

## REVIEW AND SYNTHESIS

### Freshwater biota and rising pCO<sub>2</sub>?

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#### Abstract

Rising atmospheric carbon dioxide (CO<sub>2</sub>) has caused a suite of environmental issues, however, little is known about how the partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) in freshwater will be affected by climate change. Freshwater pCO<sub>2</sub> varies across systems and is controlled by a diverse array of factors, making it difficult to make predictions about future levels of pCO<sub>2</sub>. Recent evidence suggests that increasing levels of atmospheric CO<sub>2</sub> may directly increase freshwater pCO<sub>2</sub> levels in lakes, but rising atmospheric CO<sub>2</sub> may also indirectly impact freshwater pCO<sub>2</sub> levels in a variety of systems by affecting other contributing factors such as soil respiration, terrestrial productivity and climate regimes. Although future freshwater pCO<sub>2</sub> levels remain uncertain, studies have considered the potential impacts of changes to pCO<sub>2</sub> levels on freshwater biota. Studies to date have focused on impacts of elevated pCO<sub>2</sub> on plankton and macrophytes, and have shown that phytoplankton nutritional quality is reduced, plankton community structure is altered, photosynthesis rates increase and macrophyte distribution shifts with increasing pCO<sub>2</sub>. However, a number of key knowledge gaps remain and gaining a better understanding of how freshwater pCO<sub>2</sub> levels are regulated and how these levels may impact biota, will be important for predicting future responses to climate change.

#### Keywords

Acidification, climate change, freshwater ecology.

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#### INTRODUCTION

Climate change is an ongoing phenomenon that has led to numerous environmental issues. Since the industrialisation era (beginning ~ 1850), atmospheric levels of carbon dioxide (CO<sub>2</sub>) have increased by 40% (Hartman *et al.* 2013), and this has resulted in a host of climate-related changes to the planet including increased air and surface water temperature, droughts, receding glaciers and permafrost, extreme weather events, rising sea levels and ocean acidification (Rhein *et al.* 2013). At a global scale, biota have responded to these changes and experienced altered physiological performance, phenological changes, shifts in distributions and extinctions (Sala *et al.* 2000; Walther *et al.* 2002; Parmesan & Yohe 2003; Root *et al.* 2003). The consequences of climate change are likely to persist, as anthropogenic sources of CO<sub>2</sub> continue to increase, potentially doubling the current atmospheric concentration of ~ 400 µatm by the year 2100 (Ciais *et al.* 2013). Undoubtedly, as CO<sub>2</sub> rises, the severity of climate-linked environmental issues will increase, placing further stress on the globe's biomes.

Research into the impacts of rising atmospheric CO<sub>2</sub> and climate change on aquatic systems has largely focused on the marine environment, where the majority of research has been completed (Doney *et al.* 2009). Oceans have a long water residence time, coupled with a large surface area, and an

abundance of autotrophic organisms; therefore, oceans act as a sink for atmospheric CO<sub>2</sub> (Sabine *et al.* 2004). Specifically, the partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>) in the ocean parallels atmospheric levels of CO<sub>2</sub> positively due to absorption, and future increases in atmospheric CO<sub>2</sub> over the long term are predicted to result in an increase in CO<sub>2</sub> contained within the ocean (Ciais *et al.* 1995). Upon entering the marine environment, CO<sub>2</sub> results in an unavoidable decrease in pH through the production of carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which deprotonates into bicarbonate (HCO<sub>3</sub><sup>-</sup>) and hydronium (H<sub>3</sub>O<sup>+</sup>), leading to the release of hydrogen ions and thus acidification of seawater. Ocean acidification is therefore an unavoidable outcome of rising atmospheric CO<sub>2</sub> that will persist, and likely increase, in the future (Doney *et al.* 2009; Feely *et al.* 2009).

Ocean acidification and elevated pCO<sub>2</sub> levels result in negative consequences for both ecosystem function and organismal biology (reviewed by Orr *et al.* 2005; Fabry *et al.* 2008; Munday *et al.* 2010). For example, proximate consequences of ocean acidification and elevated pCO<sub>2</sub> for tropical and temperate organisms include altered behaviour and physiology for marine fishes (e.g. Jutfelt *et al.* 2013; Munday *et al.* 2014), reduced calcification and growth rates in calcifying marine organisms (Orr *et al.* 2005), and altered performance, development and growth rates in crustaceans (Ross *et al.* 2011). Ultimate outcomes of ocean acidification may include declines in fish populations (Munday *et al.* 2010), and lower primary

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productivity due to shrinking abundance and diversity of phytoplankton (Flynn *et al.* 2012). Of note, however, is the variation in biotic responses to ocean acidification and some species have not responded as negatively as others (Doney *et al.* 2009). Taken as a whole, the biological consequences of ocean acidification and elevated pCO<sub>2</sub> may have major impacts on marine food webs, nutrient cycles and overall productivity.

Despite the research into the detrimental impacts of ocean acidification and rising pCO<sub>2</sub> on marine ecosystems, surprisingly little is known about the potential consequences of elevated atmospheric pCO<sub>2</sub> on freshwater environments. More specifically, global estimates of freshwater pCO<sub>2</sub> under future changes to atmospheric pCO<sub>2</sub> are not yet known, and the proximate and ultimate outcomes of elevated atmospheric CO<sub>2</sub> on freshwater pCO<sub>2</sub> are difficult to predict (Ciais *et al.* 2013). This lack of information with respect to how freshwater pCO<sub>2</sub> will change as atmospheric CO<sub>2</sub> rises hinders our ability to make predictions related to the future impacts of elevated CO<sub>2</sub>, which also limits attempts to develop mitigation strategies. Thus, the overall goals of this synthesis are to: (1) provide an overview of the dynamics and mechanisms regulating pCO<sub>2</sub> in the freshwater environment; (2) present details for how freshwater pCO<sub>2</sub> levels may change in the future as a result of climate change; (3) review existing literature to understand how freshwater biota may be at risk from future changes in freshwater pCO<sub>2</sub> (and where appropriate, the concomitant change in pH); and, (4) identify future research directions to fill knowledge gaps.

## CARBON DIOXIDE AND FRESHWATER ENVIRONMENTS

The total inorganic carbon in freshwater systems is comprised of free CO<sub>2</sub> (or H<sub>2</sub>CO<sub>3</sub>), HCO<sub>3</sub><sup>-</sup> and carbonate (CO<sub>3</sub><sup>2-</sup>), and the percentage of each of these compounds is dependent on the pH of the water body; basic water contains a larger proportion of CO<sub>3</sub><sup>2-</sup>, while acidic water primarily contains free CO<sub>2</sub> (Wetzel 2001). In general, the total amount of free CO<sub>2</sub> (i.e. pCO<sub>2</sub>) in freshwater is controlled by the water source (e.g. groundwater, run-off), the residence time of CO<sub>2</sub> in water, the gas transfer velocity (i.e. the ease at which atmospheric CO<sub>2</sub> dissolves into water, and *vice versa*, at the air-water interface), and the underlying geology of a water body (Wetzel 2001). In addition to these factors, pCO<sub>2</sub> in freshwater is also influenced by a number of other processes such as the presence of herbivores and fish (Atwood *et al.* 2013, 2014), the balance of heterotrophic and autotrophic activities (Sobek *et al.* 2003), terrestrial respiration (Cole *et al.* 2007), atmospheric CO<sub>2</sub> (Phillips *et al.* 2015), and landscape-scale factors such as the relative size of deciduous forests and grasslands (Maberly *et al.* 2013). For instance, the first order streams with high terrestrial respiration have high levels of dissolved CO<sub>2</sub> due to root respiration, however, should systems be in a calcium-rich landscape (e.g. limestone dominated regions), CO<sub>2</sub> is quickly transformed into calcium bicarbonate. Should similar first order systems be in a non-calcium-dominated landscape, but are highly turbulent, much of the dissolved CO<sub>2</sub> will quickly outgas. Furthermore, the pCO<sub>2</sub> in

small streams has been shown to be more closely linked to sources of groundwater, and as a watershed increases in size, the importance of subsurface flow towards supporting elevated CO<sub>2</sub> concentrations decreases (Hotchkiss *et al.* 2015). Essentially, there are several factors that can be considered when making predictions about the amount of CO<sub>2</sub> present in a freshwater system.

Due to the complexity of the interactions between biotic and abiotic factors, the amount of free CO<sub>2</sub> currently in freshwater environments varies widely. A study of global freshwater lakes found that pCO<sub>2</sub> ranged from 3.1-fold below to 16-fold above atmospheric pCO<sub>2</sub>, with a mean of ~ 1000 µatm – almost three times the current atmospheric level (Cole *et al.* 2007). Larger lakes tend to be closer to equilibrium with atmospheric CO<sub>2</sub> (~ 390 µatm) due to the long residence time and outgassing of CO<sub>2</sub>, as, for example, the annual mean pCO<sub>2</sub> measured in Lake Superior was 461 ± 171 µatm (mean ± standard deviation, SD) (Atilla *et al.* 2011) and in Lake Michigan values ranged from 250–500 µatm (Pilcher *et al.* 2015). In contrast, pCO<sub>2</sub> levels for lotic systems can vary widely depending on stream order, forest cover, precipitation and surface area (Butman & Raymond 2011; Kocic *et al.* 2015); lotic systems can also become highly supersaturated with CO<sub>2</sub>, reaching partial pressures 10–15 times higher than atmospheric levels (Amazon River – Richey *et al.* 1980; Ottawa River – Telmer & Veizer 1999). Of the 6708 stream and river systems assessed at a global scale, 95% had a median pCO<sub>2</sub> level greater than atmospheric levels, and the average pCO<sub>2</sub> was found to be ~ 3100 µatm, which is almost 8-fold above current atmospheric levels (Raymond *et al.* 2013). In addition, freshwater systems can experience daily and seasonal fluctuations in pCO<sub>2</sub> due to weather, aquatic photosynthesis and respiration, and changes in the amount of pCO<sub>2</sub> entering water bodies (Maberly 1996). For example, in a lake with high primary productivity in the English Lake District, pCO<sub>2</sub> was depleted to near zero during the day and was replenished at night (pH variation up to 1.8; Maberly 1996). Furthermore, in this same system, seasonal fluctuations resulted in levels that ranged from near zero in the late spring and summer to concentrations greater than 7-fold above the atmospheric concentration in the fall, winter and early spring (Maberly 1996; McDonald *et al.* 2013). Freshwater pCO<sub>2</sub> levels have also been shown to vary across large time spans due to climate. Diatom sediment records have demonstrated that freshwater pCO<sub>2</sub> levels, inferred from pH and alkalinity, varied across the entire Holocene period (Catalan *et al.* 2009). Clearly, with such variation in pCO<sub>2</sub> levels both within and across freshwater systems, there are a number of drivers responsible for influencing pCO<sub>2</sub> levels at present.

As shown above, inland waters are rarely, if ever, in a steady state of equilibrium with the atmosphere and are likely controlled in part by the ratio of autotrophic (producer) and heterotrophic (consumer) activity. In systems where nutrients are abundant, autotrophic photosynthesis (i.e. CO<sub>2</sub> consuming) can maintain freshwaters at pCO<sub>2</sub> concentrations below atmospheric levels. However, the balance of heterotrophic grazers, and higher trophic feeders can compensate for this autotrophy, increasing respiration (i.e. CO<sub>2</sub> producing) over short or long time scales. More importantly, biotic factors

may not always be directly involved in maintaining excess  $p\text{CO}_2$  in lakes. McDonald *et al.* (2013) found that supersaturation of lake systems in the USA that also have positive net ecosystem production are more often maintained by hydrologic inputs of inorganic carbon (i.e. free  $\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ), and not inputs of organic carbon.

Across regions, changes due to climate, anthropogenic land use and the composition of the terrestrial biosphere will also affect aquatic carbon levels and potentially influence future levels of  $p\text{CO}_2$ . Many of these changes are directly tied to the composition of dissolved organic carbon (DOC; i.e. broken down organic matter) within a stream environment, and the buffering capacity of surrounding soils. A potential major driver of freshwater  $p\text{CO}_2$  is the presence and form of dissolved organic matter (DOM), as DOM can function as a source of hydrogen ions, acting as a pH buffer. The microbial respiration of organic matter (i.e. decomposition) can saturate aquatic systems with dissolved inorganic carbon (DIC) and lead to  $p\text{CO}_2$  levels well above the level found in the open atmosphere, resulting in aquatic systems being net heterotrophic (i.e.  $\text{CO}_2$  producing) (Sobek *et al.* 2003). In aquatic systems, the processes of photosynthesis and respiration within terrestrial and aquatic environments produce DOM. The resulting form of DOC from these photosynthesis and respiration processes significantly influences the aquatic system by driving levels of productivity and altering  $p\text{CO}_2$  concentrations and the exchange of  $\text{CO}_2$  with the atmosphere (Cai *et al.* 2003). For example, high-latitude ecosystems are experiencing warming, which can increase the depth of the active layer of soil and the duration of soil microbe activity, providing an opportunity for additional organic carbon to enter aquatic systems where it then undergoes photochemical oxidation and bacterial respiration (Kling *et al.* 1992; Cory *et al.* 2014). Changes in metabolic processes that result in more organic carbon entering aquatic systems may influence  $p\text{CO}_2$  levels.

Another major factor controlling the level of carbon in freshwater and one that has been shown to influence freshwater  $p\text{CO}_2$  is the productivity of terrestrial ecosystems in the catchment. Terrestrial productivity may increase due to changes in the concentration of atmospheric  $\text{CO}_2$  and the dual effect of warming (Arneeth *et al.* 2010; Reich *et al.* 2014). In fact, the increased accumulation of terrestrial biomass due to rising atmospheric  $\text{CO}_2$  enhances the terrestrial uptake of carbon, which may lead to increases in soil carbon respiration (Arneeth *et al.* 2010) and subsequently nutrient run-off (i.e. more dissolved  $\text{CO}_2$  entering aquatic systems). However, it remains unclear whether future increases in atmospheric  $\text{CO}_2$  will produce higher rates of terrestrial productivity and total carbon sequestration in forested (Norby *et al.* 2005) and agricultural ecosystems (Long *et al.* 2006). Under future conditions of both higher temperatures and  $\text{CO}_2$  concentrations, increased rates of soil respiration (Davidson & Janssens 2006), coupled with potential shifts in water use efficiency from reduced stomatal conductance in leaves, may facilitate the movement of DIC in the form of  $\text{CO}_2$  into aquatic systems. Although the physical transport of dissolved  $\text{CO}_2$  from soils to streams has not yet been quantified, previous research suggests correlations with precipitation and stream  $p\text{CO}_2$  concentrations (Butman & Raymond 2011; Donohue *et al.* 2013).

Future precipitation conditions from shifting climate regimes are challenging to predict, but research suggests that riverine run-off will increase in northern latitudes and potentially decrease in tropical latitudes as a function of climate change (Dai *et al.* 2009). Increases in precipitation and run-off will provide an opportunity for additional DIC and DOC to enter the freshwater environment (Raymond & Saiers 2010), whereas decreases in precipitation may lead to reduced connectivity in freshwater systems, which may reduce the amount of  $p\text{CO}_2$  in downstream environments. Additions of DIC can be a result of increased soil and root respiration and weathering, and the added carbon may fuel *in situ* mineralisation and heterotroph productivity (Raymond & Saiers 2010). Furthermore, in lacustrine environments, whole catchment productivity and terrestrial respiration have been identified as the underlying driver for  $p\text{CO}_2$  in lakes (Maberly *et al.* 2013). Overall, increased terrestrial primary productivity as a result of the dual effect of warming and rising atmospheric  $\text{CO}_2$ , and the resulting increased amounts of DOC and DIC, suggest a rise in future levels of freshwater  $p\text{CO}_2$  when the connectivity of terrestrial and aquatic systems remain intact.

Factors other than direct and indirect changes to atmospheric  $\text{CO}_2$  may influence future freshwater  $p\text{CO}_2$  levels, though little is known about these factors. For example, land use patterns affect both water and carbon cycles (Howarth *et al.* 1991; Humborg *et al.* 2004), and may dictate spatial and temporal patterns in the lability and characteristics of DOM. Agricultural practices can cause the proportion of labile carbon to range from 30 to 70% (Beyer *et al.* 1993; Jacinthe *et al.* 2004), which represents a considerable influence of land use patterns on freshwater systems. The conversion of terrestrial ecosystems to intensive agriculture has introduced additional organic carbon from soils and crop residues, but the impacts of agriculture are unclear. For example, increases in freshwater DOC have been identified as primarily being a result of increased productivity due to increased nutrients, which reduces  $p\text{CO}_2$  (Wilson & Xenopoulos 2009). However, the residence time of this carbon (i.e. the increased DOC) is important to consider as seasonal stratification in lake systems and the variability in stream flow can quickly shift freshwater  $p\text{CO}_2$  concentrations (Gupta *et al.* 2008). To further understand the impact of agricultural land use on freshwater DOC, Jacinthe *et al.* (2004) used a simple rainfall simulation study to demonstrate that runoff from agricultural systems had upwards of 35% labile carbon, with large rain events producing higher photosynthetic rates. Furthermore, altering the distribution of precipitation from spring to summer or fall could possibly increase DOC flux to streams from highly productive ecosystems during periods of soil carbon accumulation, which would have downstream effects for photosynthetic rates and metabolic activity (e.g. Clair & Ehrman 1996). Additional work is needed to quantify the increase in connectivity of the hydrologic system from soils to freshwaters due to agricultural land use and whether  $p\text{CO}_2$  will be negatively or positively influenced, especially given potential trade-offs between DOC and the productivity of autotrophs and heterotrophs. The management of riparian systems within an agricultural landscape could prevent the decrease in freshwater  $p\text{CO}_2$  (e.g. Abril *et al.* 2014), suggesting that with appropriate mitigation

measures, pCO<sub>2</sub> in flowing systems controlled by agricultural practices could remain stable.

### PREDICTING WHETHER pCO<sub>2</sub> LEVELS IN FRESHWATER WILL CHANGE IN THE FUTURE

Given the above noted complexities with how freshwater levels of pCO<sub>2</sub> are governed and the wide variation observed, predicting future levels of pCO<sub>2</sub> is difficult, but focused approaches on few factors have been undertaken. Using the Laurentian Great Lakes as a case study, Phillips *et al.* (2015) predicted that rising atmospheric CO<sub>2</sub> (assuming only atmospheric CO<sub>2</sub> as a controlling factor) would cause an increase in freshwater pCO<sub>2</sub> and a resulting decline in pH. Likewise, Sobek *et al.* (2005) predicted a modest positive trend in freshwater pCO<sub>2</sub> levels as rising water temperature was found to increase DOC. The degree to which freshwater pCO<sub>2</sub> levels will change, however, is likely also dependent on more than one factor, as changes in the residence time of CO<sub>2</sub> in water and the gas transfer velocities because of rising atmospheric pCO<sub>2</sub> (e.g. changes in precipitation rates, wind patterns, etc.) may also cause a change in freshwater pCO<sub>2</sub>. It is also possible that increased atmospheric CO<sub>2</sub> will influence secondary factors (i.e. ecotype and water body type) differently. For example, differences in the response of arctic, temperate and tropical ecosystems may result in varied changes to freshwater pCO<sub>2</sub> levels (Kling *et al.* 1992; Sobek *et al.* 2003; Dai *et al.* 2009; Raymond & Saiers 2010). In general, understanding the factors that govern pCO<sub>2</sub> in freshwater and how they may change with rising atmospheric CO<sub>2</sub> are major hurdles that need to be overcome before potential outcomes can be determined (i.e. specific estimates of pCO<sub>2</sub> levels for a range of freshwater environments). Lastly, the degree to which the variation in pCO<sub>2</sub> across freshwater systems will change is also unknown, however, one might predict that given similar variation in state variables (e.g. alkalinity, temperature, etc.), the variation in pCO<sub>2</sub> currently observed in freshwater systems will continue.

### POTENTIAL EFFECTS OF CHANGING pCO<sub>2</sub> ON FRESHWATER BIOTA

As noted above, there is support from ecologists and biogeochemists that future levels of freshwater pCO<sub>2</sub> will potentially increase (Sobek *et al.* 2005; Phillips *et al.* 2015), however, given the uncertainties with predicting pCO<sub>2</sub> and the variation in pCO<sub>2</sub> across freshwater systems, it still remains unclear as to the direction of change for pCO<sub>2</sub> in freshwater, if any. Specific changes to freshwater biota following a possible increase or decrease in pCO<sub>2</sub> reflect a knowledge gap that previously has not been thoroughly studied or reviewed in freshwater-related climate change reviews. Making predictions about the biological responses of freshwater biota becomes difficult due to the lack of information about the future state of freshwater systems. Freshwater biota likely experience a wide range of pCO<sub>2</sub> levels on a daily or seasonal basis, suggesting that freshwater biota may have the capacity to tolerate fluctuations in pCO<sub>2</sub>. Furthermore, because carbon is a major factor in ecosystem metabolism, community structure and

competitive outcomes (Hessen *et al.* 2004), understanding both the biological responses and physiological tolerances to pCO<sub>2</sub> of biota will assist freshwater researchers with predictions about how trophic cascades, species distributions and population sizes may change with future levels of freshwater pCO<sub>2</sub>. For this synthesis, we have attempted to document the empirical findings directly related to freshwater changes in pCO<sub>2</sub> (and where appropriate, the concomitant change in pH) at the level of organisms, assemblages, communities and ecosystems.

Most research assessing changes in freshwater pCO<sub>2</sub> has focused on bottom-up processes, and on the consequences for freshwater phytoplankton – particularly green algae and cyanobacteria (Table 1). Algae are the basis for food webs and play key roles in aquatic energy production and respiration (Wetzel 2001). Furthermore, phytoplankton are indicators of environmental change in freshwater ecosystems (McCormick & Cairns 1994) and have been shown to specifically indicate changes in freshwater pCO<sub>2</sub> levels (Philibert & Prairie 2002; Low-Décarie *et al.* 2014). A principle concern for phytoplankton with respect to changing pCO<sub>2</sub> is how CO<sub>2</sub> will influence ecological stoichiometry (Sterner & Elser 2002) and thus, the amount of carbon (C), nitrogen (N) and phosphorus (P) available to phytoplankton (reviewed by van de Waal *et al.* 2010). In fact, several studies have found that varying levels of pCO<sub>2</sub> alter the ratio of C, N and P (Table 1); excess C relative to N and P reduces the nutritional quality of phytoplankton in nutrient-poor water bodies (Hessen *et al.* 2004). This change in the quality of phytoplankton has been shown to reduce growth in zooplankton, which may alter competitive advantages and community composition (Urabe *et al.* 2003; Urabe & Waki 2009; Verschoor *et al.* 2013; Verspagen *et al.* 2014a); however, mixed species diets appear to maintain growth in zooplankton exposed to high CO<sub>2</sub> (Urabe & Waki 2009). Should changes in pCO<sub>2</sub> alter nutritional quality at the base of freshwater food webs, there is the potential for consequences to the condition and health of higher level organisms.

Changing pCO<sub>2</sub> may impact several other important ecological processes. For example, increased primary production and biomass in nutrient-rich water bodies has been observed as a result of elevated pCO<sub>2</sub> (Schippers *et al.* 2004; Jansson *et al.* 2012; Low-Décarie *et al.* 2014, 2015), presumably because water bodies are no longer carbon limiting. Elevated pCO<sub>2</sub> also alters the phytoplankton community structure (Verschoor *et al.* 2013; Low-Décarie *et al.* 2015), and increases in the abundance of nitrogen-fixating cyanobacteria has been observed in some (Shapiro 1997; van de Waal *et al.* 2011; Verspagen *et al.* 2014a,b), but not all studies (Verschoor *et al.* 2013). Other studies have found that high pCO<sub>2</sub> results in increased photosynthesis rates (Hein 1997; Qiu & Gao 2002), as well as lower specific growth rates and oxygen generation rates in estuary phytoplankton (Ying *et al.* 2014). There is, however, the possibility that phytoplankton may abate negative consequences of elevated pCO<sub>2</sub> (reviewed by Low-Décarie *et al.* 2014). The carbon concentrating mechanism in green algae exposed to high pCO<sub>2</sub> over 1000 generations has been found to respond causing higher rates of photosynthesis and respiration (Collins & Bell 2004), and limitations to how

**Table 1** Studies that directly assess the relationships between pCO<sub>2</sub> levels and freshwater biota. Levels of pCO<sub>2</sub> (or concentrations of CO<sub>2</sub>) have been presented as described in each study

Paper	Study type	pCO <sub>2</sub> gradient	Effect
<i>Phytoplankton</i>			
Collins & Bell (2004)	Laboratory	430 ppm and 1050 ppm	Green algae grown in high pCO <sub>2</sub> had no specific adaptations Some lines showed elevated chlorophyll content and higher photosynthesis and respiration
Hein (1997)	Field	0.24–37.7 μM (calculated free CO <sub>2</sub> )	Photosynthesis by phytoplankton in low/moderate dissolved inorganic carbon (DIC) lakes was negatively influenced by low dissolved CO <sub>2</sub>
Jansson <i>et al.</i> (2012)	Field collected water manipulated in laboratory	325–4379 μatm	CO <sub>2</sub> availability enhanced primary productivity 10 times when compared to production at equilibrium Potential for unproductive, clear-water and humic lakes to be influenced by rising atmospheric CO <sub>2</sub>
Low-Décarie <i>et al.</i> (2013)	Laboratory (addition of CO <sub>2</sub> + nutrients)	380 and 1000 ppm	Long-term selection experiments did not detect an evolutionary response in several phytoplankton species
Low-Décarie <i>et al.</i> (2015)	Laboratory (addition of CO <sub>2</sub> + nutrients)	665 and 2269 ppm	Dissolved CO <sub>2</sub> concentrations can shape phytoplankton community structure in a predictable manner.
Qiu & Gao (2002)	Laboratory	5.97–11.97 μmol/L (free CO <sub>2</sub> )	Increased rate of photosynthesis and growth in carbon-limiting cyanobacteria blooms when exposed to elevated atmospheric CO <sub>2</sub>
Schippers <i>et al.</i> (2004)	Model based on C-limitation of two freshwater phytoplankton species	350–700 ppm	Doubling atmospheric CO <sub>2</sub> was shown to have a doubling effect on productivity Potential for a larger effect than in marine systems due to an increase in the number of nutrient-rich and C-limiting systems
Shapiro (1997)	Whole-lake manipulation	Low CO <sub>2</sub> vs. enriched CO <sub>2</sub> (free CO <sub>2</sub> concentrations implied by pH and not reported)	Blue-green algae reduce CO <sub>2</sub> concentrations to levels only tolerable by them Green algae have a lower ability to use CO <sub>2</sub> in low concentrations
Trobajo <i>et al.</i> (2014)	Laboratory mesocosm where pCO <sub>2</sub> was manipulated	370, 5000, 10 000 ppm	Observed responses to pH were caused by changes in CO <sub>2</sub> Higher carbon content was found in a diatom <i>Phaeodactylum tricorutum</i> (Bohlin), but not in two other diatom species Optimal range of pCO <sub>2</sub> and carbon content likely in between 5000 and 10 000 ppm for <i>P. tricorutum</i>
van de Waal <i>et al.</i> (2011)	Modelling and laboratory experiment	200, 1000–1250 ppm	Toxic blue-green algae competitively excluded non-toxic blue-green algae by reducing CO <sub>2</sub> concentrations (exclusion reverses under light-limiting conditions) Cells strongly relied on CO <sub>2</sub> as their primary source for inorganic carbon
Verschoor <i>et al.</i> (2013)	Laboratory	380, 3000, 18 800 ppm	Elevated CO <sub>2</sub> caused increased growth, leading to lower N and P concentrations, but no change in biomass Cyanobacteria did not outcompete green algae for inorganic carbon
Verspagen <i>et al.</i> (2014a)	Modelling and laboratory	200–1200 ppm (model) 0.5–2800 ppm (laboratory)	Rising CO <sub>2</sub> intensified phytoplankton blooms in nutrient-rich systems (C-limiting switched to light-limiting) N and P became limiting and nutritional quality of phytoplankton decreased (i.e. C > nutrients)
Verspagen <i>et al.</i> (2014b)	Modelling and laboratory	200, 1200 ppm	Model predictions conclude increased productivity reduces CO <sub>2</sub> more in nutrient-rich and low/moderate alkaline systems In dense blooms, C becomes limiting in low/moderate alkaline systems Rising CO <sub>2</sub> alleviates blooms from C limitation
<i>Zooplankton</i>			
Urabe <i>et al.</i> (2003)	Laboratory mesocosm where pCO <sub>2</sub> was manipulated	360, 1500, 3600 ppm	Increasing pCO <sub>2</sub> increased algal abundance, but reduced P:C resulting in lower growth rates of <i>Daphnia</i> sp.
Urabe & Waki (2009)	Laboratory mesocosm where pCO <sub>2</sub> was manipulated	360 and 2000 ppm	<i>Daphnia</i> sp. fed monospecific algae grown at high CO <sub>2</sub> had reduced growth rates, while <i>Daphnia</i> sp. fed multispecific algae grown at high CO <sub>2</sub> did not

(continued)

**Table 1.** (continued)

Paper	Study type	pCO <sub>2</sub> gradient	Effect
<i>Macrophytes</i>			
Cao & Ruan (2015)	Mesocosm where pCO <sub>2</sub> and temperature were manipulated	370, 1000 ppm	Increased pCO <sub>2</sub> led to a rise in photosynthetic performance, growth, and clonal propagation of <i>Vallisneria spiralis</i> (Hara). Stolon elongation and bud number also increased under elevated pCO <sub>2</sub> and more biomass was found in the roots, stolons, and buds
Demars & Tremolieres (2009)	Field study of multiple ground-fed river systems	3- to 43-times atmospheric level (~ 1200–15 000 µatm)	Species composition was best related to pCO <sub>2</sub> when compared to NH <sub>4</sub> and PO <sub>4</sub> and had the best predictive power
Maberly <i>et al.</i> (2015)	Field study along a natural gradient in pCO <sub>2</sub>	0.46–0.09 µmol/L (free CO <sub>2</sub> )	<i>Berula erecta</i> (Koch) dominates CO <sub>2</sub> -rich areas, as pCO <sub>2</sub> decreases downstream, species use proportionately more HCO <sub>3</sub> <sup>-</sup>
Madsen <i>et al.</i> (1996)	Laboratory mesocosm where pCO <sub>2</sub> and HCO <sub>3</sub> <sup>-</sup> were manipulated	1, 16, and 910 µM (free CO <sub>2</sub> )	Several findings, including: Increased photosynthetic performance at low pCO <sub>2</sub> , altered use of CO <sub>2</sub> and HCO <sub>3</sub> <sup>-</sup> for three species ( <i>Elodea canadensis</i> (Michx.), <i>Callitriche cophocarpa</i> (Sendtn. ex Hegelm.), and <i>Ranunculus peltatus</i> (Schrank)); Increased root/shoot ratio with increasing pCO <sub>2</sub> ; Reduced rubisco activity, protein content, and chlorophyll content as pCO <sub>2</sub> increased
Malheiro <i>et al.</i> (2013)	Laboratory aquaria where temperature, light, and pCO <sub>2</sub> were manipulated	0.01–2.50 µmol/L (free CO <sub>2</sub> )	pCO <sub>2</sub> had more of an influence on growth rate, dry matter content, and length of internodes compared to light and temperature pCO <sub>2</sub> with light and temperature also influenced total chlorophyll content and chlorophyll a : b ratio (respectively)
Titus & Andorfer (1996)	Mesocosm where pCO <sub>2</sub> and pH were independently controlled	Atmosphere, 3.2- and 10-times ambient pCO <sub>2</sub>	Al and Fe accumulated 5.5- and 7-fold (respectively) in shoots of <i>Vallisneria spiralis</i> (Michx) when exposed to elevated pCO <sub>2</sub> and pH 5 P accumulated 6- to 11-fold in shoots when plants grown in less and more fertile sediments and exposed to elevated pCO <sub>2</sub> Plant growth increased at high pCO <sub>2</sub> and low pH and reduced tissue N
Vadstrup & Madsen (1995)	Field study along a natural gradient in pCO <sub>2</sub>	500–1000 µatm above ambient	Increased growth of <i>Elodea canadensis</i> and <i>Callitriche cophocarpa</i> when CO <sub>2</sub> elevated
Yan <i>et al.</i> (2006)	Mesocosm where pCO <sub>2</sub> was manipulated	390, 1000 ppm	Elevated pCO <sub>2</sub> accounted for ~ 75% of above ground biomass and ~ 25% of below ground biomass in <i>Vallisneria spiralis</i> Clonal growth was positively affected by pCO <sub>2</sub> and a dense growth pattern was induced Phosphorous was greater in all tissues, while N was only higher in leaves and turion
<i>Macroinvertebrates</i>			
Blinn & Sanderson (1989)	Field study	550 mg/L (dissolved CO <sub>2</sub> )	Absence of Trichoptera, Lepidoptera, Megaloptera, Neuroptera, and Anisoptera larval stages suggest that either high pCO <sub>2</sub> or high alkalinity restrict the distribution of larvae in freshwater.
O'Brien & Blinn (1999)	Field study along a natural gradient in pCO <sub>2</sub> and controlled laboratory exposures of a similar range of pCO <sub>2</sub>	20–480 mg/L (dissolved CO <sub>2</sub> )	<i>Pyrgulopsis montezumensis</i> (Hershler) tolerated pCO <sub>2</sub> concentrations up to 400 mg/L for 11 days 40% mortality was found for snails exposed to pCO <sub>2</sub> of 728 mg/L after 3 days, and 100% mortality after 24 h when exposed to 1200 mg/L of pCO <sub>2</sub> Distribution of snails in the natural environment was dictated by both CO <sub>2</sub> and substratum
Roberts & Barnhart (1999)	Laboratory where pCO <sub>2</sub> was manipulated	0, 20 000, and 50 000 ppm	Low pH and high pCO <sub>2</sub> increased transformation success of <i>Anodonta suborbiculata</i> (Say) glochidia
<i>Fishes</i>			
Ou <i>et al.</i> (2015)	Laboratory where pCO <sub>2</sub> was manipulated	400, 1000, and 2000 µatm; and 400–2000 µatm (over a 24 h period)	Size, growth rates, and production efficiencies of early life stage Pink Salmon ( <i>Oncorhynchus gorbuscha</i> , Walbaum) reduced during rearing in freshwater

much elevated  $p\text{CO}_2$  will alter biomass growth have been found (Trobajo *et al.* 2014). Continuing to assess the effects of changing  $p\text{CO}_2$  on phytoplankton and the potential compensatory responses will further aid with the understanding of the impacts of changing  $p\text{CO}_2$  in freshwater ecosystems.

In addition to phytoplankton and zooplankton, the responses to changes in  $p\text{CO}_2$  of freshwater macrophytes have been examined and studies suggest that macrophytes may be less at risk compared to plankton. Macrophytes require  $\text{CO}_2$  for photosynthesis and can either obtain it directly from the surrounding environment in the form of free  $\text{CO}_2$ , or by taking up  $\text{HCO}_3^-$ . Although some species preferentially utilise free  $\text{CO}_2$  because of the increased metabolic cost associated with utilising  $\text{HCO}_3^-$  (Jones 2005), in aquatic environments with low  $p\text{CO}_2$ , species that can use  $\text{HCO}_3^-$  outcompete species that cannot (Vadstrup & Madsen 1995). Freshwater  $p\text{CO}_2$  may also be a controlling environmental factor for some macrophyte species, but responses to changes in  $p\text{CO}_2$  may vary based on alkalinity (Titus 1992). Elevated  $p\text{CO}_2$  has been found to increase the rate of photosynthesis (Madsen *et al.* 1996), influence species distributions within streams (Demars & Tremolieres 2009; Maberly *et al.* 2015), increase growth (Yan *et al.* 2006; Cao & Ruan 2015), and alter allocation of biomass (Cao & Ruan 2015). Of note, however, macrophytes have been found to self-limit based on daily fluctuations in pH (i.e. changes in  $p\text{CO}_2$ ) once biomass increases beyond specific thresholds (Maberly & Spence 1983; Sand-Jensen 1989). Some macrophytes, such as *Myriophyllum aquaticum* (Verdcourt), have shown phenotypic responses to changing  $p\text{CO}_2$  by increasing leaf, stem and root biomass (Malheiro *et al.* 2013), suggesting that *M. aquaticum* maintain normal function in variable  $\text{CO}_2$  environments. Because most macrophytes are adapted to use either free  $\text{CO}_2$  or  $\text{HCO}_3^-$ , they may respond conservatively to changing  $p\text{CO}_2$  so long as one of the two forms of DIC are available (note exception being *Vallisneria* spp. (Table 1)), and thus be less at risk to climate-induced changes in  $p\text{CO}_2$  levels compared to other more sensitive taxonomic groups.

Few studies have focused on the impacts of changing  $p\text{CO}_2$  on freshwater macroinvertebrates (Table 1), however, work on marine species is increasing and may provide at least some insight. For example, it has been shown that elevated  $p\text{CO}_2$  levels can result in a range of negative consequences for molluscs, including reduced growth, survival and recruitment (reviewed by Parker *et al.* 2013). In addition, there is evidence to suggest that several subcellular processes in molluscs will be negatively affected by elevated  $p\text{CO}_2$  levels, including the cellular stress response, energy production and protein synthesis (Thompson *et al.* 2015). Of the few studies carried out on freshwater macroinvertebrates, high  $p\text{CO}_2$  from a natural spring dictated the distribution of endemic snails (O'Brien & Blinn 1999), the presence of aquatic insects (Blinn & Sanderson 1989), and resulted in leeches being the top predator in the food web (Cole & Watkins 1977) because of the lack of fish in this system (Cole & Barry 1973). It should be noted that the high amount of  $p\text{CO}_2$  observed in these studies is likely beyond the upper limit (100 000  $\mu\text{atm}$ ) one could reasonably expect for freshwater systems in the future, but these studies do offer insight into potential outcomes for biota; further research

addressing the impacts of more ecologically relevant levels of  $p\text{CO}_2$  on freshwater macroinvertebrates is warranted.

Freshwater fish have been relatively understudied with respect to responses to climate-induced changes in  $p\text{CO}_2$ . To the best of our knowledge, Ou *et al.* (2015) have carried out the only study that explicitly links potential climate change levels of freshwater  $p\text{CO}_2$  to biological responses in a freshwater fish, pink salmon (*Oncorhynchus gobuscha*, Walbaum). In this study, Ou *et al.* (2015) characterised the effect of rising  $p\text{CO}_2$  at multiple stages of development in pink salmon, and found that growth rate and aerobic scope were reduced in fish exposed to elevated  $p\text{CO}_2$  (1000 and 2000  $\mu\text{atm}$ ). However, recently collected (August 2014) longitudinal survey data from the Sol Duc river on the Olympic Peninsula, WA, suggest that ambient  $p\text{CO}_2$  concentrations range from 383 to 3400  $\mu\text{atm}$  (D. Butman, unpublished data), with some of the highest concentrations in regions identified to contain salmonids (McMillan *et al.* 2007), suggesting that salmonids may currently be exposed to levels beyond those studied by Ou *et al.* (2015). Substantial work has also been carried out on the physiological effects of elevated  $p\text{CO}_2$  in the blood of fish (hypercapnia), and the mechanisms underlying these effects (reviewed by Heuer & Grosell 2014). Hypercapnia elicits a range of physiological responses in fish, including changes in acid–base balance, mitochondrial function, osmoregulation, reproduction, metabolism and cardiorespiratory function, but these responses have rarely been examined at environmentally relevant levels of  $p\text{CO}_2$  (1200–1900  $\mu\text{atm}$ , Heuer & Grosell 2014). To supplement the lack of understanding of how environmentally relevant  $p\text{CO}_2$  levels will affect freshwater fish, comparisons to the effects of acid rain (which also results in a decrease in pH) have been made (e.g. Heino *et al.* 2009), since this has been extensively studied (e.g. Schindler 1988). However, the applicability of comparisons between the effect of acid rain and elevated  $p\text{CO}_2$  are limited because acid rain results in the addition of strong acids (e.g. sulphuric acid [ $\text{H}_2\text{SO}_4$ ]) and reduces pH by a much greater extent than  $\text{H}_2\text{CO}_3$ , a weak acid. In general, studies on marine species, as well as work carried out on hypercapnia and acid rain in freshwater fishes, provide the bulk of the information available to biologists making predictions about the consequences of changes in  $p\text{CO}_2$  levels on freshwater fishes, presenting a clear knowledge gap that needs to be addressed.

## KNOWLEDGE GAPS

The lack of knowledge related to how freshwater  $p\text{CO}_2$  levels may change in the future and the potential impacts to freshwater biota are concerning, and thus a path forward for researchers is necessary. Below, a list of knowledge gaps that should be addressed have been compiled and commentary has been provided to suggest potential paths forward:

- (1) Gaining a detailed understanding of the abiotic and biotic factors that affect  $p\text{CO}_2$  levels in freshwater is vital for understanding how  $p\text{CO}_2$  levels will change as a result of rising atmospheric  $\text{CO}_2$  (e.g. magnitude of change, rate of change, variation, etc.), and how ecosystems may potentially respond. This may be addressed using controlled laboratory experiments and microcosms, but long-term monitoring coupled with

mechanistic modelling of carbon sources into freshwater systems is also likely the key. Studies to understand freshwater pCO<sub>2</sub> levels should not be completed in isolation of the terrestrial ecosystem and more research is needed to evaluate the magnitude of terrestrial ecosystems as a source of pCO<sub>2</sub>.

(2) Given that C cycling in freshwater systems is in part driven by freshwater biota, understanding photosynthesis and respiration rates of phytoplankton and zooplankton under altered pCO<sub>2</sub> conditions will be necessary to assess C flux. Low-Décarie *et al.* (2014) have aptly detailed changes in aquatic primary productivity should pCO<sub>2</sub> levels rise, but understanding how a range of pCO<sub>2</sub> level outcomes may influence aquatic primary productivity is needed.

(3) There is a lack of empirical data associated with the tolerance of taxa and species to deviations in environmental pCO<sub>2</sub> levels. It is unclear whether biota have the potential to acclimatise and/or adapt to changing pCO<sub>2</sub> levels, and this topic requires attention from animal and plant physiologists. Better understanding the range of pCO<sub>2</sub> levels that freshwater biota may currently be exposed to and the levels that they may experience in the future will inform how species distributions may change in the future and identify at-risk populations. To ensure ecosystem functions remain stable, our priority should be to understand how keystone species and sensitive species (e.g. calcifying organisms) respond to changes in pCO<sub>2</sub> levels. Laboratory controlled experiments offer a useful way to determine species tolerances, however, landscape-scale presence/absence studies may help to identify which species are more/less tolerant to high/low pCO<sub>2</sub>.

(4) Weak acidification as a co-factor to changing pCO<sub>2</sub> levels should be explored further, as well as understanding the independent effects of changes in pCO<sub>2</sub> and pH. Gaining a better understanding of the contribution of each of these individual factors will help to build a mechanistic understanding of the responses of biota to elevated pCO<sub>2</sub> levels and decreases in pH.

(5) Freshwater systems are complex and changes in pCO<sub>2</sub> levels will likely be coupled with other stressors. Thus, it becomes necessary to understand the impact of altered pCO<sub>2</sub> levels on biota in the context of a multiple stressor environment. For example, stressors such as limited nutrients, weak acidification, thermal changes, reduced water connectivity, desiccation, etc., are products of climate change and the additional role of pCO<sub>2</sub> as a co-stressor should be considered. Microcosm studies where different stressors can be paired and manipulated are likely the most suitable path forward to understand the role of pCO<sub>2</sub> in a multiple stressor environment. Landscape-scale studies may also help to identify the stressors that are present when pCO<sub>2</sub> levels change in particular environments.

(6) Previous studies have focused largely on the effects of an increase in pCO<sub>2</sub> levels, however, future pCO<sub>2</sub> levels may vary widely (as pCO<sub>2</sub> currently does) and as such study designs should consider assessing risk to biota across a range of pCO<sub>2</sub> levels.

## CONCLUSIONS

Overall, empirical research into changing freshwater pCO<sub>2</sub> levels has focused on the assumption that it will rise, and to

date, the validity of this is unknown and should be addressed by both biogeochemists and biologists. With respect to biota, with the exception of phytoplankton and macrophytes, little is known about the ecological and physiological consequences of changing freshwater pCO<sub>2</sub>. Perhaps the most detrimental effects found to date are the stark changes in growth and community in phytoplankton. These changes to phytoplankton growth and community structure will likely have cascading effects on the freshwater food web and water quality of inland systems. Furthermore, the few studies completed to date on freshwater fishes also suggest that significant negative consequences to behaviour and physiology can be expected should pCO<sub>2</sub> levels rise. Given the limited empirical data detailed in this review, whether or not freshwater biota are at risk to changing pCO<sub>2</sub> is difficult to assess. Certainly, changes in pCO<sub>2</sub> levels have been shown to alter key biological processes (e.g. rate photosynthesis, species distributions, community compositions, nutrient quality, etc.), but whether these changes leave freshwater ecosystems 'at risk' is still unclear. We propose that researchers use the knowledge gaps outlined in this review to grow our collective understanding of how freshwater biota are influenced by changes in pCO<sub>2</sub> levels so that predictions of how future levels of pCO<sub>2</sub> may impact species responses and overall ecosystems can be made and potentially mitigated.

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