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SUPPLEMENTARY INFORMATION

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Responses of pink salmon to CO_2-induced aquatic acidification

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1. Full Methods

1.1 Experimental design and CO_2 manipulation in freshwater

Pink salmon-eyed embryos were transported from Quinsam River Hatchery (Campbell River, Vancouver Island) to the University of British Columbia. Embryos were incubated in total darkness at 4.0 ± 1.0 °C in small containers filled with dechlorinated Vancouver City tap water ([Na^+], 0.17 mM; [Cl^-], 0.21mM; hardness, 30mgL^{-1} $CaCO_3$).). At 470 accumulated thermal units (ATU's; days x temperature in °C), embryos were pooled, sorted based on size and uniform sized embryos (weight of 169 ± 14 mg and a diameter of 7.0 ± 0.7 mm) were transferred into 1 of 4 different systems each consisting of three replicate tanks (1200 embryos per system; 450 embryos per 100 L replicate tank) and each was supplied with flow through dechlorinated tap water. The 4 systems consisted of a control treatment (477 ± 22 μatm; nominally referred to as 450 μatm), a constant medium CO_2 treatment (1036 ± 28 μatm; nominally referred to as 1000 μatm), a constant high CO_2 treatment (2031 ± 59 μatm; nominally referred to as 2000 μatm), and a 24 h oscillating CO_2 treatment (referred to as 450-2000 μatm). In each system, oxygenated tap water flowed through a reservoir tank where water was equilibrated with the respective CO_2 tension prior to being distributed to the replicate independent flow-through tanks. Dissolved oxygen in all tanks always exceeded 93% saturation. Desired pCO_2 tensions were set and maintained by mass flow controllers (Sierra Instruments, Model SmartTrak100 C100L) that bubbled either air, which had been partially stripped of CO_2, or a mixture of compressed CO_2

(5-30% CO_2 balanced with air) into the reservoir tank. Once target tensions were achieved, mass flow controller rates and water flow rates through the replicate tanks (0.3 $Lmin^{-1}$) were held constant throughout the entire 10-week experiment. For the oscillating treatment, the CO_2 mass flow controller was controlled by an Ardunio Uno circuit board (Ardunio, Ivrea, Italy), which varied the voltage output supplying the mass flow controller. The voltage was varied according to a sine function with a period of 24 h. Water pH and temperature in each system were monitored continuously in one of the replicate tanks with a glass electrode (Thermo Scientific, Orion Ross Ultra Triode) in conjunction with the Orion Star A211 pH meter (Thermo Scientific). Water pH values were recorded daily in each replicate tank and were stable over the 10 week exposure with little variation among replicates (range: ± 0.01 among replicates). All systems were housed in a temperature-controlled environmental chamber and water temperature was measured continuously at 3 h intervals in each replicate tank using iButtons (Thermochron, DS19212-F5#) and was 6.71 ± 0.9 °C over the 10 week freshwater exposure. Total dissolved inorganic carbon (TCO_2) was determined with a DIC analyzer (Apollo SciTech, Model AS-C3) using Andrew Dickson's Seawater Reference Materials (SRM; batch #117) over the duration of the experiment to verify that target pCO_2 values were achieved (Table S1). In each replicate, pH and temperature were measured in conjunction with TCO_2 every 2-3 weeks for estimates of pCO_2 (Table S1). Freshwater pCO_2 was calculated from pH, temperature and DIC with the program CO2SYS (Lewis and Wallace, 1998) using freshwater constants from Millero, 1979. In addition, water was taken directly from replicate tanks within a

given system, equilibrated with a known pCO_2 tension (410,1025 and 2050 μatm) generated using a gas-mixing pump (DIGAMIX 275 6KM, Wösthoff, Bochum, Germany) and the corresponding pH value was compared with replicate tank pH values used to calculate pCO_2 in CO2SYS (Table S2). This was used as a secondary method of water analysis to validate our measurements and ensure accuracy of our pH electrodes.

Supplementary Table S1. Freshwater parameters measured in experimental fish systems over the duration of the freshwater CO_2 exposure (week 1-10) for estimates of pCO_2. pH, TCO_2, temperature and salinity were measured directly from experimental fish tanks every 2-3 weeks. pCO_2 was calculated from these measurements in CO2SYS. Water parameters for the diurnal (450-2000 μatm) treatment were measured during peak pCO_2 tensions. Values are means of replicates ± SEM.

Treatment	450 μatm	1000 μatm	2000 μatm	450-2000 μatm
pH_{NBS}	7.26 ± 0.02	6.94 ± 0.03	6.64 ± 0.03	6.66 ± 0.03 (peak pCO_2)
TCO_2 (μmol ml^{-1})	192 ± 7	233 ± 3	292 ± 11	285 ± 4 (peak pCO_2)
pCO_2 (μatm)	477 ± 22	1036 ± 28	2031 ± 59	1979 ± 27 (peak pCO_2)
Temperature (˚C)	6.82 ± 0.58	6.76 ± 0.60	6.88 ± 0.53	6.82 ± 0.62

Supplementary Table S2. pH values (± SEM) measured with Orion Ross Ultra Triodes at specific pCO_2 tensions in freshwater (pH_{sw}) and seawater (pH_{fw}).

pCO_2 (μatm)	pH_{FW}	pH_{SW}
410	7.28 ± 0.01	8.07 ± 0.01
512	7.19 ± 0.03	7.99 ± 0.01
615	7.13 ± 0.01	7.93 ± 0.01
820	6.98 ± 0.03	7.81 ± 0.01
1025	6.92 ± 0.01	7.72 ± 0.01
1640	6.73 ± 0.01	7.52 ± 0.01
2050	6.63 ± 0.01	NA

1.2 Seawater transfer and CO_2 manipulation in seawater

Fry were transferred from their freshwater treatments at 995 ATU's (Table S3; week 9 post-hatch; week 11 post-CO_2 exposure) into 100L static aquaria containing seawater from the Vancouver Aquarium (salinity, 29.1 ± 0.3; temperature, 7.0 ± 0.2 ˚C, Total Alkalinity: 2015 μmol kg^{-1}). At this age, fry were actively swimming and were at the yolk sac absorption stage, which typically corresponds to gravel emergence and seaward migration. Time to yolk sac absorption was consistent with the age at which pink salmon fry start to emerge at the hatchery and yolk sac absorption times from previous studies (Gallagher et al. 2012). Since migrating fry still possess an internal supply of yolk reserves, fish were not fed during the entire 2-week seawater exposure. Fry reared in control freshwater conditions, were randomly transferred into 1 of 3 different seawater treatments: a control CO_2 treatment (448 ± 11 μatm; nominally referred to as 450 μatm), a high CO_2 treatment (1593 ± 30 μatm; nominally referred to as 1600 μatm),

or a 24 h oscillating CO_2 treatment (nominally referred to as 450-1600 μatm). Fry reared in high CO_2 freshwater conditions were randomly transferred to a high CO_2 seawater treatment (1593 ± 30 μatm; nominally referred to as 1600 μatm) (Fig. S1). 50 fry were transferred into each replicate tank per treatment (150 fry per treatment). Water changes in each replicate tank were performed daily with seawater equilibrated to the same pCO_2 tension. Ammonia and nitrates were measured daily and were negligible during the 2-week seawater exposure. Previous experiments suggest that transfer to 2000 μatm may be problematic and thus a high pCO_2 tension of 1600 μatm and not 2000 μatm was used. Each treatment consisted of 3 independent and static tanks, which were all housed in a temperature-controlled environmental chamber. For each treatment, desired pCO_2 tensions were set and maintained by Sierra mass flow controllers that bubbled either air, partially stripped of CO_2, or a mixture of compressed CO_2 (5-15% CO_2 balanced with air) through 3 separate lines into each of the static replicate tanks. Once target tensions were achieved, mass flow controller rates were held constant throughout the entire 2-week exposure. CO_2 oscillations were achieved using the same method as noted above. Seawater pH and temperature in each treatment was monitored continuously in one of the replicate tanks with a glass electrode (Thermo Scientific, Orion Ross Ultra Triode). Seawater pH and temperature were recorded daily from each replicate and were stable throughout the 2 week seawater exposure. Analysis of seawater samples for DIC was conducted in a manner similar to that of freshwater samples. TCO_2, along with pH, temperature and salinity, was measured in each replicate tank at the beginning and at the end of the seawater exposure and

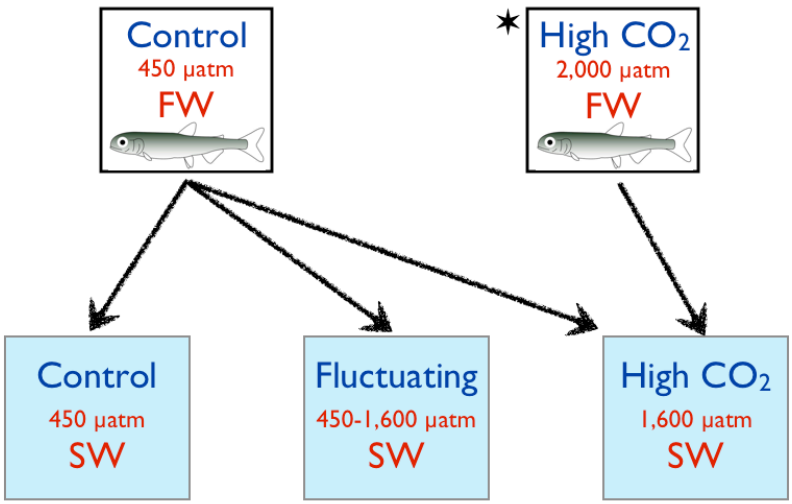
was used to calculate pCO_2 in in CO2SYS using dissociation constants from GEOSECS (NBS scale) (Table S4). Seawater samples were taken for total alkalinity (A_T) at day 14 post-transfer and were analyzed according to Dickson et al., 2007[51] at The Ocean Acidification Lab in Friday Harbor Labs, Washington. Similar to freshwater analysis, seawater from one of the replicate tanks per treatment was sampled and equilibrated with a known pCO_2 tension (410 and1640 μatm) generated using a gas-mixing pump (DIGAMIX 275 6KM, Wösthoff, Bochum, Germany) and the corresponding pH value was measured and compared with replicate tank values to ensure consistency (Table S2).

Supplementary Table S3. Time in weeks post CO_2 exposure in freshwater and following seawater transfer with corresponding age of pink salmon embryos/alevin/fry in accumulated thermal units (ATU). CO_2 exposure was initiated in this experiment at 2 weeks pre-hatch (week 0) up until 2 weeks following seawater transfer (week 13). YSA indicates yolk-sac absorption.

Time (weeks)	Age (ATU)	Notes
0	470.1	transfer into CO_2 treatments
1	529.8	
2	**586.6**	**hatch**
3	641.1	
4	692.3	
5	741.4	
6	785.4	
7	825.9	
8	868.4	
9	910.5	
10	952.6	
11	**994.7**	**seawater transfer/ YSA**
12	1037.5	
13	1072.4	end of CO_2 exposure

Supplementary Table S4. Seawater parameters measured in experimental fish systems over the duration of the seawater CO_2 exposure (week 11-13) for estimates of pCO_2. pH, TCO_2, temperature and salinity were measured directly from experimental fish tanks at the beginning and at the end of the seawater exposure. pCO_2 was calculated from these measurements in CO2SYS. Water parameters for the diurnal (450-2000 µatm) treatment were measured during peak pCO_2 tensions. Values are means of replicates ± SEM.

Treatment	450 µatm	1600 µatm	450-1600 µatm
pH_{NBS}	8.05 ± 0.01	7.54 ± 0.01	7.57 ± 0.02 (peak pCO_2)
TCO_2 (µmol ml^{-1})	1884 ± 30	2058 ± 32	2087 ± 5 (peak pCO_2)
***p*CO_2 (µatm)**	448 ± 11	1593 ± 30	1532 ± 22 (peak pCO_2)
Temperature (˚C)	6.55 ± 0.26	6.92 ± 0.19	7.37 ± 0.24
Salinity (ppt)	29.2 ± 0.4	29.0 ± 0.2	29.2 ± 0.2



Supplementary Figure S1: Transfers of pink salmon at yolk sac absorption from freshwater CO_2 treatments into seawater CO_2 treatments. Fry reared in control freshwater conditions for 11 weeks were transferred into a control, a fluctuating and a high CO_2 seawater treatment to mimic fish migrating into future CO_2 conditions in the ocean or current day local coastal CO_2 conditions. *Fry reared at high CO_2 in freshwater for 11 weeks were transferred into high CO_2 seawater to mimic future elevated CO_2 conditions in both environments.

1.3 Growth and yolk consumption

Measurements of developing pink salmon wet mass (wtm) and length (n=8 for wet mass; n=8 for length) were taken weekly from 2 weeks pre-hatch up until 2 weeks post-seawater transfer (11 weeks post-hatch). 3 embryos/fish were sampled from 2 of the 3 replicates and 2 embryos/fish were sampled from the last replicate to give a sample size of 8. Embryos/fish were then fixed in 5% neutral buffered formalin for up to 21 days before the yolk was dissected from the fish to obtain yolk wtm and tissue wtm measurements. These samples were then dried in an oven at 58 ˚C for at least 5 days to obtain dry weights for alevin tissue and yolk. Gross production efficiencies at week 10 were calculated as the increase in tissue dry mass divided by the loss in yolk dry-mass multiplied by 100. Growth rates in seawater were calculated as the difference in alevin wtm at day 14 and at day 0 post-seawater transfer.

1.4 Respirometry

Routine $\dot{M}O_2$ ($\dot{M}O_{2routine}$) and maximum $\dot{M}O_2$ ($\dot{M}O_{2max}$) were measured throughout development in freshwater and following seawater transfer. To accommodate the growing size of developing fish, 3 different-sized closed respirometers were used: 3.41 ± 0.04 ml for week 1- 4; 7.43 ± 0.10 ml for week 5-10; and 10.61 ± 0.12 ml for day 1-14 post-seawater transfer. Chambers were rinsed in Virkon ® Aquatic (Syndel Laboratories, Qualicum Beach, British Columbia, Canada) at the end of each day to reduce background bacterial respiration, which was determined to be negligible from blank experiments. To ensure adequate water

mixing, a mini stir bar was placed beneath a false bottom mesh in all the chambers. A total of 8 fish per treatment (4 treatments, where 8 fish were randomly pooled from replicate tanks) per time point (weekly) were used for measurement of $\dot{M}O_{2routine}$ and $\dot{M}O_{2max}$ in the water from the respective pCO_2 treatment in which the fish were reared.

Routine $\dot{M}O_2$ was measured weekly throughout development in freshwater and up until 14 days post-seawater transfer. Fish were placed in the chambers, where they were acclimated in darkness at an appropriate acclimation time (2 h for eggs, 3 h for alevin, 4 h for fry) in flow-through water of their respective pCO_2 treatments. Acclimation times were determined by preliminary testing, which indicated that longer periods did not significantly reduce $\dot{M}O_{2routine}$. After the acclimation period, the chamber was sealed and the decline in oxygen was monitored over time, using a fiber optic oxygen sensor (PreSens, Model NFSL) to calculate mass-specific $\dot{M}O_{2routine}$. Once O_2 levels reached 60% saturation, the measurement was terminated and the chamber was re-supplied with flow through water. The sensor was connected to a 4-channel oxygen meter (PreSens, OYY-4 Micro), allowing the simultaneous recording of 4 chambers. Data was obtained using the accompanying software (OXY-4v2_11TX), with temperature compensation. We referred to our resting MO_2 measurements as routine and not standard metabolic rate to account for spontaneous movements from the fish in the chamber.

The maximum rate of oxygen uptake ($\dot{M}O_{2max}$) immediately following an exhaustive chase protocol was used as an index of maximal O_2 uptake capacity. $\dot{M}O_{2max}$ was measured weekly from week 3-10 in freshwater and from day 1-14

post-seawater transfer, using a chase protocol similar to the one outlined in Healy and Schulte[51]. Briefly following the determination of $\dot{M}O_{2routine}$, the fish was removed from the chamber, placed in a container filled with the respective pCO_2 water and then chased to exhaustion with a plastic pipette for a maximum of 4 minutes. The inability to right itself within 3 seconds following inversion was defined as complete exhaustion. At this point the fish was immediately returned to the chamber and $\dot{M}O_{2max}$ was measured (all within 30 s). $\dot{M}O_{2max}$ was calculated from the initial decrease in oxygen (within 5 min) following closure of the chamber. Although maximum sustained swimming speed (U_{crit}) is typically used to obtain $\dot{M}O_{2max}$ in fish, previous studies have shown that chase protocols can yield similar or higher values of $\dot{M}O_{2max}$ in some species of fish[52,53,54]. Burst swimming and exhaustive chase protocols are often used to estimate $\dot{M}O_{2max}$ in larval fish and species that are poor swimmers[54,55,56,57]. During the alevin stage (yolk present), pink salmon swim poorly and exhibit burst swimming, making U_{crit} (sustained swimming) tests a poor indicator of $\dot{M}O_{2max}$. In larger and more developed pink salmon fry (~2.81 g vs. the 0.16-0.20 g fish in our study), U_{max} (constant acceleration) and U_{crit} swim tests yielded similar fatigue speeds regardless of the type of test utilized, suggesting that similar metabolic processes were occurring during both protocols[58]. Similar results have been observed in swimming small fish including other species of salmonids[59,60]. Early in development, fish may rely more on anaerobic metabolism during exercise while aerobic muscles are still developing[60] and thus an indicator of maximal oxygen uptake capacity, as conducted here, will inform on both aerobic capacity and the rate of recovery following exhaustion.

1.5 Alarm cue extraction for electro-olfactograms and two-channel choice test

Skin and muscle tissue from pink salmon fry were used as a source of the alarm cue. Approximately 500 pink salmon fry were euthanized with tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, WA, USA) (0.5 g L^{-1} of distilled water buffered with $NaHCO_3$ to pH 7) and rinsed with distilled water before skin and muscle filets were dissected from the fish. The skin and muscle fillets were homogenized, diluted with distilled water and filtered through filter floss to remove any large tissue particles. The skin extract was then diluted with distilled water to yield a stock solution with a concentration of 0.1 g of tissue per ml of water. The stock solution was frozen in 20 ml aliquots and stored at -20 ˚C until needed.

1.6 Two-channel choice test

A two-channel choice flume[61] was used to assess behavioral responses of pink salmon fry (week 11 of CO_2 exposure) to conspecific alarm cues in freshwater. The flume allowed pink salmon to freely choose between two different sides of the flume that contained water from two different sources. One water source consisted of freshwater at the same pCO_2 tension that the fish was reared in with alarm cue skin extract added to yield a final testing concentration of 0.5 mg ml^{-1}. The other source consisted of freshwater at the same pCO_2 tension with distilled water added in place of the skin extract. Similar to the protocols in Gerlach at al.[61] and Dixson et al.[1], water from two different sources were gravity fed into the flume at a rate of 100 ml min^{-1}, which was set and maintained by flow controllers. Colored dye was used to check for laminar flow at the end of trials. During each trial, a single fry was placed

at the downstream end of the flume and allowed to acclimate for 2 min. Fry that did not actively swim during this acclimation period were discarded. After acclimation, the location of the head of the fry was recorded at the end of every 5 s interval for 2 min. The steps (including acclimation) were repeated after switching the sides that the respective water sources were introduced into the choice flume to control for potential side preference. Fry were discarded after each trial.

1.7 Alarm cue electro-olfactogram recording

EOG's were conducted in freshwater on pink salmon fry at week 11 of the CO_2 exposure (n=3). Prior to EOG recording, the fish was anesthetized in 0.3 mg L^{-1} tricaine methanesulfonate (MS-222, Western Chemicals, Ferndale, WA, USA) buffered with sodium bicarbonate for ~5 seconds. Immediately afterwards, fish were placed in the EOG setup and were constantly perfused with freshwater at the respective pCO_2 tension it was reared in for the duration of the experiment. The gelatin-filled glass capillary pipettes (8% agar in 0.8% NaCl) were placed to bridge the sensory organ of the animal to silver chloride (Ag/AgCl) microelectrodes filled with 3 M potassium chloride (KCl). Nasal bridge-skin was gently removed with fine forceps to expose the olfactory epithelium. The recording and the reference electrodes (World Precision Instruments, Sarasota, FL, USA) were then positioned over the surface of the olfactory epithelium and the skin around the nasal cavity, respectively (Supplementary Fig. S2). The olfactory epithelium was constantly perfused with freshwater at the respective rearing pCO_2 tension (12.9 ml min^{-1}) while a timer-controlled solenoid valve (Model 655, GRALab, Centerville, OH, USA)

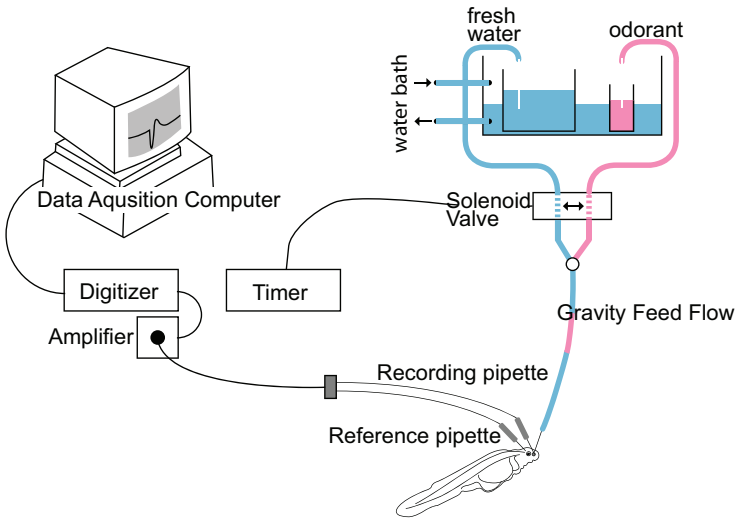
delivered test odorants (430 μl/2 s) by switching between freshwater and the alarm cue dilutions. Amplified signals (ML132, ADInstruments, Dunedin, Otago, New Zealnad) were visualized on the computer monitor through digitizing (ML866, ADInstruments) and filtering devices (low-pass, cut-off frequency 50Hz). Freshwater at the respective pCO_2 tension was used as a negative control in EOG recording (Supplementary Fig. S2). Serial dilutions of the alarm cue skin extract were prepared by diluting the stock solution (0.1 g of tissue per ml of water) with freshwater at the respective rearing pCO_2.

1.8 Amino acid electro-olfactogram recording

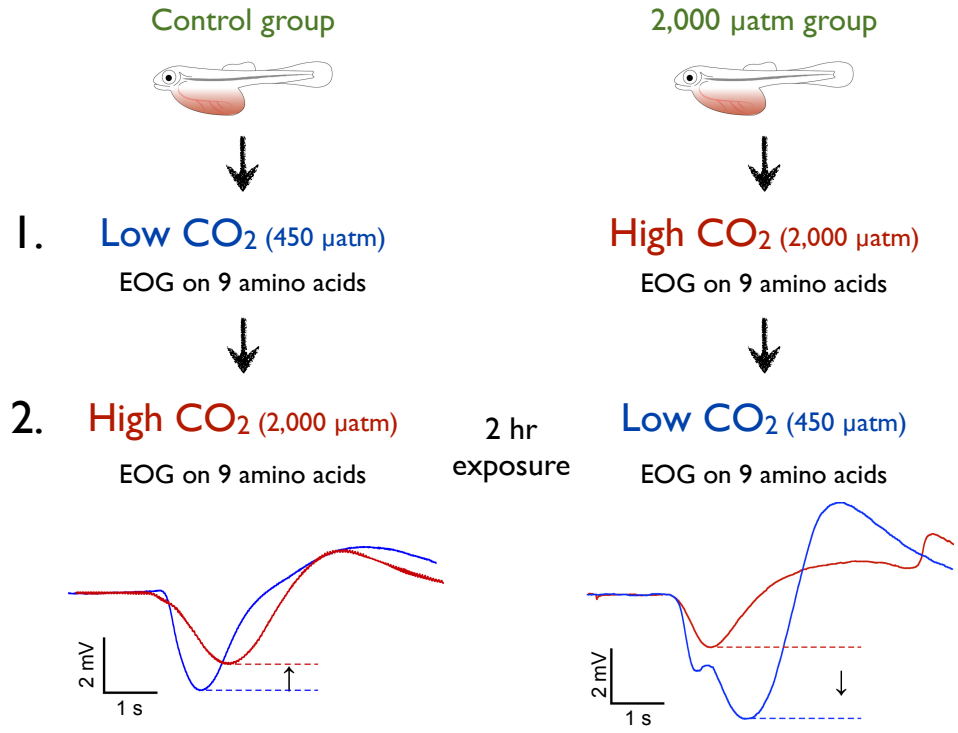
EOG's were conducted in freshwater on pink salmon alevin (yolk still present) at week 8 of the CO_2 exposure (n=5). Fish were anesthetized and prepared for EOG recordings with the same procedures and equipment described above (Supplementary Fig. S2). After positioning the recording electrodes, each fish was perfused with freshwater at the respective rearing pCO_2 tension (control or 2000 μatm) for 30 min. After this period of acclimation, electrophysiological responses to 9 different amino acids at the olfactory epithelium were recorded (Supplementary Fig. S3). The 9 amino acids selected (L-arginine, L-asparagine, L-aspartic, L-cysteine, L-glutamic, L-glutamine, L-histidine, L-lysine, L-threonine) showed relatively large response magnitudes compared to all other amino acids and thus were selected for EOG testing in pink salmon fry. The olfactory epithelium was constantly perfused with freshwater at the respective rearing pCO_2 tension (12.9 ml min^{-1}) while a timer-controlled solenoid valve (Model 655, GRALab, Centerville, OH, USA)

delivered test odorants (430 µl/2 sec) by switching between the respective freshwater type and a particular amino acid dilution. After recordings in rearing conditions, control fish were transferred into 2000 µatm freshwater and 2000 µatm -reared fish were transferred into 450 µatm freshwater for 2 h. After 2 h of acclimation, EOG responses to the same 9 amino acids were recorded on control and 2000 µatm -reared fish.

Stock solutions of amino acids (MAK002-1KT, Sigma-Aldrich, St. Louis, MO, USA) were prepared in deionized water and stored at 4 °C. Freshwater at the pCO_2 tension that was being tested was used to dilute the amino acid odorants (10^{-3}M) for all EOG experiments.



Supplementary Figure S2. Electro-olfactogram (EOG) setup. A fish was situated on copper-wire grounded EOG table after MS-222 anesthetization. After removing tissue covering the nasal cavity in larval fish, a gravity feed flow delivery system perfused CO_2 tensioned freshwater and chemical stimuli alternatively into olfactory epithelium by switching the solenoid valve. With a timer that switched the solenoid valve back and forth, the exact same amount of chemical stimuli was delivered to the olfactory epithelium. Recording and reference pipettes that were located on the olfactory epithelium and skin around nasal cavity, respectively, collected the sum of the potential difference. Finally, the sum of the action potentials was amplified and visualized on a computer monitor.

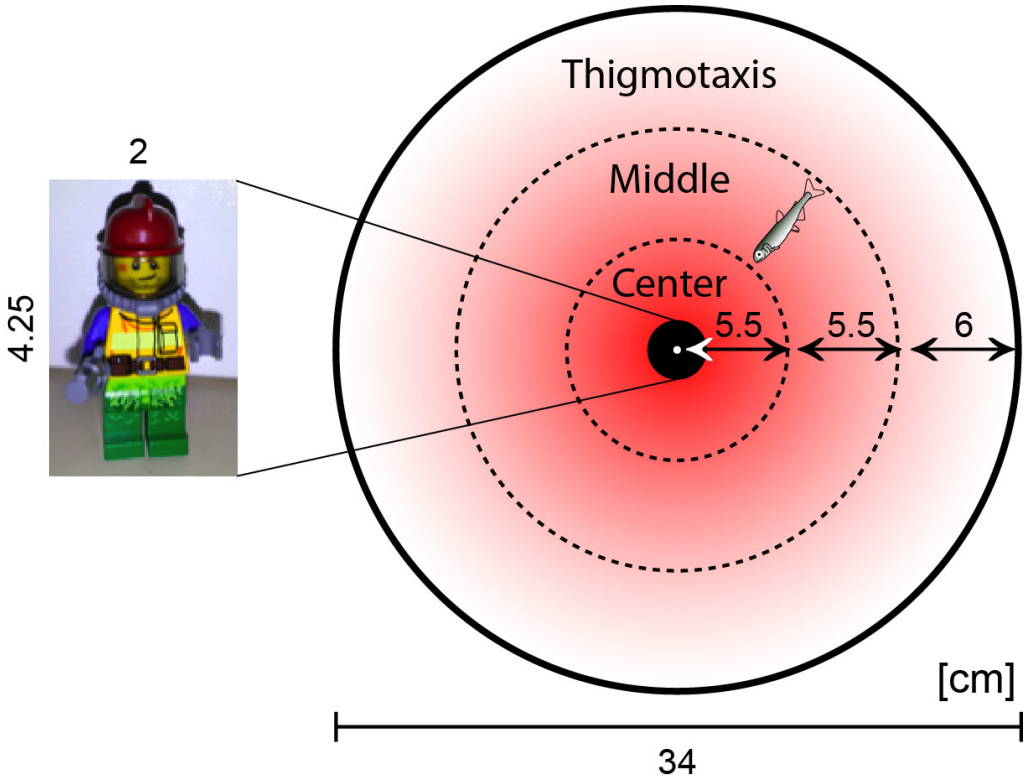


Supplementary Figure S3. Amino acid EOG recording procedure for pink salmon alevin at week 8 of the CO_2 exposure. 1) Control fish reared at 450 µatm were tested for their EOG responses to 9 different amino acids in 450 µatm freshwater and then 2) transferred into 2000 µatm freshwater for 2 h, after which EOG responses to the same amino acids were measured. 1) High CO_2 reared-fish (2000 µatm group) were tested for their EOG responses to 9 different amino acids in 2000 µatm freshwater and then 2) transferred into 450 µatm freshwater for 2 h, after which EOG responses to the same amino acids were measured (n=5). Trace lines at the bottom of the figure represent changes in olfactory sensitivities in 10^{-3}M of cysteine before and after the 2 hr exposure in either control (blue line) or high CO_2 conditions (red line). Vertical and horizontal scale bars show 2 mV and 1 s, respectively.

1.9 Novel approach test

The novel approach test consisted of an arena, 31 cm in diameter, and a small Lego figurine, which was placed in the center of the arena[5]. Before each trial, the arena was filled to a height of 10 cm with new freshwater at the respective rearing pCO_2 tension (450, 1000 or 2000 µatm). The arena was divided into 3 virtual zones

(center, middle and thigmotaxis zone; (Supplementary Fig. S4) and time spent in each zone was measured using ETHOVISION XT (v. 9.0, Noldus, Leesburg, VA) motion tracking software. A single fish was placed in the arena and released from the net into the middle zone, facing the object, and allowed to freely swim out of the net. Trials were initiated approximately 5s after the fish was placed in the arena. For the gabazine treatments, only fish reared at 450 or 2000 µatm were tested. Fish were individually placed in a solution of gabazine (4 mg l^{-1}, 10.9 µM) dissolved in the respective freshwater pCO_2 for 30 min. Following this, fish were introduced to the arena for testing at the respective pCO_2. Each fish was tested only once.



Supplementary Figure S4. Open field test for anxiety setup.

1.11 Statistical analysis

Data are expressed as means ± SEM, and were log-transformed when necessary to meet the assumptions of normality and equal variance. For all tests, a significance level of $p<0.05$ and a confidence intervals of 95% was used.

One-way nested ANOVAs were used to analyze wet mass, dry mass, length, production efficiency, growth rates and behavioral responses to alarm cue data using the software SAS JMP® 11 (Version 11.2, SAS Institute Inc., Cary, NC). Subjects were nested within their replicate tank, which was nested within their respective treatment. Pairwise comparisons among treatments and comparisons to the control were made using the TukeyHSD and Dunnett post-hoc test when significance was found.

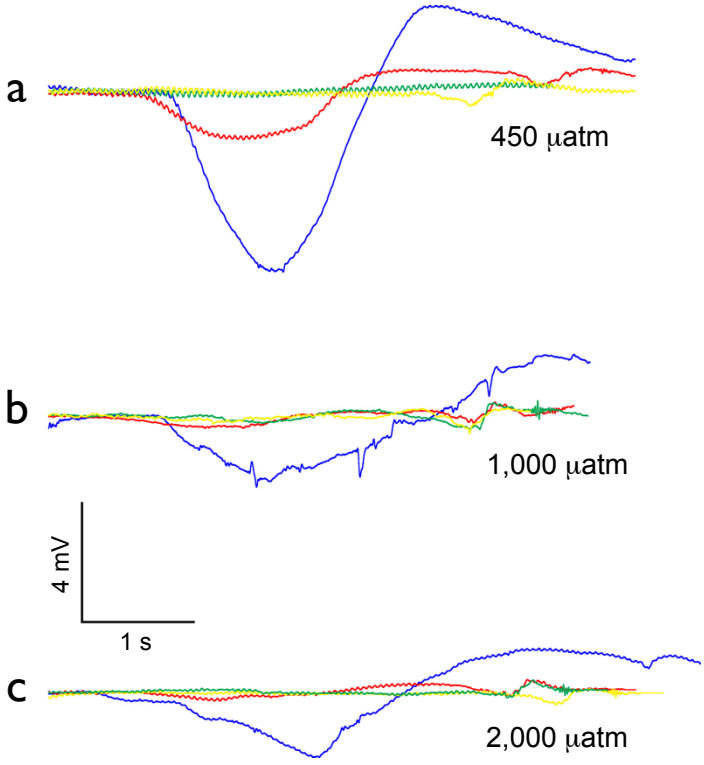
Mixed effects models with time as a repeated factor were used to analyze log-transformed mass-specific $\dot{M}O_{2routine}$ and mass-specific $\dot{M}O_{2max}$ data using the program SAS JMP® Pro 11 (Version 11.2, SAS Institute Inc., Cary, NC). Time and treatment were treated as fixed effects and tank (nested within treatment) was treated as a random effect. Since there were no significant interaction effects (time x treatment) on mass-specific $\dot{M}O_{2routine}$ and mass-specific $\dot{M}O_{2max}$, the interaction term was removed from our mixed models. Preliminary two-way ANOVAs including log-transformed mass as a factor showed no significant effects of mass on mass-specific $\dot{M}O_{2routine/max}$ and as a result, mass was dropped from our models. This is consistent with the finding that early in development, $\dot{M}O_{2routine}$ and $\dot{M}O_{2max}$ scales isometrically with body mass[44,46]. All $\dot{M}O_2$ data are expressed as mass-specific

metabolic rates. Comparisons to the control group using the Dunnett post-hoc test were made when significance was detected.

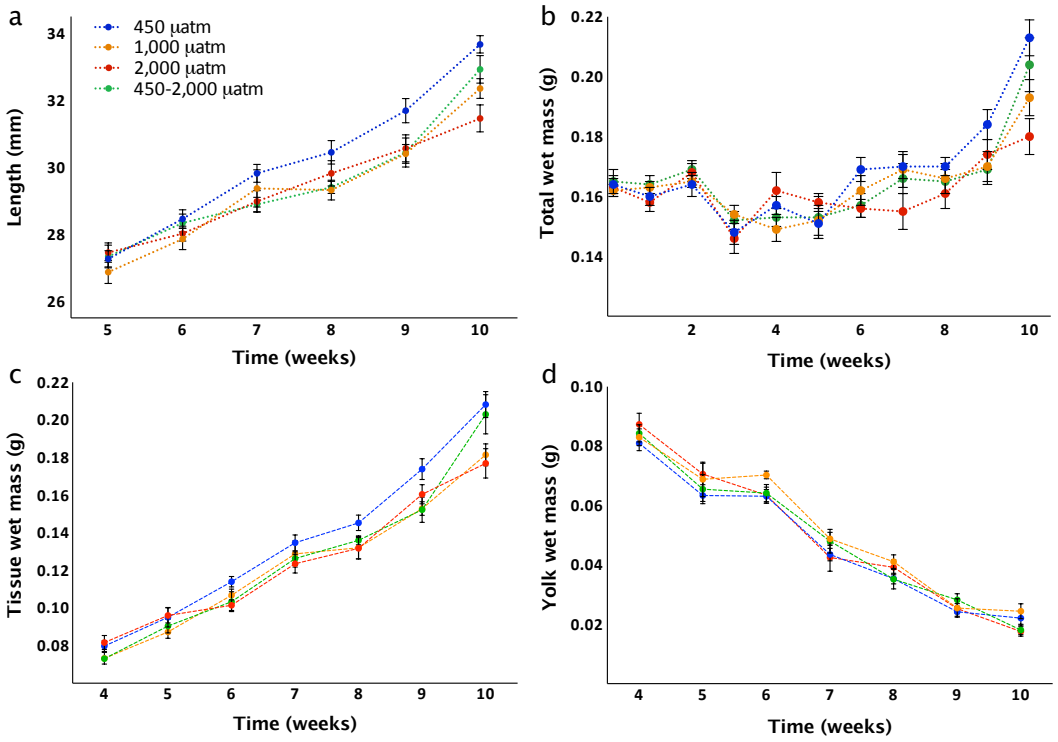
A repeated measures ANOVA was used to analyze alarm cue EOG dose-response-curve data with SPSS Statistics 21 (Version 21.0; IMB Corp., Armonk, NY). Greenhouse-Geisser adjusted F values were used because the assumptions of sphericity were not met. Comparisons among groups were made using the Sidak method for post- hoc testing. Student's paired t-tests were used to compare EOG responses to each amino acid in freshwater for control fish before and after transfer to 2000 μatm and 2000 μatm-reared fish before and after transfer to 450 μatm CO_2. Comparisons of EOG responses before transfers were also made between control and 2000 μatm-reared fish using Student's t-tests. All amino acid EOG data was analyzed with the program SigmaPlot (Version 12.0; SYSSTAT Software Inc., San Jose, CA).

Data from our novel approach test were not normally distributed and thus, non-parametric tests were performed (SAS JMP 11). Times spent in different zones were analyzed using the Dunn method (with control) for multiple comparisons or using the Wilcoxon test for gabazine treated comparisons (+ gabazine).

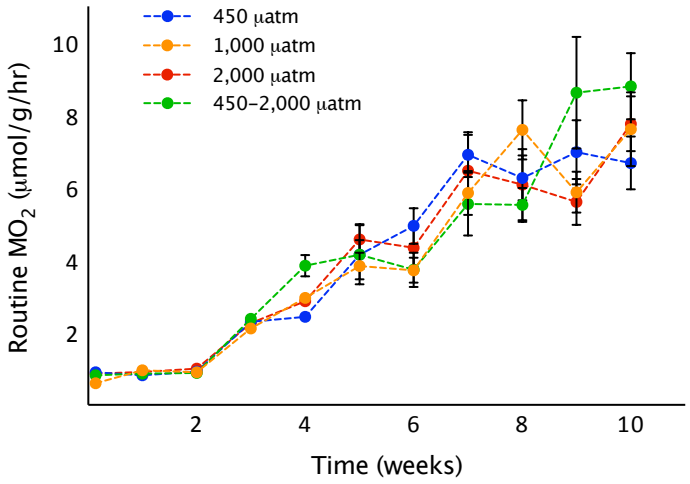
2. Results:



Supplementary Figure S5. Alarm cue EOG dose-dependent traces of pink salmon fry reared and tested at a) 450 μatm, b) 1000 μatm, and c) 2000 μatm CO_2. Diluted alarm cue extract (10, 1, 0.1, and 0.01 mg/ml for blue, red, yellow, and green, respectively) was applied on the olfactory epithelium to generate the EOG trace lines. Vertical and horizontal scale bars show 4 mV and 1 s, respectively.



Supplementary Figure S6. Pink salmon growth measurements during development in freshwater up until 1 week before seawater transfer (week 10 of CO_2 exposure) at different pCO_2 tensions (indicated in the figure where 450-2000 µatm represents a diurnal cycle and other tensions are constant throughout). **(a)** Lengths. **(b)** Total alevin wet mass (yolk and fish). **(c)** Tissue wet mass (yolk excluded). **(d)** Yolk wet mass. Values are means ± SEM (n = 7-8). There was significant effect of CO_2 treatment on fork length, total wet mass and tissue wet mass throughout freshwater development (length: $F_{3, 20}$ =6.9224, p = 0.0022; total wtm: $F_{3, 19}$ = 4.2394, p = 0.0188; tissue wtm: $F_{3, 17}$ = 8.4497, p = 0.0012). There was no significant effect of CO_2 treatment on yolk wet mass throughout freshwater development ($F_{3, 17}$ = 1.4897, p = 0.2530) (MANOVA with individuals nested within tanks, p<0.05).



Supplementary Figure S7. Routine $\dot{M}O_2$ during development in freshwater from 2 weeks pre-hatch (time 0 of CO_2 exposure) up until 1 week before seawater transfer (week 10 of CO_2 exposure) at different pCO_2 tensions (indicated in the figure where 450-2000 μatm represents a diurnal cycle and other tensions are constant throughout). Values are means ± SEM (n = 6-8). There was no significant effect of CO_2 treatment on routine $\dot{M}O_2$ throughout freshwater development ($F_{3,5}$ = 0.5085, p = 0.6935; MANOVA with individuals nested within tanks, $p<0.05$).

3. Supplementary References

(see main paper for references 1-50)

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