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Elevated CO_2 impairs olfactory-mediated neural and behavioral responses and gene expression in ocean-phase coho salmon (*Oncorhynchus kisutch*)

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Abstract

Elevated concentrations of CO_2 in seawater can disrupt numerous sensory systems in marine fish. This is of particular concern for Pacific salmon because they rely on olfaction during all aspects of their life including during their homing migrations from the ocean back to their natal streams. We investigated the effects of elevated seawater CO_2 on coho salmon (*Oncorhynchus kisutch*) olfactory-mediated behavior, neural signaling, and gene expression within the peripheral and central olfactory system. Ocean-phase coho salmon were exposed to three levels of CO_2, ranging from those currently found in ambient marine water to projected future levels. Juvenile coho salmon exposed to elevated CO_2 levels for 2 weeks no longer avoided a skin extract odor that elicited avoidance responses in coho salmon maintained in ambient CO_2 seawater. Exposure to these elevated CO_2 levels did not alter odor signaling in the olfactory epithelium, but did induce significant changes in signaling within the olfactory bulb. RNA-Seq analysis of olfactory tissues revealed extensive disruption in expression of genes involved in neuronal signaling within the olfactory bulb of salmon exposed to elevated CO_2, with lesser impacts on gene expression in the olfactory rosettes. The disruption in olfactory bulb gene pathways included genes associated with GABA signaling and maintenance of ion balance within bulbar neurons. Our results indicate that ocean-phase coho salmon exposed to elevated CO_2 can experience significant behavioral impairments likely driven by alteration in higher-order neural signal processing within the olfactory bulb. Our study demonstrates that anadromous fish such as salmon may share a sensitivity to rising CO_2 levels with obligate marine species suggesting a more wide-scale ecological impact of ocean acidification.

KEYWORDS
GABA, ocean acidification, olfactory bulb, olfactory rosette, salmon

1 | INTRODUCTION

The substantial rise in atmospheric CO_2 observed over the past 100 years has led to increased concentrations of dissolved CO_2 in marine waters, resulting in lowered pH, a process known as ocean acidification (OA). The degree of pH change and the rate at which these changes are occurring may ultimately exceed many marine organism's ability to adapt to this changing environment (Hoegh-Guldberg & Bruno, 2010). Marine biota have evolved to live in ocean waters with a consistent range in chemical composition, and therefore, even small changes in mineral content, pH, and/or temperature outside of the normal range can have large impacts on marine organisms at different life stages (Fabry, Seibel, Feely, & Orr, 2008; Kroeker et al., 2013; Marshall et al., 2017). Hard corals, hard-shelled mollusks, and plankton are among the more well-known examples of marine organisms that are sensitive to shifts in water chemistry induced by elevated CO_2 (Busch, Maher, Thibodeau, & McElhany, 2014; Hofmann et al., 2010; Orr et al., 2005).

While the effects of elevated CO_2 on calcifying organisms such as corals and mollusks have received considerable attention, the possible effects of elevated CO_2 on the neurophysiology and behavior of marine fish are an increasing concern (Ashur, Johnston, & Dixson, 2017). Elevated CO_2 has been linked to abnormal neuronal and behavioral responses in several species of marine fish including effects on auditory function (Simpson et al., 2011), vision (Chung, Marshall, Watson, Munday, & Nilsson, 2014; Ferrari et al., 2012), lateralization (Domenici, Allan, McCormick, & Munday, 2011), and elevated anxiety (Hamilton, Holcombe, & Tresguerres, 2014). In particular, a number of studies have implicated changes in CO_2 and pH levels on altered olfactory-mediated behaviors in marine fish from both tropical and temperate environments (Chivers et al., 2014; Cripps, Munday, & McCormick, 2011; Devine, Munday, & Jones, 2012; Dixson, Munday, & Jones, 2010; Ferrari et al., 2012; Hamilton et al., 2014; Leduc, Munday, Brown, & Ferrari, 2013; Miller, Watson, Donelson, McCormick, & Munday, 2012; Porteus et al., 2018).

The olfactory system is critical for many aspects of a fish's life including locating appropriate habitat, finding prey, avoiding predators, social and reproductive interactions with conspecifics, orientation, and navigation (Dittman & Quinn, 1996; Gerlach, Atema, Kingsford, Black, & Miller-Sims, 2007; Hara, 1992; McIntyre, Baldwin, Beauchamp, & Scholz, 2012; Quinn, 2011; Yambe et al., 2006). Fish rely on their olfactory system for survival, and any olfactory impairment may have profound effects on wild fish populations (Baldwin, Sandahl, Labenia, & Scholz, 2003; Sandahl, Baldwin, Jenkins, & Scholz, 2007). The olfactory system in most fish consists of a peripheral sensory epithelium (olfactory rosette) that connects directly to the olfactory bulb. Odorants in the environment bind to receptors on olfactory sensory neurons in the sensory epithelia, eliciting axon potentials that send a signal to the olfactory bulb. At the olfactory bulb, the signal is modulated and relayed to secondary neurons and higher brain centers, ultimately leading to behavioral responses (Hamdani & Doving, 2007). Neural signaling within this complex process, from odorant detection to behavioral outcome, is highly dependent upon tightly controlled ion gradients across neuronal membranes (Schild & Restrepo, 1998) and is highly sensitive to changes in water chemistry (Tierney et al., 2010).

Elevated CO_2-mediated interference of olfactory function could have profound effects on marine fish survival. For example, tropical reef fish exposed to CO_2 concentrations predicted to occur within the next 50–100 years demonstrated altered responses to odors that allowed fish to discriminate healthy reef habitat and that facilitated homing and dispersal (Devine et al., 2012; Munday et al., 2009). Furthermore, elevated CO_2 levels altered normal avoidance responses of fish to predator odors and chemical alarm cues (Dixson et al., 2010; Welch, Watson, Welsh, McCormick, & Munday, 2014) and interfered with prey detection abilities in reef predators (Cripps et al., 2011) and sharks, a group of fish known for their reliance on their highly sensitive olfactory system (Dixson, Jennings, Atema, & Munday, 2014). Finally, OA-related conditions interfered with the process of olfactory learning by reef fish (Ferrari et al., 2012). Several studies have extended these findings to directly demonstrate that CO_2-mediated interference of olfactory function may have direct effects on survival (Dixson et al., 2010; Ferrari et al., 2015). However, if a fish is exposed to elevated CO_2 and survives to successfully reproduce, recent research on multigenerational effects of parental exposure to elevated CO_2 has shown that offspring can exhibit enhanced resistance to the effects of elevated CO_2 (Allan, Miller, McCormick, Domenici, & Munday, 2014; Murray, Malvezzi, Gobler, & Baumann, 2014; Schunter et al., 2017; Welch & Munday, 2017; Welch et al., 2014).

Pacific salmon are a critical component of Pacific Northwest coastal ecosystems (Quinn, 2011). Anadromous (rear in saltwater but spawn in freshwater) salmon populations may be particularly impacted by ecosystem changes (Crozier et al., 2008) because they rely on both the freshwater and marine environment for different life cycle stages (Quinn, 2011). In this respect, salmon, and other anadromous fishes, may be particularly interesting species to study in the context of the sensitivity or resistance to the effects of elevated CO_2 because elevated CO_2 is likely to have different physiological effects in freshwater and saltwater. Some obligate marine fish species (e.g., benthic dwellers) have displayed a potential resistance to the effects of elevated CO_2 on neuronal function and behavior due to the seawater chemistry of their preferred habitat (Hamilton et al., 2017; Jutfelt & Hedgärde, 2015; Schmidt et al., 2017). While some initial studies have examined the effects of elevated CO_2 on salmon in freshwater (Ou et al., 2015), there are no studies to date that have investigated the neural and behavioral responses of ocean-phase, juvenile salmon to elevated CO_2 in the marine environment. In this study, we examined the potential effects of elevated CO_2 on olfactory-mediated behaviors and the potential mechanisms underlying these behavioral changes in coho salmon (*Oncorhynchus kisutch*) adapted to saltwater. Proper olfactory function is critical for all aspects of a salmon's life cycle, especially during their extraordinary homing migrations, wherein they use olfactory cues to identify their natal stream (Dittman & Quinn, 1996). Therefore, even minor impairment of olfactory function due to OA may ultimately have profound

effects on salmon survival and population sustainability in the Pacific Ocean. We hypothesized that elevated CO_2, at levels predicted to occur over the next 50–100 years, would significantly alter behaviors, neuronal signaling, and gene expression in the olfactory system of coho salmon.

2 | MATERIALS AND METHODS

2.1 | Animals and housing

Coho salmon for these experiments were the offspring of anadromous adults spawned at the Washington Department of Fish and Wildlife's Issaquah Creek Hatchery, Issaquah, WA, USA. Experimental fish were transferred as embryos from the Issaquah Hatchery in January 2016 and 2017, reared in freshwater at the Northwest Fisheries Science Center until undergoing the parr–smolt transformation (1.5 year of age; 15.0 g ± 5.7 g), and then transferred to saltwater at the Northwest Fisheries Science Center's Mukilteo Marine Research Station (Mukilteo, WA, USA) on May 5, 2016, and May 24, 2017. After transfer to saltwater, fish were maintained under a natural photoperiod and fed BioVita Fry Feed (Bio-Oregon, Longview, WA). Water quality, fish health, and water delivery systems were monitored daily in fresh and salt water. All animal care and procedures were in accordance with University of Washington's Institutional Animal Care and Use Committee rules and approval, protocol # 4097-1.

2.2 | Seawater chemistry/exposures

Maintenance of seawater CO_2 concentrations followed previously described methodologies (Busch et al., 2014). Exposures consisted of three different CO_2 concentrations, including a control (ambient) nominal concentration of 700 μatm, which approximates the present-day average value of CO_2 in Puget Sound Marine Waters (Reum et al., 2015), a medium CO_2 level (nominal concentration of 1,600 μatm) predicted to periodically occur over the next 50 years, and a high CO_2 level (nominal concentration of 2,700 μatm) predicted to periodically occur over the next 100 years (Busch et al., 2014). Duplicate exposure tanks (2 foot diameter × 2 foot high, 178-L cylindrical tanks) for each treatment were maintained as a flow-through system, supplied by a unique head tank for each exposure tank (Supporting Information Figure S1). Water turnover rate was approximately once every hour. Source water for the head tanks was pumped from a depth of 60 feet from Puget Sound, degassed, and filtered prior to CO_2 manipulation. A Honeywell universal data analyzer controller and Durafet pH probe monitored and maintained the pH via CO_2 injection within each head tank. Target pH levels (as measured on a total pH scale) were 7.8 for control, 7.5 for medium, and 7.2 for high CO_2 exposure levels. To ensure proper water chemistry was maintained throughout exposures, water samples were collected from each exposure tank three times during each experiment (day 0, day 7, and day 14) for measurement of total alkalinity (TA) and dissolved inorganic carbon (DIC). Water samples were analyzed at the NOAA Pacific Marine Environmental Laboratory using standard test procedures for all analyses (Dickson, Sabine, & Christian, 2007). Water temperature, pH, and salinity were checked daily throughout the experiment. Water temperature in the exposure tanks remained at 12°C for the duration of the exposures. The ambient water temperature of the source water from Puget Sound averaged 11–12°C at the time of the exposures.

The start of the exposures was staggered over a month for logistical reasons to allow for behavioral and neurophysiological testing following each of the 14-day exposures. To begin the experiment, fish were transferred from their rearing tanks to their exposure tanks (n = 4 fish/tank) and acclimated for 24 hr in 700 μatm CO_2 control water. After acclimation, fish were exposed to experimental CO_2 levels for 14 days and tested for behavioral responses (n = 48 fish/treatment). A subset of these fish (n = 24) was used for electro-olfactogram (EOG)/electroencephalogram (EEG) neurophysiological and RNA-Seq (n = 8 fish per treatment) analysis.

2.3 | Odorant preparation

To investigate the effects of elevated CO_2 on olfactory-mediated salmon behavior, we used salmon skin extract, a prototypical predation odor that elicits a reliable and measurable avoidance response (Brown & Smith, 1997; Sandahl et al., 2007; Williams et al., 2016). Salmon skin extract was prepared as described previously with minor modifications (Williams et al., 2016). Briefly, skin tissue collected from coho salmon was homogenized in artificial seawater (Instant Ocean, Blacksburg, VA), filtered, and centrifuged to remove particulates. Protein content of the skin extract was determined using the Bradford assay (Bio-Rad, Hercules, CA), and stock concentrations were normalized to 2.4 mg/ml protein concentration in artificial seawater and stored at −80°C until needed. Working stocks of L-alanine and L-serine (Sigma-Aldrich, St. Louis, MO) for use in the EOG and EEG analysis were prepared on the day of use in artificial seawater. Working concentrations of the odorants were as follows: 10 μg/L skin extract (behavioral analysis), 2.4 mg/L skin extract (EOG and EEG analysis), and 10^{-2} M L-alanine and L-serine (EOG and EEG analysis). A higher concentration of the skin extract was used for electrophysiological analysis than for behavioral analysis due to the fact that measurable neuronal signal intensity is reduced in ocean-phase salmon due to the effect of high saltwater conductivities on electrophysiological recording (Sommers, Mudrock, Labenia, & Baldwin, 2016).

2.4 | Behavioral analysis

Following the 14-day exposure, behavioral analysis was conducted as previously described (Williams & Gallagher, 2013) using two-choice mazes surrounded by a black curtain and illuminated from below with infrared light to minimize stress. Each maze (100 × 40 × 25 cm) consisted of two arms (50 cm long and 20 cm wide) that terminated at a holding chamber (40 × 40 cm). A perforated gate separated the arms from the holding chamber. A dye test

confirmed that no mixing between the arms occurred. The maze received water (flow rate of 3 L/min) from the same head tanks used to generate the exposure water, thus ensuring that salmon were tested in the same water chemistry they experienced during exposures. Individual coho salmon from each CO_2 treatment (n = 48) were allowed to acclimate for 10 min in the holding chamber, and then behaviors were recorded for 10 min prior to odorant addition. After the 10-min pre-odor period, skin extract (10 µg/L) was delivered into one arm (randomized each trial) using a peristaltic pump and behaviors were recorded for an additional 10 min. An overhead infrared light-sensitive video camera (EverFocus® EQ900, Duarte, CA) provided video recordings of the behavioral responses. Proportion of time spent on odor side of the maze was analyzed using EthoVision XT 10 behavioral software (Noldus, Leesburg, VA). Following each behavioral trial, each maze was flushed with exposure water (without odorants) for 20 min.

Differences in response to CO_2 exposure were evaluated with a beta regression model that included CO_2 exposure and pre-odor period movement as covariates using the "betareg" R package (Zeileis, Cribari-Neto, Gruen, & Kosmidis, 2016). We selected a final model based on Akaike information criterion (AIC) comparison of models with CO_2 exposure and pre-odor fraction alone and as interactions. Bootstrap 95% prediction intervals on the beta regression-modeled treatment means were calculated based on 5,000 resamples using the "boot" R package (Canty & Ripley, 2017).

2.5 | Neurophysiological analysis

EOG and EEG recordings were performed the day after behavioral testing using methods previously described with minor modifications (Baldwin & Scholz, 2005). Fish were anesthetized with 50 mg/L tricaine methanesulfonate (MS-222; Western Chemicals Inc., WA) and injected intramuscularly with gallamine triethiodide (0.3 mg/kg body weight; Sigma-Aldrich, MO). A small tube inserted in the fish's mouth delivered artificial seawater (10°C) containing MS-222 (50 mg/L) to their gills. A gravity-fed glass capillary tube perfused the rosette with artificial seawater at a rate of 2 ml/min. Fish were acclimated for 5 min before the start of electrophysiological recordings. The recording microelectrode was placed at the midline of the rosette at the base of the posterior lamella for EOGs, and against the surface of the right mediodorsal cluster of the olfactory bulb for EEGs (Figure 1). Because there is spatial variation in responsiveness to different odorants in the olfactory bulb, before the start of the experimental recording, the location of the maximal EEG responses to the odorants was determined for each individual by positioning the microelectrode at different points across the olfactory bulb. The two regions that gave the most consistent signal were used as the recording sites for the entire experiment. A reference electrode was placed on the midline of the posterior-dorsal surface of the head, and a ground electrode was placed in the caudal muscle during recordings. Odorant-induced neural signals were acquired and filtered with an AC/DC amplifier (A-M Systems Inc.® Model 3000, Sequim, WA). Seawater/odors were delivered to the rosette using gravity-assisted flow, regulated by electronic valves and into a single manifold output through a thermoelectric chiller (temp 10°C). Fish received three pulses of each odorant (skin extract, L-serine, and L-alanine) with 2-min intervals between pulses. Based on an averaged and integrated recorded response curve, the amplitude of each EOG response was measured in microvolts (µV) as the maximum evoked peak minus the prestimulus basal activity level. Based on an averaged and integrated recorded response curve, the maximum odorant-evoked response for the EEG was the peak signal amplitude minus the prestimulus basal activity level. Signal duration for the EEG responses was calculated from the moment an odorant-induced signal was detected until the moment the signal returned to basal (pre-odor) levels. Triplicate responses to each odorant were averaged to produce a single response value for each odorant. EEGs were not performed on the medium CO_2 exposure group due to the logistics of the procedure, that is, length of time needed for each fish on the rig and number of fish that could be

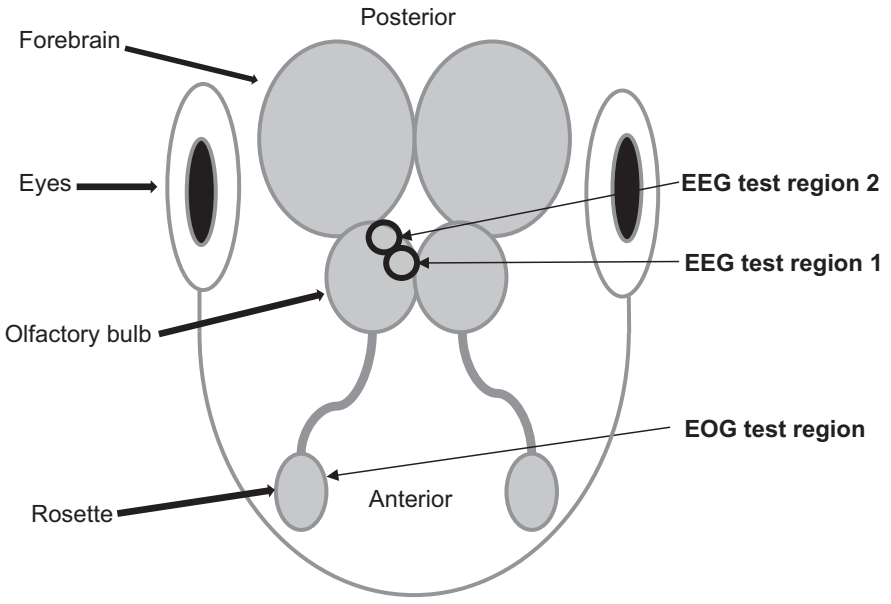

FIGURE 1 Diagram of salmon olfactory system and test sites used for EOG and EEG analysis of odorant-induced signals following exposures to varying levels of CO_2

recorded each day. Example EOG and EEG traces are located in Supporting Information Figure S2.

For the EOG analysis, a one-way ANOVA was used to test for significant differences between control and exposure groups, followed by a Dunn's multiple comparison test. For the EEG analysis, a *t* test was used to test for differences between control and high exposure groups. All analyses were done using GraphPad Prism 5 software. Differences were considered significant at $p < 0.05$.

2.6 | RNA-Seq analysis

Olfactory rosette and bulb tissues were collected from $n = 5$ individuals from the control, medium, and high CO_2 exposure groups following EOG analysis. Tissues were immediately stored in RNA*later*® before being frozen at −80°C (Thermo Fisher Scientific, Waltham, MA).

2.6.1 | RNA QC

RNA purity was assessed measuring $OD_{260/280}$ and $OD_{260/230}$ ratios with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). RNA integrity was determined using the Agilent RNA 6000 Nano Kit with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). All RNA samples were of appropriate size, quantity, and quality ($OD_{260/280}$ and $OD_{260/230}$ ratios of 1.8–2.1) and were used for RNA-Seq analysis ($n = 5$ for each exposure group/tissue).

2.6.2 | Sample processing and sequencing

cDNA libraries were prepared from 1 μg of total RNA using the TruSeq Stranded mRNA kit (Illumina, San Diego, CA) and the Sciclone NGSx Workstation (Perkin Elmer, Waltham, MA). Prior to cDNA library construction, ribosomal RNA was removed by means of poly-A enrichment. Each library was uniquely barcoded and subsequently amplified using a total of 13 cycles of PCR. Library concentrations were quantified using Qubit fluorometric quantitation (Life Technologies, Carlsbad, CA). Average fragment size and overall quality were evaluated with the DNA 1000 assay on an Agilent 2100 Bioanalyzer. Each library was sequenced with paired-end 100 bp reads to a minimum depth of 30 million reads on an Illumina HiSeq 4000. The average number of reads was 44.99 ± 6.47 million (mean ± *SE*) from olfactory rosette samples and 46.11 ± 4.41 million from olfactory bulb samples (Supporting Information Table S1).

We aligned the reads for each sample to the Atlantic salmon (*Salmo salar*) transcriptome (NCBI ICSASG_v2 build, downloaded 9/29/2017) using the Salmon aligner, accounting for GC, and sequencing bias (Patro, Duggal, & Kingsford, 2015; Patro, Duggal, Love, Irizarry, & Kingsford, 2017). Although there is a completed genome and transcriptome for coho salmon available (https://www.ncbi.nlm.nih.gov/genome/13127?genome_assembly_xml:id=309046), the functional Gene Ontology (GO) annotation for this species is not well developed relative to that for Atlantic salmon. Therefore, we chose to align the RNA-Seq data against the Atlantic salmon transcriptome, because the alignment results were similar between the two species (*S. salar*—60% of reads mapped; *O. kisutch*—73% of reads mapped). The aligned counts were imported into R (r-project.org) using the Bioconductor tximport package and then summarized at the gene level (Soneson, Love, & Robinson, 2015). We excluded any gene that was not expressed in at least four samples (i.e., any gene that had fewer than ten counts in less than four samples), to remove any data that were likely to be primarily noise. We then fit a generalized linear model with a negative binomial link function using the Bioconductor edgeR package and made comparisons between groups using likelihood ratio tests. We selected differentially expressed genes based on a false discovery rate (FDR) of 0.1 (i.e., we expect that at most 10% of the selected genes are false positives). To identify biological function that may have been perturbed due to changes in CO_2 exposure, we computed Fisher's exact tests based on GO terms, selecting those terms with a *p*-value <0.05.

3 | RESULTS

3.1 | Exposure water chemistry

Measured pH values for each exposure were consistent across the experiments and varied little within each exposure over the course of each experiment (standard deviation ≤0.03; Table 1). pH values from the Durafet sensors were consistent with discrete spectrophotometric measurements of pH from each exposure tank. Alkalinity in all exposure conditions, within and across experiments, was similar. Mean temperature in the exposure tanks ranged from 11.9–12.8°C, with small variation in each treatment over each experiment (standard deviation ≤0.2°C).

3.2 | Effects of elevated CO_2 on salmon behaviors

Using AIC analysis, the beta regression model containing only the interaction term between the CO_2 treatment and the pre-odor behavior covariate was selected ($p < 0.001$; pseudo-$R^2 = 0.24$) (Figure 2, Supporting Information Figure S3). This model indicated that fish exposed to control CO_2 levels avoided the side of the maze scented with skin extract (Figure 2, 26.7% ± 3.6% of time in odor (mean ± *SE*)), while fish that experienced the medium (Figure 2, 35.0% ± 4.5% of time in odor) and high (Figure 2, 52.3% ± 5.5% of time in odor) CO_2 treatments did not show a significant attraction or avoidance to the alarm odor. Individual fish from the medium and high CO_2 treatments tended to move around the maze less during the 20-min trials compared to controls. Conversely, fish in the control CO_2 treatment did not show a reduced tendency to explore the maze during the trial.

3.3 | Effects of elevated CO_2 on olfactory neurophysiological function

Neuronal responses in the olfactory epithelium to skin extract, L-alanine, and L-serine, as measured by EOG, were not affected by prior

TABLE 1 Water chemistry parameters

Exposure	Dates	Head tank	Salinity (psu)	Temperature (°C)	System pH		(µatm)pCO_2*	Ω_a*	TA (µmol/kg)	DIC (µmol/kg)
					Durafet setting	Spec				
1	8/18–9/23/16	A	29.9 ± 0.2	12.9 ± 0.4	7.2	7.2 ± 0.01	2,848.6 ± 143.9	0.31 ± 0.02	2,055.8 ± 11.4	2,127.4 ± 6.9
		B	29.9 ± 0.2	12.7 ± 0.2	7.8	7.8 ± 0.03	807.2 ± 16.2	0.98 ± 0.00	2,058.3 ± 12.4	2,001.4 ± 15.7
		A + B	29.9 ± 0.2	12.9 ± 0.3	7.5	7.4 ± 0.01	1,739.8 ± 28.3	0.49 ± 0.01	2,057.4 ± 12.1	2,083.1 ± 16.2
		C	29.9 ± 0.2	12.8 ± 0.3	7.2	7.3 ± 0.09	2,728.4 ± 15.6	0.32 ± 0.00	2,058.0 ± 11.9	2,137.9 ± 20.1
		D	29.9 ± 0.2	12.8 ± 0.2	7.8	7.8 ± 0.03	748.0 ± 72.0	1.05 ± 0.07	2,057.7 ± 11.9	1,994.5 ± 23.6
		C + D	29.9 ± 0.2	12.9 ± 0.2	7.5	7.4 ± 0.02	1,679.9 ± 83.1	0.51 ± 0.02	2,057.1 ± 11.8	2,078.7 ± 26.4
2	7/12–8/29/17	A	29.4 ± 0.3	11.9 ± 0.4	7.8	7.8 ± 0.03	630.1 ± 38.2	1.10 ± 0.03	2,017.5 ± 34.2	1,932.3 ± 37.3
		B	29.4 ± 0.2	12.0 ± 0.4	7.2	7.2 ± 0.08	2,698.4 ± 47.2	0.30 ± 0.01	2,016.7 ± 36.9	2,089.6 ± 29.8
		A + B	29.4 ± 0.2	12.0 ± 0.4	7.5	7.5 ± 0.05	1,424.3 ± 27.4	0.54 ± 0.00	2,019.0 ± 34.5	2,014.5 ± 34.3
		C	29.4 ± 0.2	11.7 ± 0.3	7.8	7.8 ± 0.03	636.9 ± 70.3	1.10 ± 0.08	2,005.2 ± 48.8	1,931.3 ± 40.3
		D	29.4 ± 0.2	11.8 ± 0.2	7.2	7.2 ± 0.00	2,587.7 ± 75.5	0.31 ± 0.00	2,015.4 ± 32.5	2,087.1 ± 29.0
		C + D	29.4 ± 0.2	11.9 ± 0.2	7.5	7.4 ± 0.01	1,565.9 ± 65.9	0.50 ± 0.00	2,018.4 ± 35.2	2,032.0 ± 39.3

Notes. DIC: dissolved inorganic carbon; Spec.: spectrophotometer; TA: total alkalinity.
*Ω_a and pCO_2 values were calculated via the "seacarb" package in R studio using data from DIC analysis and pH measured via spectrophotometry.

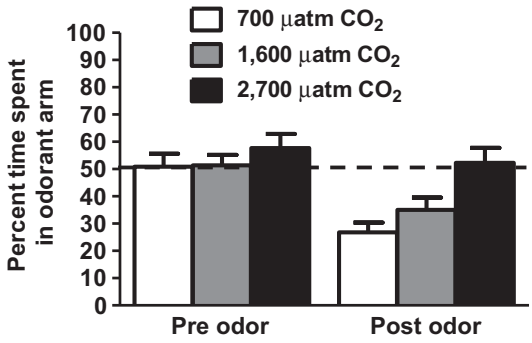

FIGURE 2 Behavioral responses to skin extract (alarm odor) odorant following CO_2 exposures. 700 µatm is the control CO_2 exposure level, 1,600 µatm is the medium CO_2 exposure level, and 2,700 µatm is the high CO_2 exposure level. Percent time juvenile coho salmon spent in the side of a two-choice maze receiving skin extract odorant before (pre-odor) and after (post-odor) introduction of the odorant. Dashed line indicates 50% level. All data represent mean ± *SEM* of n = 48 individuals

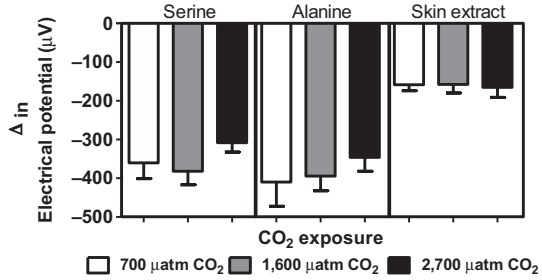

FIGURE 3 Electro-olfactogram (EOG) recorded responses of odorant-induced signaling within the olfactory rosettes of coho salmon exposed to three levels of CO_2. 700 µatm is the control CO_2 exposure level, 1,600 µatm is the medium CO_2 exposure level, and 2,700 µatm is the high CO_2 exposure level. Bars indicate the magnitude of the odorant-induced response relative to background water recorded from the olfactory epithelium. All data represent a mean ± *SEM* of n = 12 individuals

exposure to elevated CO_2 (Figure 3). However, EEG recordings revealed significant differences in peak odor-induced signaling in the right mediodorsal cluster (Figure 1, test region 1) of the olfactory bulbs of control and high CO_2 exposure coho salmon (p = 0.0068 and F = 4.754, Figure 4). High CO_2 exposure increased the mean peak signal amplitude of responses in this bulb region to skin extract (49.6% ± 39.1% increase (mean ± *SD*) and L-alanine (59.1% ± 78.7% increase) relative to responses in control fish (Figure 4a). Furthermore, the duration of EEG responses to skin extract and L-alanine tended to be longer in coho salmon exposed to high CO_2 levels compared to control fish (20.1 ± 4.0 s vs. 16.2 ± 6.5 s and 18.5 ± 4.4 s vs. 14.1 ± 5.0 s, respectively), but this difference was not significant (Figure 4b). Peak odor signal (skin extract: 0.024 ± 0.014 vs. 0.028 ± 0.015; L-alanine: 0.017 ± 0.008 vs. 0.021 ± 0.013) and duration (skin extract: 15.7 ± 4.8 s vs. 19.59 ± 5.9 s; L-alanine: 14.1 ± 4.4 s vs. 16.9 ± 9.5 s) in the right mediodorsal cluster test region 2 did not significantly differ between high CO_2 and control fish for either test odor (Figure 5) suggesting that CO_2 effects are specific to discrete bulbar regions and neurons.

3.4 | Effects of elevated CO_2 on gene expression in the salmon olfactory system

There were significant changes in gene expression in the olfactory system of coho salmon exposed to elevated CO_2. In particular, we observed considerable change in gene expression within the olfactory bulbs following exposure to the high CO_2 level (over 800 differentially expressed genes) relative to controls (Figure 6, Supporting

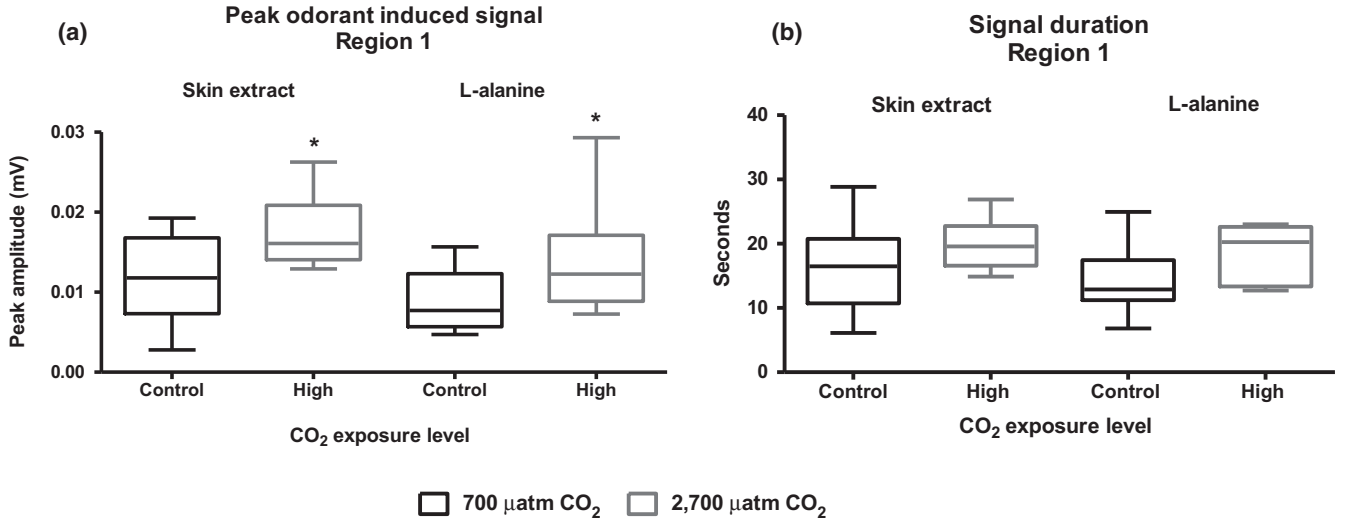

FIGURE 4 Electroencephalogram (EEG) recording data of odorant-induced signaling in test region one of the olfactory bulb from salmon exposed to two levels of CO_2. Data represented as a box and whisker plot showing median peak amplitude with whiskers representing the 5th and 95th percentile. 700 µatm is the control CO_2 exposure level, and 2,700 µatm is the high CO_2 exposure level. (a) Peak odorant-induced signaling by L-alanine and skin extract (alarm odor). (b) Duration of odorant-induced signaling by L-alanine and skin extract (alarm odor). Asterisks indicate significant differences between control and high exposure groups ($p \leq 0.05$)

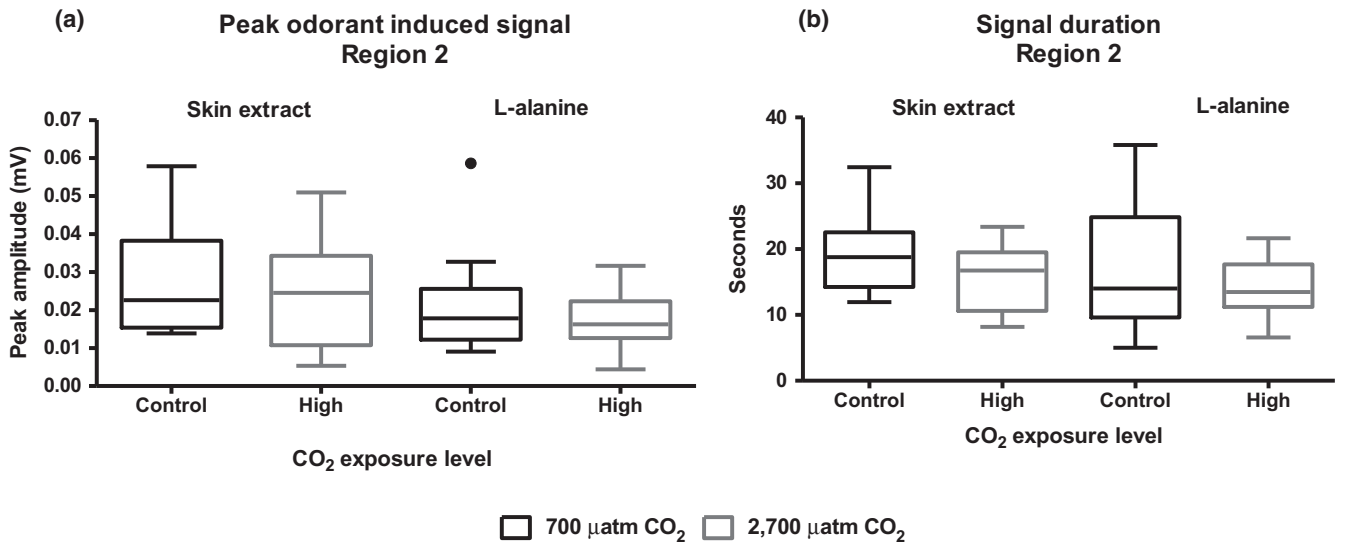

FIGURE 5 Electroencephalogram (EEG) recording data of odorant-induced signaling in test region two of the olfactory bulb from salmon exposed to two levels of CO_2. Data represented as a box and whisker plot showing median peak amplitude with whiskers representing the 5th and 95th percentile. 700 µatm is the control CO_2 exposure level, and 2,700 µatm is the high CO_2 exposure level. (a) Peak odorant-induced signaling by L-alanine and skin extract (alarm odor). (b) Duration of odorant-induced signaling by L-alanine and skin extract (alarm odor). The black dot indicates an outlier data point

Information Figure S4). A large number of these genes were involved in neural signaling/signal transduction, ion transport, and energy homeostasis (Supporting Information Figure S5). There were also significant differences in gene expression in the olfactory bulbs of medium CO_2 exposure fish relative to controls (61 differentially expressed genes) although these genes were predominantly associated with cytoskeletal function and not relevant to neural signaling. In contrast, there were relatively fewer changes in gene expression in the olfactory rosettes between control and medium (50 differentially expressed genes) or high exposure groups (20 differentially expressed genes) (Figure 6). None of the genes were significantly associated with olfactory neural signaling pathways.

We did not observe significant changes in gene expression of the GABA type A receptor, which has been hypothesized to play a role in CO_2-linked disruption of neuronal and behavioral signaling in marine fish (Schunter et al., 2017). Interestingly, however, the

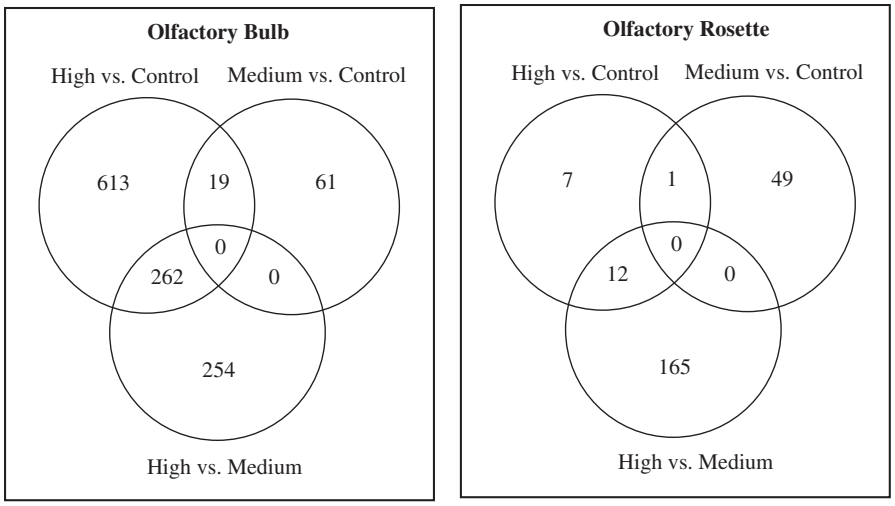

FIGURE 6 Venn diagram of RNA-Seq analysis of olfactory bulb and olfactory rosette gene expression in coho salmon exposed to three levels of CO_2. Venn diagrams show the number of significantly changed genes between each exposure group comparison. Numbers of genes listed in overlapping portion of the circles indicate number of significantly changed genes shared between each exposure comparison. Control = 700 μatm CO_2 exposure, Medium = 1,600 μatm CO_2 exposure, High = 2,700 μatm CO_2 exposure

expression of the GABA type B receptor subunit 2 (*gaba*$_{b2}$) was significantly elevated in the olfactory bulb following the high CO_2 exposure (Table 2, FDR <0.1). We also observed CO_2-induced changes in many other genes associated with GABA signaling, including increases in *hcn2*, *snap25*, and *kcc1*, which are associated with GABA-linked ion transport and synaptic activity, and significant decreases in expression of *slc6a13* and *aldh9a1*, two genes involved in GABA uptake and synthesis, respectively (Table 2). In addition to GABA signaling genes, other genes linked to neurotransmitter function (including glutamate and serotonin signaling), ion transport (*slc26a6*), G protein receptor function, neural differentiation, and melatonin production (*asmt* and *aanat*) displayed altered gene expression after elevated CO_2 exposure (Table 2). Genes important in neural energy production were also significantly altered following elevated CO_2 exposures, including a downregulation of the gene *slc22a16* (l-carnitine transport), and an upregulation of *slc2a6*, involved in glucose transport.

Interestingly, we also observed changes in gene expression of many genes associated with the photoreception system in the olfactory bulb of high exposure fish (Supporting Information Figure S5). Some of these genes included rhodopsin, parapinopsin, and various voltage-dependent ion channel genes. The reason for the inclusion of photoreception-related genes within the expression profile of the olfactory bulbs remains unclear; however, it is likely that genes involved in the olfactory and photoreception systems may share similar signal transduction function in both tissues. This hypothesis is supported by at least two other studies that reported the expression of olfactory genes in the visual system (Jovancevic et al., 2017; Pronin et al., 2014).

4 | DISCUSSION

Collectively, our results indicate that elevated CO_2 concentrations altered neural signaling pathways within the olfactory bulb and impaired olfactory-mediated behavioral responses of ocean-phase coho salmon. Given the primary need for a functional olfactory system for salmon living in the ocean to find prey, avoid predators, and ultimately find their natal stream during homing migrations, these results suggest that future predicted CO_2 concentrations in the ocean may have a profound effect on Pacific salmon and their ecosystems. Our behavioral results indicated that ocean-phase coho salmon were sensitive to acute exposures to elevated CO_2 concentrations that have been predicted to occur within the next 50–100 years. The strong avoidance behavior elicited by skin extract in the control group was decreased or eliminated in coho salmon exposed to either the medium (1,600 μatm) or high (2,700 μatm) CO_2 treatments. These results indicate that anadromous salmon may be just as sensitive to the effects of elevated CO_2 as obligate marine species that have shown behavioral impairments at similar [CO_2] levels (Chung et al., 2014; Devine et al., 2012; Hamilton et al., 2017, 2014; Munday et al., 2009; Porteus et al., 2018). While future oceanic CO_2 concentrations may not reach such high steady-state levels, exposures to transient CO_2 concentrations at these levels may already occur in some regions and will likely be more common. Juvenile coho salmon spend up to a year rearing in freshwater (Quinn, 2011) before migrating downstream to the ocean, undergoing the physiological transformation of smoltification that prepares them for life in seawater, including changes in osmoregulation and ion balance regulation (Maryoung et al., 2015; McCormick, 2012; Quinn, 2011). Our results suggest that despite having an adaptable olfactory system that functions in both marine and freshwater environments with very different pHs and water chemistries, the relative sensitivity of these anadromous fish to elevated CO_2 in the ocean is similar to other marine fish.

Tightly controlled ion balances play a key role in proper olfactory neuronal signaling, and it has been hypothesized that elevated CO_2-induced changes in transmembrane ionic gradients impair neuronal signaling and, ultimately, olfactory-mediated behaviors (Heuer, Welch, Rummer, Munday, & Grosell, 2016; Tresguerres & Hamilton, 2017). This is consistent with our analysis of neuronal signaling in the olfactory epithelium and the olfactory bulb. Elevated CO_2 did not alter neuronal responses to odorants in the olfactory epithelium suggesting that odorant-induced signaling within olfactory sensory neurons was not impacted following a shift in CO_2 concentration

TABLE 2 Significantly changed genes of relevance to neural function and signaling within the olfactory bulbs from coho exposed to high CO_2 vs. control CO_2

ENTREZID	Accession number	Gene name	Putative name	log_2 fold change	FDR
106562041	LOC106562041	Guanine nucleotide-binding protein subunit alpha-14-like	*gna14*	3.307	2.81197E−10
106574723	LOC106574723	Gamma-aminobutyric acid type B receptor subunit 2-like	*gabbr2*	2.645	9.1231E−06
106575665	LOC106575665	Cyclic nucleotide-gated channel cone photoreceptor subunit alpha-like	*cnga3*	2.660	0.000141938
106611384	LOC106611384	Synaptosomal-associated protein 25-B-like	*snap25*	1.883	0.000460991
106588157	LOC106588157	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2-like	*hcn*	3.968	0.00053587
106603743	LOC106603743	Glutamate receptor ionotropic, kainate 4-like	*grik4*	1.012	0.001089553
106569207	LOC106569207	Solute carrier family 12 member 7-like	*kcc1*	1.368	0.001601933
106602119	LOC106602119	Neuronal acetylcholine receptor subunit alpha-3-like	*chrna3*	2.201	0.001653337
106592065	LOC106592065	Neuronal acetylcholine receptor subunit alpha-3	*chrna3*	2.227	0.001665897
106573978	LOC106573978	Excitatory amino acid transporter 5-like	*slc1a7*	1.792	0.001803254
106577203	LOC106577203	Potassium voltage-gated channel subfamily H member 1-like	*kcnh7*	2.466	0.001981873
106584365	LOC106584365	Diencephalon/mesencephalon homeobox protein 1-like	*dmbx1*	4.100	0.002118515
106583073	LOC106583073	Guanine nucleotide-binding protein G(t) subunit alpha-2-like	*gnai2b*	2.588	0.002170767
106573780	LOC106573780	Solute carrier organic anion transporter family member 3A1-like	*slc21a11*	0.860	0.003012925
106572933	LOC106572933	Voltage-dependent L-type calcium channel subunit alpha-1D-like	*cacna1d*	2.282	0.003645407
106567981	LOC106567981	Neuropeptide Y receptor type 1-like	*npy1r*	−0.649	0.004204007
106605869	LOC106605869	Gamma-aminobutyric acid type B receptor subunit 2-like	*gabbr2*	1.773	0.004302948
106613596	LOC106613596	Excitatory amino acid transporter 5-like	*slc1a7*	3.902	0.004457749
106571997	LOC106571997	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	*gbb1*	0.574	0.004760336
106578273	LOC106578273	Vesicular glutamate transporter 1-like	*vglut1*	1.625	0.005044121
106607367	LOC106607367	Serotonin *N*-acetyltransferase-like	*aanat*	4.020	0.005443605
106600164	LOC106600164	Aldehyde dehydrogenase family 9 member A1-like	*aldh9a1*	−5.891	0.005486887
106573635	LOC106573635	Large neutral amino acids transporter small subunit 1-like	*slc7a5*	1.072	0.008210081
106572937	LOC106572937	Voltage-dependent L-type calcium channel subunit alpha-1F-like	*cacna1f*	1.879	0.008455018
106612651	LOC106612651	Sodium-dependent serotonin transporter-like	*slc6a4*	1.050	0.009377377
106587671	LOC106587671	Guanine nucleotide-binding protein subunit beta-5-like	*gnb5*	2.359	0.009479934
106561149	LOC106561149	Solute carrier organic anion transporter family member 3A1-like	*slc21a11*	1.019	0.011541751
106613200	LOC106613200	Short transient receptor potential channel 2-like	*trpc2*	−1.432	0.011879951
106572934	LOC106572934	Voltage-dependent L-type calcium channel subunit alpha-1D-like	*cacna1d*	1.807	0.01216225
106562494	LOC106562494	Guanine nucleotide-binding protein subunit beta-5-like	*gnb5*	1.104	0.012539596
106568477	cplx4	Complexin 4	*cplx4*	4.491	0.012892164
106611148	LOC106611148	Neurexin-1a	*nrxn1*	−0.464	0.015068892
106592915	LOC106592915	Regulator of G protein signaling 9-like	*rgs9*	3.017	0.015068892
106585038	LOC106585038	Phosphatidylinositol 4-phosphate 5-kinase type-1 beta-like	*pip5k1b*	−0.538	0.015068892
106560428	LOC106560428	Excitatory amino acid transporter 5-like	*slc1a7*	3.492	0.01547044
106612376	LOC106612376	Protein phosphatase 1A-like	*pp1*	2.488	0.017048588
106581568	LOC106581568	Guanylyl cyclase-activating protein 1-like	*guca1a*	3.316	0.018566427
106587958	LOC106587958	Sodium/potassium/calcium exchanger 1-like	*slc24a1*	2.005	0.019726988
106605751	LOC106605751	Neuronal pentraxin-1-like	*nptx1*	2.216	0.021659612
106561698	LOC106561698	Solute carrier organic anion transporter family member 1C1-like	*slco1c1*	3.137	0.022324012
106580796	slc6a4	Solute carrier family 6 member 4	*slc6a4*	2.050	0.022324012
106572384	LOC106572384	Sodium-coupled neutral amino acid transporter 3-like	*slc38a3*	2.164	0.023464354
106574495	LOC106574495	Guanine nucleotide-binding protein subunit alpha-11-like	*gna11*	−0.285	0.025017626
106579173	LOC106579173	Synaptotagmin-2-like	*syt2*	4.003	0.02540081

(Continues)

TABLE 2 (Continued)

ENTREZID	Accession number	Gene name	Putative name	log_2 fold change	FDR
106605091	LOC106605091	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3-like	*gnb3*	2.403	0.026220179
106583542	LOC106583542	Sodium- and chloride-dependent GABA transporter 2-like	*slc6a13*	0.972	0.028452986
106603834	LOC106603834	Solute carrier family 22 member 5-like	*slc22a5*	−1.252	0.029247296
106587942	LOC106587942	Sodium/potassium/calcium exchanger 1-like	*slc24a1*	2.012	0.030142477
106561912	LOC106561912	Cyclic nucleotide-gated cation channel beta-1-like	*cngb1*	3.621	0.032324991
106607984	LOC106607984	Solute carrier family 22 member 16-like	*slc22a16*	1.073	0.03395494
106561031	gpr37	G protein-coupled receptor 37	*gpr37*	1.019	0.035891712
106564793	LOC106564793	Sodium/calcium exchanger 1-like	*slc8a1*	1.535	0.037274497
106597363	LOC106597363	Guanylyl cyclase-activating protein 2-like	*gcap2*	3.478	0.037274497
106566781	LOC106566781	Solute carrier family 26 member 6-like	*slc26a6*	1.458	0.042405183
106594011	LOC106594011	Sodium/potassium/calcium exchanger 1-like	*slc24a1*	2.008	0.045357056
106577267	LOC106577267	Neuronal pentraxin-1-like	*np1*	1.727	0.047487725
106581084	LOC106581084	G protein-activated inward rectifier potassium channel 3-like	*girk3*	3.466	0.048892792
106561886	kcnk5	Potassium two-pore domain channel subfamily K member 5	*kcnk5*	1.098	0.051870554
106591467	LOC106591467	Neuronal pentraxin receptor-like	*nptxr*	−0.435	0.054754969
106570824	LOC106570824	Neuroligin-3-like	*nlgn3*	−0.609	0.068821378
106561537	slc27a4	Solute carrier family 27 member 4	*slc27a4*	−0.341	0.06932593
106610602	slc4a1ap	Solute carrier family 4 member 1 adaptor protein	*slc4a1ap*	−0.254	0.070027446
106572936	LOC106572936	Voltage-dependent L-type calcium channel subunit alpha-1S-like	*cacna1s*	1.622	0.073257288
106600499	LOC106600499	Excitatory amino acid transporter 5-like	*slc1a7*	3.387	0.075511344
106564801	LOC106564801	Potassium voltage-gated channel subfamily H member 1-like	*kcnh1*	1.124	0.076952544
106586510	asmt	Acetylserotonin O-methyltransferase	*asmt*	4.053	0.078732281
106573300	LOC106573300	Guanylyl cyclase inhibitory protein-like	—	4.062	0.079046904
106588065	LOC106588065	Synaptic vesicle glycoprotein 2B-like	*sv2b*	3.356	0.079105596
106585781	slc2a6	Solute carrier family 2 member 6	*slc2a6*	−1.210	0.08829066
106584763	LOC106584763	Potassium voltage-gated channel subfamily C member 1-like	*kcnc1*	−0.601	0.097240875

Notes. Selected based on a FDR <0.1.
FDR: false discovery rate.

that was sufficient to cause behavioral impairments. These results differ from those recently reported for European sea bass (Porteus et al., 2018). The robustness of the EOG responses to altered CO_2 levels may reflect the ability of olfactory sensory neurons to modulate ionic balances while in direct contact with the ambient water because they must be able to detect odorants in the presence of shifting ion concentrations and water chemistries. In contrast, neurons in the olfactory bulb have evolved to function in the tightly controlled fluid chemistry of the central nervous system and may be more sensitive to potential changes in extracellular fluid chemistry (Abbott, Patabendige, Dolman, Yusof, & Begley, 2010; Somjen, 2002). Our EEG recordings support this hypothesis, as elevated CO_2 exposures increased the amplitude, and tended to increase the duration of odorant-induced responses within specific regions of the olfactory bulb. This CO_2-induced increase in excitatory signaling is consistent with the hypothesis that disruption of neuronal signaling in marine fish is associated with disruption of inhibitory GABA signaling (Nilsson et al., 2012; Tresguerres & Hamilton, 2017). Specific odorant-generated signals in the olfactory bulb guide odorant perception and downstream behaviors. Alteration of this odorant specific signal, via dysregulation of the GABA signaling pathway, could lead fish to perceive odorants in an inappropriate way and thus lead to altered behavioral responses.

It has been hypothesized that the main mechanism of behavioral disruption by elevated CO_2 exposure is via alteration of GABA signaling in the central nervous system, driven by a reversal of the Cl^-/HCO_3^+ membrane gradient and a linked disruption of the normal inhibitory action of the $GABA_A$ receptor (Nilsson et al., 2012). The reversal of the Cl^-/HCO_3^+ neuronal membrane gradient results in a reversal of the intended GABA signaling. Therefore, GABA receptor activation results in hyperpolarization of the neuron rather than depolarization. This could potentially lead to inappropriate or overactivation of neurons. CO_2-induced increases in the amplitude of neuronal responses in the mediodorsal olfactory bulb in response to odorants are consistent with this hypothesis. Inhibitory GABAergic neurons in the olfactory bulb play a critical role in synchronization

and regulation of neuronal signals required for appropriate odor discrimination (Lizbinski & Dacks, 2017; Tabor, Yaksi, & Friedrich, 2008). The lack of observed effects of CO_2 in the olfactory epithelium and some discrete regions of the olfactory bulb may be due to differential spatial distribution of GABAergic neurons and GABA receptors within these tissues and the role of GABAergic neurons in regulating signaling of specific odorants and mixtures (Cocco et al., 2017; Lizbinski & Dacks, 2017; McGann, 2013; Tabor et al., 2008). For example, while G protein-coupled $GABA_B$ receptors are present in the axonal presynaptic region of the olfactory sensory neurons within the olfactory bulbs, $GABA_A$ receptors are broadly present on mitral/tufted cell secondary neurons within the olfactory bulb (McGann, 2013; Tan, Savigner, Ma, & Luo, 2010).

Consistent with the hypothesis that CO_2 effects on olfactory behaviors involve GABA signaling, our RNA-Seq analysis found that several genes involved in GABA signaling were altered at a CO_2 concentration shown to cause neurobehavioral disruption. These results are largely similar to studies that examined elevated CO_2 effects on mRNA expression of $GABA_A$ receptor genes in other fish species (Lai, Fagernes, Jutfelt, & Nilsson, 2016; Schunter et al., 2017). Interestingly, while we found no change in expression of the $GABA_A$ receptor mRNA in the olfactory bulb under high CO_2 conditions, we did observe a significant increase in $gaba_{B2}$ receptor mRNA expression. The metabotropic $GABA_B$ receptor is involved in a distinct inhibitory pathway compared to ionotropic $GABA_A$ receptor and works to modulate neural activity via presynaptic and postsynaptic signaling pathways. However, $GABA_A$ and $GABA_B$ receptors play complementary and distinct roles in modulating olfactory signaling. The $GABA_B$ receptor is a G protein-coupled receptor that, upon activation, inhibits calcium channel function (which can in turn reduce neural excitability and neurotransmitter release) and activates potassium channels to hyperpolarize neurons (Bettler, Kaupmann, Mosbacher, & Gassmann, 2004). Neuronal hyperpolarization via $GABA_A$ receptor modulation of Cl^- influx is quicker than the $GABA_B$ pathway as it does not rely on slower secondary messengers.

To our knowledge, we are the first to report changes in $GABA_B$ gene expression under elevated CO_2, which presents an interesting new component to the list of signaling molecules involved in behavioral alterations under elevated CO_2. Increased expression of the $GABA_B$ receptor could indicate a response by salmon olfactory bulb neurons to compensate for the loss of normal function of the $GABA_A$ receptor pathway. Increased expression of $GABA_A$ receptor mRNA, as a potential compensation for loss of function under elevated CO_2 conditions, is also found in three-spined sticklebacks (Lai et al., 2016). This theory is supported by the fact that several other genes associated with GABA signaling were also significantly altered in coho salmon in the present study. The significant increase in *hcn2*, which plays critical roles in membrane excitability, integration of synaptic inputs, and the generation of membrane potential oscillations within the olfactory bulb, suggests alterations in signal modulation under elevated CO_2 conditions (He, Chen, Li, & Hu, 2014). Two other genes associated with synaptic transmission and modulation of neuronal GABA signaling through Cl^- transport, *snap25* and *kcc1*, also showed significant increases in expression further suggesting altered neuronal signaling within the olfactory bulbs (Abe, Minowa, & Kudo, 2018; Delgado-Martínez, Nehring, & Sørensen, 2007; Delpire, 2000; Wang et al., 2005). The increases in *slc6a13* and *slc38a3*, which can serve roles in taurine/GABA uptake and glutamate uptake needed for GABA synthesis, respectively, potentially indicate increased production or uptake of GABA as a compensatory response by the bulb neurons (Chan et al., 2016; Scimemi, 2014). There was also a significant decrease in *aldh9a1*, which is involved in the production of GABA, and was reported to be overexpressed in fish tolerant of elevated CO_2 exposures (Schunter et al., 2016). Furthermore, the significant changes in expression of the multitude of other genes involved in signal transduction, ion transport (such as *slc26a6* which serves a vital role in transporting HCO_3^+/Cl^-), and machinery related to neurotransmitters such as glutamate, serotonin, and acetylcholine also indicate a potential compensatory response to restore normal neural signaling within the olfactory bulbs.

We found increased expression of major genes involved in melatonin and the circadian rhythm, *asmt* and *aanat*, genes that play key roles in the production of melatonin and its precursor *N*-acetylserotonin. Melatonin production has been linked to modulation of ion regulation in rainbow trout in response to changes in salinity (López-Patiño, Rodríguez-Illamola, Gesto, Soengas, & Míguez, 2011). Schunter et al. (2016) found similar results in damselfish wherein offspring from parents sensitive to elevated CO_2 also had elevated levels of *asmt* mRNA expression, as opposed to offspring from CO_2-tolerant parents. GABA signaling has also been linked to circadian rhythm regulation, and the alteration of expression of genes central to $GABA_B$ function could be driving these changes in genes linked to the circadian rhythm as well (DeWoskin et al., 2015). In total, the RNA-Seq data indicate that olfactory bulb neural signaling pathways experienced major changes on a wide scale in response to the elevated CO_2 exposure, potentially as a mechanism to restore normal function, albeit unsuccessful during the exposure window given our behavioral and neurophysiology results.

The results of our study highlight the fact that salmon, once acclimated to saltwater, are susceptible to neurophysiological changes that can influence behavioral function under shifts in pH similar to those expected with OA. These results are worrisome as the native range of coho salmon in the North East Pacific Ocean is characterized by strong upwelling currents and is predicted to be impacted by elevated CO_2 and low pH projected for the foreseeable future. Indeed, many areas in the Salish Sea (encompassing the Strait of Georgia, Strait of Juan de Fuca, and Puget Sound in Washington State, USA, and British Columbia, CDN) already experience CO_2 and pH levels, at certain times of the year, that are similar to those affecting fish in our study (Feely et al., 2010). Olfaction plays a central role in the salmon life history, and the impairment of normal olfactory-driven behaviors in juvenile salmon can jeopardize their survival. Furthermore, the GABA signaling system hypothesized to be impaired under elevated CO_2 conditions is critical in many other areas of the central nervous system, including vision, mechanoreception, and control of anxiety. However, the effects of elevated CO_2

on these critical neuronal systems, remain to be investigated and are largely unknown in salmon (Ou et al., 2015).

While future real-world exposures to CO_2 concentrations at 2,700 μatm are likely to only occur in a transient scenario similar to our exposure paradigm, longer term exposures would be informative to investigate a potential for salmon to acclimate to the changed chemistry and regain normal neural function. Furthermore, while our study did not investigate recovery of normal behavioral function following cessation of the exposures, there is evidence that such recovery does happen in fish (Chivers et al., 2014; Jarrold, Humphrey, McCormick, & Munday, 2017). The environment that salmon reside in (i.e., open ocean vs. nearshore environment, time of year they reside in each environment, and the water depth they reside at) is important to consider going forward as the degree of neural impairment driven by elevated CO_2 could vary (Jarrold et al., 2017; Pacella, Brown, Waldbusser, Labiosa, & Hales, 2018).

In conclusion, juvenile ocean-phase coho salmon are sensitive to neurobehavioral disruption induced by exposure to elevated CO_2 associated with climate change predictions in the Puget Sound region. Salmon are a keystone species in many aquatic ecosystems in the North Eastern Pacific Ocean and already face substantial pressure from other anthropogenic and nonanthropogenic factors. The potential effects of elevated CO_2 on their mortality will only add to this pressure for long-term survivorship of Pacific salmon.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

C.W., A.D., E.G., P.M., T.B., and S.B. all participated in the design of the experiment. C.W. and M.M. conducted the study. T.B. and J.M. conducted the bioinformatics. C.W. wrote the paper with editorial input from A.D., E.G., P.M., S.B., T.B., J.M., and M.M.

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REFERENCES

Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood–brain barrier. *Neurobiology of Disease*, *37*(1), 13–25. https://doi.org/10.1016/j.nbd.2009.07.030

Abe, T., Minowa, Y., & Kudo, H. (2018). Molecular characterization and gene expression of synaptosome-associated protein-25 (SNAP-25) in the brain during both seaward and homeward migrations of chum salmon Oncorhynchus keta. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *217*, 17–25. https://doi.org/10.1016/j.cbpa.2017.12.006

Allan, B. J. M., Miller, G. M., McCormick, M. I., Domenici, P., & Munday, P. L. (2014). Parental effects improve escape performance of juvenile reef fish in a high-CO_2 world. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1777), 20132179. https://doi.org/10.1098/rspb.2013.2179

Ashur, M. M., Johnston, N. K., & Dixson, D. L. (2017). Impacts of ocean acidification on sensory function in marine organisms. *Integrative and Comparative Biology*, *57*(1), 63–80. https://doi.org/10.1093/icb/icx010

Baldwin, D. H., Sandahl, J. F., Labenia, J. S., & Scholz, N. L. (2003). Sublethal effects of copper on coho salmon: Impacts on nonoverlapping receptor pathways in the peripheral olfactory nervous system. *Environmental Toxicology and Chemistry*, *22*(10), 2266–2274. https://doi.org/10.1897/02-428

Baldwin, D. H., & Scholz, N. L. (2005). The electro-olfactogram: An in vivo measure of peripheral olfactory function and sublethal neurotoxicity in fish. *Techniques in Aquatic Toxicology*, *2*, 257–276.

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure and physiological functions of GABAB receptors. *Physiological Reviews*, *84*(3), 835–867. https://doi.org/10.1152/physrev.00036.2003

Brown, G. E., & Smith, R. J. F. (1997). Conspecific skin extracts elicit antipredator responses in juvenile rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Zoology*, *75*(11), 1916–1922. https://doi.org/10.1139/z97-821

Busch, D. S., Maher, M., Thibodeau, P., & McElhany, P. (2014). Shell condition and survival of puget sound pteropods are impaired by ocean acidification conditions. *PLoS ONE*, *9*(8), e105884. https://doi.org/10.1371/journal.pone.0105884

Canty, A., & Ripley, B. (2017). Package boot: Bootstrap R (S-Plus) Functions. R package version 1.3-20. Retrieved from https://cran.r-project.org/web/packages/boot/

Chan, K., Busque, S. M., Sailer, M., Stoeger, C., Bröer, S., Daniel, H., … Wagner, C. A. (2016). Loss of function mutation of the Slc38a3 glutamine transporter reveals its critical role for amino acid metabolism in the liver, brain, and kidney. *Pflügers Archiv - European Journal of Physiology*, *468*(2), 213–227. https://doi.org/10.1007/s00424-015-1742-0

Chivers, D. P., McCormick, M. I., Nilsson, G. E., Munday, P. L., Watson, S.-A., Meekan, M. G., & … Ferrari, M. C. O. (2014). Impaired learning of predators and lower prey survival under elevated CO_2: A consequence of neurotransmitter interference. *Global Change Biology*, *20*(2), 515–522. https://doi.org/10.1111/gcb.12291

Chung, W.-S., Marshall, N. J., Watson, S.-A., Munday, P. L., & Nilsson, G. E. (2014). Ocean acidification slows retinal function in a damselfish through interference with GABAA receptors. *Journal of Experimental Biology*, *217*(3), 323–326. https://doi.org/10.1242/jeb.092478

Cocco, A., Rönnberg, A. C., Jin, Z., André, G. I., Vossen, L. E., Bhandage, A. K., … Winberg, S. (2017). Characterization of the γ-aminobutyric acid signaling system in the zebrafish (*Danio rerio* Hamilton) central nervous system by reverse transcription-quantitative polymerase chain reaction. *Neuroscience*, *343*, 300–321. https://doi.org/10.1016/j.neuroscience.2016.07.018

Cripps, I. L., Munday, P. L., & McCormick, M. I. (2011). Ocean acidification affects prey detection by a predatory reef fish. *PLoS ONE*, *6*(7), e22736. https://doi.org/10.1371/journal.pone.0022736

Crozier, L. G., Hendry, A., Lawson, P. W., Quinn, T., Mantua, N., Battin, J., … Huey, R. (2008). Potential responses to climate change in organisms with complex life histories: Evolution and plasticity in Pacific salmon. *Evolutionary Applications*, *1*(2), 252–270.

Delgado-Martínez, I., Nehring, R. B., & Sørensen, J. B. (2007). Differential abilities of SNAP-25 homologs to support neuronal function. *Journal of Neuroscience*, *27*(35), 9380–9391. https://doi.org/10.1523/JNEUROSCI.5092-06.2007

Delpire, E. (2000). Cation-chloride cotransporters in neuronal communication. *Physiology*, *15*(6), 309–312. https://doi.org/10.1152/physiologyonline.2000.15.6.309

Devine, B. M., Munday, P. L., & Jones, G. P. (2012). Homing ability of adult cardinalfish is affected by elevated carbon dioxide. *Oecologia*, *168*(1), 269–276. https://doi.org/10.1007/s00442-011-2081-2

DeWoskin, D., Myung, J., Belle, M. D. C., Piggins, H. D., Takumi, T., & Forger, D. B. (2015). Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. *Proceedings of the National Academy of Sciences*, *112*(29), E3911–E3919. https://doi.org/10.1073/pnas.1420753112

Dickson, A. G., Sabine, C. L., & Christian, J. R. (2007). Guide to best practices for ocean CO_2 measurements.

Dittman, A., & Quinn, T. (1996). Homing in Pacific salmon: Mechanisms and ecological basis. *Journal of Experimental Biology*, *199*(Pt 1), 83–91.

Dixson, D. L., Jennings, A. R., Atema, J., & Munday, P. L. (2014). Odor tracking in sharks is reduced under future ocean acidification conditions. *Global Change Biology*, *21*(4), 1454–1462.

Dixson, D. L., Munday, P. L., & Jones, G. P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters*, *13*(1), 68–75. https://doi.org/10.1111/j.1461-0248.2009.01400.x

Domenici, P., Allan, B., McCormick, M. I., & Munday, P. L. (2011). Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biology Letters*, *8*, 78–81. Retrieved from http://rsbl.royalsocietypublishing.org/content/early/2011/08/10/rsbl.2011.0591.abstract.

Fabry, V. J., Seibel, B. A., Feely, R. A., & Orr, J. C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science: Journal Du Conseil*, *65*(3), 414–432. https://doi.org/10.1093/icesjms/fsn048

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., … Maloy, C. (2010). The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*, *88*(4), 442–449. https://doi.org/10.1016/j.ecss.2010.05.004

Ferrari, M. C., McCormick, M. I., Allan, B. J., Choi, R., Ramasamy, R. A., Johansen, J. L., … Chivers, D. P. (2015). Living in a risky world: The onset and ontogeny of an integrated antipredator phenotype in a coral reef fish. *Scientific Reports*, *5*, 15537–16000. https://doi.org/10.1038/srep15537

Ferrari, M. C. O., Manassa, R. P., Dixson, D. L., Munday, P. L., McCormick, M. I., Meekan, M. G., … Chivers, D. P. (2012). Effects of ocean acidification on learning in coral reef fishes. *PLoS ONE*, *7*(2), e31478. https://doi.org/10.1371/journal.pone.0031478

Gerlach, G., Atema, J., Kingsford, M. J., Black, K. P., & Miller-Sims, V. (2007). Smelling home can prevent dispersal of reef fish larvae. *Proceedings of the National Academy of Sciences*, *104*(3), 858–863. http://www.pnas.org/content/104/3/858.abstract

Hamdani, E. H., & Doving, K. B. (2007). The functional organization of the fish olfactory system. *Progress in Neurobiology*, *82*(2), 80–86. https://doi.org/10.1016/j.pneurobio.2007.02.007

Hamilton, S. L., Logan, C. A., Fennie, H. W., Sogard, S. M., Barry, J. P., Makukhov, A. D., … Bernardi, G. (2017). Species-specific responses of juvenile rockfish to elevated pCO_2: From behavior to genomics. *PLoS ONE*, *12*(1), e0169670. https://doi.org/10.1371/journal.pone.0169670

Hamilton, T. J., Holcombe, A., & Tresguerres, M. (2014). CO_2-induced Ocean Acidification Increases Anxiety in Rockfish via Alteration of GABAA Receptor Functioning (Vol. *281*).

Hara, T. J. (1992). *Fish chemoreception*. London: Chapman & Hall.

He, C., Chen, F., Li, B., & Hu, Z. (2014). Neurophysiology of HCN channels: From cellular functions to multiple regulations. *Progress in Neurobiology*, *112*, 1–23. https://doi.org/10.1016/j.pneurobio.2013.10.001

Heuer, R. M., Welch, M. J., Rummer, J. L., Munday, P. L., & Grosell, M. (2016). Altered brain ion gradients following compensation for elevated CO(2) are linked to behavioural alterations in a coral reef fish. *Scientific Reports*, *6*, 33216. https://doi.org/10.1038/srep33216

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*(5985), 1523–1528.

Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., & Sewell, M. A. (2010). The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 127–147. https://doi.org/10.1146/annurev.ecolsys.110308.120227

Jarrold, M. D., Humphrey, C., McCormick, M. I., & Munday, P. L. (2017). Diel CO_2 cycles reduce severity of behavioural abnormalities in coral reef fish under ocean acidification. *Scientific Reports*, *7*(1), 10153–11000. https://doi.org/10.1038/s41598-017-10378-y

Jovancevic, N., Wunderlich, K. A., Haering, C., Flegel, C., Maßberg, D., Weinrich, M., … Gelis, L. (2017). Deep sequencing of the human retinae reveals the expression of odorant receptors. *Frontiers in Cellular Neuroscience*, *11*, 03. https://doi.org/10.3389/fncel.2017.00003

Jutfelt, F., & Hedgärde, M. (2015). Juvenile Atlantic cod behavior appears robust to near-future CO_2 levels. *Frontiers in Zoology*, *12*(1), 1–7. https://doi.org/10.1186/s12983-015-0104-2.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., … Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*(6), 1884–1896. https://doi.org/10.1111/gcb.12179

Lai, F., Fagernes, C. E., Jutfelt, F., & Nilsson, G. E. (2016). Expression of genes involved in brain GABAergic neurotransmission in three-spined stickleback exposed to near-future CO_2. *Conservation. Physiology*, *4*(1), cow068-cow068. https://doi.org/10.1093/conphys/cow068.

Leduc, A. O., Munday, P. L., Brown, G. E., & Ferrari, M. C. (2013). Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: A synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1627), 20120447.

Lizbinski, K. M., & Dacks, A. M. (2017). Intrinsic and extrinsic neuromodulation of olfactory processing. *Frontiers in Cellular Neuroscience*, *11*, 424. https://doi.org/10.3389/fncel.2017.00424

López-Patiño, M. A., Rodríguez-Illamola, A., Gesto, M., Soengas, J. L., & Míguez, J. M. (2011). Changes in plasma melatonin levels and pineal organ melatonin synthesis following acclimation of rainbow trout (*Oncorhynchus mykiss*) to different water salinities. *Journal of Experimental Biology*, *214*(6), 928–936. https://doi.org/10.1242/jeb.051516

Marshall, K. N., Kaplan, I. C., Hodgson, E. E., Hermann, A., Busch, D. S., McElhany, P., … Fulton, E. A. (2017). Risks of ocean acidification in the California Current food web and fisheries: Ecosystem model projections. *Global Change Biology*, *23*(4), 1525–1539. https://doi.org/10.1111/gcb.13594

Maryoung, L. A., Lavado, R., Bammler, T. K., Gallagher, E. P., Stapleton, P. L., Beyer, R. P., … Schlenk, D. (2015). Differential gene expression in liver, gill, and olfactory rosettes of *Coho salmon* (*Oncorhynchus kisutch*) after acclimation to salinity. *Marine Biotechnology*, *17*(6), 703–717. https://doi.org/10.1007/s10126-015-9649-5

McCormick, S. D. (2012). *Smolt physiology and endocrinology fish physiology*, Vol. *32* (pp. 199–251). Oxford, UK: Elsevier.

McGann, J. P. (2013). Presynaptic inhibition of olfactory sensory neurons: New mechanisms and potential functions. *Chemical Senses*, *38*(6), 459–474. https://doi.org/10.1093/chemse/bjt018

McIntyre, J. K., Baldwin, D. H., Beauchamp, D. A., & Scholz, N. L. (2012). Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecological Applications*, *22*(5), 1460–1471. https://doi.org/10.1890/11-2001.1

Miller, G. M., Watson, S.-A., Donelson, J. M., McCormick, M. I., & Munday, P. L. (2012). Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. *Nature Climate Change*, *2*(12), 858–861. https://doi.org/10.1038/nclimate1599

Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V., & Døving, K. B. (2009). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences*, *106*(6), 1848–1852. https://doi.org/10.1073/pnas.0809996106

Murray, C. S., Malvezzi, A., Gobler, C. J., & Baumann, H. (2014). Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series*, *504*, 1–11. https://doi.org/10.3354/meps10791

Nilsson, G. E., Dixson, D. L., Domenici, P., McCormick, M. I., Sørensen, C., Watson, S.-A., & Munday, P. L. (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change*, *2*(3), 201–204.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., … Yool, A. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, *437*(7059), 681–686. https://doi.org/10.1038/nature04095

Ou, M., Hamilton, T. J., Eom, J., Lyall, E. M., Gallup, J., Jiang, A., … Brauner, C. J. (2015). Responses of pink salmon to CO_2-induced aquatic acidification. *Nature Climate Change*, *5*, 950–955. https://doi.org/10.1038/nclimate2694

Pacella, S. R., Brown, C. A., Waldbusser, G. G., Labiosa, R. G., & Hales, B. (2018). Seagrass habitat metabolism increases short-term extremes and long-term offset of CO_2 under future ocean acidification. *Proceedings of the National Academy of Sciences*, *115*(15), 3870–3875.

Patro, R., Duggal, G., & Kingsford, C. (2015). *Salmon: Accurate, Versatile and Ultrafast Quantification from rna-seq Data Using lightweight-alignment*. Biorxiv, 021592.

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*, 417–419. https://doi.org/10.1038/nmeth.4197

Porteus, C. S., Hubbard, P. C., Uren Webster, T. M., van Aerle, R., Canário, A. V. M., Santos, E. M., & Wilson, R. W. (2018). Near-future CO_2 levels impair the olfactory system of a marine fish. *Nature Climate Change*, *8*(8), 737–743. https://doi.org/10.1038/s41558-018-0224-8

Pronin, A., Levay, K., Velmeshev, D., Faghihi, M., Shestopalov, V. I., & Slepak, V. Z. (2014). Expression of olfactory signaling genes in the eye. *PLoS ONE*, *9*(4), e96435. https://doi.org/10.1371/journal.pone.0096435

Quinn, T. P. (2011). *The behavior and ecology of Pacific salmon and trout*. Vancouver, Canada: UBC Press.

Reum, J. C., Alin, S. R., Harvey, C. J., Bednaršek, N., Evans, W., Feely, R. A., … McElhany, P. (2015). Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. *ICES Journal of Marine Science: Journal Du Conseil*, *73*(3), 582–595.

Sandahl, J. F., Baldwin, D. H., Jenkins, J. J., & Scholz, N. L. (2007). A sensory system at the interface between urban stormwater runoff and salmon survival. *Environmental Science and Technology*, *41*(8), 2998–3004. https://doi.org/10.1021/es062287r

Schild, D., & Restrepo, D. (1998). Transduction mechanisms in vertebrate olfactory receptor cells. *Physiological Reviews*, *78*(2), 429–466. https://doi.org/10.1152/physrev.1998.78.2.429

Schmidt, M., Gerlach, G., Leo, E., Kunz, K. L., Swoboda, S., Pörtner, H. O., … Storch, D. (2017). Impact of ocean warming and acidification on the behaviour of two co-occurring gadid species, Boreogadus saida and Gadus morhua, from Svalbard. *Marine Ecology Progress Series*, *571*, 183–191. https://doi.org/10.3354/meps12130

Schunter, C., Welch, M. J., Nilsson, G. E., Rummer, J. L., Munday, P. L., & Ravasi, T. (2017). An interplay between plasticity and parental phenotype determines impacts of ocean acidification on a reef fish. *Nature Ecology & Evolution*, *2*, 334–342. https://doi.org/10.1038/s41559-017-0428-8

Schunter, C., Welch, M. J., Ryu, T., Zhang, H., Berumen, M. L., Nilsson, G. E., … Ravasi, T. (2016). Molecular signatures of transgenerational response to ocean acidification in a species of reef fish. *Nature Climate Change*, *6*, 1014–2000. https://doi.org/10.1038/nclimate3087

Scimemi, A. (2014). Structure, function, and plasticity of GABA transporters. *Frontiers in Cellular Neuroscience*, *8*, 161. https://doi.org/10.3389/fncel.2014.00161.

Simpson, S. D., Munday, P. L., Wittenrich, M. L., Manassa, R., Dixson, D. L., Gagliano, M., & Yan, H. Y. (2011). Ocean acidification erodes crucial auditory behaviour in a marine fish. *Biology Letters*, *7*, 917–920. https://doi.org/10.1098/rsbl.2011.0293

Somjen, G. G. (2002). Ion regulation in the brain: Implications for pathophysiology. *The Neuroscientist*, *8*(3), 254–267. https://doi.org/10.1177/1073858402008003011

Sommers, F., Mudrock, E., Labenia, J., & Baldwin, D. (2016). Effects of salinity on olfactory toxicity and behavioral responses of juvenile salmonids from copper. *Aquatic Toxicology*, *175*, 260–268. https://doi.org/10.1016/j.aquatox.2016.04.001

Soneson, C., Love, M. I., & Robinson, M. D. (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research*, *4*, 1521. https://doi.org/10.12688/f1000research.7563.2

Tabor, R., Yaksi, E., & Friedrich, R. W. (2008). Multiple functions of GABAA and GABAB receptors during pattern processing in the zebrafish olfactory bulb. *European Journal of Neuroscience*, *28*(1), 117–127.

Tan, J., Savigner, A., Ma, M., & Luo, M. (2010). Odor information processing by the olfactory bulb analyzed in gene-targeted mice. *Neuron*, *65*(6), 912–926. https://doi.org/10.1016/j.neuron.2010.02.011.

Tierney, K. B., Baldwin, D. H., Hara, T. J., Ross, P. S., Scholz, N. L., & Kennedy, C. J. (2010). Olfactory toxicity in fishes. *Aquatic Toxicology*, *96*(1), 2–26. https://doi.org/10.1016/j.aquatox.2009.09.019

Tresguerres, M., & Hamilton, T. J. (2017). Acid–base physiology, neurobiology and behaviour in relation to CO_2-induced ocean acidification. *Journal of Experimental Biology*, *220*(12), 2136–2148. https://doi.org/10.1242/jeb.144113

Wang, C., Ohno, K., Furukawa, T., Ueki, T., Ikeda, M., Fukuda, A., & Sato, K. (2005). Differential expression of KCC2 accounts for the differential GABA responses between relay and intrinsic neurons in the early postnatal rat olfactory bulb. *European Journal of Neuroscience*, *21*(5), 1449–1455. https://doi.org/10.1111/j.1460-9568.2005.03975.x

Welch, M. J., & Munday, P. L. (2017). Heritability of behavioural tolerance to high CO_2 in a coral reef fish is masked by nonadaptive phenotypic plasticity. *Evolutionary Applications*, *10*(7), 682–693. https://doi.org/10.1111/eva.12483

Welch, M., Watson, S., Welsh, J., McCormick, M., & Munday, P. (2014). Effects of elevated CO_2 on fish behaviour undiminished by transgenerational acclimation. *Nature Climate Change*, *4*, 1086–1089.

Williams, C. R., & Gallagher, E. P. (2013). Effects of cadmium on olfactory mediated behaviors and molecular biomarkers in coho salmon (*Oncorhynchus kisutch*). *Aquatic Toxicology*, *140*, 295–302. https://doi.org/10.1016/j.aquatox.2013.06.010

Williams, C. R., MacDonald, J. W., Bammler, T. K., Paulsen, M. H., Simpson, C. D., & Gallagher, E. P. (2016). From the Cover: cadmium exposure differentially alters odorant-driven behaviors and expression of olfactory receptors in juvenile Coho salmon (*Oncorhynchus kisutch*). *Toxicological Sciences*, *154*(2), 267–277. https://doi.org/10.1093/toxsci/kfw172.

Yambe, H., Kitamura, S., Kamio, M., Yamada, M., Matsunaga, S., Fusetani, N., & Yamazaki, F. (2006). L-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. *Proceedings of the National Academy of Sciences*, *103*(42), 15370–16000. http://www.pnas.org/content/103/42/15370.abstract

Zeileis, A., Cribari-Neto, F., Gruen, B., & Kosmidis, I. (2016). betareg: Beta regression. [Computer software manual].

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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