



# Physiology underlies the assembly of ecological communities

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Edited by Nils Chr. Stenseth, University of Oslo, Oslo, Norway, and approved May 4, 2018 (received for review February 4, 2018)

**Trait-based community ecology promises an understanding of the factors that determine species abundances and distributions across habitats. However, ecologists are often faced with large suites of potentially important traits, making generalizations across ecosystems and species difficult or even impossible. Here, we hypothesize that key traits structuring ecological communities may be causally dependent on common physiological mechanisms and that elucidating these mechanisms can help us understand the distributions of traits and species across habitats. We test this hypothesis by investigating putatively causal relationships between physiological and behavioral traits at the species and community levels in larvae of 17 species of dragonfly that co-occur at the landscape scale but segregate among lakes. We use tools borrowed from phenotypic selection analyses to show that physiological traits underlie activity rate, which has opposing effects on foraging and predator avoidance behaviors. The effect of activity on these behaviors ultimately shapes species distributions and community composition in habitats with either large-bodied fish or invertebrates as top predators. Remarkably, despite the inherent complexity of ecological communities, the expression of just two biomolecules accounts for a high proportion of the variation in behavioral traits and hence, dragonfly community composition between habitats. We suggest that causal relationships among traits can drive species distributions and community assembly.**

odonata | activity rate | causality | animal personality | phenotype

Ecologists have long understood that phenotypic differences among species play a central role in structuring ecological communities (1). This reasoning has led to trait-based views of communities and from a practical perspective, the use of species traits for predicting community composition (2). Notably, changes in species composition are rarely associated with a single trait. Rather, suites of traits that act independently or in conjunction with each other temper species responses to environmental differences, stressors, and biotic interactions (2–4). For instance, in plants, suites of chemical and physical traits may determine the abundance of a species across temperature or precipitation gradients (5), and species that are dissimilar with respect to some traits may be more likely to coexist, because they compete less strongly (3). A growing body of literature is testing the role that functional traits play in structuring ecological dynamics, with the goal of providing mechanistic predictions of how species and communities should respond to abiotic and biotic environments.

Hierarchical structures of trait development and expression can create phenotypic cascades, whereby one trait and its ecological influence are dependent on another trait or even arise as a result of an underlying causal trait. For example, more active amphibians often develop faster and emerge earlier than less active amphibians, meaning that activity rate is causal to development rate and ultimately, the timing of emergence (6, 7). These conceptual models that evoke causal links are distinct from many traditional ecological analyses and selection analyses that focus on collinearity of these traits but often assume that they do not cause one another (4, 8). However, a vast literature in microevolution has shown that this assumption is untrue; individual traits are often causal to one another, and this causality can shape the trajectory of phenotypic

change (9). By analogy, we may expect equivalent dynamics to occur in communities when some species-level traits underlie others. For instance, causality could allow traits with no clear functional role, beyond affecting other traits, to impact species' performance and the distribution of traits across environmental gradients (9). If communities are predominantly structured by differences in species performance (i.e., absolute fitness) (10, 11), then these causal relationships will ultimately affect the composition of ecological communities.

Before exploring the consequences of trait causality, we have to determine what traits are most likely to influence community dynamics and how they are likely to be causally linked. Ecologically, any suite of behavioral, morphological, and physiological traits that directly influence vital rates through energy consumption (birth and growth rates) and predator or disease avoidance (mortality rate) is expected to influence community assembly (4). Within such a suite of traits, those at lower levels of biological organization are likely to be causally related to those at higher levels of organization (9). For example, physiological traits may affect the expression of morphological or behavioral traits, an assertion lent credence by fields such as evolutionary developmental biology (i.e., “evo-devo”) (12–14). Ultimately, physiology is likely to underlie key behavioral and morphological traits that are ultimately responsible for determining species distributions.

Differences in physiologically determined habitat distributions may, in turn, affect community composition, causing convergent species and hence, trait composition in environmentally similar communities (15–17). Given the functional connections between traits within the organism itself, we expect that the community-level

## Significance

**Ecology aims to understand the distribution of species and the processes that assemble communities. One common strategy is to use species traits to predict interaction with the abiotic and biotic environment, thus gaining an understanding of ecological communities. Here, we show that considering causal relationships among physiological and behavioral traits can help elucidate patterns of species distribution and community assembly in larval dragonflies across ponds differing in predation risk. Beyond a conceptual understanding of trait causality, we also show a striking ecological pattern: the activity of two biomolecules accounts for >80% of the variation in community composition. Together, our conceptual framework and demonstration of a strong ecological pattern suggest that causal relationships are key to advancing trait-based community ecology.**

Author contributions: D.S., S.M., and B.G. designed research; D.S. performed research; D.S. analyzed data; and D.S., S.M., and B.G. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1802091115/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1802091115/-DCSupplemental).

Published online May 21, 2018.

convergence of those traits is caused by the corresponding and underlying convergence of physiology in species occupying similar habitats. Some evidence supports this prediction, including that many physiological pathways differ consistently with environmental conditions (18, 19), suggesting that selection is acting similarly on these pathways (20). For example, ref. 13 showed how physiological pathways underlying plant net assimilation rate can determine a species' ability to grow under different light regimes. When species share common physiological pathways, up- or down-regulation of particular biomolecules will have predictable impacts on morphological or behavioral traits (18, 20–22). While untested, such physiological trait–performance relationships (9) are likely to mediate patterns of community assembly across environmental gradients (2, 3).

Systems that are strongly structured by predation provide some of the best-understood and most mechanistic examples linking community-level trait distributions to environmental variation. Communities with and without predators or with different intensities or types of predation often differ markedly in composition (23–25), with the relative abundance of particular prey species being predicted by suites of antipredator traits (26, 27). For instance, while many factors can affect the distributions of larval odonates (dragonflies and damselflies) (16), many species segregate between ponds with large game fish and those where game fish are absent but invertebrate predation dominates (25, 28). Species that are more frequently found in ponds with large game fish tend to avoid being seen by and encountering predators by having low activity rates, whereas higher activity rates appear advantageous when predators are absent, likely due to higher consumption rate and shorter development times (6, 7). Differences in burst swimming speed may also allow faster species to escape. However, this pattern appears to be more varied—studies have alternatively shown that fast burst swimming speed is important for facilitating escape from fish (22) or dragonfly predators (18, 20). Nevertheless, many studies have shown that activity rate and burst swimming speed are favored under some predator regimes vs. others (18, 20, 22, 29). In short, differences in behavioral traits underlie species' performance in fish vs. fishless habitats and, thus, have the potential to mediate community assembly across this biotic gradient (25, 30).

The ecological importance of activity rate and burst swimming speed suggests that, if their causal physiological pathways are shared among species, they could underpin ecological patterns of distribution. Work in a number of species has shown that arginine kinase (*Ak*) phosphorylates ADP to quickly produce the ATP needed for rapid muscle contractions (31). In two odonate genera (one dragonfly and one damselfly), high levels of *Ak* have been shown to correspond with fast burst swimming speeds (18, 20), likely facilitating escape from fish (22) or invertebrate predators (18, 20). Overall, differences in *Ak* are known to impact phenotypic selection (20) and macroevolution of damselflies (18), but there is limited evidence for *Ak* influencing the distributions of odonates (18, 22). Lactate dehydrogenase (*Ldh*) is a key enzyme mediating glycolysis, and increased *Ldh* activity is associated with an increase in overall activity rate in some vertebrate species (32–34). Despite a lack of evidence of *Ldh* activity altering movement or species distributions in the only odonate genus tested (28), its link to metabolism and movement in other animals, including insects, suggests that it could underpin a causal pathway for movement across a broader diversity of odonates. In sum, the effects of *Ak* on burst swimming speed and the general link between *Ldh*, metabolic rate, and movement suggest that these molecules are likely candidates to test whether physiological pathways scale up to determine the composition and trait distributions of entire intact communities.

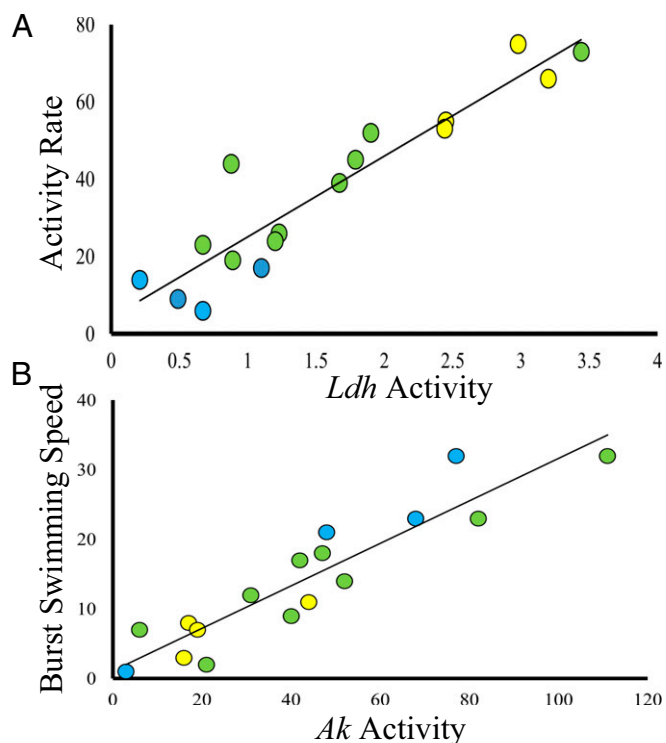
We tested for the effects of physiology on behavioral traits and further tested if these traits predict species distributions and community composition of larval dragonflies. Specifically, we test for the role of *Ak* and *Ldh* in mediating burst swimming

speed and activity rate of 17 species of dragonfly; then, we link these species-level trait differences to species distributions, building on previous work that related species' behavioral traits to their distributions. However, our work is able to go further than previous studies by determining the underlying physiological mechanisms that shape the distribution of behavioral traits across ponds with and without fish and structure the composition of the dragonfly community in these habitats. This work advances our understanding of physiological ecology and more broadly shows that considering causality among traits can provide a powerful tool for testing evolutionary and community ecology.

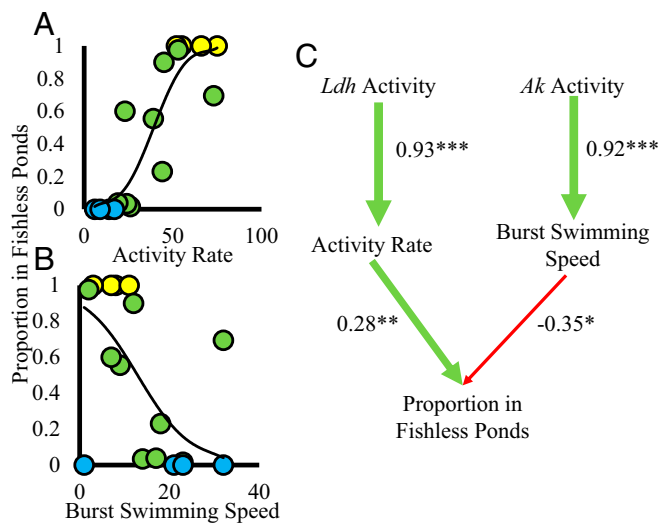
## Results

Physiology predicted morphological and behavioral traits of the 17 species of dragonfly encountered in our survey, the distribution of those 17 species, and ultimately, community composition of the study ponds. Two species were Aeshnids that are likely dominant predators in ponds lacking game fish (*SI Appendix*). Four species were found only in fish ponds, four were found only in fishless ponds, and nine were generalists, occurring in varying abundances in both habitat types (*SI Appendix*). Species with higher *Ldh* activity had correspondingly high activity rates (Fig. 1A) ( $R^2 = 0.88$ ,  $P < 0.001$ ), and high *Ak* activity was associated with fast burst swimming speeds (Fig. 1B) ( $R^2 = 0.82$ ,  $P < 0.001$ ). In univariate analyses, greater *Ldh* activity (pseudo- $R^2 = 0.61$ ,  $P < 0.001$ ), high activity rates (Fig. 2A) (pseudo- $R^2 = 0.74$ ,  $P < 0.001$ ), low *Ak* activity (pseudo- $R^2 = 0.18$ ,  $P = 0.04$ ), and slow burst swimming speeds (Fig. 2B) (pseudo- $R^2 = 0.23$ ,  $P = 0.03$ ) were associated with species found more commonly in ponds lacking large game fish.

Structural equation modeling analyses supported the hypothesized role of physiology in mediating species distributions. *Ldh* and *Ak* activities predicted activity rate and burst swimming



**Fig. 1.** Relationships between enzymatic activity and corresponding behavioral traits. *Ldh* activity increased activity rate (A), and *Ak* activity predicted burst swimming speed (B). Blue, green, and yellow points show species found in fish ponds only, both pond types, and fishless ponds only, respectively. Lines show predicted values from LMs.



**Fig. 2.** Physiological and behavioral traits predict species' habitat distributions. Active species (A) and those with low-burst swimming speed (B) tended to be found in fishless ponds. (C) *Ldh* and *Ak* predicted activity rate and burst swimming speed, respectively, ultimately determining the frequency with which a species was found in fish vs. fishless habitats. Lines in A and B show that predicted values form logistic regressions. Yellow, blue, and green points represent fishless pond specialists, fish pond specialists, and habitat generalists, respectively. In C, arrows show regression links. Numbers represent standardized regression coefficients, and arrows are scaled to the magnitude of those coefficients. Red and green arrows show negative and positive effects, respectively. Significance codes: \* $<0.05$ ; \*\* $<0.001$ ; \*\*\* $<0.001$ .

speed, respectively (Fig. 2C) (both  $P < 0.001$ ). In turn, active species ( $P < 0.01$ ) and those with low-burst swimming speed ( $P = 0.02$ ) were more often found in ponds without large fish (Fig. 2C). As with the results above, the structural equation modeling results were qualitatively identical when using random effects to partially control for phylogeny. In sum, enzymatic activity determined behavioral phenotypes, which in turn, predicted a species' habitat distribution with respect to predator regime.

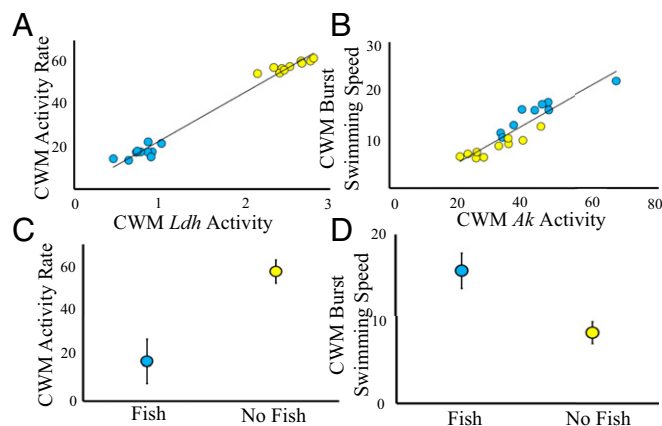
Beyond species-level effects, physiological and behavioral traits differed between fish and fishless ponds at the community level. Community-weighted mean *Ldh* activity predicted community-weighted activity rate (Fig. 3A) ( $R^2 = 0.95$ ,  $P < 0.001$ ), and community-weighted mean *Ak* predicted community-level burst swimming speed (Fig. 3B) ( $R^2 = 0.85$ ,  $P < 0.001$ ). Consistent with species-level patterns, lakes with game fish supported communities that were less active (Fig. 3C) ( $R^2 = 0.92$ ,  $P < 0.001$ ), had higher-burst swimming speeds (Fig. 3D) ( $R^2 = 0.63$ ,  $P < 0.001$ ), reduced *Ldh* ( $R^2 = 0.89$ ,  $P < 0.001$ ), and increased *Ak* activity ( $R^2 = 0.33$ ,  $P = 0.004$ ).

Multivariate approaches supported these findings—physiological and behavioral traits were important predictors of dragonfly community composition. The presence/absence of large game fish predicted 41% of the variation in dragonfly community composition [canonical correspondence analysis 1 (CCA1) axis in Fig. 4A] ( $P < 0.001$ ; the second canonical correspondence axis shows unconstrained variation in community composition). Mirroring species-level patterns, community-weighted physiological traits predicted corresponding behavioral traits (Fig. 4B) (both  $P < 0.001$ ), which, in turn, described multivariate community composition (Fig. 4B) (activity rate:  $P < 0.01$ ; burst swimming speed:  $P = 0.03$ ). Overall, the role of physiology in structuring species' distributions and community composition is pervasive, and it is mediated through its effect on behavioral traits.

## Discussion

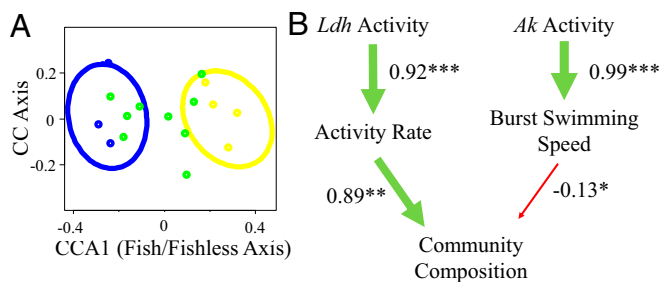
Our study shows that physiological mechanisms shared by multiple species scale up to impact the structure of ecological communities and, more generally, suggests that the hierarchical nature of trait relationships may allow for a simple but powerful understanding of how traits structure the distributions of species and community composition across landscapes. Species-level differences in enzymatic activity accurately predict corresponding differences in activity rate and burst swimming speed (Fig. 1), key traits affecting predator–prey interactions in larval dragonfly communities (15, 24, 25, 30). Physiological and, hence, behavioral variation was structured between habitat types. In contrast to previous work (18), species more frequently found in habitats without game fish had higher levels of *Ldh* and, consequently, were more active (Fig. 2A and C). Similarly, high *Ak* and correspondingly high-burst swimming speeds were associated with game fish habitats (Fig. 2B and C), a result that is consistent with only one of two studies on odonates (18, 22). Importantly, these differences affected not only species distributions but ultimately scaled up to create trait differences among communities (Fig. 3). Structural equation modeling analyses confirmed our a priori understanding of causality in this system, showing that enzymatic activity was responsible for the expression of behavioral traits and ultimately, multivariate community composition (Fig. 4). We suggest that physiology may underlie many common community-level patterns and argue that incorporating causality into trait-based community ecology can simplify and improve our understanding of ecological dynamics.

The mapping of physiological traits onto corresponding behavioral traits in the 17 species studied suggests that common physiological mechanisms should be investigated more broadly in work seeking to understand the forces structuring communities. The association between increased activity rate and higher *Ldh* activity (Fig. 1) was a particularly important finding for these species (Fig. 4); while *Ldh* and activity rate have been linked in other species (32, 33), this relationship has never been detected among odonates, of which only damselflies have been tested (18). In contrast, two studies have shown that *Ak* levels in odonates are correlated with burst swimming speed, although among many fewer species and genera (18, 22), suggesting that literature on physiological processes may provide rich ground for hypotheses of community composition. Many traits known or suspected to be important in structuring species' distributions have physiological underpinnings,



**Fig. 3.** Community-level patterns of enzymatic activity and behavioral traits between habitat types. (A) Community-weighted mean (CWM) *Ldh* activity predicted the same measure of activity rate. (B) Average *Ak* similarly predicted burst swimming speed. Beyond species-level differences, communities in fishless ponds were more active (C) but had slower burst swimming speeds (D). Blue and yellow points represent communities occurring in ponds with and without fish, respectively. Lines represent predicted values from corresponding LMs.





**Fig. 4.** Physiological and behavioral traits predicted multivariate community composition. (A) Forty-one percent of all of the variation in community composition was predicted by habitat type (fish vs. fishless). Ellipses show 95% confidence intervals about the centroid representing an average fish (blue) or fishless (yellow) pond. Blue, green, and yellow points show species found in fish ponds only, both pond types, and fishless ponds only, respectively. (B) In the structural equation modeling analysis, community-weighted mean *Ldh* and *Ak* predicted the same metrics of activity rate and burst swimming speed. These behavioral traits in turn predicted multivariate community composition measured as community scores from a CCA (A). Note that high scores represent communities associated with fishless ponds. As such, the structural equation modeling analysis shows that high activity rate is associated with fishless ponds but that high-burst swimming speed is consistent with communities found in fish ponds. Arrows show regression links. Numbers represent standardized regression coefficients, and arrows are scaled to the magnitude of those coefficients. Red and green arrows show negative and positive effects, respectively. Significance codes: \* $<0.05$ ; \*\* $<0.001$ ; \*\*\* $<0.001$ .

and investigations of shared physiological mechanisms among species that structure communities may be more widespread than commonly appreciated.

The effect of physiology on behavioral traits and how those behaviors in turn influence species' habitat distributions provide insights into the ecological limits of species distributions. In line with previous work (6), active species tended to be found in fishless ponds more frequently (Fig. 2 A and C). This result supports the hypothesis that high activity is advantageous, because it increases growth rates and competitive ability (6), but it also increases encounter rates with predators and the ability of visual predators to detect prey, causing the loss of active species from a community with fish predators (25, 30). However, our study also had a notable exception; nearly one-third of all *Anax junius*, the second most active species (SI Appendix), were found in game fish ponds. *Anax* are unusual in that they are themselves dominant predators of smaller dragonflies in fishless ponds. As such, the need to capture relatively fast-moving prey may necessitate high activity rates, despite the potential costs arising from predation (6). An alternative explanation for the occurrence of *Anax* with fish is that they were at relatively early instars during the collection period, suggesting that many of those individuals in game fish ponds may be consumed before emerging. In this case, ponds with game fish may serve as a habitat sink for *Anax*, causing our survey to detect *Anax* in game fish ponds where they may nevertheless have fitness below replacement. The latter hypothesis is more likely given that many *Anax* larvae in our region are the offspring of adult migrants, which will oviposit in both fish and fishless habitats but suffer from high levels of predation when exposed to fish (35). Overall, while interesting idiosyncrasies occur, activity rate clearly maps to species' habitat distributions, illustrating the impact that predation has on active individuals (Fig. 2 A and C).

In our study, species with high-burst swimming speeds were found in ponds with game fish (Fig. 2 B and C), although other studies have shown mixed outcomes. Studies on damselflies suggest that the fastest swimmers are instead found in fishless ponds, allowing escape from invertebrate predators, but that no species can swim quickly enough to escape fast-moving fish predators (18, 20). Our results and those of other studies on dragonflies (22)

contrast with this work by showing that fast burst swimming is associated with fish ponds, likely because rapid swimming facilitates escape to refugia (e.g., vegetation). These contrasting outcomes may come about, because dragonflies and damselflies occupy different phenotypic space—dragonflies can burst swim an order of magnitude more quickly than damselflies (18, 22), which may permit dragonflies to burst away from fish, even if the fastest damselflies are unable to do so (22). In other words, whether burst swimming speed is favored depends on the speed of the prey but also, the speed of the predator (i.e., a dependent trait) (sensu ref. 11). The tight matching between predator regime and trait values in this study and others suggests that, in contrast to other well-studied patterns of trait–environment matching, biotic factors (i.e., predation) are overwhelmingly the dominant structuring force of these communities. More broadly, given the commonality of observed trait–environment matching in many systems, the physiological and community-level patterns observed here may be common.

While physiology determines ecological strategies and has been shown to shape habitat distributions for a number of species and systems (12–14), the scaling of physiology to community composition remains largely unexplored. In our study, physiological traits of communities predicted behavioral variation among communities (Fig. 3), ultimately predicting multivariate community composition (Fig. 4). Consistent with species-level analyses, these changes were seemingly driven by the divergent effects of contrasting predator regimes on species differing in activity rate and burst swimming speed (Fig. 3) (15, 17, 25, 30). In many ways, this is unsurprising; we detected strong species-level effects of physiology on habitat distributions (Fig. 2), and the community-level effects simply expand these analyses to include differences in species' relative abundances (Figs. 3 and 4). However, the opposite argument is often made, namely that population-level processes do not scale up to communities because of the noise generated when many interactions play out over multiple dimensions (10, 36, 37). Contrary to this view, we have shown that changes in the activity of just two biomolecules predict differences in community composition among habitats (Fig. 4) with far greater explanatory power than is typically observed in trait-based studies (2, 18, 20, 22). Given the inherent complexity of natural ecological systems and the often high dimensionality of functional traits (2–4), the strength of our results is extremely surprising. The considerable explanatory power of these traits suggests that, in this system, predation is pervasive and differs in intensity between pond types (25), that enzymatic activity is a strong predictor of behavioral traits (Figs. 1 and 3 A and B), and that predation is strongly biased with respect to activity rate and burst swimming speed (i.e., high trait–fitness covariance) (Figs. 2, 3 C and D, and 4B) (15, 20, 30). In sum, a few key physiological differences can have important consequences not only for species but also for the processes governing the assembly of entire communities in the wild. Given the often complex relationships between organisms and their environment, it is surprising that community assembly can be understood by investigating so few traits (3).

Causal relationships between traits have important implications for community assembly. In our study, where physiology determined behavioral traits, the inferred causality is similar to performance and fitness gradients—some traits underlie others, and it is this mapping from one trait to another and then ultimately, to fitness that determines patterns of selection (9). Analogously, physiological traits may underlie behavioral or morphological traits, ultimately determining species performance (i.e., absolute fitness or population growth rate) (11) and thus, species' distributions and community composition. Reciprocally, changes in species relative abundances will act directly on proximate traits (e.g., behavior), but in so doing, they will also alter patterns of underlying physiological traits (e.g., enzymatic activity). While our study did not measure changes in species relative abundances through time directly, selection can be inferred

when phenotypes are consistently associated with a particular habitat type (38) and a clear mechanism explaining the relationship between trait values and absolute fitness is understood (25). In sum, a separate understanding of the causal effects of some traits on others and the effects of proximate traits on species performance or community composition could help to elucidate the complexity with which traits and environmental differences jointly produce ecological patterns (2–4).

In our study, there is a clear physiology–trait–ecology mapping (Figs. 2C and 4B), but an open question remaining is to what degree this is a general phenomenon. The physiological underpinnings of many traits are likely to be far more complex than those observed here, and organisms may arrive at the same trait through different physiological pathways. Interestingly, in our study, the same physiological mechanisms underlie movement traits across 17 species distributed among five families. This is surprising, given that *Ldh* is only one of several glycolytic enzymes, with the rate of glycolysis likely limited by the least abundant/slowest enzyme; *Ldh* may simply proxy the overall rate of glycolysis. More broadly, given that many traits are a product of several genes (i.e., quantitative or polygenic traits), it follows that many largely independent physiological pathways should influence traits, muddying the link between common differences in biomolecule concentrations and communities. Interestingly, this may cause complex patterns of species-level selection, as the effect of changing any given pathway and hence, the performance of a species in a given environment will depend on concurrent shifts in other pathways (8). For example, if a different glycolytic enzyme is limiting activity rate, increased *Ldh* will have no effect on activity rate and thus, no impact on species distributions or community composition. In short, the genetic and physiological underpinnings of traits can have important consequences for the ability of species and communities to respond to environmental differences (4), but those underpinnings may typically be more complex than those presented here because of both complex physiological underpinnings and typically high trait dimensionality (3). Ultimately, these complexities at the molecular and trait levels could create correspondingly complex patterns of species-level selection and community composition (4). Nonetheless, when links between physiology and phenotype can be established, they provide a powerful tool for testing the evolutionary ecology of entire communities.

Our study did not directly measure the effects of fish on species survival and reproduction and instead, inferred that community-level trait distributions are a result of differences in species performance between environments. This inference relies on two important assumptions. Species-level physiological and behavioral traits must be relatively constant across fish and fishless ponds. Behavioral traits, including activity level, are often thought to be plastic, including in response to predation risk, suggesting some flexibility in the behavior of species. We expect this plasticity to be mediated by the underlying up- or down-regulation of particular biomolecules (in our case, *Ak* and *Ldh*). However, there is considerable evidence that the magnitude of the species-level behavioral differences is much larger than equivalent plastic changes (15, 30, 39). Indeed, there is only minimal evidence that activity rate is plastic in these larval dragonflies under differing predation conditions. For example, McCauley (40) showed that only 3 of 10 species of larval dragonfly changed their activity rate in the presence of fish or invertebrate predators compared with their activity in no predator trials, and those changes were of much smaller magnitude than fixed species-level differences. Thus, activity rate may vary plastically, but this seems to be limited, and evidence suggests that species-level differences in activity are overwhelmingly larger than the level of plasticity in this trait.

By considering the physiological basis of common antipredator traits, we show that specific biomolecules have predictable consequences for the assembly of entire communities. In line with

previous work, enzymes impacting metabolic pathways had strong effects on predator avoidance behaviors (Figs. 1 and 3A and B), and those behaviors in turn determined how frequently species co-occurred with fish (Fig. 2). More interestingly, these species-level effects scaled up to impact entire assemblages (Fig. 3), such that community-level physiology was ultimately responsible for community composition across ponds with and without large-bodied fish (Fig. 4). We suggest that our understanding of ecological dynamics could be improved by studies that focus on mapping that investigates the pathways from physiology to traits to ecological patterns, particularly in systems where traits have clear functional roles. More broadly, we assert that causal relationships among traits should be considered when investigating traits and their role in community assembly.

## Methods

**Study System.** Dragonflies are a diverse group of odonates (Odonata: Anisoptera) with larvae that are important members of many aquatic communities, acting both as prey to large fish and invertebrates and also, as important predators, especially in ponds lacking large game fish (16, 25). Notably, larval dragonfly communities are thought to be predominantly structured by predation from large game fish (Centrarchids) when they are present or else from other dragonflies and invertebrates in ponds where large game fish are absent (16, 17). In this article, we investigate trait-based community assembly by focusing on intact dragonfly communities found in ponds with and without large game fish near the Koffler Scientific Reserve (KSR; 44.03, –79.53) in King City, Ontario, Canada.

**Collection.** We aimed to determine the distribution of individual species and the composition of dragonfly communities with respect to predator regime. We began by identifying 10 ponds of each type, all within ~30 km of the KSR (*SI Appendix*). We then used dip nets to sample up to 50 dragonfly larvae from the littoral zone of each pond, sampling equally from floating and emergent vegetation. After collection, all larvae were identified to species (*SI Appendix*) using keys and known species distributions, and they were stored individually in 473-mL cups, where they were fed zooplankton ad libitum.

**Behavioral Trait Assays and Biochemical Characterization.** To link behavioral traits to species distributions and community composition, we measured two distinct behavioral traits: activity rate and burst swimming speed. We measured both traits on the same 10–14 individuals of each species. Individuals of each species were randomly selected from all of those collected, meaning that tested individuals originated in fish or fishless ponds with roughly the same frequency at which they occurred in the wild. All assays were performed within 3 d of collection, after which all individuals were immediately frozen at –80 °C.

We measured activity rate using an open field test following the methods from ref. 24. Briefly, we introduced each individual to a 9-cm petri dish filled with pond water but without food. After allowing 24 h for acclimation, we recorded the position of each individual every 20 min for 3 h and then calculated the minimum distance between each position. The sum across all 10 observations represents our estimate of activity rate.

Should an individual encounter a predator, it may escape by swimming away quickly by ejecting water from a specialized rectal chamber (22). We assayed for burst swimming speed using a modified version of the protocol developed in ref. 22. Each larva was introduced into the center of a 75 × 58-cm tank filled to a height of 3 cm with filtered pond water. We then touched the larvae at the end of the abdomen with a wooden rod to simulate a predator attack while video recording the subsequent swimming burst. In some instances, larvae had to be touched repeatedly to induce a response. After the assay, we calculated burst swimming speed by dividing the distance moved by the larva by the time during which the behavior occurred. All video analyses were conducted in VLC Media Player.

We used standard photometric analyses to characterize the activity of *Ak* and *Ldh* following ref. 18 and *SI Appendix*.

**Statistical Analyses.** Our ultimate aim was to investigate differences in community composition among habitat types and test whether those differences were caused by proximate behavioral traits and ultimate physiological mechanisms. We began by calculating mean trait values for all trait by species combinations using species mean values in all subsequent analyses. We then explored a priori predicted links between enzymatic activity and behavioral traits. We used a linear model (LM) to test for the effect of *Ldh*

activity on activity rate. We repeated this analysis while estimating burst swimming speed using *Ak* activity as the predictor variable.

We next aimed to determine the habitat distributions of species differing in physiological and behavioral traits. We quantified habitat distribution as the proportion of individuals of a given species that were found in fishless ponds. We used this metric to investigate relationships between traits and distributions, beginning with the simplest possible analyses. Specifically, we used a series of logistic regressions, with one trait predicting the proportion of individuals of a given species found in fishless ponds. We repeated this analysis independently for each physiological and behavioral trait. Unfortunately, a well-characterized phylogeny is lacking for dragonfly species, precluding complete phylogenetic control. The possibility of building a phylogeny is limited, because only ~70% of species had any sequence data, usually only for a single genomic region. In an effort to provide a level of phylogenetic control, we nevertheless repeated all above analyses while including genus nested in family as a random effect. We found that all results were qualitatively identical.

Despite the utility of these analyses for showing differences in trait-mediated habitat distributions, they do not consider our purported causal trait relationships. Because we hypothesized that traits were causally structured (e.g., *Ak* levels determine burst swimming speed), we chose to make use of structural equation modeling, which can explicitly account for such relationships (9). We began by linking *Ldh* and *Ak* activity rates to their corresponding behavioral traits and then linked those traits to our measure of habitat distribution. We optimized the model by sequentially adding/removing links, retaining only significant relationships. In all cases, structural equation modeling models were fit using regression rather than covariance links using the package *lavaan* (41), and all variables were standardized before analyses.

After exploring the species-level effects of physiological and behavioral traits on species distributions, we tested for the scaling of these relationships to intact dragonfly communities. We began by calculating community-

weighted means for each trait value by summing species-level mean trait values for each individual and then dividing by the number of individuals collected in that community. We first investigated these data by testing for community-level trait differences using a series of univariate analyses. We used LMs to predict community-weighted behavioral traits using their corresponding community-weighted physiological traits. We then related community-level traits to habitat types. Specifically, we ran independent LMs for each community-weighted trait using habitat type (fish vs. no large fish) as the predictor variable.

Again, these simple univariate analyses can adequately describe trait distributions but do not represent our hypothesized causality or consider the actions of multiple traits. As such, we next quantified differences in multivariate community composition using CCA, constraining the ordination by pond status (Centrarchid fish vs. no large fish). This constraint should cause clustering of community scores by pond type, such that high values would be associated with one or the other habitat type.

CCA scores are then a multivariate representation of dragonfly community composition, while community-weighted means represent a community-level measure of trait distributions. We, therefore, tested for relationships between CCA scores and corresponding community-weighted mean trait values using structural equation modeling. We predicted community scores along the first axis from the CCA using community-weighted mean values of activity rate and burst swimming speed, in turn linking the latter two variables to their corresponding community-weighted enzymatic activity. Again, we selectively added/removed links based on *P* values, and all links were fit as regressions. Note that the postulated causality of traits in our dataset precluded an ordination analysis where we simply use traits as predictor variables, which tends to be the norm in trait-based assessments of community composition (2). Such an analysis would likely show the collinearity between traits but would not allow us to distinguish causality with respect to community composition. All analyses were conducted in R (42) using the *lavaan* (41), *lme4* (43), and base packages.

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