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## Warming alters food web-driven changes in the CO<sub>2</sub> flux of experimental pond ecosystems

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1 **Title:** Warming alters food web-driven changes in the CO<sub>2</sub> flux of experimental pond  
2 ecosystems

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21 **Evidence shows the important role biota play in the carbon cycle, and strategic**  
22 **management of plant and animal populations could enhance CO<sub>2</sub> uptake in aquatic**  
23 **ecosystems. However, it is currently unknown how management-driven changes to**  
24 **community structure may interact with climate warming and other anthropogenic**  
25 **perturbations to alter CO<sub>2</sub> fluxes. Here we show that under ambient water temperatures,**  
26 **predators (Three-Spined Stickleback) and nutrient enrichment synergistically increased**  
27 **primary producer biomass, resulting in increased CO<sub>2</sub> uptake by mesocosms in early**  
28 **dawn. However, a 3°C increase in water temperatures counteracted positive effects of**  
29 **predators and nutrients leading to reduced primary producer biomass, and a switch from**  
30 **CO<sub>2</sub> influx to efflux. This confounding effect of temperature demonstrates that climate**  
31 **scenarios must be accounted for when undertaking ecosystem management actions to**  
32 **increase biosequestration.**

33

34 Keywords: trophic cascades, biosequestration, carbon cycling, climate change

## 35 1. INTRODUCTION

36 In freshwater ecosystems, projected increases in water temperatures (3-5°C) [1] are likely  
37 to interact with bottom-up and top-down processes to modify community structure [2] and CO<sub>2</sub>  
38 dynamics [3,4]. Heterotrophs contribute to the net carbon balance of ecosystems by consuming  
39 organic matter and respiring it as CO<sub>2</sub>. Predators and herbivores can further influence carbon  
40 balance by directly or indirectly (via trophic cascades) shifting the balance between heterotrophic  
41 respiration and photosynthesis. However, warming and eutrophication can modify food web  
42 structure by increasing species extinctions, especially at higher trophic levels [5], altering species

43 interactions. Phytoplankton blooms caused by eutrophication and trophic cascades can only  
44 enhance long-term carbon storage if plant matter escapes mineralization and is buried in  
45 sediments; however, higher water temperatures increase metabolism and remineralization rates.  
46 Because temperature modifies food web structure with consequences for CO<sub>2</sub> assimilation and  
47 remineralization [6], alterations to animal and plant populations could cause complex climate  
48 feedbacks in a warmer world.

49 We tested two hypotheses of how elevated water temperatures could alter the effects of  
50 top-down and bottom-up manipulations on the CO<sub>2</sub> flux of freshwater pond mesocosms.  
51 Mesocosm food webs contained phytoplankton, periphyton, zooplankton, benthic  
52 macroinvertebrates and in treatments containing fish, *Gasterosteus aculeatus* (Three-Spined  
53 Stickleback; Table S1). First, warming would increase the strength of trophic cascades and  
54 decrease mesocosm CO<sub>2</sub> emissions. We predicted that warming would increase indirect positive  
55 effects of predators on primary production [7,8] leading to higher CO<sub>2</sub> influx in treatments  
56 containing predominantly odd-numbered food chains with top predators. Second, warming  
57 would alter interactive effects between eutrophication and predators on primary producers and  
58 CO<sub>2</sub> flux. In a previous study we described the temporal response of phytoplankton to the same  
59 three variables, showing that warming reduced the positive effects of nutrients on primary  
60 producers [7]. We predicted that this interaction between nutrients and warming would lead to  
61 increased CO<sub>2</sub> emissions [9]. However, because our previous study also showed that warming  
62 enhanced top-down control [7], it is difficult *a priori* to predict the combined effects of all three  
63 stressors on CO<sub>2</sub> flux.

## 64 2. MATERIALS AND METHODS

### 65 (a) *Experimental set-up*

66 We manipulated water temperature, nutrients, and the presence of Stickleback in a 2x2x2 design  
67 using 40, open-air, well mixed, 1,136 L Rubbermaid plastic mesocosms (0.6 m deep, 1.5 m in  
68 diameter) to test their independent and interactive effects on consumer biomass, producer  
69 biomass and CO<sub>2</sub> flux. Each treatment was replicated 5 times. Water temperatures were either  
70 ambient or 3.04° ± 0.05° C (mean ± s.e.) above ambient. Mesocosms had either ambient nutrient  
71 levels or were meso-eutrophic (N:P ratio of 22) with monthly additions of both nitrogen and  
72 phosphorus (264 µg of nitrogen/L as NaNO<sub>3</sub> and 27 µg of phosphorus/L as KH<sub>2</sub>PO<sub>4</sub>). Finally,  
73 mesocosms contained either five Stickleback (54.4 ± 0.05 mm standard body length) or no fish.  
74 Detailed experimental methods can be found in Supplementary Material.

#### 75 ***(b) Organism sampling and CO<sub>2</sub> measurements***

76 Primary producer biomass, consumer biomass (not including fish), and water CO<sub>2</sub> concentrations  
77 were collected one year (May) and 16 months (October) following the start of the study.  
78 Phytoplankton and periphyton biomasses and benthic and pelagic consumer biomasses were  
79 combined for total primary producer and total consumer biomasses, respectively.

80 Water samples for dissolved CO<sub>2</sub> were extracted at dawn using 50-mL gas-tight syringes  
81 and headspace equilibrium analysis. CO<sub>2</sub> concentrations were measured on a 5890 Series II gas  
82 chromatograph within 24 h.

83 CO<sub>2</sub> flux (mg C m<sup>-2</sup> d<sup>-1</sup>) to the atmosphere was calculated as follows:

$$84 \quad \text{CO}_{2\text{flux}} = (\text{CO}_{2\text{water}} - \text{CO}_{2\text{sat}}) * k$$

85 Here CO<sub>2water</sub> is the temperature corrected CO<sub>2</sub> concentration of the water, CO<sub>2sat</sub> is the  
86 concentration the water would have if it were at equilibrium with the atmosphere (390 ppm), *k* is

87 the CO<sub>2</sub> exchange velocity coefficient (0.63 m d<sup>-1</sup>), which was estimated using literature values  
88 for our study sites average wind speed (2.8 ± 0.09 m s<sup>-1</sup>)[10].

### 89 *(c) Statistical analyses*

90 Treatment effects on primary producer biomass, consumer biomass, and CO<sub>2</sub> flux were tested  
91 using linear mixed-effects models ( $\alpha= 0.05$ ) in R 3.1.1 (R Development Core Team, 2014).  
92 Individual mesocosms and date were treated as random factors. Date was included as a random  
93 factor to account for non-independence between dates and because our sampling design did not  
94 have sufficient power to describe temporal differences. In order to understand how treatment-  
95 mediated changes to food web structure influenced CO<sub>2</sub> flux, it was imperative to analyse only  
96 dates where pelagic and benthic organisms and CO<sub>2</sub> were sampled at the same time. Because of  
97 the highly destructive nature of benthic sampling on the community (see supplementary for  
98 detailed methods) we limited our collections to only two occasions.

## 99 **3. RESULTS & DISCUSSION**

100 Under ambient water temperatures, we detected independent and interactive effects of nutrients  
101 and predators on total consumer and producer biomass that led to increased CO<sub>2</sub> influx (Table 1,  
102 Fig. 1). Increased CO<sub>2</sub> influx was likely the result of predator- (in predator only) and nutrient-  
103 induced (in nutrient addition treatments only) increases in primary production [9,11]. In the  
104 absence of predators, nutrient additions doubled consumer and plant biomass, which would have  
105 increased both primary production and respiration (Tables S2). However, because increases in  
106 CO<sub>2</sub> influx were still observed in these treatments, the effect of nutrients on CO<sub>2</sub> assimilation  
107 appears greater than their effect on respiration. Fish reduced consumer biomass by 70% and  
108 increased primary producer biomass by 32% (Table S2). Trophic cascades were enhanced by

109 nutrient enrichment, with a 27-fold increase in producer biomass (Table S2). Similar to Cole et  
110 al. [10], mesocosms with both nutrient additions and top predators had the greatest CO<sub>2</sub> influx.

111 In general, warming had strong negative effects on trophic cascade strength and the  
112 influence of nutrients on primary producers, generating top-heavy food webs with reductions in  
113 CO<sub>2</sub> influx (Table 1; Fig. 1). Higher emissions in warmed mesocosms likely resulted from  
114 positive effects of warming on respiration rates [3] and observed negative effects of warming on  
115 primary producers. Contrary to our prediction, warming only strengthened trophic cascades in  
116 non-fertilized mesocosms. In fertilized mesocosms, warming dampened consumer effects  
117 resulting in similar CO<sub>2</sub> fluxes as mesocosms without predators (Fig. 1, Table S2). The negative  
118 effect of warming on primary producers was strongest in mesocosms with nutrient additions and  
119 especially large in mesocosms with nutrients and predators. Warmed mesocosms containing both  
120 added nutrients and predators had 90% less primary producer biomass compared to ambient  
121 temperature mesocosms (Table S2). The negative effect of warming on primary producer  
122 biomass in our study and others [3,12] may have been the result of increased consumption by  
123 herbivores under warmer temperatures [6,8], or the replacement of productive phytoplankton  
124 taxa by stress tolerant, but less productive taxa. Although further studies are needed to  
125 understand the mechanisms by which increased temperatures alter CO<sub>2</sub> cycling, our results  
126 suggest that future increases in temperatures could reduce biosequestration by aquatic  
127 ecosystems.

128 Mesocosms are well suited to explore complex interactions between global change  
129 drivers that are otherwise difficult to study in natural systems. Nevertheless, the use of  
130 mesocosms and our context-dependent results should be considered. First, our mesocosms did  
131 not receive large terrestrial subsidies, which can make up > 50% of the dissolved organic C pool

132 in natural ecosystems [13]. Thus, respiration in natural lentic ecosystems is not constrained by *in*  
133 *situ* primary production, as was the case in our mesocosms. Because respiration rates have a  
134 stronger temperature-dependence than photosynthetic rates [3], the positive effects of warming  
135 on CO<sub>2</sub> efflux seen in our study may be conservative. Second, the effects seen on communities  
136 and ecosystem process in this study may be only short-term, transient responses to perturbations  
137 that could greatly differ from long-term ones. Future studies should investigate whether the  
138 effects seen in our study are consistent across diurnal cycles and longer time scales within a  
139 myriad of aquatic ecosystems. Finally, the direction and magnitude of predator effects on food  
140 webs and carbon cycling may depend on predator identity, food chain length, and species  
141 diversity [8]. Despite these limitations our study is the first to our knowledge to unravel the  
142 cumulative effects of eutrophication, warming, and alterations to top predators on the CO<sub>2</sub> flux  
143 of an ecosystem.

144 Our findings support other studies [9,14] which suggest that maintaining viable predator  
145 populations in odd-numbered food webs and/or adding nutrients could increase natural  
146 biosequestration. However, our results reveal how previously unknown interactions between  
147 warming, nutrients, and changes to top predators could create positive climate feedbacks by  
148 reducing the capacity of top-down and bottom-up forces to reduce the production of *in situ* CO<sub>2</sub>.  
149 The number of freshwater ecosystems experiencing negative or undesirable anthropogenic  
150 impacts is likely to increase in the future due to a combination of a 2.4-2.7 fold increase in  
151 eutrophication [15] and increased rates of trophic downgrading [16]. As most freshwater  
152 ecosystems are sources of CO<sub>2</sub>, our results suggest that CO<sub>2</sub> emissions from aquatic systems  
153 could increase as temperatures rise.

154



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203

204 **Ethics** This work was conducted with the approval by the University of British Columbia's  
205 Animal Ethics Committee (animal ethics permit #A08-0220)

206 **Data accessibility** Raw data are available from the Dryad digital repository  
207 (<http://dx.doi.org/10.5061/dryad.1f090>)

208 **Author contributions** T.B.A., E.H., H.S.G., P.K., J.B.S., J.S.R contributed to study design and  
209 writing of the manuscript. Data were collected by T.B.A., E.H., H.S.G. and P.K. All authors gave  
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216 **Competing interests statement** We have no competing interests.

217

For Review Only

218 **Figure Legend**

219 **Figure 1** Interaction plots illustrating the impacts of warming, nutrient additions, and predators  
220 on community biomass and CO<sub>2</sub> flux of mesocosms. Effects of nutrients and warming on  
221 consumer biomass (a), primary producer biomass (c), and CO<sub>2</sub> flux (e) in food webs where fish  
222 are absent. Effects of nutrients and warming on consumer biomass (b), primary producer  
223 biomass (d), and CO<sub>2</sub> flux (f) in food webs containing fish. Dotted lines in graphs (e) and (f)  
224 represent CO<sub>2</sub> source/sink boundaries. Means  $\pm$  95 % C.I.s represent fixed effects and were  
225 approximated using the “predictSE.lme()” function in the “AICcmodavg” package in *R*.

226

227 **Tables**

228 **Table 1** Summary statistics of linear mixed-effects models for individual and interactive  
 229 effects of warming (W), nutrient additions (N), and predators (P) on consumer biomass,  
 230 primary producer biomass, and CO<sub>2</sub> flux of mesocosms. *P*-values in bold are statistically  
 231 significant.

	<b>Consumer biomass</b>		<b>Primary producer biomass</b>		<b>CO<sub>2</sub> flux</b>	
	<i>F</i> <sub>1,32</sub>	<i>P</i>	<i>F</i> <sub>1,32</sub>	<i>P</i>	<i>F</i> <sub>1,32</sub>	<i>P</i>
N	55.796	< <b>0.001</b>	148.673	< <b>0.001</b>	31.950	< <b>0.001</b>
P	26.756	< <b>0.001</b>	28.483	< <b>0.001</b>	44.814	< <b>0.001</b>
W	3.218	0.081	32.620	< <b>0.001</b>	77.174	< <b>0.001</b>
N:P	1.775	0.191	3.815	0.060	0.061	0.807
W:N	0.102	0.751	18.839	< <b>0.001</b>	6.712	<b>0.014</b>
W:P	0.035	0.852	2.378	0.133	13.024	<b>0.001</b>
W:P:N	0.072	0.790	11.901	<b>0.002</b>	11.744	<b>0.002</b>

232

233

## Predator absent

## Predator present

