Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial ecosystems

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Abstract

The exchange of organisms and energy among ecosystems has major impacts on food web structure and dynamics, yet little is known about how climate warming combines with other pervasive anthropogenic perturbations to affect such exchanges. We used an outdoor freshwater mesocosm experiment to investigate the interactive effects of warming, eutrophication, and changes in top predators on the flux of biomass between aquatic and terrestrial ecosystems. We demonstrated that predatory fish decoupled aquatic and terrestrial ecosystems by reducing the emergence of aquatic organisms and suppressing the decomposition of terrestrial plant detritus. In contrast, warming and nutrients enhanced cross-ecosystem exchanges by increasing emergence and decomposition, and these effects were strongest in the absence of predators. Furthermore, we found that warming advanced while predators delayed the phenology of insect emergence. Our results demonstrate that anthropogenic perturbations may extend well beyond ecosystem boundaries by influencing cross-ecosystem subsidies. We find that these changes are sufficient to substantially impact recipient communities and potentially alter the carbon balance between aquatic and terrestrial ecosystems and the atmosphere.

Keywords: allochthonous resources, amphibians, climate warming, detritus decomposition, global change, insect emergence, spatial subsidies, top-down control

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Introduction

The movement of nutrients and organisms across ecosystem boundaries is a ubiquitous property of many natural systems (Polis *et al.*, 2004). Adjacent ecosystems are linked by fluxes of resources that subsidize primary producers (Gratton *et al.*, 2008; Hocking & Reynolds, 2011), consumers (Marczak *et al.*, 2007), and alter direct and indirect species interactions in recipient food webs (Knight *et al.*, 2005; Piovia-Scott *et al.*, 2011). Moreover, theory indicates that the magnitude, composition, and phenology of these cross-ecosystem fluxes can contribute to food web stability (Huxel *et al.*, 2002; Takimoto *et al.*, 2002; Leroux & Loreau, 2008). Consequently, changes in the structure and dynamics of one ecosystem are likely to influence the structure and stability of adjacent ecosystems (Polis *et al.*, 2004; Massol *et al.*, 2011).

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Freshwaters and adjacent terrestrial habitats are tightly coupled by the reciprocal exchange of resources (Nakano & Murakami, 2001). Aquatic ecosystems export organic matter to the terrestrial environment in the form of organisms with complex life cycles that inhabit freshwaters as larvae and emerge into the terrestrial environment following metamorphosis. Aquatic insects and amphibians in particular become important resources for terrestrial consumers (Baxter *et al.*, 2005), act as predators that alter terrestrial food webs (Knight *et al.*, 2005; McCoy *et al.*, 2009), and also become detritus inputs that supply carbon and nitrogen to terrestrial plants (Gratton *et al.*, 2008). Reciprocally, the accumulation of dissolved and particulate organic carbon from terrestrial detritus and organisms forms the basis of secondary production in many aquatic food webs (Wallace *et al.*, 1997; Rubbo *et al.*, 2006; Solomon *et al.*, 2008; Cole *et al.*, 2011). Terrestrial carbon that does not enter consumer pathways through microbes and macro-consumers is either exported down the catchment or stored within sediments (Clymo *et al.*, 1998). This long-term storage of terrestrial carbon in freshwaters, especially lentic systems, plays a critical role in global carbon sequestration (Cole *et al.*, 2007; Tranvik *et al.*, 2009),

demonstrating how cross-ecosystem exchanges can influence major biogeochemical cycles.

Although there has been substantial progress in quantifying the magnitude and effects of subsidies among ecosystems (Marczak et al., 2007), we know little about the influence of accelerating levels of anthropogenic change on cross-ecosystem resource fluxes. Natural systems are being dramatically altered by a combination of perturbations including nutrient deposition, altered predator guilds, and climate warming (Falkowski et al., 2000; Sala et al., 2000; Tylianakis et al., 2008; Ferretti et al., 2010). Freshwaters are especially vulnerable as they accumulate nutrients applied to the surrounding catchment (Carpenter et al., 1998), are subject to warming associated with climate change (Schindler et al., 1990), and are threatened by strong cascading effects of predator loss or invasions (Pace et al., 1999; Simon & Townsend, 2003). These perturbations may influence the size and timing of resource fluxes between aquatic and terrestrial ecosystems (Riley & Jefferies, 2004), potentially altering the strength of cross-ecosystem coupling and role of freshwaters in global biogeochemical cycles (Boyero et al., 2011).

Changes in temperature, predator abundance, and nutrient availability can have a range of effects on the exchange of materials between aquatic and terrestrial ecosystems. Predatory fish can reduce insect and amphibian developmental rates (Benard, 2004) and emergence (Pope et al., 2009; Wesner, 2010), and indirectly reduce detritus decomposition rates by feeding on benthic detritivores (Greig & Mcintosh, 2006). Consequently, fish may decouple aquatic and terrestrial food webs by moderating biomass fluxes across the land-water interface. In contrast, both nutrients and warming may stimulate algal primary production (Yvon-Durocher et al., 2010), fuelling an increased abundance of adult aquatic organisms (Michelutti et al., 2005) and accelerating the metabolism and respiration of terrestrial detritus (Gessner et al., 1999; Boyero et al., 2011). Furthermore, warmer temperatures can also advance the phenology of insect and amphibian metamorphosis (Harper & Peckarsky, 2006). However, such bottom-up effects of nutrients or warming may be counteracted by strong top-down control from predatory fish. Alternatively, accelerated larval development under warming may reduce the risk of predation prior to metamorphosis, thereby dampening the negative effects of fish. The cumulative effects of variation in temperature along with top-down and bottom-up forces on movements of biomass between aquatic and terrestrial ecosystems are largely unknown.

Predicting interactive effects of different perturbations is difficult (Sala et al., 2000), especially when their independent effects are also poorly understood. Given the potential for complex interactions, direct experimental manipulations are required to understand the direct and indirect effects of anthropogenic perturbations on cross-ecosystem dynamics. Herein we report on an outdoor aquatic mesocosm experiment in which we quantified the subsidies of emerging adult aquatic organisms to the terrestrial environment, and the incorporation of terrestrial resources into aquatic food webs through decomposition processes. Our factorial manipulation of temperature, nutrients, and predators presents the first experimental evidence that warming interacts with top-down and bottom-up perturbations to alter the magnitude, composition, and phenology of cross-ecosystem resource fluxes.

Materials and methods

Experimental design and sampling

We manipulated a factorial combination of temperature, nutrients, and the presence of predatory fish in outdoor freshwater mesocosms (1136 L tanks; Rubbermaid®, Atlanta, GA, USA) located at the University of British Columbia's experimental pond facility, Vancouver, Canada. Tanks were filled with municipal water that was left to dechlorinate by evaporation for 1 week prior to the application of treatments. Each of the eight treatment combinations was randomly assigned and replicated five times, resulting in 40 mesocosms. Submersible water heaters (300 W; Hagen®, Montreal, Canada) in half of the tanks maintained a continuous 3 °C increase above the ambient diurnal and seasonal temperature fluctuations of unheated tanks (Appendix S1). This temperature difference falls within the conservative projections of increase in North American surface water temperatures over the next century (IPCC, 2007). We added 264 μg of nitrogen L^{-1} (as $NaNO_3$) and 27 μg of phosphorus L^{-1} (as KH_2PO_4) monthly to nutrient addition tanks. The experiment started on 9 June 2010 when we introduced five threespine stickleback (Gasterosteus aculeatus) to each tank to initiate the predation treatments. Stickleback are generalist predators that can exert strong top-down control on zooplankton and benthic macroinvertebrates (Bell et al., 2003; Feuchtmayr et al., 2007).

Mesocosms were inoculated with zooplankton, phytoplankton, and macroinvertebrates 1 month prior to the start of the experiment by adding benthic sediments (1 L) and standard aliquots of live plankton collected from a nearby pond with a 64 μm mesh net. Two species of snails (Physa and Menetus, 20 individuals of each) were also added to each tank. As a result of these inoculations and oviposition by insects naturally dispersing from surrounding water bodies, mesocosms contained diverse benthic and pelagic communities within 2 months following the initiation of the experiment. In the following spring (May 2010) we also introduced 70 newly hatched Pacific chorus frog tadpoles (Pseudacris regilla, Gosner stage: 24–25) to each tank in two separate additions (26 tadpoles on 7 May and 44 tadpoles on May 17). This tadpole density falls within the range observed in natural ponds in

British Columbia (Govindarajulu & Anholt, 2006). Frogs were collected as embryos from adjacent ponds and incubated until hatching in outdoor mesocosms.

Adult aquatic insects emerging from the mesocosms were sampled from 15 March 2010 until the end of the experiment on the 24 September 2010. Insects emerging from the water surface were collected using a floating conical emergence trap (33 cm diameter, 700 μm mesh) tethered to the center of each tank. Traps were cleared every 2–4 days with a hand-held motorized aspirator (BioQuip, Rancho Dominguez, CA, USA). Samples were pooled each week, and all individuals were identified to family level and measured with an ocular micrometer to calculate dry mass from length-mass regressions (Rogers *et al.*, 1976; Sabo *et al.*, 2002). Emerging odonates were quantified by collecting and identifying exuviae attached to the tanks walls above the water surface. Odonate biomass was estimated by applying mean dry masses of confamilial adults collected at nearby ponds. During the frog metamorphosis period (29 June–23 August 2010), tanks were inspected every 2 days for metamorphosed frogs, which were subsequently removed, measured, weighed, and released to the natal pond. Overhanging edges surrounding each tank were constructed of heavy duty aluminum foil to contain metamorphosing frogs between census dates.

We assessed the incorporation of terrestrial resources into aquatic food webs by measuring decomposition rates of plant detritus, which is a critical resource supplement to aquatic systems (Wallace *et al.*, 1997; Cole *et al.*, 2006). We measured leaf breakdown by securing two leaf packs [2 g of air-dried bigleaf maple (*Acer macrophyllum*) enclosed in 10 mm mesh] to the benthos of each mesocosm. One leaf pack was removed after 4 weeks and the other after 8 weeks. Trials were conducted in both spring (March–April) and in summer (July–August). Collected leaf packs were washed over a 1 mm sieve, sorted for macroinvertebrates, and the remaining leaf material was dried at 60 °C for 48 h, weighed, ashed at 500 °C, and reweighed to calculate ash-free dry mass. Invertebrates from leaf packs were enumerated, identified to genus where possible, and measured to determine dry mass from length-mass regressions (Benke *et al.*, 1999; H.S. Greig, unpublished data).

Our mesocosm approach enabled the controlled manipulations necessary to separate the individual and combined effects of multiple perturbations in diverse systems experiencing levels of environmental variation comparable to natural shallow ponds. Mesocosms were, however, impermeable to inputs of dissolved organic carbon which can be an important subsidy for pelagic communities (Cole *et al.*, 2006). We were also not able to quantify the consumption or decomposition of terrestrial arthropods entering the mesocosms. Nevertheless, by focusing on aquatic emergence and leaf decomposition our experiment addressed two critical pathways of biomass exchange between freshwater and terrestrial ecosystems (Baxter *et al.*, 2005; Richardson *et al.*, 2010).

Data analyses

We quantified the combined effects of warming, eutrophication, and predator removal on the $\log_e$-transformed total biomass of insect emergence using three-way ANOVA. We then calculated the flux of aquatic insect emergence per mesocosm (mg × m^{-2} × surface area^{-1} × day^{-1}) using the data pooled across 2-week periods to investigate the timing of insect emergence. The data were $\log_e$-transformed to equalize variances and analyzed with a linear mixed effects (LME) model in which warming, nutrients, fish, and time were fixed factors. We set individual mesocosm as a random factor and accounted for temporal autocorrelation among repeated measurements using the AR(1) function (Bence, 1995; Pinheiro & Bates, 2000). Model comparison by AIC indicated that the AR(1) error structure improved the model fit (Appendix S3).

Leaf decomposition rates in 4 and 8 week litter bags were estimated by fitting a linearized negative exponential decay model (Benfield, 2007), where the decay constant (k) was the slope of a linear regression of the $\log_e$-transformed proportion of ash-free dry mass lost over time (days). Leaf litter decay rate (k) and the biomass of primary consumers in leaf packs (mean biomass from 4 and 8 week leaf bags) in spring and in summer were analyzed using the same LME model structure as for emergence, but without the AR(1) error structure because of the lack of autocorrelation (Appendix S5). To account for the strong negative effect of fish presence on frog survival, the export of amphibians to the terrestrial ecosystem was analyzed using a three-way general linear model with a binomial error distribution on the presence/absence of metamorphic frogs. For fishless tanks only, we also conducted a two-way ANOVA on the $\log_e$-transformed total biomass of metamorphic frogs, with nutrients and warming included as fixed effects. This combination of analyses accounted for the binomial distribution of total frog biomass caused by high tadpole mortality in treatments containing fish predators.

The effect of treatments on multivariate species composition of emerging adult insects was analyzed using redundancy analysis (RDA) ordination of a community matrix consisting of the total biomass (mg × m^{-2} tank surface area) of each of the 19 taxa emerging over the duration of the experiment (Appendix S2). Biomass data were Hellinger-transformed to reduce the influence of outliers (Legendre & Gallagher, 2001). Significance of each treatment and their interactions were determined using Monte Carlo permutation tests on the RDA results. All statistical analyses were performed in R 2.11.1 (R Development Core Team, 2010) using packages '*nlme*' and '*vegan*'.

Results

Fluxes of aquatic organisms to the terrestrial ecosystems

We detected dramatic effects of predation on total biomass of adult aquatic organisms emerging from the ponds. Fish reduced insect emergence by 75% (Table 1; Fig. 1a and b) and prevented nearly all chorus frogs (97%) from surviving to metamorphosis (Table 1; Fig. 1c and d). In contrast, nutrient addition increased adult insect biomass by 87% but only in the absence of

Table 1 Summary of linear models and redundancy analysis (RDA) on the biomass and composition of aquatic organisms emerging from the mesocosms exposed to factorial manipulations of warming, nutrients, and fish

	Total insect biomass[†]			Insect community composition[‡]		Frog metamorphs[§]			Frog biomass[¶]		
	Effect direction	$F_{1,32}$	P	$F_{1,32}$	P	Effect direction	Deviance	P	Effect direction	$F_{1,16}$	P
Warming	+	5.99	0.020*	3.44	0.009**	+	0.10	0.749	−	1.31	0.269
Nutrients	+	9.48	0.004**	1.24	0.262	−	0.10	0.749	+	3.50	0.079
Fish	−	52.66	<0.001***	9.26	0.001**	−	38.24	<0.001***			
Warming : Nutrients	−	0.11	0.743	2.84	0.015*	+	1.09	0.296	−	1.95	0.182
Warming : Fish	−	0.70	0.410	0.95	0.439	+	0.00	1.000			
Nutrients : Fish	−	5.95	0.020*	4.65	0.001**	−	0.00	1.000			
Warming : Nutrients : Fish	+	1.32	0.259	2.41	0.032	+	0.00	1.000			

Significance is indicated by asterisks: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[†]Three-way ANOVA on total adult insect biomass summed over the 6 month emergence period.
[‡]Permutation test (999 randomizations) of an RDA on the Hellinger-transformed total biomass of 17 insect taxa.
[§]Binomial general linear model on presence/absence of frogs surviving to metamorphosis. Significance was tested with chi-square approximation. Deviance for null model: 54.55.
[¶]Two-way ANOVA on total biomass of frogs in fishless treatments only.
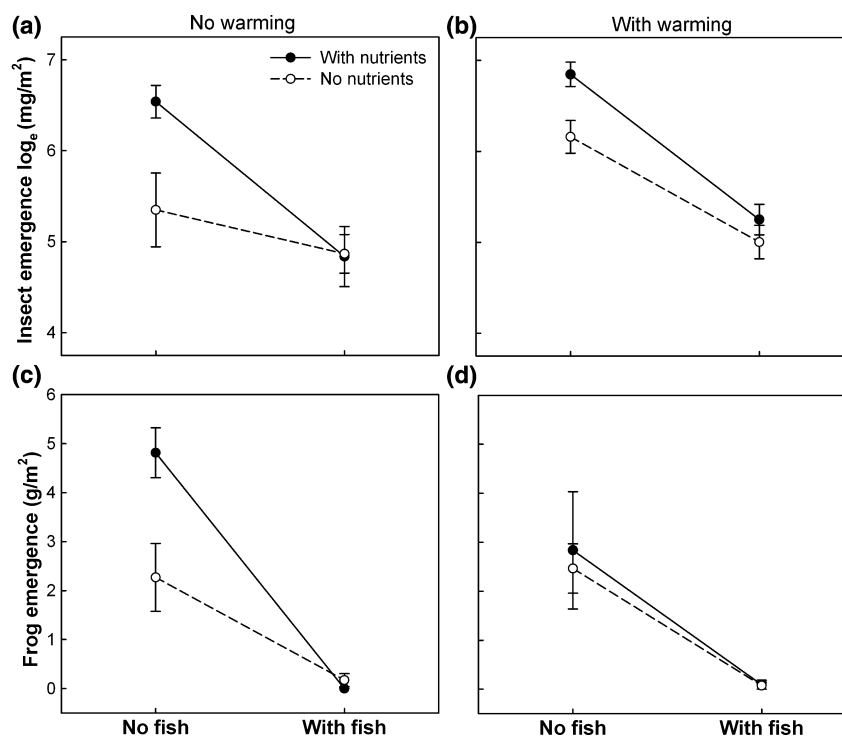
Fig. 1 Three-way interaction plots illustrating the impacts of warming, fish predation, and nutrient enrichment on the total biomass of (a, b) adult aquatic insects and (c, d) tree frog metamorphs emerging from experimental mesocosms to terrestrial ecosystems over the 7-month sampling period. Data points represent the mean of five replicates, error bars are ± 1 SE.

fish (Table 1; Fig. 1a and b). Nutrients also increased total frog biomass in fishless treatments (Fig. 1c and d) although the effect was only marginally significant and did not vary with warming (Table 1). Warming increased the biomass of insect emergence overall (38% increase; Fig. 1a and b) and this effect was not modified
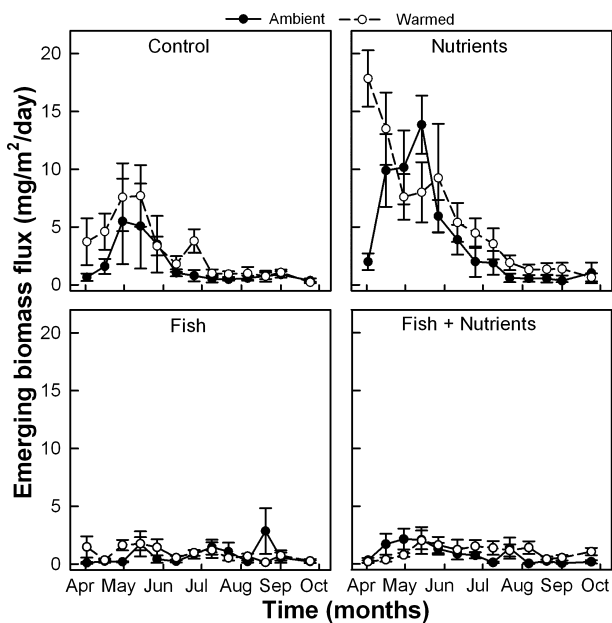
Fig. 2 Seasonal patterns of emerging adult aquatic insects from mesocosms exposed to factorial manipulations of warming, fish predation, and nutrient enrichment. Filled circles represent control treatments and open circles are mesocosms exposed to increased warming (3 °C). Data points represent the mean of five replicates, error bars are ± 1 SE.

Fig. 3 Redundancy analysis (RDA) ordination on Hellinger-transformed total biomass data of the community composition of aquatic insect emergence. Points represent taxa but only those away from the origin are labelled. Taxa not labelled are: Dytiscidae, *Chaoborus*, *Caenis*, *Helophorus*, Sciomyzidae, Hydrophilidae, Hydraenidae, unidentified Brachycera, and Nymphomidae. Arrows shown were significant ($P < 0.05$) main effects or interactions of treatments following a permutation test of three-way model with 999 randomizations. Species aligned with the direction of arrows show positive associations with those treatments. The treatments together explained 45.6% of the variation in emergence composition. Axis 1 and 2 explains 23.7% and 13.2% of the total variation in community composition, respectively.

by fish (Table 1). By contrast, warming had no effect on the total biomass of frogs (Fig. 1c and d; Table 1).

We found that treatments shifted the phenology of aquatic insect emergence (Fig. 2; Appendices S3 and S4). Warming stimulated the earlier emergence of adult insects (Fig. 2; warming × time: $P = 0.0005$; Appendices S3 and S4), and this effect was stronger in the presence of fish (Fig. 2; fish × warming × time: $P = 0.018$). The early spring peak of emergence was much less apparent in fish tanks (Fig. 2), resulting in a low biomass of emergence that extended into the summer (fish × time: $P = 0.0003$; Appendices S3 and S4).

In addition to influencing the magnitude and phenology of emergence, warming, nutrients and fish also modified the composition of the adult insect community (Fig. 3; Table 1). Fish substantially reduced the emergence of *Callibaetis* mayflies (97%) but the emergence of ephydrid flies increased (45%) in the mesocosms with fish (Fig 3). Chironomid midges were also negatively affected by fish (62% reduction; Univariate ANOVA, fish effect: $F_{1,32} = 48.38$, $P < 0.0001$), but chironomids did not contribute to the separation of treatments in the RDA (Fig 3) as they numerically dominated emergence across all treatments (Appendix S2). *Mystacides* caddisflies were prevalent in the emergence from warmed, enriched tanks (Fig. 3) and more libellulid
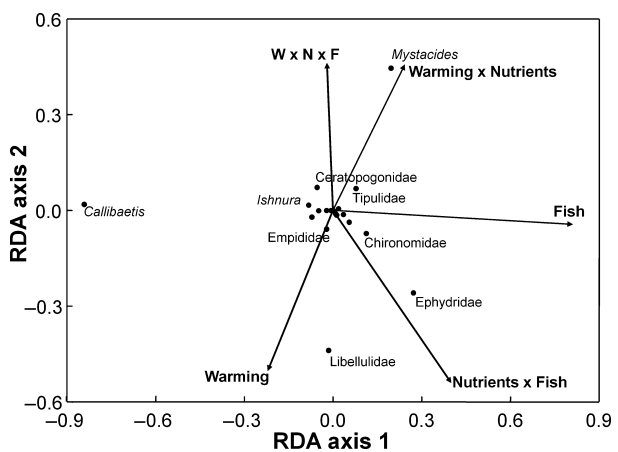
dragonflies emerged from warmed, unfertilized tanks (Fig. 3).

Aquatic decomposition of terrestrial detritus

The treatments interactively affected the decomposition rate of terrestrial leaves in aquatic ecosystems. Fish reduced leaf decomposition in spring and summer (Fig. 4; Appendix S5; fish: $P = 0.0007$), and this effect was stronger in the presence of nutrient enrichment (fish × nutrient: $P = 0.013$). Warming increased leaf decomposition (Fig. 4; Appendix S5; warming: $P = 0.0001$) consistently in the spring and the summer (warming × season, $P = 0.24$). Leaf decomposition increased from spring to summer, regardless of warming (season: $P = 0.0001$). The effect of nutrient enrichment varied seasonally (nutrient × season: $P = 0.0002$; Appendix S5). Nutrients had a marginally significant positive effect on leaf decomposition in the spring (spring nutrient effect: $P = 0.051$), but decreased decomposition in summer (summer nutrient effect: $P < 0.0001$; Appendix S5).

Fish reduced the biomass of primary consumers in leaf packs during the spring (Fig. 5a and b; spring fish effect: $P = 0.009$), but not the summer (Fig. 5c and d; fish × season: $P = 0.007$; Appendix S6). Similarly,
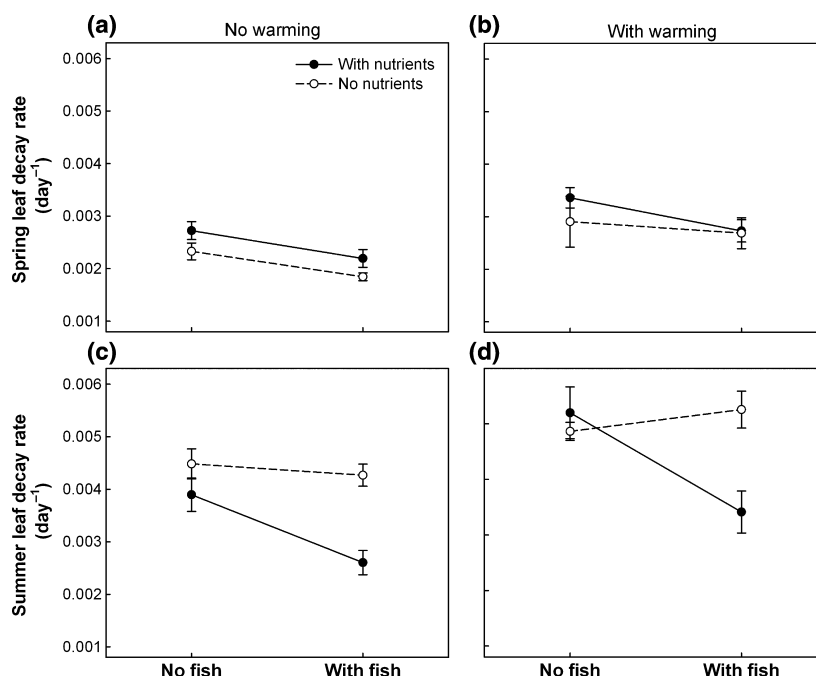
Fig. 4 Three-way interaction plots illustrating the impact of warming, fish predation, and nutrient enrichment on the mean (± 1 SE) decay rates of terrestrial leaf detritus in experimental mesocosms during (a, b) the spring and (c, d) summer. Decay rate (k) is the slope of a negative exponential decay curve describing the proportion of leaf ash-free dry mass lost over time (days).

nutrients increased the biomass of primary consumers (nutrient effect: $P = 0.0013$; Appendix S6), but this effect was strongest in spring (Fig. 5a and b; nutrient × season: $P = 0.0058$). Warming had a weak negative effect on consumer biomass in spring (Fig. 5a; spring warming effect: $P = 0.018$), but this effect was not apparent in summer (Fig. 5d; warming × season: $P = 0.0041$) because of the positive interaction between warming and nutrients in this season (warming × nutrient × season: $P = 0.012$; Appendix S6).

Discussion

Despite the widespread recognition that food webs are open to the movement of resources and organisms from adjacent ecosystems, our understanding of the effects of multiple anthropogenic perturbations on cross-ecosystem fluxes is rudimentary. This uncertainty is compounded by a lack of experiments that investigate how community structure affects the export and receipt of cross-ecosystem subsidies. We demonstrate that fish predators diminish the otherwise strong connection between aquatic and terrestrial ecosystems by reducing the emergence of aquatic organisms and slowing the decomposition of terrestrial material. In contrast, warming and nutrient enrichment strengthened the coupling of terrestrial and aquatic food webs through increasing emergence and leaf decomposition; although

the effect of nutrients on decomposition changed seasonally. Furthermore, warming, nutrient enrichment, and predator loss often combined synergistically to determine magnitude, composition, and phenology of cross-ecosystem fluxes. This indicates that the impacts of global changes such as rising temperatures on cross-ecosystem exchange will depend on local conditions including nutrient status and the abundance of predators.

Multiple perturbations alter aquatic emergence

We observed striking effects of anthropogenic perturbations on fluxes of aquatic biomass to terrestrial ecosystems, particularly with the removal of predatory fish. The suppression of aquatic emergence by fish may have resulted from two complimentary mechanisms: direct predation on benthic larvae and pupae, and reduced colonization from adult insects avoiding oviposition in mesocosms with fish (Vonesh *et al.*, 2009). *Callibaetis* mayflies, which were the most heavily affected by predators, have not been found to avoid habitats with fish (Caudill, 2003), suggesting that direct predation is the most likely explanation for the fish effects. In the presence of fish, nutrients had little effect on the biomass of emerging insects and frogs, despite increased primary production in the benthic (Appendix S7) and limnetic zone (P. Kratina & H. Greig, unpublished data).
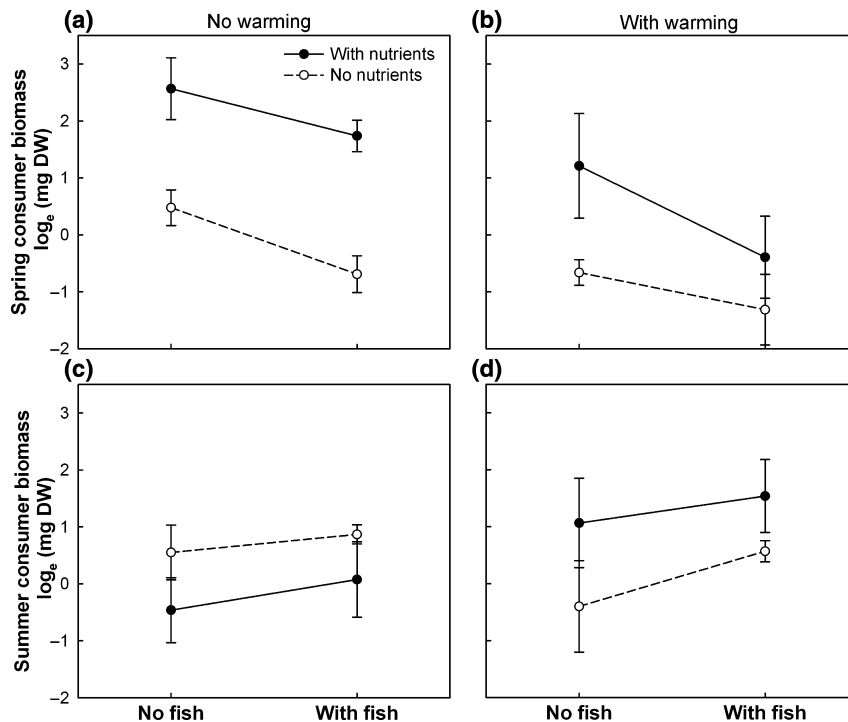
Fig. 5 Three-way interaction plots illustrating the impact of warming, fish predation, and nutrient enrichment on the mean (± 1 SE) biomass of primary consumers in leaf packs during (a, b) the spring and (c, d) summer.

However, when fish were absent, both warming and nutrient enrichment increased the biomass flux of aquatic insects to the terrestrial environment. These results indicate synergisms between predator loss and bottom-up perturbations on cross-ecosystem subsidies, whereby the loss of predatory fish may erode top-down control of aquatic ecosystems, and intensify the impact of eutrophication on both aquatic and adjacent terrestrial ecosystems.

The magnitude of the changes we observed in cross-ecosystem biomass transfer (up to a 97% reduction in frog biomass by fish) can have substantial consequences for recipient food webs. For example, experimental reductions in aquatic insect emergence of ca. 60% decreased the density and growth rates of riparian consumers (Sabo & Power, 2002; Marczak & Richardson, 2007) and altered the strength of trophic interactions in riparian ecosystems (Sabo & Power, 2002). In addition, theoretical studies suggest that while weak to moderate subsidies can contribute to food web stability through weakening trophic interactions (Huxel *et al.*, 2002), large increases in the biomass of subsidies, such as we observed with nutrient addition in the absence of fish, may decouple consumer–resource interactions in the recipient environment and reduce ecosystem stability (Huxel & McCann, 1998; Huxel *et al.*, 2002).

Although our results indicate that predatory fish disconnect aquatic and terrestrial food webs, fish can utilize terrestrial subsidies by consuming falling terrestrial arthropods (Baxter *et al.*, 2005). However, in the absence of fish, terrestrial arthropods can be rapidly incorporated into pond and stream food webs as detritus (Nowlin *et al.*, 2007; Menninger *et al.*, 2008). Thus, consumption of terrestrial arthropods by fish may be more important in determining the trophic level at which terrestrial subsidies are incorporated than the magnitude of subsidy inputs.

Changes in the biomass of aquatic subsidies were accompanied by shifts in taxonomic composition that may influence uptake rates and alter the trophic level at which subsidies are incorporated. For example in our study, fish disproportionally reduced the abundance of small-bodied, non-feeding adult insects (mayflies and chironomids) emerging from aquatic habitats. These weakly flying taxa can constitute the majority of riparian bird and arthropod diets (Hering & Plachter, 1997; Epanchin *et al.*, 2010) and they can also be easily captured by the full range of web architectures and body sizes of riparian spiders (Kato *et al.*, 2004; Akamatsu *et al.*, 2007). Compositional shifts toward large predatory insects may therefore decrease the uptake of subsidies by many riparian consumers and potentiality alter direct and indirect effects of predators

in recipient food webs (Knight *et al.*, 2005; McCoy *et al.*, 2009).

Our study revealed important changes to the phenology of resource subsidies due to warming and predation. Warming advanced the spring pulses of aquatic emergence, probably by accelerating larval growth rates and advancing cues for metamorphosis (Harper & Peckarsky, 2006). In contrast, predators usurped the spring pulse of emergence (primarily *Callibaetis* mayflies) that dampened seasonal fluctuations and delayed peak insect emergence to late summer. These phenological shifts are likely to alter the dynamics of consumer–resource interactions in recipient food webs. Pulses of subsidies that are out of phase with *in situ* resources stabilize recipient consumer populations, but these effects rely on precise asynchronies between resources (Takimoto *et al.*, 2002). In temperate zones where our experiment was conducted, many terrestrial consumers time migration and reproductive activity to take advantage of the spring emergence of aquatic insects when terrestrial prey is otherwise scarce (Nakano & Murakami, 2001; Uesugi & Murakami, 2007; Epanchin *et al.*, 2010). Consequently, changes in the timing and magnitude of spring pulses of emergence are likely to have implications for the growth and reproduction of recipient consumers (e.g., Marczak & Richardson, 2008). Phenological mismatches that occur when interacting organisms respond to different life history cues are disrupting species interactions where seasonal temperature cues have changed (Visser & Both, 2005; Yang & Rudolf, 2011). Our findings indicate that the same mechanisms may impact the temporal dynamics of cross-ecosystem subsidies under climate warming. Long-term field patterns, such as advancing adult dragonfly flight periods with climate warming in Europe (Hassall *et al.*, 2007), indicate that phenological shifts in subsidies are already occurring globally.

Decomposition of terrestrial subsidies in aquatic ecosystems

Warming and predatory fish both influenced the uptake of terrestrial carbon in aquatic food webs. Fish predation indirectly reduced leaf decomposition rates, and these cascading effects were stronger with nutrient enrichment. Reduced macroinvertebrate biomass in spring leaf packs with fish is consistent with a trophic cascade mediated by predation on macroconsumers (Greig & Mcintosh, 2006). However, fish did not influence the biomass of macroinvertebrates in summer. It is possible that the effects of fish on summer decomposition resulted from feedbacks between phytoplankton and detritus energy pathways (Leroux & Loreau, 2010). Sedimentation from phytoplankton blooms associated

with pelagic trophic cascades (P. Kratina & H. Greig, unpublished data) may have generated anoxic conditions within the benthic sediments that reduced decomposition. Overall, our results indicate that reduced top-down control from predator loss should increase the uptake of terrestrial carbon into aquatic consumer pathways.

Warming increased leaf decomposition rates in both seasons. Although we cannot directly compare decomposition caused by microbes and macroinvertebrates, the lack of warming effects on macroinvertebrate biomass suggests elevated microbial decomposition was the primary mechanism. This result agrees with laboratory and field experiments that show decomposition by microbes is strongly temperature dependent (Petchey *et al.*, 1999; Boyero *et al.*, 2011). Microbial biofilms are often targeted by macroinvertebrate consumers, so it possible that warming, by elevated microbial biomass, also enhanced decomposition by macroinvertebrates (Gessner *et al.*, 1999).

Recent advances in the understanding of global carbon cycles have highlighted the critical role of lakes and wetlands in long-term carbon storage (Cole *et al.*, 2007; Tranvik *et al.*, 2009). Changes in carbon sequestration in aquatic systems can therefore have important consequences for the global biogeochemical cycles. Our results support the contention that warming accelerates the decomposition and mineralization of particulate carbon in aquatic sediments (Flanagan & Mccauley, 2010; Gudasz *et al.*, 2010; Yvon-Durocher *et al.*, 2010; Boyero *et al.*, 2011) which has the potential to liberate substantial quantities of carbon to the atmosphere and form a positive feedback to accelerate future warming. Fluxes of organic matter to freshwaters are expected to increase with warming (Larsen *et al.*, 2011), fueling further carbon emissions from inland waters. Predicting the response of landscape level biogeochemical cycles to warming and other perturbations will benefit from considering ecosystems as interacting patches within a broader meta-ecosystem (Massol *et al.*, 2011), where dynamics within patches, and spatial flows between them are equally important.

We designed our study to mimic the loss of predators in aquatic ecosystems, but our experiment also provides insight to predator invasions. Invading piscivorous fish generally depress the abundance planktivorous and benthivorous fish (Carpenter & Kitchell, 1993), likely enhancing cross-ecosystem exchanges in a similar fashion to predator loss in our experiment. Conversely, the introduction of fish into previously fishless habitats is likely to increase top-down control on aquatic organisms. Our results indicate that fish invasions are likely to disrupt previously strong aquatic-terrestrial linkages from the abundant aquatic

emergence and rapid breakdown of terrestrial subsidies. Such invasions may become more prevalent as warming facilitates range expansions of predators to high latitude waterways (Lassalle & Rochard, 2009) and their consequences are already evident in previously fishless alpine lakes (Knapp *et al.*, 2007; Pope *et al.*, 2009; Epanchin *et al.*, 2010).

Conclusions

Our experiment suggests that anthropogenic impacts can operate at broad spatial scales by influencing the transmission and receipt of subsidies across ecosystems. Moreover, effects of simultaneous perturbations were often synergistic rather than independent, indicating that the effects of global changes such as warming will depend on local resource supply or top-down control. In particular the erosion of top-down control from predator loss will likely enhance the effects of bottom-up perturbations on cross-ecosystem interactions. Predicting the future state of ecosystems not only rests on understanding interactions among multiple anthropogenic perturbations, but also interactions among adjacent ecosystems that are mediated by spatial flows of resources.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Mean daily water temperature in mesocosms with (warmed) and without (unwarmed) continuous heating with a 300 W submersible aquarium heater, over the course of the 17-month experiment (June 2009–September 2010). Warming increased the daily average water temperature by 2.98 ± 0.07 °C (mean ± SE).

Appendix S2. The taxonomic composition of adult insects emerging from the mesocosms over the duration of the experiment.

Appendix S3. Mixed effects linear model of the biomass flux of emerging adult aquatic insects (mg × m² × day) over 7 months. Time refers to each of the fourteen 2-weekly intervals. The model included an AR(1) error structure to account for first-order temporal autocorrelation. Comparisons of models with AIC and plots of temporal autocorrelation indicated the AR(1) error structure significantly improved model fit.

Appendix S4. Cumulative proportional aquatic insect emergence for (a) control, (b) nutrient, (c) fish, and (d) nutrients + fish treatments with and without warming. Curves are drawn from mean (± SE) cumulative emergence proportions of five replicate mesocosms at each date. Warming accelerated emergence (steeper curve) in all treatments except nutrients + fish (d), and the effect was strongest in the presence of fish (c). Fish delayed insect emergence [lower slopes in (c) and (d)] especially in the absence of nutrients and warming (c).

Appendix S5. Summary of the mixed effects linear model of log_e-transformed leaf decomposition rates (k day^{-1}) in spring and summer (Season main effect). Models were run with and without an AR(1) error structure to account for first-order temporal autocorrelation. Plots of temporal autocorrelation indicated weak autocorrelation between seasons (<0.4) which did not change when an AR(1) error structure was included. Consequently, the AR(1) error term was not retained in the final model.

Appendix S6. Mixed effects linear model of the mean biomass of primary consumers in leaf packs in spring and summer (Season main effect). Models were run with and without an AR(1) error structure to account for first-order temporal autocorrelation. Plots of temporal autocorrelation indicated weak autocorrelation between seasons (<0.4) which did not change when an AR(1) error structure was included. Consequently, the AR(1) error term was not retained in the final model.

Appendix S7. Mean biomass of periphyton (as chlorophyll-*a*) on benthic tiles. One 25 cm² terracotta tile was added to each tank on 9 June 2009 and was removed on 4 February 2010. Tiles were brushed and filtered on to a Whatman GF/F filter paper, which was then immersed in 90% acetone to extract pigments. Biomass of chlorophyll-*a* was estimated with the nonacidification method on a Turner Trilogy Flourometer. Factorial ANOVA revealed strong positive effect of nutrients ($P < 0.0001$) on periphyton biomass. No other effects were significant.

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