

RESEARCH ARTICLE

Functional Ecology



Past selection impacts the strength of an aquatic trophic cascade

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Abstract

1. In complex food webs, interactions among species in different trophic levels can generate cascading indirect effects that couple top predators with primary producers, thereby affecting ecosystem functioning. Natural selection imposed by top predators on intermediate predators may play a role in shaping the strength of these trophic cascades, but this conjecture remains largely untested.
2. To determine the effects of natural selection on the strength of trophic cascades, we conducted a two-part experiment in a four-level aquatic trophic system involving a top predator (fish), an intermediate predator (damselflies), herbivores (zooplankton) and primary producers (algae). We first quantified how predation by fish generated selection on damselfly activity levels after controlling for phenotypic plasticity. We then measured the indirect effects of this selection on primary production (phytoplankton biomass). In both experiments, we varied the density of predators, allowing us to elucidate both trait-mediated and density-mediated indirect effects.
3. We found that as fish density increased, damselfly survivorship declined, which generated natural selection favouring less active damselflies. These results are robust after taking into account latent effects of plasticity in response to fish predator cues. The surviving damselflies likely foraged less, freeing herbivores from predation, which in turn reduced primary production. This selection-driven trait-mediated indirect effect was only apparent at low damselfly densities, because the consumptive effect of damselflies at high densities overwhelmed the effects of past selection.
4. These results demonstrate how the past action of natural selection imposed by predators affects the phenotypes of prey that consume herbivores, which ultimately influences primary productivity—a selective trophic cascade. Natural selection can therefore act as a mechanism coupling ecological dynamics across trophic levels, which ultimately influences ecosystem functioning.

KEYWORDS

eco-evolutionary, ecosystem function, indirect effect, natural selection, odonate, predation, trophic cascade

1 | INTRODUCTION

The discovery that predators can indirectly shape primary production in ecosystems was a watershed moment in ecology (Hairston, Smith, & Slobodkin, 1960). This finding was paramount to the establishment of a rigorous research programme focused on understanding the importance of species interactions (Schmitz, 2010). Trophic cascades, whereby predators indirectly affect primary producers, have become a cornerstone of ecological theory (Estes et al., 2011; Paine, 1966; Shurin et al., 2002). In systems with intermediate predators (i.e., a four-level interaction chain; Figure 1), top predators can generate negative indirect effects on primary producers (Schmitz, Krivan, & Ovadia, 2004). This indirect coupling between predators and primary producers can arise through both density- and trait-mediated indirect effects (Abrams, Menge, Mittelbach, Spiller, & Yodzis, 1996; Preisser, Bolnick, & Benard, 2005). Density-mediated indirect effects occur when top predators reduce the abundance of intermediate predators, freeing herbivores from predation and thus reducing primary production. Trait-mediated indirect effects are often generated through behavioural plasticity, wherein the threat of predation by top predators causes intermediate predators to reduce consumption of herbivores, which then generates a similar trophic cascade (Abrams et al., 1996; Preisser et al., 2005).

Studies concerning trait-mediated indirect effects are typically conducted by removing the direct consumptive effects of predators (e.g., using caged predators or rendering them unable to kill) to allow only behaviourally mediated changes to occur (Beckerman, Uriarte, & Schmitz, 1997; Schmitz et al., 2004). While insightful, this approach obscures the possible effects of natural selection by top predators on intermediate predators and thus on the strength of a trophic cascade (Figure 1). Such a possibility opens up the potential for eco-evolutionary dynamics in shaping the indirect interactions among species in complex food webs (Bassar et al., 2010; Harmon et al., 2009; Hendry, 2016; Siepielski & Benkman, 2008). Individual prey that are consumed by predators are typically not a random subset of most populations, and the traits that mediate the risk of predation are often the same traits that affect foraging by intermediate consumers (Lima & Dill, 1990). Previous studies demonstrating density-dependent effects may therefore have captured the effects of natural selection. Indeed, intraspecific variation in foraging traits is emerging as a potentially key factor shaping the strength of trophic cascades (Bolnick et al., 2011; Pettolelli, Hilborn, Duncan, & Durant, 2015; Sih, Cote, Evans, Fogarty, & Pruitt, 2012; Start & Gilbert, 2017). Moreover, the strength of selection imposed by predators should also vary with their densities, as the strength of selection for predator–prey interactions is expected to increase with greater prey mortality (Benkman, 2013). These associations imply that the past action of a key evolutionary process—natural selection—may have a role in shaping the strength of trophic cascades in complex food webs. However, this conjecture remains largely untested.

Here, we use an experimental approach to test this “selective trophic cascade” hypothesis in a four-level trophic system involving a top predator (fish), an intermediate predator (*Enallagma* damselflies),

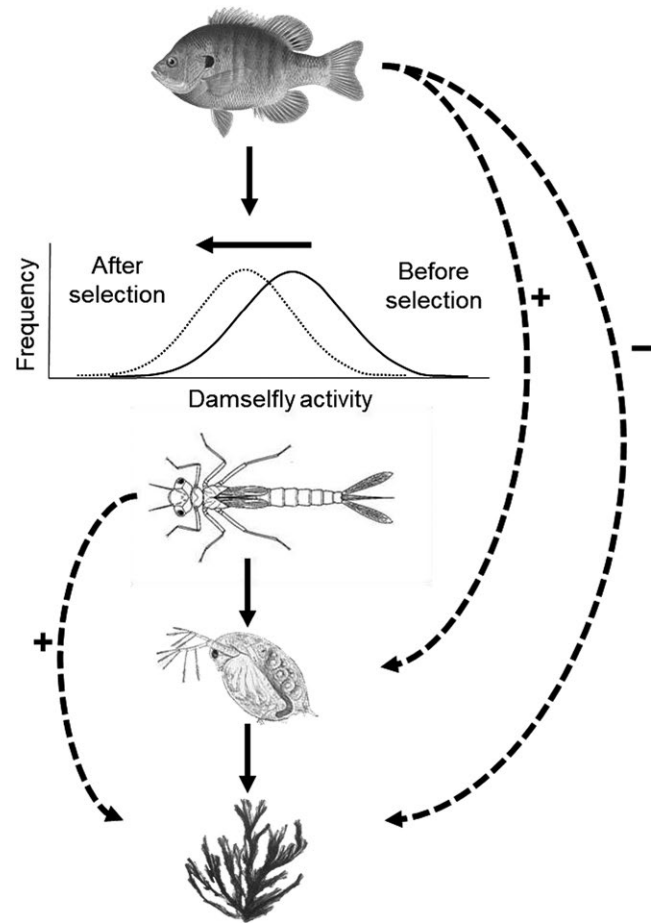


FIGURE 1 Hypothesized indirect trait-mediated effects generated because of natural selection on intermediate predators by a top predator in a four-level trophic system. Dashed lines show indirect effects generated by top predators (fish) and intermediate predators (damselflies). The direct effect of consumption by fish on damselflies generates natural selection for reduced activity in damselflies, causing them to forage less on zooplankton, which results in increased herbivory and therefore reduced primary productivity. Selection by fish thus weakens the indirect benefit that damselflies have on primary producers, and alters the strength of the trophic cascade. Image credits (top to bottom): Hugh McCormick Smith, CSIRO Australian National Insect Collection, William Thomas Calman, Pierre-Louis Crouan and Hippolyte-Marie Crouan

herbivores (zooplankton) and primary producers (algae) (Figure 1). These four trophic levels dominate the littoral zone of many lakes and ponds in temperate regions. Previous work has established that fish preferentially consume active damselflies, selecting for relatively inactive damselflies that forage less on zooplankton (Strobbe, McPeck, De Block, & Stoks, 2011) and allow zooplankton populations to increase their rates of algal grazing. Because damselflies cannot swim away to evade fish, minimizing activity helps damselflies avoid detection (Strobbe et al., 2011). This leads to the prediction that as fish density increases, increased selective predation by fish will indirectly result in reduced primary productivity, and that this effect should be greater when damselflies are less abundant, as

would be the case if predators have strong consumptive effects reducing damselfly densities. These inferences, however, require that we take into account behavioural plasticity for reduced activities that *Enallagma* typically exhibit (McPeck, 1990a; Stoks, McPeck, & Mitchell, 2003; Strobbe et al., 2011). Overall, our results support the selective trophic cascade hypothesis, and demonstrate that natural selection acts as a mechanism coupling ecological dynamics across trophic levels.

2 | MATERIALS AND METHODS

We tested the selective trophic cascade hypothesis using a two-step experiment designed to parse out possible density- and selective trait-mediated indirect effects of fish predation on primary production.

2.1 | Fish predation and selection

We first quantified how fish density shapes damselfly mortality and the strength of selection on damselfly activity levels. At the University of Arkansas greenhouse facility, we established 22 19-L mesocosms with dechlorinated tap water, macrophytes (coontail *Ceratophyllum* spp.) as a foraging substrate for damselflies, and concentrated aliquots of zooplankton from a natural lake. After 4 days, we added 27 Orange Bluet (*Enallagma signatum*) larvae, as an intermediate predator. *Enallagma* are predatory insects found in many lakes and ponds, where the larvae inhabit plants found in the littoral zones of lakes, feed on small invertebrates and are fed upon themselves by larger predators (principally fish) (McPeck, 1990b, 1998; McPeck & Brown, 2000; Siepielski, Hung, Bein, & McPeck, 2010). All damselflies were collected from Lake Fayetteville, Arkansas; during this time, most damselflies were near their final instar. After an additional 4 days, we manipulated the density of fish predators by randomly assigning 0, 1, 2 or 4 Bluegill fish (*Lepomis macrochirus*), a common predator of *Enallagma* (McPeck, 1998; McPeck & Brown, 2000; Siepielski et al., 2010) (standard length: mean = 50.7 mm $\pm$ 4.6 1 SD), to each mesocosm. We included four replicates of 0 fish, otherwise each treatment had six replicates. After 20 days, we collected surviving damselflies, digitally recorded them with videos in activity trials, and then counted the number of times an individual groomed itself, moved limbs, swam or walked over a 10-min period. These activities constitute the bulk of routine damselfly activity behaviours. We did not record damselfly prey attacks because the frame rate was too low relative to the strike speed of damselflies; however, some limb movements were likely prey attacks because damselfly attacks typically result in a rapid jerking response (A. M. Siepielski, personal observation). Although we only conducted a single trial per individual, previous work has shown that damselfly larvae activity levels are generally consistent through time (Johansson, Stoks, Rowe, & De Block, 2001). Trials were conducted in plastic dishes (3.9 cm diameter $\times$ 5.5 cm height) with a wooden dowel as a perch, dechlorinated tap water at room temperature and approximately

TABLE 1 Regression of behaviour on time since removal indicates that movement increased with time since removal from fish predator cues. However, there was no indication of latency affecting walks, grooms or swims

Behaviour	Slope	SE	F	p
Movements	3.28	1.11	7.12	.023
Walks	-0.26	1.58	0.02	.883
Grooms	0.02	0.04	0.25	.620
Swims	0.24	0.23	0.93	.355

30 *Artemia* as prey. Trials were conducted after damselflies fasted for ca. 24 hr to ensure consistent motivation for foraging (Siepielski, Wang, & Prince, 2014).

An important assumption in our analysis is that damselflies display no latent behavioural plasticity in response to previous exposure to fish. Although all of the activity trials were completed in the absence of fish cues, it is possible that damselflies could have exhibited a latent plastic response to fish cues. Such latent behavioural responses after removal from predator cues are exhibited in other species, such as tadpoles (Buskirk, 2002; Relyea, 2003) and fish (Pilakouta & Alonzo, 2013). *Enallagma* species coexisting with fish reduce foraging activity as a plastic response to the threat of predation (McPeck, 1990a; Stoks et al., 2003; Strobbe et al., 2011), and thus, if we did not take this possibility into account our estimates of selective responses could simply reflect a latent plastic response. If such latency effects were present, damselfly activity levels should consistently increase with time since removal from fish cues at the end of the experiment. To evaluate this, we regressed individual activity on time since removal (h) with a random effect of mesocosm in a linear mixed-effects model using the lme4 package (Bates, Maechler, Bolker, & Walker, 2014) in R (R Core Team, 2014). We excluded the no fish treatment from this analysis, because those damselflies never encountered fish cues during the experiment, and thus could obscure any latency effects. This analysis revealed that movement behaviour, but no other behaviours, increased slightly with time since removal from fish cues (Table 1, Figure S1). To account for this latency effect, we extracted the residuals from the linear mixed-effects models of each measure of activity regressed on time since removal. In all subsequent analyses with behaviour as the response variable, we present results from models with both the raw data and using the residuals from this model.

We modelled damselfly survival in relation to fish density with an exponential model using the nls function in base R (R Core Team, 2014). Because damselflies grow through moulting, we could not individually mark them and use standard approaches for quantifying selection by tracking the fate of individuals (McPeck, 1997). Instead, we compared the distributions of phenotypes among predator treatments. Such a design is appropriate and sufficient for detecting selection, even though it is not the most commonly used approach of selection gradient analysis (Siepielski et al., 2014). Evidence consistent with directional selection imposed by fish would be manifested as increased differences between the phenotypic means in the

no-predator and predator treatments, whereas nonlinear selection (i.e., selection on variances—potentially stabilizing/disruptive selection) would be manifested as a change in the variance among treatments. Thus, if the strength of directional selection increases with greater fish densities, we would expect that mean values of activity phenotypes from surviving damselflies would decline. Likewise, the magnitude of variation in activity phenotypes may also change with increasing fish densities. Therefore, linear regression was used to test for the strength of directional selection in relation to fish density by regressing mean activity phenotypes of each experimental replicate against fish density. Potential nonlinear selection (selection on trait variances) was tested by regressing the standard deviation of activity phenotypes of each replicate on fish density.

We calculated variance standardized direction selection differentials (i , the intensity of selection) for the effects of fish predation as the difference in means of the activity phenotypes between the no-predator treatment, and the predator treatments, $i: \bar{z}_p - \bar{z}_n / \sqrt{v_n}$ where $\bar{z}_p$ is the mean of the activity phenotype of surviving individuals from the predator treatment and $\bar{z}_n$ is the mean of individuals from the no-predator treatment, and v_n is the variance of the phenotype from the no-predator treatment (see Perez & Munch, 2010). We calculated a standardized index of nonlinear selection (j , potentially stabilizing/disruptive selection; Perez & Munch, 2010) as, $j = [v_p - v_n + (\bar{z}_p - \bar{z}_n)^2] / v_n$ where v_p is the variance in the predator treatment, and the rest of the terms are as described above. Negative values of j are consistent with potentially stabilizing selection, whereas positive values are consistent with disruptive selection. We note that these values are only consistent with these forms of selection because we cannot visualize the fitness surfaces (the relationship between individual trait values and survival). However, these measures still capture the expected changes in the means and variances of traits if selection is operating (Price 1971, Falconer and Mackay 1996, Perez & Munch, 2010). We therefore exercise caution in interpreting these nonlinear selection coefficients (Perez & Munch, 2010). We used bootstrapping with 1,000 replicates to calculate standard errors for the selection coefficients. Activity measures were not significantly correlated, so each variable was analysed separately.

2.2 | Selection and density effects in a trophic cascade

To isolate the relative effects of density- (i.e., consumption by fish reducing damselfly density) and potential selective trait-mediated indirect effects of predation on damselflies, we examined how primary producer biomass changed through time (a proxy for primary productivity) in response to the phenotypes of surviving damselflies under low and high damselfly densities. Low damselfly density treatments create a situation where consumption by fish was high, and high damselfly treatments a situation where fish consumption was lower. This design, therefore, allowed us to understand the potential roles of fish predation depressing damselfly abundances, relative to the effect of natural selection imposed by fish predation.

We established 34 5.5-L mesocosms with filtered lake water (as an algae source; 0.77-mm mesh), macrophytes (*Ceratophyllum* spp.) and concentrated aliquots of zooplankton (primarily *Daphnia* and ostracods). After allowing these trophic levels to establish for 8 days, we sampled initial concentrations of phytoplankton by measuring chlorophyll-*a* (hereafter chl-*a*) content ($\mu\text{g/ml}$) in the water column with a fluorometer (Turner Designs, Sunnyvale, CA, U.S.A.) after a standard ethanol digestion (Siepielski & McPeck, 2013). We then randomly assigned two (low density) or four (high density) damselfly larvae, which translates to approximately 28 or 56 damselflies/ m^2 , to each mesocosm from the pooled surviving damselflies from each fish density treatment. These densities are on the low end of *Enallagma* densities typically found in our study area (A.M. Siepielski, unpublished data), but typical of *Enallagma* densities elsewhere (Siepielski, Nemirov, Cattivera, & Nickerson, 2016). Because our goal was to determine the effects of differences in damselfly density, we did not include a no-damselfly treatment. For this reason, our analysis only allows us to compare the relative effects of differences in damselfly density, not an absolute effect of damselflies. Forty-eight hr transpired between the end of the fish experiment and placing damselflies in the mesocosms. After 10-d exposure to the damselfly treatments, we sampled final phytoplankton concentration to determine the change in chl-*a* between the start and end of the experiment. We used the change in chl-*a* over this time period as a proxy for primary productivity, and therefore the strength of the trophic cascade (Start & Gilbert, 2017), because damselflies consume filter-feeding zooplankton in the water column that consume phytoplankton (Strobbe et al., 2011).

To determine the effects of past exposure to varying fish densities, and the resulting selection it may have generated on damselfly phenotypes, as well as the effect of fish reducing damselfly density, we used a linear model with the change in chl-*a* between the start and end of the experiment as the response variable, and fish density, damselfly density, and their interaction as predictors. Because survival was low in the four-fish density treatment (see Results, Figure 2a), we pooled two- and four-fish density treatment surviving damselflies together for statistical analyses. The trends and statistical results were nearly identical before pooling (Figure S2). All statistical analyses were performed in R v3.3.3 (R Core Team, 2014).

3 | RESULTS

Damselfly survival decreased exponentially as fish density increased (Figure 2a, $0.68e^{-1.12 \times \text{Fish density}}$, $p < .001$ for both estimated parameters). With the exception of the number of walks, all other behaviours of surviving damselflies declined, albeit weakly, as fish density increased, indicating directional selection; however, only the reduction in the number of movements was statistically significant (Table 2; Figure S3), with the reduction greatest at the highest fish density (Figure 2b). These findings were robust to latent behavioural plasticity, with both the raw data and the analysis based on the residuals (e.g., after taking into account latency effects; Table 1), showing

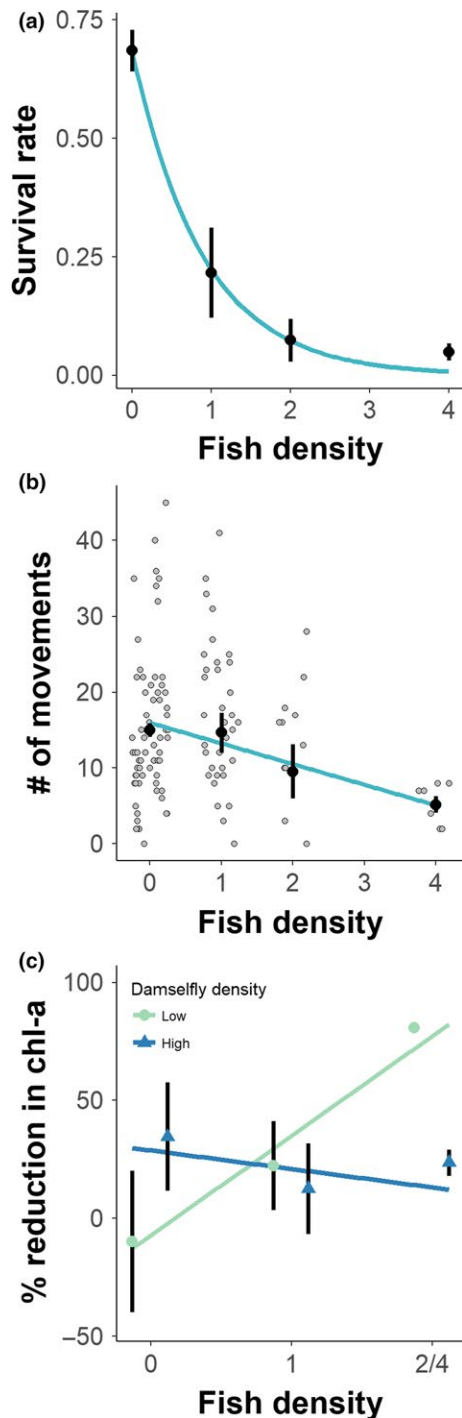


FIGURE 2 Natural selection drives the strength of a trophic cascade. As fish density increased (a) damselfly survivorship declined exponentially ($n = 22$ mesocosms), with (b) selection for reduced activity being most apparent at the highest fish density ($n = 22$) (c). At low, but not high damselfly densities, the effects of reduced primary productivity because of the past action of natural selection by fish on damselflies were stronger ($n = 34$). In (c), the values were jittered horizontally; the error bar for the low damselfly density two- or four-fish density treatment are smaller than the point denoting the mean, and therefore obscured. In all panels, large points shown are mean $\pm$ SE, and in panel (b), small grey points are individual damselfly observations ($n = 122$ individuals)

nearly identical patterns. Estimates of the directional selection coefficients for the number of movements were also especially high at the highest fish density (Table 3).

The variance in all behavioural measures also tended to decline as fish density increased, but only significantly so for the number of walks and the number of movements (Table 2). Again, these results were supported by both the raw data and the analysis using the residuals from the latency models (Table 2). Visual inspection of the trait distributions reveals, however, that for some traits (grooms and swims; Figure S3) there was little variation among any fish density treatments. Estimates of the index of nonlinear selection coefficients also indicated reduced trait variation (Table 3). Although the reduced trait variation is consistent with stabilizing selection, the accompanying directional shifts in means imply that the overall pattern is more indicative of directional selection, as extremely low activity level individuals were not strongly selected against (Figures 2b and S3). Indeed, without a visual assessment of the fitness surface, it is important to note that these later results only reveal a pattern consistent with, but not necessarily indicative of, stabilizing selection on these traits.

The change in primary productivity (chl-*a* content) over the duration of the experiment was explained by an interaction between previously experienced fish density (e.g., generating natural selection, Tables 2 and 3) and damselfly density (Figure 2c, $F_{2,22} = 5.58$, $p = .011$). The main effects of both damselfly density ($F_{1,22} = 6.62$, $p = .017$) and fish density ($F_{2,22} = 4.82$, $p = .018$) were significant. However, given the interaction between these two factors, an analysis with separate models for each damselfly density showed that while the reduction in chl-*a* increased strongly linearly with increasing past fish density at low damselfly density ($F_{2,10} = 8.25$, $p = .008$), this effect was not present at high damselfly density ($F_{2,10} = 1.21$, $p = .331$) (Figures 2c and S2).

4 | DISCUSSION

After accounting for the effects of latent plasticity in response to fish cues, our results support the hypothesis that trait-mediated indirect effects driven by natural selection can affect the strength of a trophic cascade (Figure 1). Because our study occurred within a generation of damselflies, the results demonstrate the effects of natural selection, but not adaptive evolution (Bassar et al., 2010; Harmon et al., 2009; Hendry, 2016; Palkovacs et al., 2009), by top predators on lower trophic levels. However, activity-mediated traits in damselflies are heritable (Swaegers, Strobbe, McPeck, & Stoks, 2017), and selection by fish predators has repeatedly driven adaptive evolution of such traits in damselflies (McPeck, 1990a, 2000; Stoks et al., 2003; Swaegers et al., 2017). Fish are visually hunting predators, so reduced activity levels make damselflies less likely to be attacked and eaten (Wellborn, Skelly, & Werner, 1996). Several experimental studies have also shown that fish predators select for reduced activity in damselflies (Strobbe et al., 2011; Swaegers et al., 2017). Selection was not, however, consistent across fish densities, and

TABLE 2 Results from linear regressions of surviving damselfly activity levels against fish density suggest that fish typically exert selection favouring less active individuals. The mean column denotes the results from an analysis of mean activity levels, reflecting directional selection, whereas the standard deviation column denotes results using the standard deviation as a response, reflecting selection on the variance. Both regressions used the mesocosm mean or standard deviation as the unit of replication (see Figure S3). The data column indicates whether raw counts (Count) were used in the analysis or residuals (Resid) extracted from the latency regression

Behaviour	Data	Mean				SD			
		Slope	SE	F	p	Slope	SE	F	p
Movements	Count	-2.72	0.91	8.88	.008	-1.62	0.62	6.91	.027
	Resid	-2.42	0.83	8.02	.012	-1.61	0.62	6.78	.029
Walks	Count	0.28	1.64	0.03	.865	-2.02	0.64	10.16	.011
	Resid	0.38	1.64	0.05	.821	-2.03	0.63	10.19	.011
Grooms	Count	-0.04	0.06	0.43	.519	-0.13	0.08	2.40	.156
	Resid	-0.70	0.06	1.41	.252	-0.13	0.08	2.54	.146
Swims	Count	-0.05	0.06	0.77	.394	-0.11	0.24	0.22	.649
	Resid	-0.02	0.05	0.21	.654	-0.11	0.24	0.22	.649

TABLE 3 Directional (*i*) and variance (*j*) estimates of standardized selection coefficients on damselfly behavioural phenotypes in relation to fish density. SEs based on 1,000 bootstrap replicates

Behaviour	Term	Fish density					
		1		2		4	
		Coefficient	SE	Coefficient	SE	Coefficient	SE
Movements	<i>i</i>	0.24	0.23	-0.11	0.28	-1.03	0.15
Movements	<i>j</i>	0.18	0.46	-0.22	0.35	0.16	0.32
Walks	<i>i</i>	0.03	0.19	-0.36	0.23	0.03	0.3
Walks	<i>j</i>	-0.06	0.26	-0.29	0.35	-0.43	0.29
Grooms	<i>i</i>	-0.26	0.1	-0.13	0.28	-0.39	0.06
Grooms	<i>j</i>	-0.75	0.15	-0.39	0.6	-0.84	0.05
Swims	<i>i</i>	^a	^a	-0.39	0.06	-0.13	0.28
Swims	<i>j</i>	^a	^a	-0.84	0.05	-0.46	0.55

^aValues for selection coefficients of the number of swims were poorly estimated because of small sample sizes and considerable variation among individuals at this treatment level (see Figure S3).

there was no simple linear response between the extent of mortality and the resulting strength of selection (Figure 2). Instead, strong directional selection was only detected for reduced activity at the highest fish density. Indeed, the strength of selection on movements generated by a high density of fish was almost an order of magnitude higher than in most experimental selection studies (Caruso et al., 2017). These results therefore support recent work predicting that the strength of selection should increase nonlinearly with the strength of antagonistic species interactions (Benkman, 2013).

Although we detected strong selection on some traits, we suspect that the lack of selection on other traits, such as the number of walks, may have arisen for several reasons owed primarily to the effects of standing phenotypic variation. First, the damselfly species used in this study has much lower average activity levels than other damselfly species coexisting with fish (McPeck, 2000). Second, unlike previous experiments, which were conducted in the fall with earlier instar larvae (damselflies lay eggs in the summer, and larvae hatch and grow during the late summer and fall), our experiment was conducted in the spring with later instar larvae.

It may be that this prolonged duration of selection in the natural field conditions had already reduced the amount of phenotypic variation. Indeed, for some traits (i.e., grooms and swims) there was very little variation (Figure S3). As a result, more extreme fish density treatments were necessary to detect directional selection. Although we largely detected directional selection, the variation in activity traits also declined (consistent with weak potential stabilizing selection) with increased fish density, especially for the number of movements and walks. This later finding may reflect a physiologically imposed lower limit to the reduction in activity possible (e.g., damselflies must still move to attack prey). In the same way that past selection can make competition difficult to detect (Connell, 1980), the same issue may plague studies attempting to determine how natural selection affects ecosystem functioning. More generally, these results, along with a growing body of work (Post, Palkovacs, Schielke, & Dodson, 2008; Schmitz et al., 2004; Sih et al., 2012; Start & Gilbert, 2017), point to the importance of intraspecific variation as a key determinant underlying the strength of trophic cascades.

While our results are consistent with fish driving natural selection to reduce damselfly activity, they also support other studies showing that phenotypic plasticity plays a role (Strobbe et al., 2011; Swaegers et al., 2017). *Enallagma* species coexisting with fish exhibit reduced foraging activity as a plastic response to the threat of predation (McPeck, 1990a; Stoks et al., 2003; Strobbe et al., 2011). We likewise found evidence of latent behavioural plasticity in one activity trait in our analysis of activity following removal from fish treatments. If such phenotypic plasticity was entirely responsible for our results, our findings would support previous work demonstrating that non-lethal predator effects on prey traits (i.e., predator-induced plasticity) can induce trophic cascades (Beckerman et al., 1997; Byrnes et al., 2006; Schmitz et al., 2004; Werner & Peacor, 2003). However, after taking into account this latency effect we still found detectable selection, suggesting that both plasticity and selection can shape damselfly activity phenotypes. Regardless, our results are also consistent with numerous other examples in this same study system documenting selection on damselfly activity-related traits after accounting for the contribution of plasticity in mediating behavioural responses (McPeck, 1997; Siepielski et al., 2014; Strobbe et al., 2011; Swaegers et al., 2017). Finally, Swaegers et al. (2017) have shown that activity-related traits in other *Enallagma* populations adapted to living with fish predators do exhibit significant broad-sense heritabilities, indicating genetic variation for such traits. While we do not have estimates of genetic variation in the traits we investigated in the species here, it is reasonable to expect similar patterns. Therefore, the changes in activity that we observed are likely the result of selection on some genetic variation, rather than plasticity alone.

The effects of natural selection for reduced activity in damselflies generated cascading effects on primary productivity (Figure 2c). However, this effect varied with damselfly density. At low densities, primary production was reduced more in mesocosms with damselflies from greater fish densities, which were less active because of past selection by fish. This effect presumably arises because as fish density increased, generating strong selection for less active damselflies, fewer zooplankton were likely consumed which led to greater grazing on primary producers; that is, selection by fish on damselflies weakened the indirect benefit they have to primary producers (Figures 1 and 2). Although we did not directly quantify zooplankton consumption by damselflies, this inference is supported by experiments which showed that fish generated selection on damselflies resulting in reduced damselfly foraging on zooplankton (Strobbe et al., 2011). By contrast, at high damselfly densities, the effects of past selection had little impact. This dissociation of selection likely occurred because damselflies consumed sufficient numbers of zooplankton so that the effects of selection were essentially muted. This suggests that the selective impacts of consumption at upper trophic levels on lower trophic levels may only be released when predators reduce intermediate predators to sufficiently low densities (Figure 2). Such interactions between trait- and density-mediated indirect effects may be a general feature shaping trophic cascades (Start & Gilbert, 2017).

While our results demonstrate the role of natural selection in generating trait-mediated indirect effects and their consequences for the strength of a trophic cascade, two caveats are important for their interpretation. First, the exclusion of fish for our estimates of primary production in response to the indirect effects of damselflies may exaggerate the potential effects of selection on damselflies. Fish are intraguild predators of damselflies. As a result, although damselfly consumption of zooplankton likely declined because of selection, zooplankton abundances would still be reduced even further by fish predation. Thus, our results demonstrate the relative, but not absolute impacts of past selection. Second, our results were a short-term experiment in a simplified mesocosm environment, and thus reflect only a snapshot of the dynamic feedback between predators and primary producers via density- and trait-mediated indirect effects. For instance, the drastic reduction in chl-*a* we detected (Figure 2c) because of the past action of selection on damselflies would eventually lead to a decline in zooplankton, and thus a decline in damselfly abundances. Nevertheless, our results establish a mechanistic basis and demonstrate how natural selection can generate variation in the strength of a trophic cascade (Bassar et al., 2010; Harmon et al., 2009).

Rather than trait-mediated indirect effects being driven by fear and non-consumptive behavioural effects, we show that natural selection imposed by top predators on intermediate predators can also affect a trophic cascade. The effects of such selection were only important, though, when the effects of consumption reduced damselfly densities sufficiently. Our study therefore demonstrates that investigating the role of predators in shaping trophic cascades will benefit from more explicit consideration of the effects of selection-driven trait-mediated indirect effects and how they interact with density. Understanding the past action of natural selection in complex food webs is necessary for developing a more complete and predictive theory defining how species interactions are coupled in nature and the consequences of this coupling for key ecosystem processes (Matthews et al., 2011).

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AUTHOR CONTRIBUTION

A.M.S. and B.H.O. designed the experiments; B.H.O. ran the experiments, conducted statistical analyses and helped to draft the manuscript; S.R.G., A.Z.H. and M.S. collected data and revised the manuscript; A.M.S. conceived of the study, analysed data and drafted the manuscript.

DATA ACCESSIBILITY

Data are available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.7mc7r30>) (Ousterhout, Graham, Hasik, & Siepielski, 2018).

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