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Multiple anthropogenic stressors cause ecological surprises in boreal lakes

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Abstract

The number of combinations of anthropogenic stressors affecting global change is increasing; however, few studies have empirically tested for their interactive effects on ecosystems. Most importantly, interactions among ecological stressors generate nonadditive effects that cannot be easily predicted based on single-stressor studies. Here, we corroborate findings from an *in situ* mesocosm experiment with evidence from a whole-ecosystem manipulation to demonstrate for the first time that interactions between climate and acidification determine their cumulative impact on the food-web structure of coldwater lakes. Interactions among warming, drought, and acidification, rather than the sum of their individual effects, best explained significant changes in planktonic consumer and producer biomass over a 23-year period. Further, these stressors interactively exerted significant synergistic and antagonistic effects on consumers and producers, respectively. The observed prevalence of long- and short-term ecological surprises involving the cumulative impacts of multiple anthropogenic stressors highlights the high degree of uncertainty surrounding current forecasts of the consequences of global change.

Keywords: acidification, boreal lakes, climate warming, drought, global change, nonadditive effects, phytoplankton, zooplankton

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Introduction

Multiple anthropogenic stressors drive the cumulative impacts of global change on biodiversity and ecosystem function (Sala *et al*., 2000; Steffen *et al*., 2004). In particular, stressors are expected to exert complex interactive effects given the ubiquity of ecological trade-offs (Kneitel & Chase, 2004), stress-induced tolerances (Blanck, 2002), and differences in environmental sensitivity among trophic levels (Vinebrooke *et al*., 2003; Petchey *et al*., 2004; Raffaeli, 2004). Unfortunately, interactions among multiple stressors cannot be easily modelled because they generate net impacts that either exceed (i.e. synergism) or fall below (i.e. antagonism) their expected additive effects (Folt *et al*., 1999). Our understanding of the ecological effects of global change remains limited by lack of both theory (see Vinebrooke *et al*., 2004) and empirical evidence from only a few multifactorial studies of aquatic (e.g. Doyle *et al*., 2005; Persaud & Williamson, 2005; Przeslawski *et al*., 2005) and terrestrial (e.g. Zavaleta *et al*., 2003; Hanson *et al*., 2005; Henry *et al*., 2005) ecosystems. Consequently, global change is often characterized by nonadditive *ecological surprises* (sensu Paine *et al*., 1998).

Several scenarios demonstrate the high probability of interactions among ecological stressors causing nonadditive impacts on lake ecosystems. For instance, warming antagonistically suppresses ultraviolet-B (UV-B) effects in certain organisms by enhancing photo-enzymatic repair (Williamson *et al*., 2002; MacFadyen *et al*., 2004). Conversely, warming may synergistically amplify the adverse effects of UV-B on organisms that rely on cold-induced photoprotective pigmentation (Hairston, 1979). Further, acidification may antagonistically dampen biological effects of other stressors by inducing a shift towards smaller body size, which is a common trait of many stress-tolerant organisms (Cattaneo *et al*., 1998; Vinebrooke *et al*., 2004). Also, differential stressor effects on adjacent trophic levels can alter their interac-

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tions, thereby further increasing the probability of complex nonadditive cumulative impacts. Therefore, a high degree of uncertainty exists in predicting the cumulative impacts of anthropogenic stressors on an ecosystem because stress-induced species tolerances result in antagonistic responses, while ecological trade-offs generate synergy.

Boreal landscapes around the world contain the largest number of lakes that are susceptible to the interactive effects of climate change and acid deposition (Schindler *et al*., 1990, 1996; Schindler, 1998). Warmer and drier climatic conditions together with acid rain suppress concentrations of terrestrially derived, light-attenuating dissolved organic carbon (DOC) in lakes, thereby increasing their sensitivities to warming events (Snucins & Gunn, 2000) and biologically damaging UV-B (Schindler *et al*., 1996). Drought-induced declines in DOC also expectedly stress northern lake ecosystems because of their heterotrophic dependency on terrestrial subsidies (del Giorgio *et al*., 1999). Drought also synergistically increases the impacts of acidic sulphur deposition on lakes by causing pronounced reacidification events and loss of UV-attenuating DOC (Yan *et al*., 1996; Clark *et al*., 2005). However, it is unknown whether these well-documented interactions among abiotic stressors cause simple additive or relatively unpredictable nonadditive impacts on ecosystem functioning.

We hypothesized that interactions among warming, drought, and acidification, rather than the sum of individual effects, determine their cumulative impact on planktonic consumers and producers in boreal lakes. Our rationale for these expected nonadditive effects involved how ecological trade-offs (Kneitel & Chase, 2004) and stress-induced tolerances (Blanck, 2002) amplify or suppress the additive impacts of stressors, respectively (Vinebrooke *et al*., 2004). Also, we hypothesized that these stressors would exert a more pronounced synergistic impact effect on consumers based on several lines of evidence. Taxonomic, physiological, and genetic diversity typically decline with increasing trophic rank (Rosenzweig, 1995; Pace, 1997; Feldgarden *et al*., 2003), resulting in higher trophic levels containing less *biological insurance* (sensu Yachi & Loreau, 1999) against the impacts of environmental stressors (Petchey *et al*., 2004; Raffaeli, 2004). For example, several studies have demonstrated that consumers are adversely impacted while producers benefit from exposure to single stressors, such as higher temperatures (Beisner *et al*.,

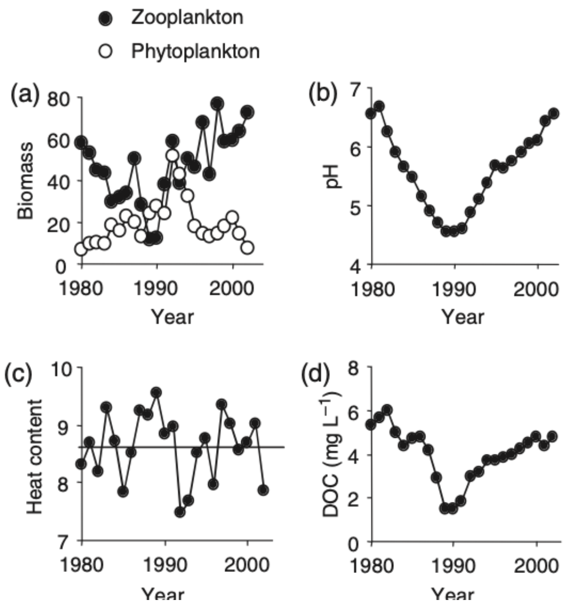
Materials and methods

Study area

Our investigation of the ecological impacts of anthropogenic stressors focused on Lake 302 South (49°40′N, 94°45′W) at the experimental lakes area (ELA) in northwestern ON, Canada. Lake 302S was the site of a whole-lake experiment from 1980 to 1990 when additions of sulphuric acid lowered its pH from 6.8 to 4.5 and reduced DOC from 6 to 1.5 mg L^{-1} (Fig. 1). Chemical and biological recovery occurred during the 1990s (Findlay *et al*., 1999) with pH returning to preacidification levels by 2003. In addition, climate warming during the 1980s resulted in warmer and drier conditions in the ELA, which further decreased DOC and increased underwater warming and penetration of UV-B (Schindler *et al*., 1996; Fig. 1). Thus, this ecosystem-level investigation simulated the effects of acidification, drought, and warming, which similarly affect a wide range of other experimentally (Frost *et al*., 1999) and atmospherically acidified lakes (Arnott *et al*., 2001).

Biomonitoring of Lake 302S

Lake 302S was sampled on a bi-weekly basis during the ice-free season (May–October) from 1980 to 2003 at a



2003 for details). For the purposes of this paper, we examined the abiotic variables of temperature, DOC, and pH, and both planktonic consumer and producer biomass. To account for the movement of some biota throughout the water column, we used the lake's heat content as an integrated measurement of lake temperature. Increases in heat content corresponded to higher air temperatures of 1–2 °C, and increased hypolimnetic warming of ~2 °C (Findlay *et al.*, 2001; data not shown). Each variable was calculated as an annual average.

Mesocosm experiment

A three-factor experimental design consisting of two treatment levels of acidification (pH 6.7 vs. 5.0), DOC (5 vs. 3 mg L^{-1}), and temperature (control vs. an average increase equalling 32° days) was performed in triplicate, and randomly assigned among 24 mesocosms (1500 L capacity). All polyethylene-lined mesocosms were suspended from a floating wooden frame in Lake 302S, and filled with 64 μm sieved lake water on 9 July 2004. Sulphuric acid was gradually added over a 3-day period to acidify 12 mesocosms. Warming was achieved by trapping solar heat using 98% UV-transparent acrylic canopies, which were positioned on top of 12 double-walled insulated mesocosms. Greenhouse canopies were sealed on top of warmed mesocosms using weather stripping, and suspended 2 cm above the other 12 noninsulated units to allow for convective heat loss. Supplementary warming was performed at regular 3-day intervals using 20 L water carboys filled with heated water from a propane water tank positioned on the shoreline. Drought was simulated in mesocosms by diluting with 10 μm sieved lake water that had been passed through a polyethylene bucket containing 75 kg of activated charcoal, which removed 40% of the DOC and doubled the penetration of UV-B from 0.75 to 1.5 m depth without significantly affecting low ambient concentrations of total dissolved phosphorus (<10 μg L^{-1} in all mesocosms).

All mesocosms were then inoculated with equal densities of plankton that were representative of ambient lake conditions at the start of the experiment. The planktonic inoculum was concentrated at the midlake station by pooling the contents obtained from vertical 9 m hauls with 10 and 64 μm mesh-sized plankton nets. Abiotic and biotic response variables were sampled on Days 0, 10, 30, and 50. Inverted light microscopy was used to enumerate phytoplankton, and total biomass calculated by converting precise geometrically derived taxonomic volumes using a specific gravity of one. Zooplankton taxa were counted using a stereomicro-

Statistical analyses

Backward stepwise regressions using temperature (heat content), DOC, and pH were performed to identify significant environmental predictors of log-transformed annual total planktonic consumer and producer biomass from the 23-year experiment in Lake 302S. An α value of 0.05 was used as the criterion for removing variables from the model. To determine the role of interactions among multiple stressors and the relative utility of an additive vs. nonadditive approach, a subsequent regression analysis involving both individual predictors and their interactions (i.e. temperature-DOC, temperature × pH, DOC × pH, and temperature-DOC × pH) was performed, and the two models were compared using adjusted R^2 values to determine the most parsimonious model. As expected, interaction terms were highly correlated with single predictors. To reduce colinearity, we rescaled the independent variables by centering (i.e. subtracting the mean from each value) (Aiken & West, 1991). Tolerance values and Durbin–Watson statistics were subsequently used to test for colinearity and autocorrelation, respectively.

Repeated-measures analyses of variance (RM-ANOVAS) were conducted to test for the interactive effects of warming, drought, and pH on log-transformed total zooplankton and phytoplankton biomass during the mesocosm experiment. Although detection of significant higher order interactions precluded using the same statistical results to examine lower order interactions and main effects (Zar, 1999), these were quantified to determine the nature (i.e. synergy or antagonism) of higher-order interactions. Therefore, significant three-way interactions were examined using a series of protected (sensu Scheiner, 2001) lower-order ANOVAS. For example, a significant three-way interaction was followed-up with ANOVAS using only controls and groups exposed to single stressor to determine the effect of each stressor. The observed combined impact of all three stressors was then compared with their expected net additive effect, which was based on the sum of their individual effects. If the observed combined impact of stressors exceeded their expected additive effect [e.g. (stressor$_1$−control) + (stressor$_2$−control)], then the interaction was defined as being synergistic. In contrast, if the observed impact was less than the additive effect, then the interaction was denoted by antagonism. To illustrate the nature of the interactive impact, we show graphically the difference between the observed [(stressor$_{1\times2}$−control)] and predicted additive effects, which indicates the direction and magnitude of the interaction.

Consumer biomass	Additive	pH	0.204	1.000	0.001	0.001	0.411	1.301
	Interactive	pH	0.176	0.963	< 0.001	< 0.001	0.615	2.018
		Temp. × DOC	0.147	0.963	0.002			
Producer biomass	Additive	Temp.	−0.120	0.972	0.048	< 0.001	0.545	1.382
		pH	−0.245	0.972	0.001			
	Interactive	pH	−0.249	0.982	< 0.001	< 0.001	0.689	1.728
		Temp. × pH	0.279	0.982	0.001			

Additive models considered only three independent variables (DOC, pH, temp.), while interactive models contained both the single predictors and all of their possible combinations (temp. × DOC, temp. × acid, DOC × acid and temp. × DOC × acid). Temp. = Heat content of lake.

D–W, Durbin–Watson statistic; DOC, dissolved organic carbon.

Results and discussion

Interactions among temperature, DOC, and pH were significant predictors of planktonic consumer and producer biomass in Lake 302S (Table 1). Inclusion of interaction terms increased the predictability of additive models by 20.4% for consumers, and 14.4% for producers. Partial regression coefficients showed that the interaction between warming and DOC together with higher pH best explained increases in total consumer biomass, particularly during the late 1990s (Fig. 1). In contrast, a significant positive temperature–pH interaction term suggested that warming reversed the positive influence of acidification on total producer biomass, which was most evident as phytoplankton was more abundant under acidified conditions during the colder early 1990s than in the warmer late 1980s (Fig. 1). Although these results revealed the potential direction of the impacts of interactions among ecological stressors in Lake 302S, they did not identify the nature (i.e. synergy vs. antagonism) of the interactive effects, and therefore, a multifactorial experiment was warranted.

Our mesocosm experiment also demonstrated that interactions among stressors, rather than individual direct effects, determined their net impact on consumer abundance. Specifically, warmer temperatures stimulated total zooplankton biomass only under conditions of simulated drought and high acidity towards the end of the experiment (Fig. 2a; RM-ANOVA, time–temperature–drought–acid interaction, $F_{2,32} = 11.59$, $P < 0.001$). Interactions among the three stressors synergistically amplified total biomass by 130–240% above that predicted based on the sum of their individual main effects (i.e. additive model) during the experiment (Fig. 3a). Most importantly, these experimental results agreed with the transient increase in zooplankton abundance that was observed in Lake 302S during the advanced stages of acidification, which coincided with warmer and drier conditions in the late 1980s (Fig. 1).

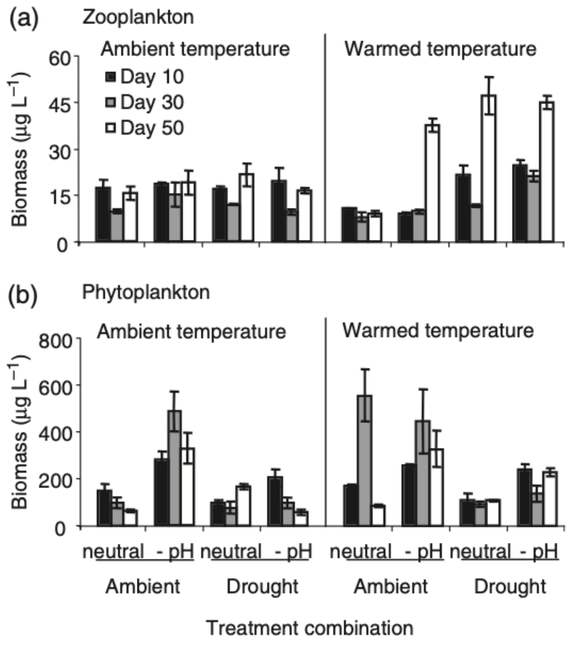


Fig. 2 Interactive and direct effects of warming, drought, and acidification on total biomass of (a) planktonic consumers and (b) producers during a 50-day mesocosm experiment. Error bars denote ±1 SE ($n = 3$).

The synergistic positive impact of warming, drought-induced UV-B exposure, and acidity on consumer biomass was, however, unexpected based on earlier reports of their individual negative effects on zooplankton (Moore *et al.*, 1996; Williamson *et al.*, 2002; Vinebrooke *et al.*, 2003; Strecker *et al.*, 2004). The positive response of consumers to these triple stressors

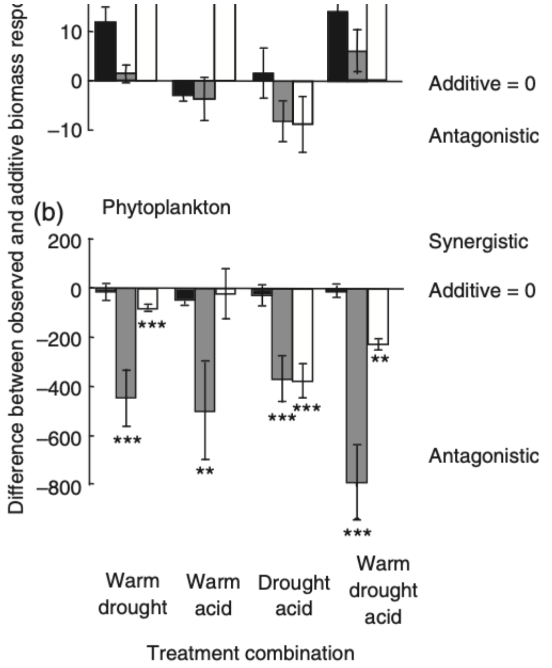


Fig. 3 The nature (synergy vs. antagonism) and magnitude of the observed interactive impacts of warming, drought, and acidification on total planktonic (a) consumer and (b) producer biomass compared with the sum of their individual effects (i.e. additive model). Error bars denote ± 1 SE ($n = 3$ for the three-way interaction; $n = 6$ for all two-way interactions). $^{**}P < 0.05$, $^{***}P < 0.01$.

was primarily attributable to stimulation of a single stress-tolerant herbivore species (*Daphnia catawba*). *D. catawba* had also become the most abundant planktonic consumer in Lake 302S (Vinebrooke *et al.*, 2003) when lake temperatures, acidity, and risk of UV-B damage were elevated during the late 1980s (Fig. 1). Warming enhances photoenzymatic repair in this species (Williamson *et al.*, 2002), offering a potential partial explanation for the positive effect of temperature under simulated drought conditions and elevated exposure to UV-B. Further, *D. catawba* is also highly efficient under conditions of severe food limitation (Tessier, 1986), possibly enabling it to benefit more than its acid-sensitive competitors from the positive effects of warming on feeding rates (Moore *et al.*, 1996) and growth (Gillooly, 2000). Warming can also increase the amplitude of fluctuations in daphnid populations, resulting in dramatic boom-bust cycles (Beisner *et al.*, 1997). Therefore,

interactive effect on producer biomass during the mesocosm experiment (Fig. 2b; RM-ANOVA, temperature–drought-acid interaction, $F_{2,32} = 13.79$, $P = 0.002$). Warming stimulated the interactive effect of drought and acidification on total phytoplankton biomass. However, protected ANOVA revealed that both the three-way and lower-order interactions involved warming and drought antagonistically suppressing the otherwise positive effect of acidification on producer biomass (Fig. 3b). These antagonistic interactions reduced the net impact of the three stressors on phytoplankton abundance by 170–750% below their potential additive effect, especially during the latter half of the experiment (Fig. 3b).

The positive triple-stressor impact on producer biomass in the mesocosms corresponded with increases in phytoplankton abundance during the acidification of Lake 302S (Fig. 1). In both cases, filamentous green algae (*Mougeotia* spp.) proliferated under conditions of increased acidity, warmer lake temperature, and elevated exposure to UV-B. Similarly, warmer and drier conditions during the late 1980s promoted the abundance of filamentous green algae along the shoreline of the nearby circumneutral reference Lake 239 (Schindler *et al.*, 1990). We expect that the positive effect of moderate warming on photosynthetic rates (Davison, 1991) stimulated this acidophilic primary producer. Also, drought- and acidification-induced declines in DOC improved light availability in both the mesocosms (data not shown) and Lake 302S (Schindler *et al.*, 1996), likely further stimulating acid-tolerant producers that were cotolerant of concomitant increases in biologically damaging UV-B. However, producers in the mesocosms were suppressed by the combined effect of drought and acidification. This antagonistic impact could reflect photo-inhibition of producers owing to the synergistic positive effect of drought and acidification on light and UV-B exposure (Schindler *et al.*, 1996; Yan *et al.*, 1996).

The contrasting synergistic and antagonistic triple-stressor impacts on consumers and producers could not be attributed to the mediating influence of a trophic interaction. Lack of a significant correlation between total consumer and producer biomass during the experiment ($r^2 = 0.002$ $P = 0.74$) and in Lake 302S ($r^2 = 0.04$, $P = 0.35$) supported other reports that consumer populations are too sparse to exert a significant impact on producers in unproductive ecosystems (Elser & Goldman, 1991). The weak linkage between consumers and producers in the mesocosms was not surprising given the predominance of large inedible algae and small filter-feeding herbivores. Instead, contrasting impacts of ecological stressors on consumers and producers likely reflect the greater sensitivity of higher trophic levels owing to their relative lack of physiological acclimation, genetic adaptation, and species diversity (Vinebrooke *et al*., 2003; Petchey *et al*., 2004; Raffaeli, 2004).

Our results highlight the prevalence and magnitude of interactions among anthropogenic stressors, and how their cumulative impact can generate ecological surprises in lakes. Similarly, other studies have demonstrated the complex nonadditive impacts of multiple stressors in marine ecosystems (Hoffman *et al*., 2003; Przeslawski *et al*., 2005). Likewise, interactive effects of ecological stressors have been reported for terrestrial ecosystems. For example, experimentally derived physiological responses by hardwood forests to the interactive effects of temperature, precipitation, ozone, and CO_2 did not support predictions of increased carbon exchange derived from additive models (Hanson *et al*., 2005). In grassland ecosystems, excess nutrients synergistically amplified the impact of drought on plant communities by causing competitive extirpation of stress-tolerant species (Tilman & Downing, 1994). In contrast, other studies have demonstrated that ecologically distinct stressors can cause more predictable additive effects on terrestrial ecosystems. For example, elevated nitrogen and CO_2 levels exerted additive effects on total plant biomass (Reich *et al*., 2001) and grassland biodiversity (Zavaleta *et al*., 2003) likely because each stressor independently affected a different biological property. Nevertheless, our findings reveal that unexpected ecological surprises can occur in ecosystems that are perturbed by a single major stressor (e.g. acidification) owing to interactions with other concomitant stressors (Frost *et al*., 1999; Arnott *et al*., 2001). Clearly, forecasts of the cumulative impacts of global change on biodiversity and related ecosystem processes will remain highly unreliable without further investigation into the nature of interactions among key anthropogenic stressors in different biomes around the world (Sala *et al*., 2000; Steffen *et al*., 2004; Hanson *et al*., 2005).

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