

Multitrophic diversity sustains ecological complexity by dampening top-down control of a shallow marine benthic food web

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Abstract. Biodiversity is typically considered as a one-dimensional metric (e.g., species richness), yet the consequences of species loss may be different depending on where extinctions occur in the food web. Here, I used a manipulative field experiment in a temperate subtidal marine system to explore the implications of diversity loss at multiple trophic levels for ecosystem functioning and food web structure. The four manipulated predators included the small painted goby and common prawn, which are also fed on by the larger black goby and shore crab. Antagonistic interactions between the manipulated predators (e.g., intraguild predation, intimidation, interference competition) limited their negative effects on the rest of the food web. Top-down control was so suppressed at the highest level of multitrophic diversity that the resulting food webs were as complex and productive as those containing no manipulated predators. Negative interactions between the predators weakened as multitrophic diversity decreased, however, resulting in stronger consumption of lower trophic levels and a simpler food web with lower rates of two key ecosystem processes: primary production and decomposition. These results show how indirect interactions between predators on multiple trophic levels help to promote the complexity and functioning of natural systems.

Key words: connectance; food chain length; indirect interactions; marine; multiple predator effects; net effects; subtidal.

INTRODUCTION

It is now well established that species loss reduces the functioning and stability of natural ecosystems and the services that humans derive from them (Isbell et al. 2017). Yet all species are not equal, with the impact of extinctions depending in part on the trophic level from which they occur (Petchey et al. 2004, Soliveres et al. 2016). While the loss of plant species directly lowers the productivity of an ecosystem (Cardinale et al. 2007), predator diversity plays a crucial role in dampening trophic cascades that can lead to either the suppression or release of basal resource biomass (Finke and Denno 2004, Bruno and O'Connor 2005, Frank et al. 2006, Haggerty et al. 2018). Predators are also more susceptible to anthropogenic actions than lower trophic levels (Estes et al. 2011) and their effects typically percolate further through food webs than those of bottom-up processes (Borer et al. 2006, Scherber et al. 2010). Predator effects are propagated via altered densities or behavioral traits of intermediate species (Bernot and Turner 2001), with trait-mediated effects often the stronger of the two (Dill et al. 2003, Werner and Peacor 2003, Preisser et al. 2005, Trussell et al. 2006b). The net effects of a predator

tend to be greatest in simplified systems (Montoya et al. 2005, O'Gorman et al. 2010), suggesting that biodiversity plays a key role in attenuating top-down control of resources.

One of the best-studied mechanisms by which this occurs is the prevalence of antagonistic interactions between multiple predator species. These interactions can happen within a trophic level, if predators spend less time foraging due to intimidation and competition for shared resources (Sih et al. 1998, Griffen and Byers 2006a), or between trophic levels, through intraguild predation and predator avoidance (Finke and Denno 2004, Schmitz et al. 2004, Griffen and Byers 2006a, b, Trussell et al. 2006a, O'Connor et al. 2008). Here, intraguild predation refers to a top predator who feeds on an intermediate predator, with both species also sharing lower-trophic-level prey (Crumrine and Crowley 2003). The loss or even addition of a trophic level can trigger a state change in the entire system (Estes et al. 2011). Thus, diversity on multiple trophic levels could be vital for ecosystem functioning and stability, by providing insurance against the loss or gain of trophic levels. Yet biodiversity is typically treated as a one-dimensional metric in most research on the topic (i.e., total species richness or evenness), rather than partitioning it out across different trophic levels. This underscores the need for better awareness of how diversity on multiple trophic levels (i.e., multitrophic diversity) contributes to the

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overall structure and functioning of ecological systems (Duffy et al. 2007, Terborgh 2015, Soliveres et al. 2016).

Studies that consider diversity as a two-dimensional entity describe horizontal diversity as the number of species within a trophic level and vertical diversity as the number of trophic levels (Duffy et al. 2007, Srivastava and Bell 2009, Jabiol et al. 2013). Simultaneous manipulation of diversity in these two dimensions are rare, but tend to show interactive effects of species loss across multiple trophic levels. For example, loss of insect detritivore diversity can trigger secondary extinctions, but only in the absence of predators (Srivastava and Bell 2009). Loss of entire trophic levels are not even necessary for such effects to emerge, with reduced ecosystem functioning (Jabiol et al. 2013) and community biomass (Gamfeldt et al. 2005) after species loss at consecutive trophic levels. Such studies tend to be carried out in closed systems (i.e., microcosms), however, which restrict potential (re)colonization of organisms in response to diversity manipulations. In situ studies are thus needed to more accurately quantify the effects that may dissipate or propagate through the myriad interactions in complex natural systems.

Here, I manipulated the diversity of fish and crustacean predators on consecutive trophic levels in a shallow subtidal caging experiment. The manipulated predators all feed on a diverse array of benthic invertebrates (e.g., amphipods, gastropods, polychaetes) that were free to assemble into the cages, but with a clear separation in dietary composition between the top and intermediate predators (Appendix S1: Fig. S1). The top predators that were manipulated (a goby and a crab) can also feed on the intermediate predators (a smaller goby species and a prawn; see Appendix S1: Table S1). My goals were to: (1) understand how changes in multi-trophic diversity affect ecosystem functioning and food web complexity; and (2) determine the underlying mechanisms by quantifying the strength of indirect interactions between the manipulated predators and their overall net effects on the rest of the food web.

MATERIALS AND METHODS

Experimental design

The experiment was performed at Lough Hyne marine reserve in southwest Ireland (51°29'59" N, 9°17'48" W; Fig. 1a,b), which is a highly sheltered, yet fully marine sea lough that is ideal for manipulative experiments (O'Gorman and Emmerson 2009, Jochum et al. 2012) and broadly representative of temperate, shallow-water, Atlantic communities (O'Gorman and Emmerson 2010). Four predator species were manipulated in the experiment: black goby (*Gobius niger*, Linnaeus), shore crab (*Carcinus maenas*, Linnaeus), painted goby (*Pomatoschistus pictus*, Malm), and common prawn (*Palaemon serratus*, Pennant), which are four of the most common benthic predators in the lough and surrounding

coastline (O'Gorman et al. 2010). The black goby and shore crab both consume the painted goby and common prawn (though not each other) and thus are top predators in the experiment, while the painted goby and common prawn do not feed on each other or the top predators and thus are intermediate predators here (see Appendix S1: Table S1; O'Gorman et al. 2010). The manipulated predators were constrained within cages constructed from extruded plastic mesh (42 × 41 × 10 cm) with an inner layer of fine nylon netting (1 mm mesh size). The cages controlled the density of the manipulated predators, while allowing organisms from lower trophic levels to assemble through the mesh over the two-month duration of the experiment (2 May to 25 June 2008).

The experimental design consisted of three levels of top predator diversity (0, 1, and 2 species) crossed with three levels of intermediate predator diversity (0, 1, and 2 species) for a total of nine treatments (Fig. 1c). Replication differed among treatments because all possible combinations of the four manipulated predators were used to prevent species identity effects from influencing the treatments effects. This constituted 3 replicates of 16 different species combinations for a total of 48 cages. A substitutive experimental design was employed, with two individuals of the black goby (14.2 ± 1.13 mg wet mass) or shore crab (27.0 ± 6.00 mg) used in single-species top predator treatments and one individual of each species used in two-species treatments. In a similar fashion, four individuals of the painted goby (0.42 ± 0.010) or common prawn (1.74 ± 0.047 mg) were used in single-species intermediate predator treatments, with two individuals of each species used in two-species treatments. The substitutive experimental design ensured that increasing species richness was not confounded with total density within trophic levels (Balvanera et al. 2006). While biomass differed between species (due to heavier hard parts in the crustaceans compared to the fish), gut content analysis revealed that the larger predators within each trophic level consumed fewer prey and thus were unlikely to have stronger trophic impacts due to their larger size (Appendix S1: Fig. S2).

Replicate cages for each treatment were prepared in a randomized order: a cylindrical black refuge (15 cm length; 5 cm diameter) was cable tied to the rear side of the cage and a 1 cm layer of clean coarse gravel was added, followed by three loose glass slides (5.5 × 2.6 cm), a litter bag (1 mm mesh size) containing a circular disc of kelp frond (*Laminaria digitata*, Hudson), and the manipulated predators for that treatment. The cage lid was cable-tied shut and the cage was immediately placed in the shallow subtidal between 1 and 2 m depth at low tide. The location of the experiment was dominated by stony substrate, algal turf (particularly *Corallina officinalis*, Linnaeus), and filamentous brown algae (particularly, *Stilophora tenella* Silva), but the kelp used in the litter bags represents a locally available

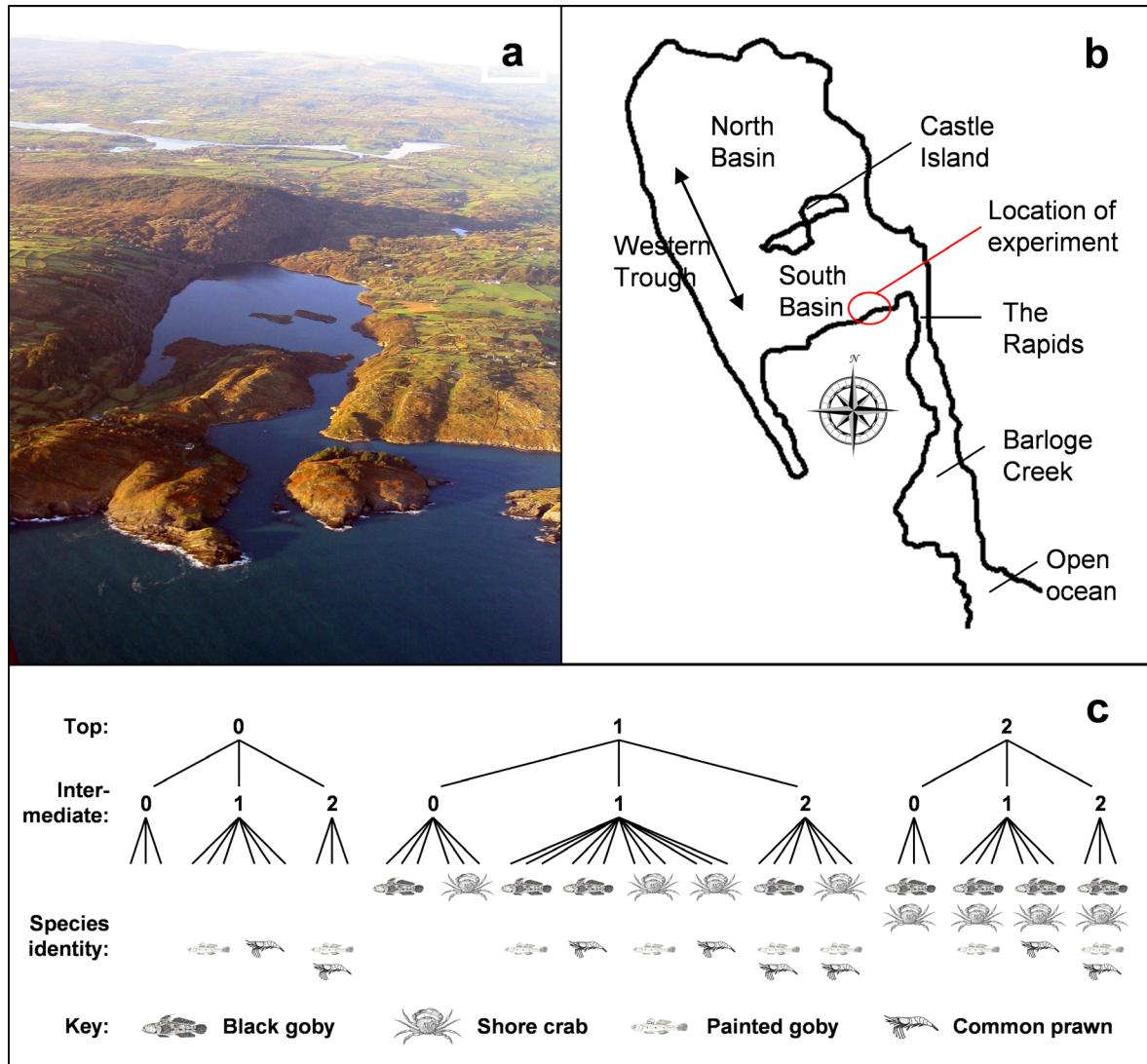


FIG. 1. Study site and experimental design. (a) Aerial view of Lough Hyne, Ireland marine reserve. (b) Schematic of Lough Hyne with main features and location of the caging experiment. (c) Three levels of top predator diversity (0, 1, and 2 species) were crossed with three levels of intermediate predator diversity (0, 1, and 2 species) for a total of nine treatments. Replication differed between treatments due to the various combinations of species identity in each treatment, with each combination represented three times.

organic material that was easily standardized for quantifying decomposition rates. The dominant macroalgae also have low palatability for most grazers (Watson and Norton 1985, Andersson et al. 2009), so biofilms of microphytobenthos on sediment and stony surfaces are a key source of primary production in the shallow subtidal.

At the end of the experiment, the manipulated predators were removed for gut content analysis to provide insights into treatment effects on their feeding behavior (see Appendix S1: Supplementary Analysis). All top predators were recovered, but the number of missing intermediate predators was recorded to determine

mortality rates (e.g., due to predation by top predators). The glass slides were frozen for later quantification of chlorophyll *a* using the spectrophotometric method (Parsons et al. 1984). The remaining kelp was dried in an oven at 60°C for 48 h and weighed. The gravel substrate was thoroughly washed in a 250 µm sieve, with the contents stored in 70% ethanol for later identification of mobile benthic invertebrates (e.g., amphipods, gastropods, isopods). Sessile species on the inner lid of each cage (e.g., bivalves, bryozoans, calcareous polychaetes) were also identified and enumerated. Note that all four manipulated predators, though predominantly benthic in nature, have been observed to access the inner lid of

the cage through swimming or climbing, so it does not provide any refuge from predation. All benthic invertebrates were identified to species level where possible ($n = 56,240$), with their density (individuals/m²) estimated by scaling counts to the area of the cage.

Ecosystem functioning

From 136 animal species that colonized the cages during the experiment, 83 are known to graze on microphytobenthos and 101 consume coarse particulate organic matter (CPOM). Thus, production of microphytobenthos and decomposition of CPOM are likely to be key processes associated with these benthic invertebrate communities. The accumulation of microphytobenthos on the glass slides over the duration of the experiment was taken as a proxy for primary production (P) and estimated as

$$P = \frac{\text{chl } a}{t}.$$

Here, chl a was the average chlorophyll a measured on the three individual slides in each cage (mg/m²) and t was the duration of the experiment (54 d). The decomposition rate of CPOM (D) was estimated as

$$D = \frac{M_I - M_F}{t}.$$

Here, M_I and M_F were the initial and final dry mass of kelp (mg dry mass). M_I was estimated as $2,207 \pm 138$ mg (mean \pm SE) from 30 control discs of kelp frond, which were not used in the experiment.

Food web structure

A database of trophic interactions was established for the Lough Hyne system (around the same time as this experiment) through a combination of intensive gut content analysis and literature research (O'Gorman and Emmerson 2009, 2010). Food webs were constructed for each cage in the experiment by taking the species list for that cage (excluding the manipulated predators) and including any trophic interactions between those species from the Lough Hyne database. It is important to note that this approach does not account for potential flexibility in the diet of individual consumers in response to treatments. Nevertheless, most organisms that colonized the cages were primary consumers (113 of 136 animal species) whose resources are unlikely to change (i.e., grazers of biofilm are unlikely to become predators), while the diet of predators should be considered as their fundamental rather than realized niche (Morlon et al. 2014). Four food web properties were computed for each cage using the cheddar package in R 3.5.0 (Hudson et al. 2013): species richness, link richness, connectance (using the DirectedConnectance function), and mean

food chain length (using the count.chain.length column in the trophic.chains data frame returned by the NvMTriTrophicStatistics function). The first two properties describe the overall size of the food web, connectance is the proportion of possible links that are realized, while mean food chain length is the average height of the food web.

Interaction strengths

Predators do not simply alter the abundance of lower-trophic-level species through direct consumption, but rather a mixture of direct and indirect effects (e.g., feeding on their competitors for shared resources). The sum of these direct and indirect effects is the predator's net effect (NE) on a species (Montoya et al. 2009), which was estimated using the dynamic index (Wootton 1997)

$$\text{NE} = \frac{\ln\left(\frac{X_i^+}{X_i^-}\right)}{X_j}.$$

Here, X_i^+ is the density of species i in the presence of predator j , X_i^- is the density of species i in the absence of predator j , and X_j is the density of predator j . NE was calculated for predator j on every species in the pair of cages and then the mean net effect, $\overline{\text{NE}}$, was taken as the average of all these values. Note that $\overline{\text{NE}}$ equates to the average change in abundance of a benthic invertebrate species in the community when a predator is present, compared to when it is absent (expressed as a log ratio). The mean net effect of each predator on its own was calculated by comparing the single species treatments to the predator-free cages. The mean net effect of each predator was also calculated in the presence of each of the other manipulated predators by comparing the 2, 3, or 4 species treatment containing that predator to the corresponding 1, 2, or 3 species treatment without it. This procedure enabled the quantification of the mean net effect that every manipulated predator had on the abundance of benthic invertebrates in the community that assembled into every cage during the experiment.

Predators can alter each other's net effects through nonlethal interactions such as competition and facilitation. Since these interactions do not directly alter the density of the predators, but rather their traits (e.g., time spent foraging), they can be termed indirect effects. The indirect effect (IE) of predator k on predator j was estimated as

$$\text{IE} = \overline{\text{NE}}_j^- - \overline{\text{NE}}_j^+.$$

Here, $\overline{\text{NE}}_j^-$ and $\overline{\text{NE}}_j^+$ are the mean net effects of predator j on the rest of the cage community in the absence and presence of predator k , respectively. Thus, if predator j had a strong negative mean net effect on its own and a less negative mean net effect in the presence of predator k , the latter would have dampened its

suppression of lower trophic levels (i.e., a negative indirect effect). Similarly, if predator j had a strong positive mean net effect on its own and a less positive mean net effect in the presence of predator k , the latter would have strengthened its suppression of lower trophic levels (i.e., a positive indirect effect). Note that the IE of top predators on intermediate predators may be a combination of direct and indirect effects because the former can potentially feed on the latter.

Statistical analysis

All statistical analyses were carried out in R 3.5.0. The effects of multitrophic diversity on ecosystem functions, food web properties, mean net effects, and indirect effects (as separate response variables) were examined with two-way Analysis of Variance (ANOVA), where top and intermediate predator diversity were the explanatory variables. Note that the number of levels for each explanatory variable depended on the response variable being explored, e.g., when analyzing the mean net effect of a top predator, intermediate predator diversity had three levels (0, 1, and 2 species) and top predator diversity had two levels (0 and 1 other species), but when analyzing the mean net effect of an intermediate predator, intermediate predator diversity had two levels (0 and 1 other species) and top predator diversity had three levels (0, 1, and 2 species). Additionally, one-way ANOVA was sufficient to explore the effect of top (or intermediate) trophic level diversity (three levels: 0, 1, and 2 species) on indirect effects among intermediate (or top) predators, and on the mortality rate of intermediate predators. Tukey's post-hoc test was performed to assess which treatment levels were significantly different from one another. Additional analyses were also carried out to examine potential species identity effects in the experiment (see Appendix S1: Supplementary Analysis). All indirect interactions between the manipulated predators and their net effects on the food webs that assembled into each cage are visualized in Fig. 2 (for the highest multi-trophic diversity treatment) and Appendix S1: Fig. S3 (for the remaining treatments).

RESULTS

There was a significant effect of top predators on the mortality rate of the painted goby ($F_{3,20} = 6.635$; $P = 0.0027$; Fig. 3a) and common prawn ($F_{3,20} = 3.273$; $P = 0.0425$; Fig. 3b). Both intermediate predators experienced mortality in the absence of top predators (4–17% on average), suggesting that some natural mortality occurred in the experiment. Nevertheless, there was a significant increase in their mortality rate when both top predators were present together (45–60% on average), indicating a high probability of consumption during the experiment. Both intermediate predators were also detected in the gut contents of both top predators at the end of the experiment (Appendix S1: Table S1).

There was an interactive effect of multitrophic diversity on all the ecosystem functions and food web properties that were studied. Primary production ($F_{4,39} = 8.066$; $P < 0.0001$; Fig. 4a) and decomposition rate ($F_{4,39} = 4.668$; $P = 0.0036$; Fig. 4b) were greatest in the absence of all manipulated predators and when multitrophic diversity was highest, with significantly lower process rates when intermediate predators occurred in the absence of top predators (90% reduction in primary production and 60% reduction in decomposition rate, on average). There was significantly more species ($F_{4,39} = 20.83$; $P < 0.0001$; Fig. 4c) and trophic links ($F_{4,39} = 24.03$; $P < 0.0001$; Fig. 4d) in the absence of all manipulated predators and when multiple top and intermediate predators were present, compared to all other treatments (30–60% reduction in species and link richness, on average). The loss of benthic invertebrate species in these other treatments was not biased toward a particular trophic group, with similar patterns found for both primary consumers and predatory invertebrates (Appendix S1: Fig. S4). The food web was also less connected ($F_{4,39} = 8.463$; $P < 0.0001$; Fig. 4e) and had longer food chains ($F_{4,39} = 9.384$; $P < 0.0001$; Fig. 4f) in the absence of all manipulated predators and when multitrophic diversity was highest (42–87% increase in connectance and 1–18% reduction in food chain length, on average).

There was an interactive effect of multitrophic diversity on the mean net effect that top predators had on the lower trophic levels ($F_{2,42} = 10.29$; $P = 0.0002$). Here, top predators had negative net effects at all levels of intermediate predator diversity when they occurred on their own, but their effects became more positive as intermediate predator diversity increased when a second top predator was present (Fig. 5a). In contrast, there was no interactive effect of multitrophic diversity on the mean net effect that intermediate predators had on the lower trophic levels ($F_{2,42} = 2.077$; $P = 0.1380$). Rather, their net effects became increasingly more positive when both intermediate ($F_{1,42} = 5.275$; $P = 0.0267$) and top ($F_{2,42} = 20.73$; $P < 0.0001$) predator diversity increased (Fig. 5b). These effects were generally consistent for each of the manipulated species, although the intermediate predators had much more negative mean net effects when they were both present with black gobies (Appendix S1: Fig. S5). The results were also supported by gut content analysis, which showed that the manipulated predators consumed a lower species richness and abundance of prey when multitrophic diversity was highest (Appendix S1: Fig. S6).

Indirect effects were consistently negative between multiple predators (Fig. 6), indicating that the manipulated species had largely antagonistic effects on one another (see also Fig. 2 and Appendix S1: Fig. S3). These negative effects also became stronger as multitrophic diversity increased. For example, indirect effects between top predators were 3–7 times more negative when multiple intermediate predators were present

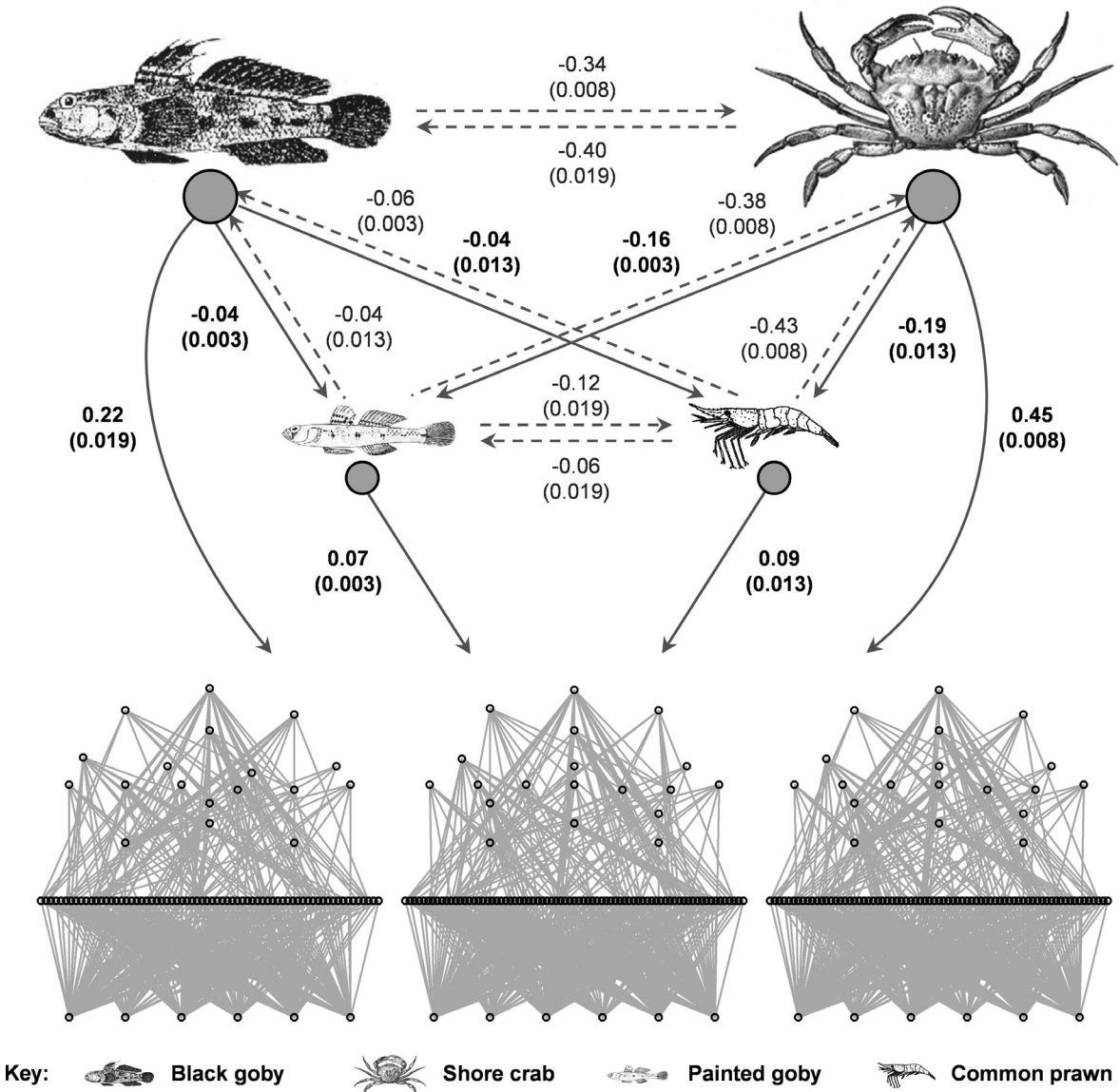


FIG. 2. Interactions between manipulated predators and their mean net effects on the food webs for the highest multi-trophic diversity treatment. Indirect interactions are indicated by dashed lines and gray text, while net effects (a mixture of direct and indirect interactions) are indicated by solid lines and black text. Mean indirect and net effects (with standard errors in brackets) are given for the three replicates of the treatment. Food webs for each replicate are also shown, with species and feeding links represented as circles and lines, respectively. Note that all interactions between manipulated predators are negative and all net effects on the food webs are positive. See Appendix S1: Fig. S3 for a visualization of interactions in the remaining treatments.

($F_{2,21} = 26.20$; $P < 0.0001$; Fig. 6a) and indirect effects between intermediate predators were 3.5 times more negative when multiple top predators were present ($F_{2,21} = 3.757$; $P = 0.0403$; Fig. 6b). Similarly, the effect of top predators on intermediate predators was 2–5 times greater when multiple predators were present on both trophic levels ($F_{1,32} = 11.74$; $P = 0.0017$; Fig. 6c). The only positive indirect effects were of multiple intermediate predators on single top predators ($F_{1,32} = 9.355$; $P = 0.0045$; Fig. 6d), which was driven

by the specific case of both intermediate predators in the presence of black gobies (Appendix S1: Fig. S7).

DISCUSSION

These results highlight the importance of multitrophic diversity for the structure and functioning of ecological communities. Loss of either horizontal or vertical diversity resulted in weaker antagonistic interactions between predator species and thus stronger negative effects of the

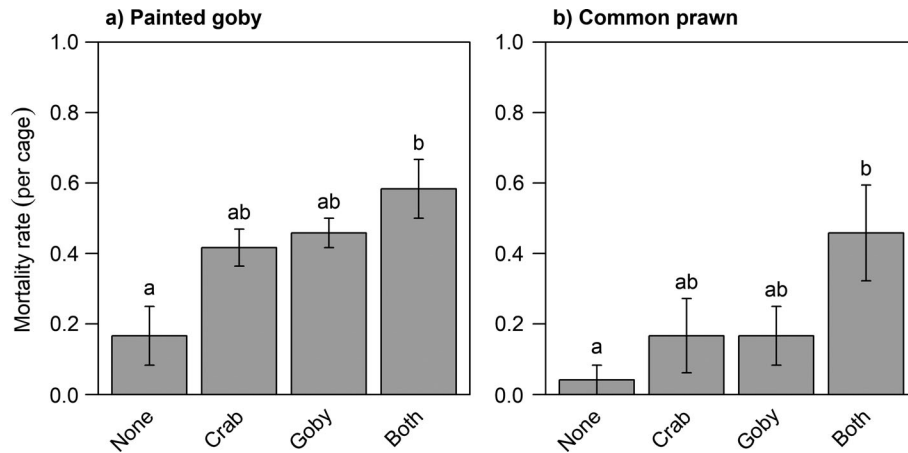


FIG. 3. Mortality rate of the intermediate predators during the experiment. All top predators were recovered in the experiment, but some mortality of the (a) painted goby and (b) common prawn occurred in all treatments (None, no top predators; Crab, shore crab only; Goby, black goby only; Both, shore crab and black goby). Mortality rate refers to the proportion of intermediate predators that were not recovered at the end of the experiment. Means \pm SE are shown; bars not sharing a common letter are significantly different from each other (Tukey test; $P < 0.05$).

predators on lower trophic levels. This led to a reduction in primary production and decomposition, as key ecosystem processes fueling green and brown pathways of energy flow through ecosystems (Wolkovich et al. 2014). The net result was a food web that had fewer species and trophic linkages and shorter food chains, albeit with more connectivity between the constituent species. In fact, the structure and functioning of a community with a highly diverse predator assemblage on multiple trophic levels was equivalent to one where no large predators were present to exert any top-down control on the system. This phenomenon emerged consistently in the results as an interactive effect between top and intermediate predator diversity, highlighting the value of multitrophic diversity for dampening top-down control of these shallow subtidal food webs.

Weaker top-down control in the highest multitrophic diversity treatment equates to a reduction in the risk imposed by multiple predators on their shared benthic invertebrate prey. Previous research has identified three main mechanisms driving risk reduction in such situations: (1) prey switching by top predators, (2) reduced density of intermediate predators, and (3) behavioral changes among the predators (Crumrine and Crowley 2003, Griffen and Byers 2006b). There was evidence for all three mechanisms in this experiment. Support for prey switching comes from gut content analysis and the higher mortality rate of intermediate predators in the presence of top predators (Fig. 3), indicating that the latter consumed the former. Thus, top predators are likely to have allocated less time to foraging on lower trophic levels in the experiment whenever they were satiated after consuming the much larger intermediate predators. Nevertheless, all the intermediate predator treatments still contained some individuals at the end of the experiment, indicating that predation rates by the top predators were very low (1.6 individuals consumed

out of 4, on average). Regular feeding by the top predators on the benthic invertebrate community would thus have been required to meet their energy demands over the 2-month duration of the experiment.

Direct consumption by top predators also reduced the density of intermediate predators, leaving fewer individuals to consume benthic invertebrate prey, i.e., a density-mediated indirect effect. Such effects lead to the classic concept of a trophic cascade, e.g., sea otters reducing the density of sea urchins through predation, thus releasing their algal resources from grazing pressure (Estes et al. 2011). The magnitude of density-mediated indirect effects is much weaker when intraguild predation occurs, however, i.e., when the top predator also feeds on the lower trophic levels (Bruno and O'Connor 2005, Griffen and Byers 2006b). Thus, trait-mediated indirect effects were likely to be the stronger driver of changes in the benthic invertebrate community here (Preisser et al. 2005, Trussell et al. 2006b). Partitioning out density- and trait-mediated indirect effects would require additional treatments that simulate the threat of predation without reduction in density of intermediate predators, and reduction in their density without the risk cue from top predators (Griffen and Byers 2006b, Trussell et al. 2006b). That was beyond the scope of this study, which focused instead on quantifying the total magnitude of all indirect effects among the manipulated predators. Elimination of density-mediated indirect effects could have been achieved by replacing any manipulated predators that were consumed during the experiment, but this was impractical due to the disturbance it would have caused to the cage communities.

Behavioral changes were certainly at play among the top predators, i.e., where there was no reduction in density of either species over the course of the experiment. Here, presence of the black goby reduced the consumptive pressure exerted by the shore crab on the benthic

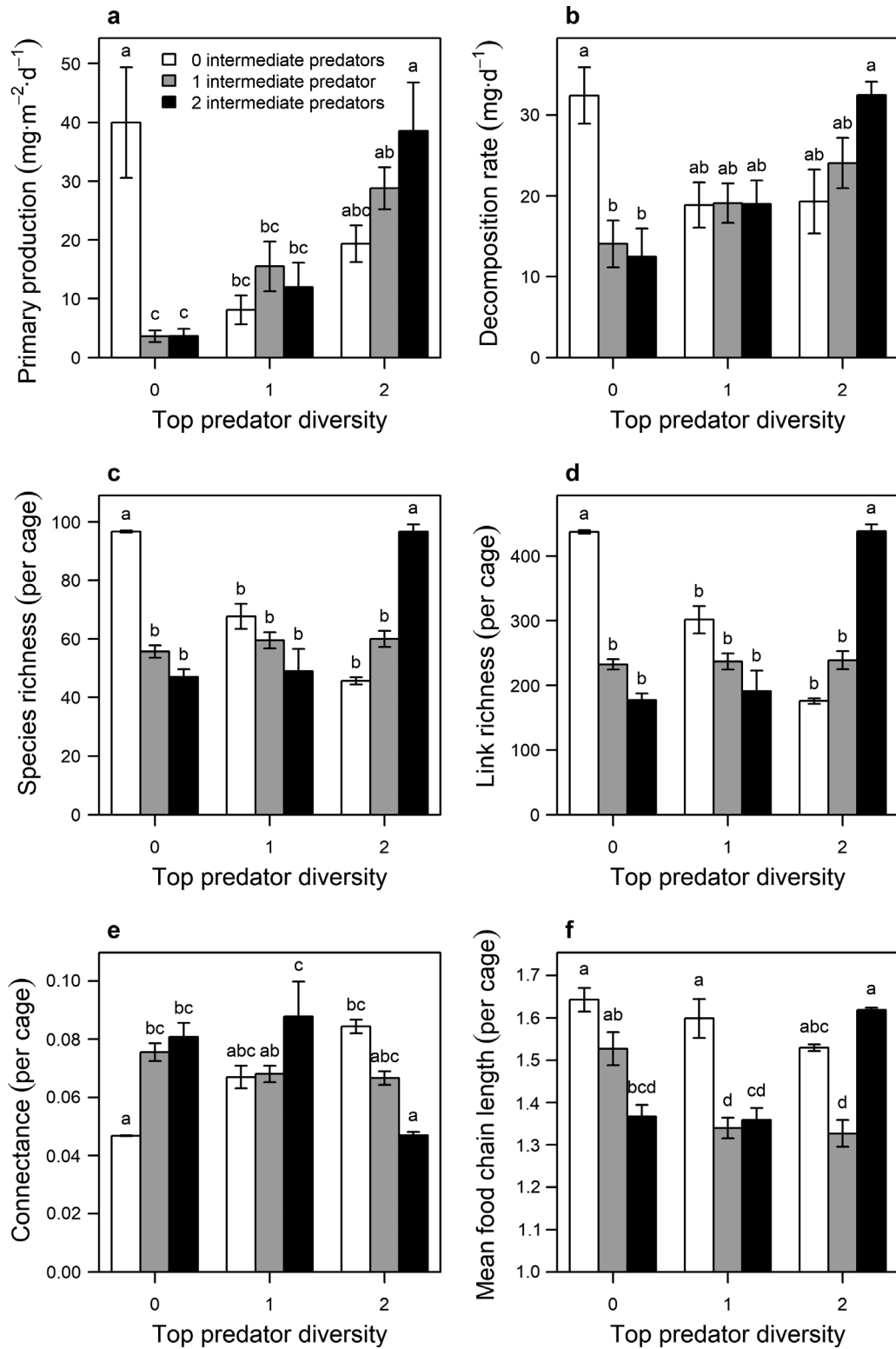


FIG. 4. Ecosystem functioning and food web properties for the communities that assembled into the cages during the experiment. Ecosystem functions included (a) primary production and (b) decomposition rate, which were estimated as a per day rate from beginning and end measurements taken during the experiment. Food web properties included (c) species richness, (d) link richness, (e) directed connectance, and (f) mean food chain length. Means \pm SE are shown; bars not sharing a common letter are significantly different from each other (Tukey test; $P < 0.05$).

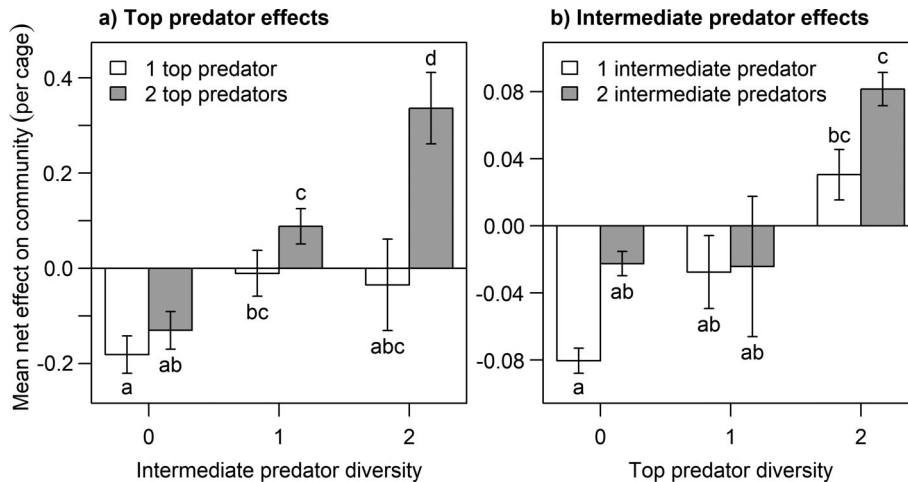


FIG. 5. Mean net effects of the manipulated predators on the community that assembled into the cages during the experiment. Here, net effects equate to the log ratio abundance of a species in the presence and absence of a manipulated predator, and the mean net effect across all species in the community was estimated. (a) Top predator net effects and (b) intermediate predator net effects. Means \pm SE are shown; bars not sharing a common letter are significantly different from each other (Tukey test; $P < 0.05$). Note the difference in y-axis scale between the two panels, indicating that top predator effects were much greater than intermediate predator effects in the experiment.

invertebrate community, and vice versa (Fig. 6a; Appendix S1: Fig. S7a,b). The most likely explanation for this response is a disruption to the time spent feeding on invertebrate prey due to interference competition (Connell 1983, Schoener 1983). Here, direct aggression among individuals of each species reduces their predation rate, or simply limits their physical establishment in a portion of the available habitat (Young 2004). Interestingly, interspecific competition was several times stronger in this experiment than competition between conspecifics, in contrast to some previous studies (Connell 1983, Griffen 2006), which may be due in part to the larger body mass of the shore crabs here (Young 2004). Similar effects were found between the intermediate predators (Fig. 6b), indicating the prevalence of antagonistic behavioral interactions in the system. Indeed, antagonistic multiple predator effects have been widely reported from experiments across the marine, freshwater, and terrestrial realms (Eklov and Werner 2000, Finke and Denno 2004, Siddon and Witman 2004, Schmitz 2007, O’Gorman et al. 2008, O’Connor et al. 2013).

Previous experiments in the study system have shown that behavioral interactions between trophic levels also play a major role in moderating top-down control, with intermediate predators actively avoiding the space occupied by larger predators (O’Gorman et al. 2008). This suggests that they allocate more time to predator avoidance than to feeding, with intimidation by predators often shown to elicit stronger responses in prey than their consumptive effects (Preisser et al. 2005). Interestingly, there were similar negative effects of intermediate predators on their higher trophic level consumers here (Fig. 6d), which suggests the top predators may have allocated more time to pursuing them than feeding on

other organisms further down the food web. The potential for prey to effectively “harm” predators has been proposed in population dynamics modelling (Abrams 1992), but rarely explored in experimental ecology.

Nevertheless, risk enhancement for prey is also commonplace (Sih et al. 1998, Schmitz et al. 2004) and was observed in one particular predator combination in this experiment (Appendix S1: Fig. S7). This illustrates that species identity effects can also lead to facilitative interactions between multiple predators, although the underlying mechanism here would require further study. Knowledge of predator hunting modes and habitat use by key prey species can help to predict these seemingly idiosyncratic effects (Schmitz et al. 2004). Environmental context is also crucial for anticipating the effects of multitrophic diversity loss, with nutrient enrichment (O’Connor and Donohue 2013) and warming (Barton and Schmitz 2009, Antikueira et al. 2018) shown to alter the impact of predator diversity on lower trophic levels.

Weakening of antagonistic interactions through the loss of multitrophic diversity systematically shifted predator impact on the lower trophic levels from a positive net effect to a negative one (Fig. 5). Indirect effects are known to contribute substantially to the net effects of predators (Peacor and Werner 2001), but the altered sign of the impact on lower trophic levels demonstrated here is particularly striking. This suggests that nontrophic interactions can alter the role of a predator from a net stimulator to a degrader of ecological complexity. Other experiments have shown that a reduction in nontrophic interactions, including competition for space and predator-avoidance behavior, can trigger cascading extinctions following predator diversity loss (Donohue et al. 2017). This clearly illustrates the importance of

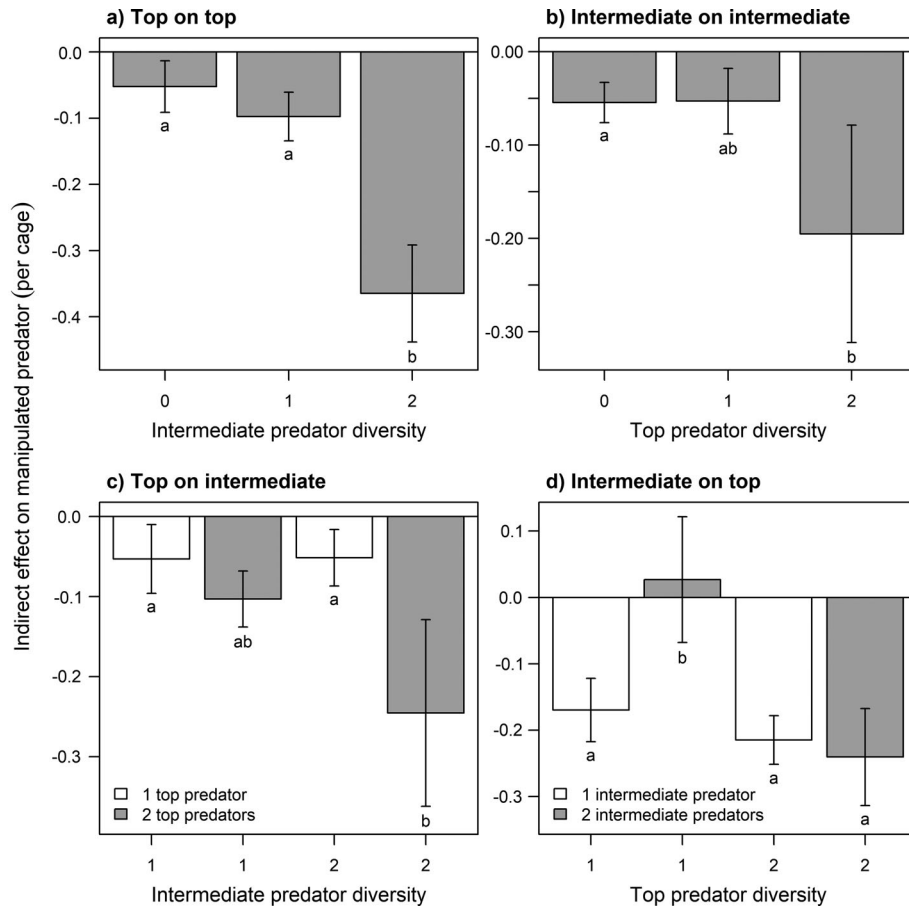


FIG. 6. Indirect effects between the manipulated predators in the experiment. Here, indirect effects equate to the difference in the mean net effect a manipulated predator has on the community in the presence and absence of another manipulated predator. The indirect effect of (a) top predators on each other, (b) intermediate predators on each other, (c) top predators on intermediate predators, and (d) intermediate predators on top predators. Means \pm SE are shown; bars not sharing a common letter are significantly different from each other (Tukey test; $P < 0.05$). Note that panel c may contain a mixture of direct and indirect effects because top predators also consume intermediate predators.

quantifying non-trophic interactions in natural systems if we are to accurately anticipate the effects of biodiversity loss on ecosystem processes and ecological stability. Note that the positive net effect of top predators was four times higher than that of intermediate predators in the highest multitrophic diversity treatment (Fig. 5). Interaction strength typically increases with body size due to the higher metabolic demands of larger organisms (Brown et al. 2004, Rall et al. 2012). Thus, antagonistic interactions that limit feeding by larger top predators are likely to have disproportionately greater effects on lower trophic levels, highlighting the importance of maintaining predator diversity at the top of the food chain.

The shift from positive to negative net effects of the manipulated predators on the lower trophic levels resulted in a clear reduction in ecosystem functioning and food web complexity. Lower primary production following the loss of horizontal or vertical diversity

(Fig. 4a) was likely driven by increased grazing pressure from herbivorous invertebrates. This could be an indirect effect of stronger feeding by the manipulated species: (1) on predatory invertebrates that consume the grazers, i.e., a trophic cascade (Jochum et al. 2012); and/or (2) on detritivorous invertebrates that compete with the grazers for space, i.e., apparent competition (Bonsall and Hassell 1997). In support of the first mechanism, food chains tended to be shorter after reductions in multitrophic diversity (Fig. 4f), driven by the loss of predatory benthic invertebrate species (Appendix S1: Fig. S4b). Here, predatory crustaceans (e.g., *Galathea squamifera*, *Pilumnus hirtellus*, *Siriella armata*) were generally only found in the highest multitrophic diversity treatment and in the absence of any manipulated species. Their exclusion from lower multitrophic diversity treatments would have reduced consumption on their primary consumer prey, leading to increased grazer abundance, stronger grazing pressure, and thus lower

primary production. This was particularly clear for the snails *Bittium reticulatum*, *Rissoa parva*, and *Rissoa sarsi*, which were three of the most abundant species in the system (Appendix S1: Fig. S8). Shorter food chains are also indicative of a lower productivity environment, as seen in these treatments (Kaunzinger and Morin 1998). The observed reduction in decomposition rates after loss of manipulated predators (Fig. 4b) offers support for the second mechanism, with detrital processing rates similarly maximized by high multitrophic diversity in freshwater microcosms (Jabiol et al. 2013).

A third (though not mutually exclusive) mechanism explaining lower primary production following multitrophic diversity loss could be a reduction in grazer diversity through direct consumption by the manipulated species. This may have facilitated stronger grazing pressure, in the same way that multitrophic diversity loss strengthened top-down control by the manipulated predators. In support of this, there were fewer species of primary consumers following loss of either top or intermediate predators (Appendix S1: Fig. S4a). Here, herbivorous chitons (*Acanthochitona crinitus*) and sea slugs (*Eubrancheus farrani* and *Cuthona* sp.) were generally only found in the highest multitrophic diversity treatment and in the absence of any manipulated species. Indeed, species richness in general and the total number of trophic links both declined following multitrophic diversity loss (Fig. 4c and d), suggesting an overall reduction in interference competition throughout the food web (Loeuille and Loreau 2005).

Despite the smaller web size and shorter food chains, the connectivity of the food web was higher after the removal of manipulated predators (Fig. 4e). Meta-analyses of high-quality food web datasets indicate that connectance generally increases with biodiversity loss (Schmid-Araya et al. 2002, Riede et al. 2010), suggesting that this may be a macroecological property of complex natural systems (but see Winemiller 1989). Increasing connectance following species loss could help to stabilize ecosystems (Allesina and Tang 2012), with higher connectance typically associated with increased resistance to invasion (Smith-Ramesh et al. 2017) and robustness against biodiversity loss (Dunne et al. 2002, Gilbert 2009). Ecological stability has been shown to consist of multiple dimensions (e.g., variability, resistance, recovery, persistence), however, and the correlations between these components of stability tend to break down following species extinctions (Donohue et al. 2013). Thus, it would be unwise to make inferences about ecological stability from a single metric (e.g., robustness) or proxy (e.g., connectance). Temporal and spatial variability in ecosystem process rates and the persistence of species in the food web should also be measured for a more holistic understanding of how multitrophic diversity alters ecological stability.

As with all mesocosm experiments, there are limitations that must be acknowledged. The cages would have restricted natural movements of the predators,

potentially altering their behavior and even magnifying their impacts on the prey community compared to an open system. The experimental design examined predator impacts on a prey community that assembled into the cages, but the conclusions could be different for predators colonizing an area that already contained prey. This could be tested by allowing prey communities to assemble before introducing the manipulated predators, although the starting conditions are unlikely to be the same for all cages in this case. The mesocosms also necessarily restricted the scale of the food webs that were studied. The top predators in this experiment would only be meso-predators in the context of an open coastal environment, where even single species of apex predator could help to promote ecosystem complexity and function (Estes et al. 2011). Thus, the results of the experiment are likely to be most relevant for nearshore ecosystems dominated by small benthic predators. Finally, only two species were manipulated on each trophic level in the experiment to facilitate quantification of all possible indirect interactions among the predators in the 16 species combinations. A more complex experimental design would have been logistically infeasible in this context, but natural systems are likely to have even greater predator diversity, so future studies should consider greater numbers of manipulated species when exploring multitrophic diversity effects.

This study highlights the importance of non-trophic interactions between predators on multiple trophic levels for determining the sign and magnitude of their effects on the rest of the food web. It also demonstrates a causal link between the loss of horizontal or vertical diversity and the simplification of food webs and subsequent suppression of ecosystem process rates. Future studies should determine how these general findings are altered by trophic role (i.e., predators, primary consumers, basal resources), movement type (i.e., active, sit-and-wait, sedentary), and environmental context (i.e., warming, nutrient enrichment, chemical pollution). Experiments should also explore the potential for prey to “harm” their predators through non-trophic interactions that may reduce their impacts on the food web or their overall fitness.

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