Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

PROCEEDINGS OF THE ROYAL SOCIETY B

FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2011.0606
Published online

Scaling-up anti-predator phenotypic responses of prey: impacts over multiple generations in a complex aquatic community

Scott D. Peacor[1,*], Kevin L. Pangle[2], Luis Schiesari[1,3] and Earl E. Werner[4]

[1]*Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA*
[2]*Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH 43212, USA*
[3]*Environmental Management, School of Arts, Sciences and Humanities, University of São Paulo (EACH-USP), São Paulo, SP 03828-080, Brazil*
[4]*Department of Ecology and Evolutionary Biology, The University of Michigan, Ann Arbor, MI 48109-1048, USA*

Non-consumptive effects (NCEs) of predators owing to induced changes in prey traits are predicted to influence the structure of ecological communities. However, evidence of the importance of NCEs is limited primarily to simple systems (e.g. two to four species) over relatively short periods (e.g. less than one generation). We examined the NCEs of a fish predator, arising from phenotypic plasticity in zooplankton prey traits, over multiple generations of a diverse zooplankton community. The presence of fish, caged to remove consumptive effects, strongly influenced zooplankton community structure, through both direct and indirect NCE pathways, altering the abundance of many taxa by magnitudes as large as 3 to 10-fold. Presence of fish affected different species of cladocerans and copepods both positively and negatively. A particularly striking result was the reversal of dominance in copepod taxa: presence of fish reduced the ratio of calanoids to cyclopoids from 6.3 to 0.43. Further, the NCE of fish had a strong negative trophic cascade to zooplankton resources (phytoplankton). To our knowledge, this is the first experiment to show that NCEs can influence the abundance of multiple prey species over time spans of multiple prey generations. Our findings demonstrate that adaptive phenotypic plasticity of individuals can scale-up to affect the structure of ecological communities.

Keywords: non-consumptive; trait-mediated; zooplankton; predator–prey; phenotypic plasticity; interaction modification

1. INTRODUCTION

Abstracting complex ecological communities as food webs highlights the trophic interactions that link species and the potential for indirect effects to propagate through communities [1–4]. Traditionally, these trophic interactions or linkages have been viewed as direct consumptive effects (CEs). However, the net effect of predators on prey is composed not only of CEs but also of important non-consumptive effects (NCEs) that result from phenotypic plasticity (NCEs *sensu* [5], also non-lethal effects and interaction modifications [6]). NCEs are principally owing to prey-modifying traits in response to changes in predation risk (e.g. in behaviour, physiology, morphology or life history), which in turn affect prey survival and growth rate [7–9]. The magnitudes and consequences of such NCEs in food webs are predicted to greatly influence community structure and dynamics by introducing strong nonlinearities and higher order interactions [10,11]. Experiments suggest that these NCEs and associated trait-mediated indirect effects (i.e. indirect effects of the predator on other species through induced changes in traits of the intervening prey, *sensu* [4]) can be as or more important than the direct and indirect effects arising from CEs of predators (reviewed in [12–16]).

The great preponderance of evidence for the impacts of NCEs comes from experimental studies of very simple food webs (e.g. two to four species) typically conducted on short-term within-generational timescales of the prey, assessing individual parameters (e.g. somatic growth rates). Although this body of literature abundantly documents strong NCEs, it leaves open the critical question of whether these NCEs are translated to effects over longer time horizons (i.e. multiple generations of prey) and in more complex assemblages. For example, Abrams [17] argued that short-term experiments can overestimate the influence of NCEs, and Bolker *et al.* [11] emphasized that although short-term effects of NCEs have been shown to be important, we do not know if they are weakened or strengthened over longer time-scales. Similarly, Persson & DeRoos [18] argued, based on field-parametrized models showing that population feedbacks reduce potential effects of NCEs, that

* Author for correspondence (peacor@msu.edu).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2011.0606 or via http://rspb.royalsocietypublishing.org.

Received 21 March 2011
Accepted 27 April 2011

This journal is © 2011 The Royal Society

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

extrapolating from short-term experiments may overemphasize or misrepresent long-term, population level effects. Further, although many theoretical studies of the dynamics of large food webs (i.e. more than 10 species) include adaptive behaviour of predators through prey selection (e.g. [19]), far fewer include adaptive responses of prey to predation risk [20,21]. A need therefore exists to examine whether NCEs have impacts on larger communities over longer timescales [11,22,23].

Here, we address this central issue through an experiment assessing the impacts of NCEs of a predator on a complex community of prey species (more than 10 prey spp.), over multiple generations and across multiple trophic levels. Hereafter, we use the term 'NCEs' to include all effects resulting from predator-induced trait changes in prey (i.e. via phenotypic plasticity of prey), including both direct effects on individual prey abundance and indirect effects on species with which the prey interacts (i.e. trait-mediated indirect interactions). In particular, we tested for the NCEs of a fish species on a community of zooplankton prey, many species of which are known to exhibit phenotypic responses to the presence of fishes [24,25] that lead to potentially large NCEs [26]. The relatively short generation times of the zooplankton enabled us to quantify NCEs on species abundance over multiple generations.

2. MATERIAL AND METHODS

Experiments were conducted in mesocosms placed in an open field at the E.S. George Reserve (ESGR) of the University of Michigan near Pinckney, MI, USA (42°28′ N, 84°00′ W). Mesocosms consisted of cylindrical cattle-watering tanks 1.9 m in diameter, 0.75 m high and filled to a depth of 45 cm with 1300 l of well water, with washed sand as a bottom substrate. Tanks were covered with fibreglass window screen lids in order to deter colonization by aquatic insects. On particularly sunny days, 60 per cent shade cloth lids were used to reduce heating.

A randomized block design was used with fish (non-consumptive fish presence) and no-fish treatments with six replicates. We manipulated the NCE of fish on zooplankton communities by maintaining one zooplanktivorous fish (bluegill sunfish, *Lepomis macrochirus*, standard deviation mean $\pm$ s.d. 4.7 ± 0.4 cm) in each of three floating cages within each treatment tank. No-fish treatments consisted of tanks with three empty floating cages.

Cages were constructed from $41 \times 27 \times 26$ cm plastic boxes with polystyrene foam glued to the sides to ensure flotation. Midge netting glued to wide windows cut on all sides and the bottom allowed diffusion of fish chemical cues (i.e. kairomones), which were predicted to affect zooplankton traits [25], without allowing escape of the *Daphnia* used as fish food. Mesh windows were kept clean by spraying with water and keeping three large snails (*Planorbella* cf. *trivolvis* >11.2 mm in diameter) inside each cage to graze on periphyton.

On 30 May we added an initial pulse of nutrients (see below) and a phytoplankton inoculum to each tank. This inoculum consisted of a mixed sample of water collected from four semi-permanent and permanent ponds in the ESGR. On 4 and 5 June we added a zooplankton inoculum from four semi-permanent and permanent ponds and three lakes to create a diverse zooplankton community. Zooplankton samples were collected with zooplankton nets (64 μm mesh) and undesirable animals such as insects (e.g. *Chaoborus*) were removed. To ensure zooplankton homogeneity, on 25 June we collected zooplankton from each tank with a zooplankton net, mixed all samples in a container and redelivered subsamples of this mixed community to all tanks. To increase the chances of establishment of multiple zooplankton species, on 24–28 July we added a second zooplankton inoculum with samples originating from six ponds and three lakes. *Hydra*, which prey on zooplankton, were noticed in the tanks prior to fish addition. We monitored densities of *Hydra* to ensure that they occurred in relatively equal frequency in no-fish and fish tanks.

Nutrients were supplied to the tanks to support phytoplankton growth as a resource for zooplankton. On 30 May an initial dose of 5.06 g of NH_3NO_3 and 0.37 g of KH_2PO_4 was added to each tank. Afterwards, a maintenance dose of 1.20 g of NH_3NO_3 and 0.14 g of KH_2PO_4 was supplied twice a week to each tank, which was then reduced by 25 per cent of the original dosage on 23 June and 25 July. To reduce periphyton growth and to cycle nutrients back to the water column, we added 15 individuals of the snail *Planorbella* cf. *trivolvis* >11.2 mm in diameter to each tank on 23 June. The snails reproduced and grew in number throughout the experiment.

After a 40 day period enabling establishment of zooplankton communities (14 July), the experimental treatments were initiated by adding one sunfish to each fish-treatment cage. Fish originated from Patterson Lake, Livingston County, MI, USA. In order to ensure fish health, once a week we rotated the fish from the experimental tanks to a culture tank where they were fed zooplankton. Fish in culture tanks were not fed ('starved') for 24 h before being rotated back into the experiment. While in the cage, each fish was fed twice a week, including the day they were added to the tank, with, on average, 200 *Daphnia* >700 μm sieved from zooplankton cultures. No-fish cages received equal amounts of *Daphnia* that were first killed by microwaving. Killing ensured that populations did not build in the cages. We concluded that any nutrient input by fish excretion would be overwhelmed by other factors affecting nutrient supply, including external supply, and recycling by zooplankton, snails and hydra (electronic supplementary material, appendix A).

Chlorophyll *a* concentrations were collected to estimate phytoplankton abundance through pigment analysis. On 3 September, phytoplankton in the seston in each tank was collected from 50 ml of water collected 5 cm below the water surface in the middle of the tank on a glass fibre filter, immediately stored in dry ice and taken to a deep-freezer the next day. Chlorophyll *a* was extracted using an ethanol solution and measured fluorometrically following Nusch [27].

The experiment terminated on 10 September, when zooplankton were sampled in all tanks. Zooplankton were collected from three strata: 'high' (just below the surface), 'middle' (at the midwater level) and 'low' (right above the bottom). Six samples (hexaplicates) were collected at each stratum with a 15 cm long 2.2 l horizontal water sampler (Wildco), four by the walls and two near the centre of the tank, combined, passed through a 53 μm mesh and preserved in sugar formalin. All zooplankton in each sample were identified to species or genus. To test for a behavioural response to caged fish, we calculated a vertical position index for each zooplankton taxa in each tank by summing the product of zooplankton density in the high, middle and low strata by 1,

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

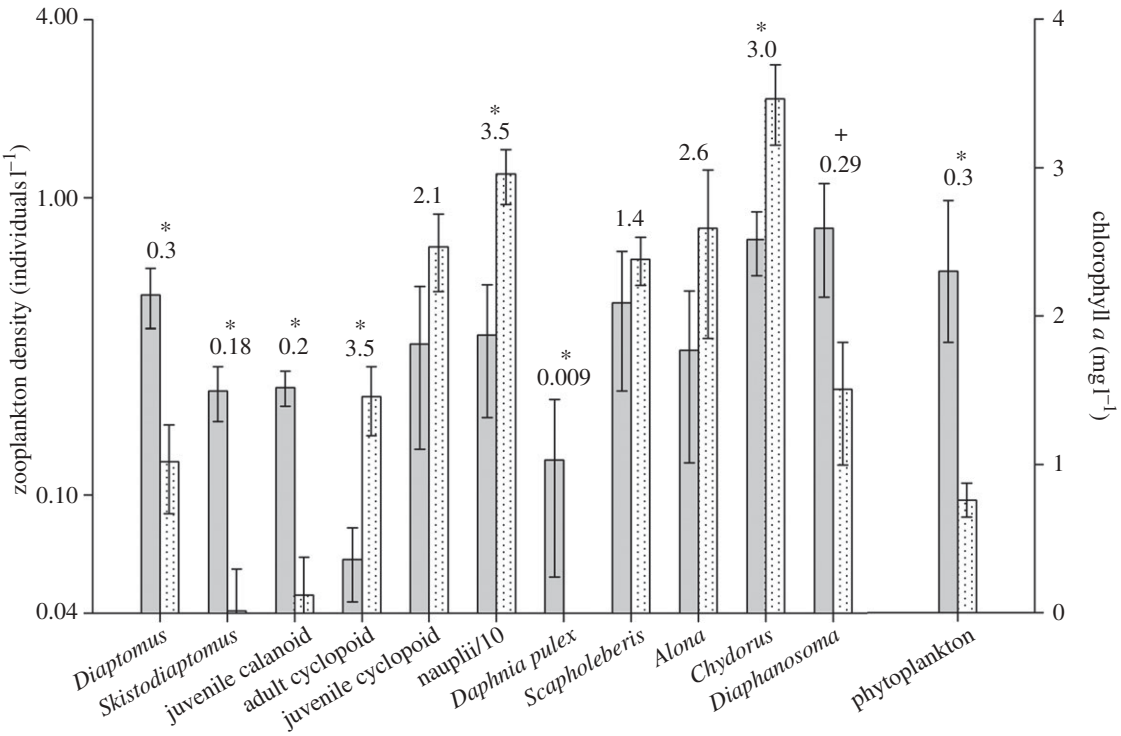

Figure 1. Density (individuals per litre) of zooplankton taxa (logarithmic scale), and of chlorophyll *a* concentration (linear scale) in the absence (no-fish) and presence of caged fish. Numbers above bars indicate the ratio of the density in the fish treatment (bars with dots) relative to the no-fish (grey-shaded bars) treatment. Asterisks indicate that the effect of fish was significant ($p < 0.05$) and crosses indicate that it was marginally significant ($p < 0.10$). Rare taxa such as *Macrothrix* and *Daphnia retrocurva* (which accounted for less than 1% of total zooplankton density), ostracods, rotifers and *Ceriodaphnia* (the most abundant species with 5.2 ± 1 and 4.6 ± 2 individuals l^{-1} in the no-fish and fish treatments, respectively) are not included for clarity. *Daphnia pulex* density in the presence of fish was 0.001 ± 0.0009, and therefore does not appear on the figure.

0 and −1, respectively. Zooplankton biomass (micrograms of dry mass per litre) was estimated using taxa-specific length–mass relationships, for which zooplankton lengths were measured using a drawing tube and digitizer [28].

(a) *Statistical analyses*

To determine if fish significantly affected zooplankton community composition, we conducted a multiple response permutation procedure (MRPP) that measured the dissimilarity in zooplankton abundance between no-fish and fish treatments in a multivariate context. We also performed additional MRPPs on cladoceran and copepod zooplankton separately, to determine the effect of fish specifically within these taxa. We chose MRPP because it is appropriate for datasets that contain rare species and are not normally distributed [29]. All MRPPs were performed using Bray–Curtis distance measures of $\log(x + 1)$-transformed abundance data. In addition to multivariate tests of abundance data, we also ran an MRPP using vertical distribution data (untransformed Bray–Curtis distance measures) to determine if fish significantly affected the spatial distribution of the zooplankton community.

When a significant fish treatment effect was found by MRPP, we used univariate tests to determine the effect of fish on abundance or vertical distribution of individual zooplankton taxa. Specifically, we used analysis of variance (ANOVA) for zooplankton taxa that did not violate assumptions of normality and homogeneity of variance based on Shapiro–Wilks and Levene's tests, respectively, and Mann–Whitney tests for taxa that violated either of these assumptions.

We evaluated the difference in chlorophyll *a* concentration between no-fish and fish treatments using ANOVA. Chlorophyll data met assumptions of normality and homogeneity of variances.

We also evaluated correlations among zooplankton taxa, independent of any fish effect, by using residuals taken from ANOVAs of the effect of fish on the log-transformed abundance of each zooplankton taxon. The significance of each correlation was determined using an alpha value corrected for multiple comparisons using the sequential Bonferroni method.

3. RESULTS

(a) *Zooplankton community composition*

MRPPs indicated that caged fish affected total zooplankton community composition (chance-corrected within-group agreement $A = 0.15$, $p < 0.01$), copepods as a group ($A = 0.24$, $p = 0.01$) and cladocerans as a group ($A = 0.09$, $p = 0.02$). Presence of fish affected the abundance of many taxonomic groups, with some increasing and others decreasing. The largest effects were seen in the copepods. The effect of fish on calanoids was strongly negative; *Diaptomus*, *Skistodiaptomus* and juvenile calanoids decreased by 73 per cent ($F_{1,10} = 12.2$, $p = 0.01$), 82 per cent ($F_{1,10} = 14.4$, $p < 0.01$) and 80 per cent ($F_{1,10} = 25.0$, $p < 0.01$), respectively (figure 1). By contrast, cyclopoids exhibited the opposite pattern; adult cyclopoid density increased 2.1-fold (figure 1; $F = 6.8$, $p = 0.03$) in the fish treatment, and juvenile cyclopoids exhibited a trend in the same direction (by 3.5-fold, $F_{1,10} = 2.95$, $p = 0.12$). Nauplii also were significantly affected ($F_{1,10} = 10.22$, $p = 0.01$), increasing by 3.5-fold in the presence of fish.

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

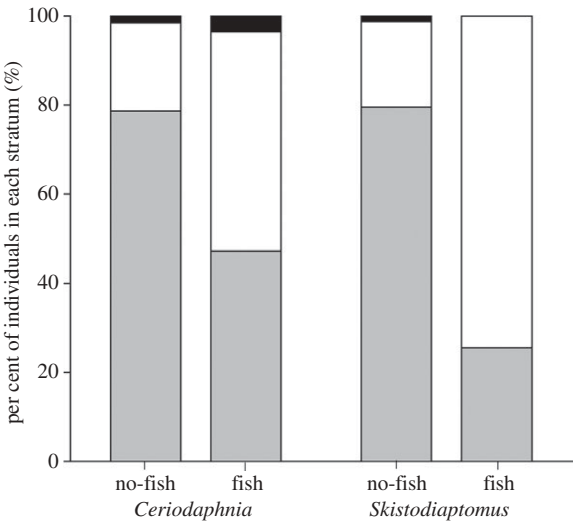

Figure 2. Per cent of *Ceriodaphnia* (left bars) and *Skistodiaptomus* (right bars) found at the end of the experiment at three different vertical levels in the mesocosms. With fish, both species were much more common in the middle region of the tank. Black shade, high; no shade, middle; grey shade, low.

Caged fish also strongly affected cladocerans (figure 1). For example, *Daphnia pulex* was less than 10 per cent as dense (Mann–Whitney statistic $[U] = 30.5$, $p = 0.04$) in the presence of caged fish, while *Diaphanosoma* showed a marginally non-significant trend in the same direction ($F_{1,10} = 3.55$, $p = 0.09$). By contrast, chydorids were three times more common ($F_{1,10} = 8.23$, $p = 0.02$) in the presence of caged fish, and *Daphnia retrocurva* exhibited a non-significant trend in the same direction ($U = 9$, $p = 0.06$). The density of *Ceriodaphnia*, which was the most common cladoceran, was unaffected by caged fish, as were the less common *Alona*, *Bosmina*, *Daphnia parvula*, *Eurycerus* and *Scapholeberis*.

Despite changes in community composition, total zooplankton biomass did not significantly differ ($F_{1,10} = 1.11$, $p = 0.32$) between no-fish (mean ± s.e., 523 ± 64 $\mu g\, l^{-1}$) and fish (412 ± 83 $\mu g\, l^{-1}$) treatments.

Many zooplankton taxa exhibited positive and negative correlations independent of the effect of caged fish. When all species and stages were grouped, cladocerans were negatively correlated with cyclopoids ($p = 0.009$, for corrected alpha value (α_c) of 0.05); there was a marginally significant negative correlation between calanoids and cyclopoids ($p = 0.064$); and a positive correlation between calanoids and cladocerans ($p = 0.074$). Looking at more defined taxonomic groups, there was a negative correlation between *Ceriodaphnia* and juvenile cyclopoids ($p = 0.003$, α_c of 0.017), and positive correlations between *Skistodiaptomus* and *Ceriodaphnia* ($p = 0.007$), and between *Skistodiaptomus* and juvenile calanoids ($p = 0.012$).

(b) *Zooplankton vertical position*

Caged fish affected the distribution of the zooplankton community in the mesocosms (MRPP: $A = 0.06$, $p = 0.04$). A higher proportion of individuals were found in the middle layer and fewer on the low layer in the presence of fish (figure 2). For example, 1.3, 19 and 80 per cent of *Skistodiaptomus* individuals were found in the high, middle and low layers in the absence of fish; but 0, 75 and 25 per cent were found in these layers in the presence of fish. This pattern led to *Ceriodaphnia* ($F_{1,10} = 8.8$, $p = 0.01$) and *Skistodiaptomus* ($U = 0.5$, $p < 0.01$) being statistically higher in the water column in the presence of fish, and *Diaptomus* and *Diaphanosoma* exhibited a similar trend ($F_{1,10} = 4.4$, $p = 0.06$ and $F_{1,10} = 3.6$, $p = 0.09$, respectively).

(c) *Phytoplankton*

Caged fish had a strong negative effect (threefold lower) on chlorophyll *a* measured at the end of the experiment ($F_{1,10} = 9.88$, $p = 0.01$) (figure 1).

4. DISCUSSION

To our knowledge, this is the first study that demonstrates NCEs extending multiple generations in a complex community involving many (i.e. >5) competing species and multiple trophic levels. The presence of fish, caged to ensure no consumptive effects, had profound effects on both dominant zooplankton groups (copepods and cladocerans), with, for example, a striking reversal of dominance in copepod taxa by the fish NCE reducing the calanoid/cyclopoid ratio from 6.3 to 0.43. A large body of literature has shown that presence of predators has effects on fitness correlates of species, such as individual growth rate and survivorship, and some recent studies have shown population level effects of NCEs in controlled laboratory settings with a few species [30,31]. Further, in a multiple year experiment in which the density of a consumer was manipulated experimentally each generation, Schmitz [32] found strong effects of spider-induced changes in grasshopper behaviour on plant composition and ecosystem function. In our experiment, zooplankton responses were owing to dynamics over three to 10 generations. The generation times of the longest living taxa, cyclopoids and calanoids, are approximately 18 and 20 days, respectively, given the water temperature in our tanks (average, 23°C) and the average sizes observed for these taxa [33–35]. Therefore, we expect that all species experienced at least three generations during the course of the experiment, and that the majority of species, including *Daphnia*, *Ceriodaphnia*, *Alona* and *Chydorus*, experienced at least four to 10 generations (see [36–38] for species-specific generation times).

A number of short-term experimental studies have shown that predator-induced changes in prey traits can lead to larger indirect effects than those originating from consumption (i.e. trait-mediated indirect effects can be larger than density-mediated indirect effects [12–16]). We did not test for the relative CE and NCE of the fish in this study, but the large NCEs on zooplankton community structure are suggestive that these effects will be in the same order of magnitude as CEs. We observed differences in densities in the predator–no predator treatments in the order of 3 to 10-fold for a number of species. Although studies with free (i.e. uncaged) fish have found even larger effects than this, we must keep in mind that such results are the consequence of CEs and NCEs combined, which can strongly interact in ways that cause stronger effects than the sum of their individual impacts [12,39]. That is, NCEs may have played a large role in the previous studies with free fish, though CEs

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

are implicitly assumed to be the principal cause of observed effects. Whereas our experiment is suggestive that NCEs could be a large component of the net effect of the predator, experiments examining the isolated and combined impacts of the NCEs and CEs will be required to establish their relative importance.

Numerous studies have demonstrated strong phenotypic responses of zooplankton species to fishes and other predators [25]. For example, many zooplankton are known to respond to the presence of predators to reduce predation risk by modifying morphological characteristics or altering habitat use by swimming lower or high in the water column, or moving further or closer to structure [25,40]. Further, copepods have been shown to reduce daytime foraging in the presence of fishes, presumably to empty their gut and lower visibility [25,41]. Such phenotypic changes can affect the growth rate of the responding prey, and species it interacts with. In our study, several zooplankton taxa inhabited different regions of the tank in the presence of fish, however, it was beyond the scope of this experiment to examine the potential myriad-specific trait changes and ensuing dynamical changes that caused the observed effects of caged fish on zooplankton community structure.

The presence of both positive and negative effects of fish on different taxonomic groups, combined with the negative correlations we found among species or groups of species, independent of caged fish presence, suggests that there were strong interactions among zooplankton that affected abundance. For example, the negative correlation between cladocerans and cyclopoids could arise from competition for resources, or predation from adult stages on nauplii or early instars. Therefore, if one species responded phenotypically to the presence of fish, the response could affect other species through changes in competitive or predator–prey interactions. For example, whereas we did not find an effect of fish on *Ceriodaphnia* density (the dominant cladoceran), we did observe a difference in habitat use by *Ceriodaphnia* with fish. Such a response could affect a number of other species that *Ceriodaphnia* competes with for resources. In this case, the phenotypic response could affect the growth rate of a competitor of the responding prey, but not that of the responding prey itself, as predicted by theory [42].

The direction of the NCEs of fishes on zooplankton density is difficult to predict given the complexity of the problem. It is inviting to suggest that the NCEs seen here are in the same direction as CEs. For example, fishes are known to have negative CEs on large-bodied cladocerans, and we observed a large negative effect on *D. pulex*. Further, fishes have frequently been shown to increase the cyclopoid/calanoid ratio, as we found in this study, although some studies show the opposite pattern (reviewed in [43]). However, experiments have frequently demonstrated the context-dependence and complexity of such effects. For example, fishes that prey more heavily on cylopoids than calanoids may increase the calanoid/cyclopoid ratio owing to indirect interaction spanning different life-history stages [43]. Soto & Hurlbert [44] found that strong interactions between cyclopoids and calanoids in mesocosm experiments caused cyclopoids to have an initial negative effect on calanoids that reversed over time, possibly because of an effect on resource edibility. Thus, the effects of fishes on the zooplankton assemblage in our experiment, e.g. the cyclopoid/calanoid ratio, are likely to be strongly context-dependent. One important factor that could affect the relative magnitude of NCEs on different species is the recent diet of fishes, as fish diet has been shown to affect the magnitude of phenotypic responses of zooplankton [45].

In our experiment, the NCEs of fish strongly suppressed phytoplankton density, in contrast to a planktivorous fish initiating a trophic cascade that increases phytoplankton density [46,47]. There are two probable mechanisms that could be responsible for this positive effect. First, a four-level cascade will lead to a positive effect, and is plausible in our system. For example, a fish-induced reduction in calanoid foraging and density (observed in experiment) could lead to an increase in small zooplankton, that they potentially prey on, which could lead to a decrease in phytoplankton. Indeed, densities of the three smallest common zooplankton taxa in the tanks (i.e. copepod nauplii (average mass, 0.12 μg), juvenile cyclopoids (0.93 μg) and chydroids (1.03 μg)) were negatively related to densities of calanoid taxa, even after controlling for the effect of fish (partial Mantel test: Mantel $r = 0.23$, $p = 0.041$). Further, owing to allometric scaling relationships, small zooplankton ingests phytoplankton at a much greater rate relative to their mass. For example, nauplii ingest phytoplankton at a 10-fold greater rate (per unit biomass) than adult copepods [48]. Thus, any mechanisms by which fish would lead to a shift in the community towards smaller zooplankton, including a four-level trophic cascade, would cause an increase in the rate zooplankton reduces algae. This mechanism is supported by the fact that the average body size of zooplankton in the fish treatment (mean $\pm$ s.d. 3.6 ± 0.8 μg) was about half (treatment effect: $F_{1,10} = 6.55$, $p = 0.03$) the average size of the no-fish treatment (7.1 ± 1.1 μg). Note that small zooplankton represented a sizeable fraction of the total zooplankton biomass, with, for example, the three smallest taxa making up 32 per cent of the biomass in the fish tanks. A second explanation for how fish could lead to an increase in phytoplankton arises when considering a range of edibility of the resource. When herbivory reduces a more vulnerable resource which is a better competitor but achieves an overall lower biomass, the presence of the herbivore can favour a less-competitive resource species that can achieve higher biomass, as has been examined in zooplankton systems in which different phytoplankton taxa (e.g. some blue-green algae) and larger phytoplankton species are less edible [49]. Consequently, a predator-induced reduction in herbivore-foraging rate could lead to a net decrease in phytoplankton biomass. Indeed, we observed this response in experiments using another pond system: presence of predatory dragonfly larvae caused a reduction in tadpole foraging rates, which in turn led to a net reduction in periphyton biomass when competitively inferior (but less vulnerable) species were replaced with more vulnerable (but more competitive) species that achieved lower standing crops [50]. Whereas both of these mechanisms are plausible, each may contribute, and a more intense investigation that examines dynamical changes would be required to determine their contributions.

In conclusion, the importance of NCEs of predators is becoming increasingly recognized, and numerous

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

experiments in diverse systems have demonstrated that trait responses to predators can affect fitness of both the reacting prey and indirectly other species in the system. Though ecological theory has indicated that including these adaptive responses to predator density can affect predator functional responses, and in turn, population dynamics and community structure, there has been little empirical documentation that these responses propagated to important population dynamic and community changes. This study indicates that these short-term effects on prey traits indeed can translate to dramatic effects on community structure over timescales that encompass many generations. Although conducted in an artificial environment, the different community compositions exhibited in our mesocosms fall within the range of compositions found in Michigan pond communities [51,52], suggesting that the communities observed here are real possibilities in nature. We are just beginning to understand the manner in which NCEs propagate through a community and potentially lead to emergent effects on community structure, but it is clear that these effects can be large and will probably interact strongly with CEs of predators. The effects of predators on community structure plays a central role in ecological theory, and the vast majority of this impact has been assumed to be owing to the patterns in consumption of prey by predators. However, the impacts of predators on community structure, and specific community attributes such as biodiversity and species richness, may be strongly influenced by the NCEs of predators resulting via trait changes in prey in these communities.

Animal care was covered by the University of Michigan's Committee on the Use and Care of Animals under approval number 07765.

We thank Z. Schulman and R. Lanfear for field assistance. We thank B. Peters and C. Davies for help with zooplankton enumeration, and N. Bosch and T. Johengen for help with nutrient analysis. We thank P. Geddies, S. Hall, O. Sarnelle and J. Shurin for consultations in experimental design and data interpretation. We thank P. McIntyre and M. Vanni for assistance in nutrient analysis and comments on the electronic supplementary material, appendix A. Comments by P. Bourdeau and three anonymous reviewers improved this manuscript. Funding for this study was provided by the National Science Foundation (DEB-0089809 and OCE-0826020). S.D.P. acknowledges support from the Michigan Agricultural Experiment Station. L.S. was funded by FAPESP (2008/57939-9) during the final preparation of the manuscript.

REFERENCES

1 Schoener, T. W. 1993 On the relative importance of direct versus indirect effects in ecological communities. In *Mutualism and community organization: behavioral, theoretical and food web approaches* (eds H. Kawanabe, J. E. Cohen & K. Iwasaki), pp. 365–411. Oxford, UK: Oxford University Press.

2 Wootton, J. T. 1994 The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.* **25**, 443–466. (doi:10.1146/annurev.es.25.110194.002303)

3 Polis, G. A. & Winemiller, K. O. 1996 *Food webs: integration of patterns and dynamics*. New York, NY: Chapman and Hall.

4 Abrams, P. A., Menge, B. A., Mittelbach, G. G., Spiller, D. & Yodzis, P. 1996 The role of indirect effects in food webs. In *Food webs: dynamics and structure* (eds G. Polis & K. Winemiller), pp. 113–121. New York, NY: Chapman and Hall.

5 Peckarsky, B. L. *et al.* 2008 Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology* **89**, 2416–2425. (doi:10.1890/07-1131.1)

6 Wootton, J. T. 1993 Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. *Am. Nat.* **141**, 71–98. (doi:10.1086/285461)

7 Peckarsky, B. L., Cowan, C. A., Penton, M. A. & Anderson, C. 1993 Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. *Ecology* **74**, 1836–1846. (doi:10.2307/1939941)

8 Van Buskirk, J. & Yurewicz, K. L. 1998 Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. *Oikos* **82**, 20–28. (doi:10.2307/3546913)

9 Relyea, R. A. 2000 Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology* **81**, 2278–2289. (doi:10.1890/0012-9658(2000)081[2278:TMIEIL]2.0.CO;2)

10 Abrams, P. A. 1984 Foraging time optimization and interactions in food webs. *Am. Nat.* **124**, 80–96. (doi:10.1086/284253)

11 Bolker, B., Holyoak, M., Krivan, V., Rowe, L. & Schmitz, O. 2003 Connecting theoretical and empirical studies of trait-mediated interactions. *Ecology* **84**, 1101–1114. (doi:10.1890/0012-9658(2003)084[1101:CTAESO]2.0.CO;2)

12 Peacor, S. D. & Werner, E. E. 2001 The contribution of trait-mediated indirect effects to the net effects of a predator. *Proc. Natl Acad. Sci. USA* **98**, 3904–3908. (doi:10.1073/pnas.071061998)

13 Werner, E. E. & Peacor, S. D. 2003 A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84**, 1083–1100. (doi:10.1890/0012-9658(2003)084[1083:AROTII]2.0.CO;2)

14 Schmitz, O. J., Křivan, V. & Ovadia, O. 2004 Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecol. Lett.* **7**, 153–163. (doi:10.1111/j.1461-0248.2003.00560.x)

15 Trussell, G. C., Ewanchuk, P. J. & Matassa, C. M. 2006 Habitat effects on the relative importance of trait- and density-mediated indirect interactions. *Ecol. Lett.* **9**, 1245–1252. (doi:10.1111/j.1461-0248.2006.00981.x)

16 Peacor, S. D. & Werner, E. E. 2008 Nonconsumptive effects of predators and trait-mediated indirect effects. In *Encyclopedia of life sciences* (eds T. Ohgushi, O. J. Schmitz & R. D. Holt), pp. 1–8. Chichester, UK: John Wiley and Sons.

17 Abrams, P. A. 2010 Implications of flexible foraging for interspecific interactions: lessons from simple models. *Funct. Ecol.* **24**, 7–17. (doi:10.1111/j.1365-2435.2009.01621.x)

18 Persson, L. & De Roos, A. M. 2003 Adaptive habitat use in size-structured populations: linking individual behavior to population processes. *Ecology* **84**, 1129–1139. (doi:10.1890/0012-9658(2003)084[1129:AHUISP]2.0.CO;2)

19 Drossel, B., McKane, A. J. & Quince, C. 2004 The impact of nonlinear functional responses on the long-term evolution of food web structure. *J. Theor. Biol.* **229**, 539–548. (doi:10.1016/j.jtbi.2004.04.033)

20 Dambacher, J. M. & Ramos-Jiliberto, R. 2007 Understanding and predicting effects of modified interactions through a qualitative analysis of community. *Q. Rev. Biol.* **82**, 227–250. (doi:10.1086/519966)

Downloaded from http://rspb.royalsocietypublishing.org/ on May 22, 2018

21 Kondoh, M. 2007 Anti-predator defence and the complexity-stability relationship of food webs. *Proc. R. Soc. B* **274**, 1617–1624. (doi:10.1098/rspb.2007.0335)

22 Beckerman, A., Petchey, O. L. & Morin, P. J. 2010 Adaptive foragers and community ecology: linking individuals to communities to ecosystems. *Funct. Ecol.* **24**, 1–6. (doi:10.1111/j.1365-2435.2009.01673.x)

23 Loeuille, N. 2010 Consequences of adaptive foraging in diverse communities. *Funct. Ecol.* **24**, 18–27. (doi:10.1111/j.1365-2435.2009.01617.x)

24 Tollrian, R. & Harvell, C. D. (eds) 1999 *The ecology and evolution of inducible defenses*. Princeton, NJ: Princeton University Press.

25 Lass, S. & Spaak, P. 2003 Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia* **491**, 221–239. (doi:10.1023/A:1024487804497)

26 Pangle, K. L., Peacor, S. D. & Johannsson, O. E. 2007 Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology* **88**, 402–412. (doi:10.1890/06-0768)

27 Nusch, E. A. 1980 Comparison of different methods for chlorophyll and phaeopigment determination. *Arch. Hydrobiol.* **14**, 14–36.

28 Roff, J. C. & Hopcroft, R. R. 1986 High precision microcomputer based measuring system for ecological research. *Can. J. Fish. Aquat. Sci.* **43**, 2044–2048. (doi:10.1139/f86-251)

29 McCune, B. & Grace, J. B. 2002 *Analysis of ecological communities*. Gleneden Beach, OR: MjM Press.

30 Verschoor, A. M., Vos, M. M. & Van der Stap, I. 2004 Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. *Ecol. Lett.* **7**, 1143–1148. (doi:10.1111/j.1461-0248.2004.00675.x)

31 Van der Stap, I., Vos, M., Verschoor, A. M., Helmsing, N. R. & Mooij, W. M. 2007 Induced defenses in herbivores and plants differentially modulate a trophic cascade. *Ecology* **88**, 2474–2481. (doi:10.1890/07-1731.1)

32 Schmitz, O. J. 2008 Effects of predator hunting mode on grassland ecosystem function. *Science* **319**, 952–954. (doi:10.1126/science.1152355)

33 Gehrs, C. W. & Robertson, A. 1975 Use of life tables in analyzing the dynamics of copepod populations. *Ecology* **56**, 665–672. (doi:10.2307/1935500)

34 Hansen, A. & Hairston Jr, N. G. 1998 Food limitation in a wild cyclopoid copepod population: direct and indirect life history responses. *Oecologia* **115**, 320–330. (doi:10.1007/s004420050523)

35 Da Graça Gama Melão, M. & Rocha, O. 2004 Life history, biomass and production of two planktonic cyclopoid copepods in shallow subtropical reservoir. *J. Plankton Res.* **26**, 909–923. (doi:10.1093/plankt/fbh080)

36 Arnold, D. E. 1971 Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. *Limnol. Oceanogr.* **16**, 906–920. (doi:10.4319/lo.1971.16.6.0906)

37 Bottrell, H. H. 1975 Generation time, length of life, instar duration and frequency of moulting, and their relationship to temperature in eight species of cladocera from the River Thames, Reading. *Oecologia* **19**, 129–140. (doi:10.1007/BF00369097)

38 Klüttgen, B., Kuntz, N. & Ratte, H. T. 1996 Combined effects of 3,4-dichloroaniune and food concentration on life-table data of two related cladocerans, *Daphnia magna* and *Ceriodaphnia quadrangular*. *Chemosphere* **32**, 2015–2028. (doi:10.1016/0045-6535(96)00081-1)

39 Griffin, C. A. M. & Thaler, J. S. 2006 Insect predators affect plant resistance via density- and trait-mediated indirect interactions. *Ecol. Lett.* **9**, 335–343. (doi:10.1111/j.1461-0248.2005.00880.x)

40 Burks, R. L., Jeppesen, E. & Lodge, D. M. 2001 Littoral zone structures as *Daphnia* refugia against fish predators. *Limnol. Oceanogr.* **46**, 230–237. (doi:10.4319/lo.2001.46.2.0230)

41 Cieri, M. D. & Stearns, D. E. 1999 Reduction of grazing activity of two estuarine copepods in response to the exudate of a visual predator. *Mar. Ecol. Prog. Ser.* **177**, 157–163. (doi:10.3354/meps177157)

42 Peacor, S. D. & Werner, E. E. 2000 Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. *Ecology* **81**, 1998–2010. (doi:10.1890/0012-9658(2000)081[1998:PEOAAO]2.0.CO;2)

43 Hurlbert, S. H. & Mulla, M. S. 1981 Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* **83**, 125–151. (doi:10.1007/BF02187157)

44 Soto, D. & Hurlbert, S. H. 1991 Long-term experiments on calanoids cyclopoids interactions. *Ecol. Monogr.* **61**, 245–265. (doi:10.2307/2937108)

45 Laforsch, C., Beccara, L. & Tollrian, R. 2006 Inducible defenses: the relevance of chemical alarm cues in *Daphnia*. *Limnol. Oceanogr.* **51**, 1466–1472. (doi:10.4319/lo.2006.51.3.1466)

46 Carpenter, S. R., Kitchell, J. F. & Hodgson, J. R. 1985 Cascading trophic interactions and lake productivity. *BioScience* **35**, 634–639. (doi:10.2307/1309989)

47 Mehner, T. 2010 No empirical evidence for community-wide top-down control of prey fish density and size by fish predators in lakes. *Limnol. Oceanogr.* **55**, 203–213. (doi:10.4319/lo.2010.55.1.0203)

48 Paffenhofer, G. A. 1971 Grazing and ingestion rates of nauplii, copepodids, and adults of the marine planktonic copepod *Calanus helgolandicus*. *Mar. Biol.* **11**, 286–298. (doi:10.1007/BF00401275)

49 Leibold, M. A. 1989 Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. *Am. Nat.* **134**, 922–949. (doi:10.1086/285022)

50 Werner, E. E. & Peacor, S. D. 2006 Interaction of lethal and non-lethal effects of a predator on a guild of herbivores mediated by system productivity. *Ecology* **87**, 347–361. (doi:10.1890/05-0091)

51 Shurin, J. B. 2000 Dispersal limitation, invasion resistance, and the structure of pond zooplankton communities. *Ecology* **81**, 3074–3086. (doi:10.1890/0012-9658(2000)081[3074:DLIRAT]2.0.CO;2)

52 Steiner, C. F. & Roy, A. H. 2003 Seasonal succession in fishless ponds: effects of enrichment and invertebrate predators on zooplankton community structure. *Hydrobiologia* **490**, 125–134. (doi:10.1023/A:1023470730397)