Appendix 1: Supplementary tables

Table S1: Overview of reported pCO_2 concentrations from literature. Mean (or median) pCO_2 with standard error (s.e.m.) or standard deviation (s.d.) and minimum - maximum range was reported when present. N represents number of measurements in different freshwater types.

Source	Freshwater type and region (no. lakes)	No. measurements (N)	Mean/median pCO_2 (µatm)	Minimum pCO_2 (µatm)	Maximum pCO_2 (µatm)
Cole et al. (1994)	Global lakes (37)	390	801 ± 67 s.e.m.	10	4,128
Cole et al. (1994) and citations herein	Full seasonal cycles of global lakes (69)	2,395	680	5	7,991
Cole et al. (1994) and citations herein	African lakes (59)	79	2296	32	20,249
Sobek and Tranvik (2005)	Global lakes (4902)	12,898	1287 ± 41 s.d.	17	65,250
Kortelainen et al. (2006)	Subset of Nordic Lakes Survey, Finland (177)	/	1400	290	4,500
Alin and Johnson (2007) and citations herein	Global large lakes (41)	/	850	0	27,600
Lazzarino et al. (2009)	Florida lakes, USA (948)	/	3,550	0	81,000
Balmer and Downing (2010)	Agriculturally eutrophic lakes, USA (131)	3049	322 (median)	0.1	40,392
Raymond et al. (2013)	Global streams and rivers	6,708	3,100	/	/
Raymond et al. (2013)	Global lakes and reservoirs (7,939)	20,632	839	340	1,906
Raymond et al. (2013)	Global non-tropical lakes and reservoirs	/	1,410	/	/
Raymond et al. (2013)	Tropical lakes and reservoirs	/	4,390	/	/
Abril et al. (2015)	Global rivers and watersheds	761	3,707	36	23,047
Abril et al. (2015)	Leyre River, France	92	4,429	901	23,047
Abril et al. (2015)	Meuse River, Belgium	50	2292	179	10,033
Abril et al. (2015)	Amazon river, Brazil	155	4,204	36	18,400
Holgerson (2015) and citations herein	Global small temporary ponds (73)	/	7,717 ± 343 s.e.m.	2,279	22,670
Crawford et al. (2017)	Headwater streams, USA	>40,000	/	236	9,894
Weiss et al. (2018)	German reservoirs (4)	/	923.25 ± 49.14 s.d.	/	/

Table S2: Output of linear mixed models (LMM's) testing the effects of treatment, time (experimental interval or day) and the treatment x time interaction on somatic (som.) growth rate and body size of the water flea *D. magna* and the seed shrimp *H. incongruens* and population (pop.) growth rate and population size of the rotifer *B. calyciflorus*. Seed shrimp body size and rotifer population size was split in two periods for analysis (seed shrimp: period 1 = day 3-10, period 2 = day 10-24; rotifer: period 1 = day 2-13, period 2 = day 13-23) because of high mortality in the extreme treatment (T2).The models of period 1 include all three treatments, in period 2 they only include control (C) and the elevated treatment (T1). For the water flea, T2 was not included in any model and also the effect of clone and the treatment x clone interaction was tested.

	Water flea			**Seed shrimp**			**Rotifer**		
LMM	χ^2	Df	*p*	χ^2	Df	*p*	χ^2	Df	*p*
Som./pop. growth rate									
Treatment	6.667	1	**0.010**	101.910	1	**<0.001**	8.920	1	**0.003**
Time	18.096	2	**<0.001**	868.400	2	**<0.001**	181.813	2	**<0.001**
Treatment x Time	0.891	2	0.640	11.920	2	**<0.001**	181.813	2	**<0.001**
Clone	4.034	2	0.133						
Treatment x Clone	2.646	2	0.266						
Body/ pop. size									
Treatment	11.474	1	**<0.001**						
Time	406.350	1	**<0.001**						
Treatment x Time	9.893	1	**0.002**						
Clone	31.881	2	**<0.001**						
Treatment x Clone	8.172	2	**0.017**						
Period 1									
Treatment				3.406	2	0.182	2.0649	2	0.356
Time				503.206	1	**<0.001**	360.934	1	**<0.001**
Treatment x Time				185.589	2	**<0.001**	62.198	2	**<0.001**
Period 2									
Treatment				185.589	2	**<0.001**	39.138	1	**<0.001**
Time				30.749	1	**<0.001**	65.213	1	**<0.001**
Treatment x Time				15.187	1	**<0.001**	45.992	1	**<0.001**

Table S3: Mineral composition of the pond water in the control (C) and elevated (T1) and extreme (T2) pCO_2 treatment in our experiment. Elemental concentrations were measured with inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700x).

Component	Control	T1	T2
Na (mg/L)	79.00	83.79	71.68
Mg (mg/L)	4.32	4.55	4.22
Al (µg/L)	35.60	11.82	14.27
P (µg/L)	344.45	206.38	170.78
K (mg/L)	8.39	9.19	8.012
Ca (mg/L)	14.64	18.27	15.76
Cr (µg/L)	0.31	0.37	0.28
Mn (µg/L)	2.05	1.80	0.28
Fe (µg/L)	11.49	6.47	2.92
Co (µg/L)	0.13	0.11	0.10
Ni (µg/L)	0.95	0.93	0.92
Cu (µg/L)	11.27	5.48	10.18
Zn (µg/L)	12.05	11.59	29.37
Ga (µg/L)	1.95	1.69	1.80
As (µg/L)	1.20	1.27	1.18
Sr (µg/L)	100.98	121.13	112.00
Zr (µg/L)	1.93	3.31	2.95
Mo (µg/L)	0.78	0.82	0.70
^{111}Cd (µg/L)	0.54	10.31	0.06
^{114}Cd (µg/L)	0.55	10.90	0.05
In (µg/L)	0.02	0.01	0.01
Sn (µg/L)	0.29	0.11	0.12
Cs (µg/L)	0.04	0.05	0.04
Ba (µg/L)	14.09	12.28	12.89
Pb (µg/L)	0.59	0.39	0.55
U (µg/L)	0.16	0.12	0.06

Table S4: Comparison of pCO_2 calculation (mean ± standard deviation) according to Fasching et al., 2014 (**Appendix 4**) and the CO2SYS program (Pierrot et al., 2011) for the control (C), elevated (T1) and extreme (T2) pCO_2 treatments in our experiment. Also included are the values for the different carbon ions (HCO_3^-, CO_3^{2-} and CO_2) and their relative percentages.

Variables	**C**	**T1**	**T2**
pCO_2 (ppm)*	1,520 ± 702	25,609 ± 4,541	83,201 ± 15,533
pCO_2 (ppm)**	1,560 ± 725	26,262 ± 4,689	85,355 ± 16,061
HCO_3^- (µmol/kg)**	2,766 ± 18.00	2,858 ± 0.31	2,860 ± 0.18
CO_3^{2-} (µmol/kg)**	16.3 ± 9.00	0.8 ± 0.14	0.3 ± 0.05
CO_2 (µmol/kg)**	58 ± 27	1,004 ± 178	3,227 ± 585
% HCO_3**	97.37	73.99	46.99
% **CO_3^{2-}****	0.57	0.02	0.004
% **CO_2****	2.06	25.98	53.01

*Calculated according to Fasching et al. 2014.

**Calculated according to the CO2SYS program.

Table S5: Overview of different life history traits with frequency and technique of measurement per species. Growth rate/population growth was calculated for 3 intervals (int.) over the period of the experiment

Study species	Life history trait	Frequency	Technique
Water flea *Daphnia magna*	Body size	3x/week	Stereo-microscope
	Growth rate	Int. 1: day 1-6 Int. 2: day 6-17 Int. 3: day 17-24	Calculated from body size using formula: $\ln(BS_1)-\ln(BS_0)/(t_1-t_0)$
	Mean daily fecundity Lifetime fecundity	3x/week	Counting #neonates per female per clutch
	Mortality	Daily	No observed movement
Seed shrimp *Heterocypris incongruens*	Body size (BS)	2x/week	Stereo-microscope
	Growth rate (GR)	Int. 1: day 0-7 Int. 2: day 7-17 Int. 3: day 17-24	Calculated from body size using formula: $\ln(BS_1)-\ln(BS_0)/(t_1-t_0)$
	Mean daily fecundity Lifetime fecundity	2x/week	Counting #neonates per female
	Size at maturity Age at maturity	Daily (starting from day 14)	Stereo-microscope, when first eggs laid
	Mortality	Daily	No observed movement
Rotifer *Brachionus calyciflorus*	Population size	2x/week	Stereo-microscope/ Sedwick-Rafter counting chamber
	Population growth	Int. 1: day 0-6 Int. 2: day 6-16 Int. 3: day 16-23	Calculated from pop. size using formula: $\ln(PS_1)-\ln(PS_0)/t_1-t_0$
	Mean daily fecundity Lifetime fecundity	2x/week	Stereo-microscope/ Sedwick-Rafter counting chamber, counting #females with eggs
	Max. population size Max. population size timepoint	/	Calculated from pop. size
	Population mortality	Daily	No observed individuals

Supplementary references (**Table S1**; rest in main manuscript)

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Appendix 2: Supplementary figures

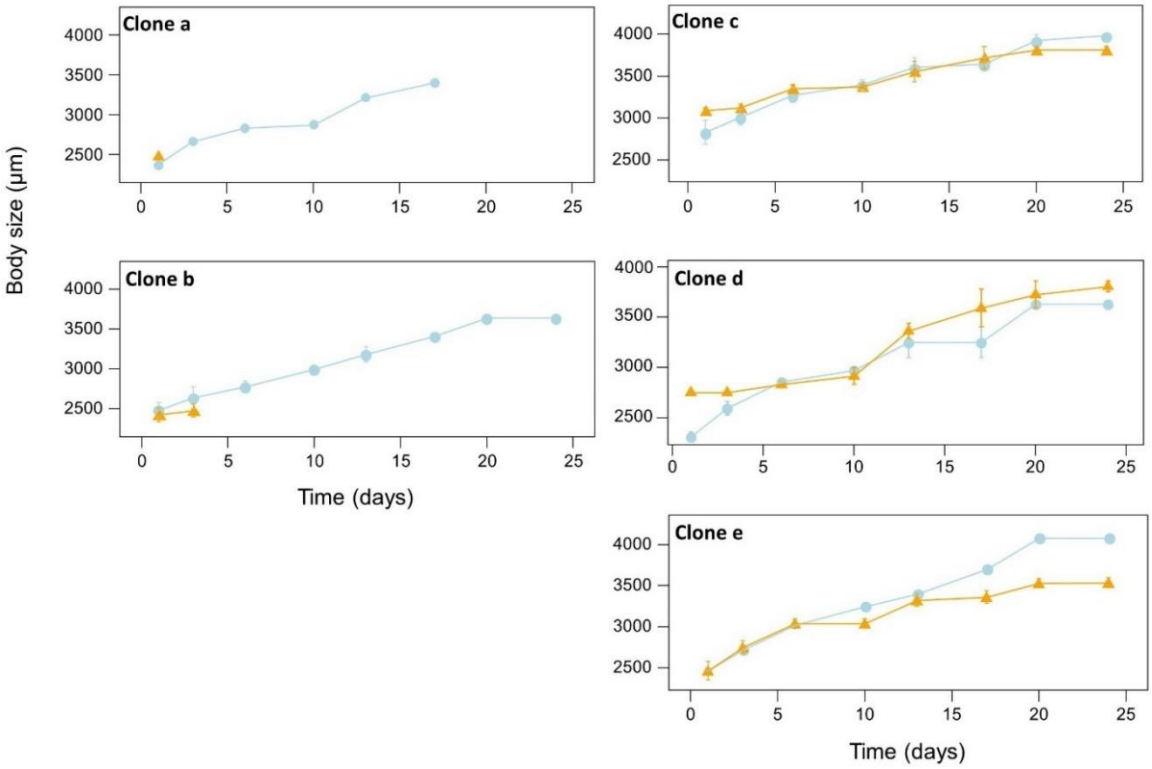

Figure S1: Differential response in body size of *D. magna* water flea clones (a-e) subjected to a control (C = 1,520 ppm; ●) and an elevated (T1 = 25,609 ppm; ▲) pCO_2 treatment. Error bars represent standard errors. Clone a and b were excluded from analysis since insufficient data was present in T1 due to high mortality.

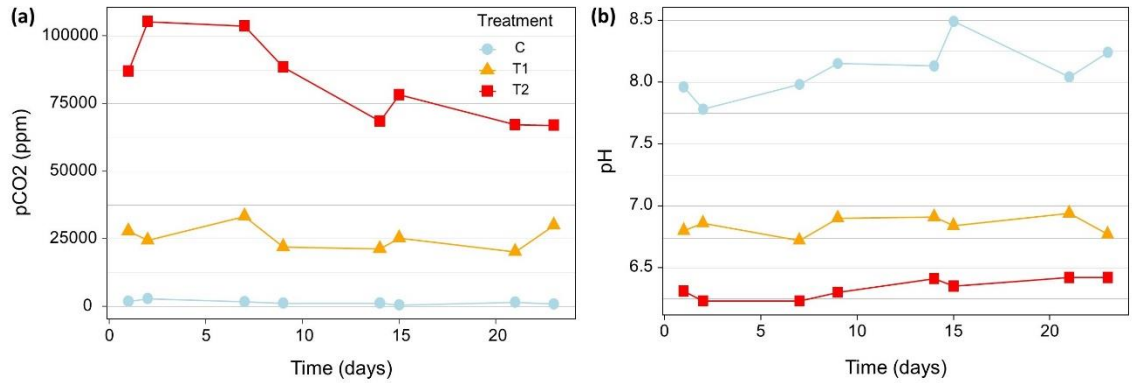

Figure S2: Time series of (a) calculated pCO_2 (ppm) and (b) pH over the duration of the experiment. Treatments include a control (C = 1,520 ppm ; ●), an elevated (T1 = 25,609 ppm ; ▲) and an extreme (T2 = 83,201 ppm; ■) pCO_2 treatment. Treatments were adjusted to stay within a range of ~ 20,000 – 30,000 ppm (pH 6.9 - 6.7) for T1 and ~ 70,000 – 120,000 ppm (pH 6.4 - 6.1) for T2.

Appendix 3: Supplementary methods

Animal culture and medium

Three freshwater model organisms were chosen to investigate the effects of elevated pCO_2 on different functional groups of primary consumers. Functional groups differently affect the food web and the broader ecosystem because of differences in functional traits such as feeding mode and size. The water flea *Daphnia magna* (Crustacea: Cladocera; 1.5 - 6 mm, [1]) is a large efficient pelagic filter feeder of phytoplankton and a dominant competitor in many small pond ecosystems [2]. Ostracods or seed shrimp are generally more benthic opportunistic collector gatherers. However, several species such as *Heterocypris incongruens* (Crustacea: Ostracoda; 1.5-2.5 mm, [3]) can be found free swimming in the pelagic consuming phytoplankton [4]. Finally, the rotifer *Brachionus calyciflorus* (Rotifera; 0.18-0.57 mm, [5]), is a small pelagic filter feeder in a wide range of freshwater systems consuming individuals algal cells [6]. *Daphnia* species typically have a high calcium content associated with their exoskeleton compared to other water flea species [7]. While seed shrimp also have highly calcified valves [8], rotifers are less reliant on calcium since their lorica consists mainly of keratin-like proteins [9]. The three selected species have broad distributions and can alternate between asexual and sexual reproduction. Sexual reproduction is typically restricted to stressful conditions e.g. at the onset of winter, under food limitation or when water levels drop [10].

Water fleas were sampled from two ponds on agricultural land in the province of West-Flanders, Belgium (Vleteren: 50°55'06.7" N, 2°43'27.0" E and De Haan 51°13'53.8" N, 3°01'49.2") in June 2018 and cultured in the lab for at least eight months under optimized laboratory conditions (20 $\pm$ 1 °C, 16:8 h light:dark cycle). Five different clonal lineages were reared separately and individually in 210 ml jars in incubators under similar conditions (at 20 $\pm$ 1 °C, 14:10 h light:dark cycle). They were fed frozen *Acutodesmus obliquus* green algae three times per week (500 µL, 100 x 10^6 cells/ml) and 70% of the medium was refreshed twice a week. Offspring was removed from the jars to avoid crowding. Fourth brood neonates were isolated and reared to maturity (i.e. the moment when a first clutch of eggs is released in the brood pouch), under similar standardized conditions as described

above, before treatment exposure. Seed shrimp and rotifer resting eggs were obtained from a commercial supplier (MicroBioTests Inc., *H. incongruens* strain MBT/1999/10, product code TB36; *B. calyciflorus*, product code TK21, Belgium). The seed shrimp originated from Ghent, Belgium and the rotifers from Florida, USA. Both are lab cultured and represent single clonal lineages. All resting eggs were inundated with EPA medium [11], which is demineralized water with 0.096 g/L $NaHCO_3$, 0.06 g/L $CaSO_4$-$2H_2O$, 0.06 g/L $MgSO_4$ and 0.004 g/L KCl, to reach a conductivity of 160 µS/cm, in petri dishes. The eggs were incubated at 25 °C and under permanent light conditions for 24 h for the rotifer and 52 h for the seed shrimp, before hatching. Hatchlings were transferred to 20 °C pond water for acclimatization and less than 24 h old when transferred to experimental conditions.

Natural pond water was used as medium to establish ecologically relevant conditions that mimic the complex water chemistry of ponds and a realistic level of buffering (i.e. the chemical ability to resist pH changes) that will lead to future pCO_2 induced acidification. The water was extracted from a typical Western European fish pond in the 'Midden-Limburg' pond complex (Grote vijver, 50°59'00.92" N, 5°19'55.85" E, Zonhoven, Belgium) with soft, poorly buffered water in November 2018. These ponds have been used for intensive fish farming for many decades and therefore most suffer from eutrophication, with trophic states varying between mesotrophic and eutrophic [12,13]. The pond complex was chosen since it is representative for many similar ponds in the wider region including neighboring countries. The pond water was filtered three times through a 64 µm plankton net to exclude zooplankton. The mineral composition of the pond water is reported in **Table S3** (**Appendix 1**). Regular checks of the culture medium never revealed any metazoan zooplankton. It was stored in a 500 L container in a climate-controlled room at 20 °C, aerated and kept in darkness to prevent algal growth. Medium was refreshed (70 %) twice a week.

Supplementary references

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Appendix 4: pCO_2 calculation formulas

$$xCO_2 = \frac{pCO_2}{P_{field}}$$

$$pCO_2 = \left(\frac{CO_{2_{aq}}}{K_H}\right) * 10^6$$

$$CO_{2_{aq}} = \frac{DIC}{1 + \frac{K_1}{[H^+]} + \frac{K_1 * K_2}{[H^+]^2}}$$

$$DIC = (A_T - \frac{K_w}{[H^+]} + [H^+]) * (\frac{[H^+]^2 + K_1 * [H^+] + K_1 * K_2}{K_1 * [H^+] + 2 * K_1 * K_2})$$

xCO_2 = partial pressure of CO_2 as mole fraction (or volume) in ppmv (assuming ideal gas)
pCO_2 = partial pressure of CO_2 (µatm)
P_{filed} = air pressure (atm)
CO_{2aq} = concentration of dissolved CO^2 (M/L)
K_H = Henry's constant (adjusted for temperature after Weiss 1974)
DIC = dissolved inorganic carbon (M C/L)
K_1 = Equilibrium constant for dissociation of H_2CO_3 (1^{st} acidity constant), adjusted for temperature (Stumm and Morgan, 1996)
K_2 = Equilibrium constant or dissociation of HCO_3^- (2^{nd} acidity constant), adjusted for temperature (Stumm and Morgan, 1996)
$[H^+]$ = proton concentration
A_T = total alkalinity (M/L)
K_w = ion product of water, adjusted for temperature (Stumm and Morgan, 1996)
Modified from Fasching et al. 2014.

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