norm shape  $R_z$  as the angle formed between the upright and horizontal portions of negative or L-shaped reaction norms. Based on the Day and Rowe (12) model, we expect this angle to vary from nearly  $180^{\circ}$  when the threshold size is large (a shallow negative slope toward a horizontal asymptote) to somewhat less than  $90^{\circ}$

when the threshold size is small (a slightly forward-leaning L-shaped reaction norm) (Fig. 1A). To quantify  $R_L$  for each species, we first modeled the relationship between age and size at maturity using piecewise regression. For species showing greater variance in age at maturity than in size at maturity, we modeled size as a function of age (Fig. 2 G and H). Conversely, for species showing greater variance in size than in age, we modeled age as a function of size (Fig. 2 F, I, and J). This approach allowed us to identify the main axes of variation in age–size space without making strong assumptions about the functional form of this relationship. All analyses of reaction norm shape were performed using data from high-food individuals. Two individuals of *E. pollutum* that emerged 34 d before any other individuals were identified as outliers and excluded from the analysis (two left-most open circles in Fig. 2H). We plotted the predictions from the models in age–size space, together with a horizontal asymptote at the 2.5% quantile of adult sizes (18, 19), and measured the angle formed between the upright and horizontal portions of the resulting reaction norms (Fig. 2). This angle  $R_L$  represents our quantitative estimate for the shape of reaction norms.

Theory predicts that the magnitude of developmental thresholds ( $\hat{w}_s$ ) will be positively correlated with both the shape reaction norms (expressed as the angle  $R_L$ ) and habitat permanence, but the form of these relationships is unknown. We therefore used (partial) Spearman's rank order tests to test for correlations between these variables, using genus as a covariate when appropriate. All statistical analyses were performed in R 3.4.3 (48).

**Data Availability.** Data and R-code associated with this article have been deposited in Dryad (<https://doi.org/10.5061/dryad.xpnvx0kds>) (49).

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