Apr 5th, 3:30 PM - 3:45 PM

Elevated carbon dioxide alters neural signaling and anti-predator behaviors in ocean phase coho salmon (Oncorhynchus kisutch)

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Speaker
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Chase Williams, UW/NOAA

University of Washington
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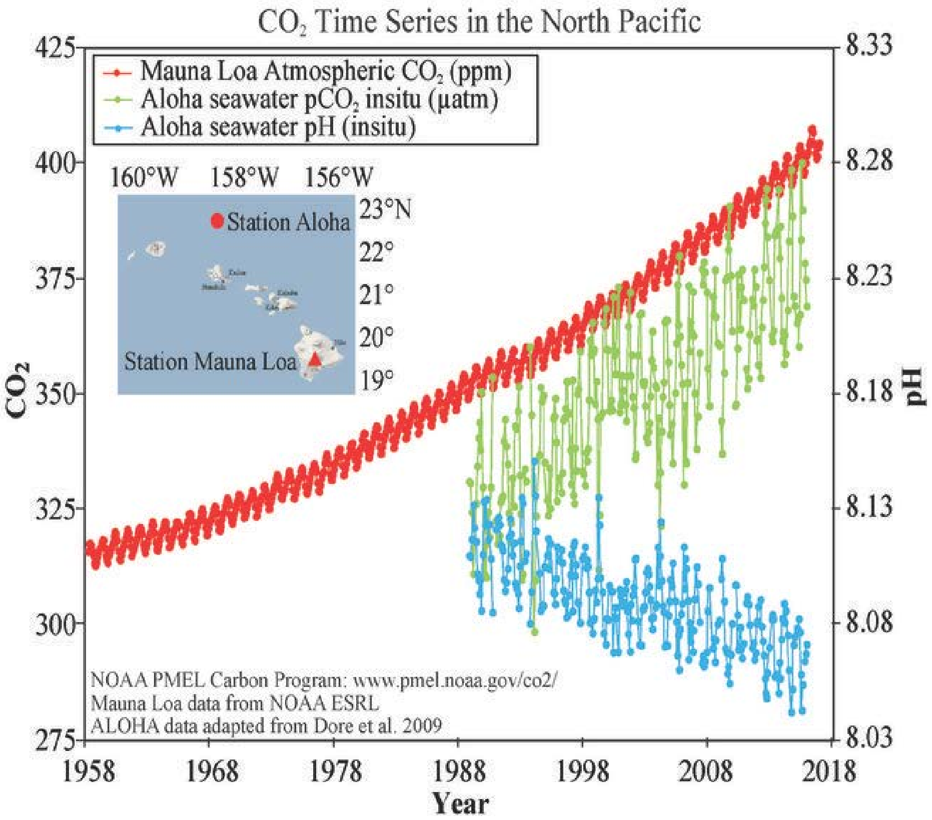
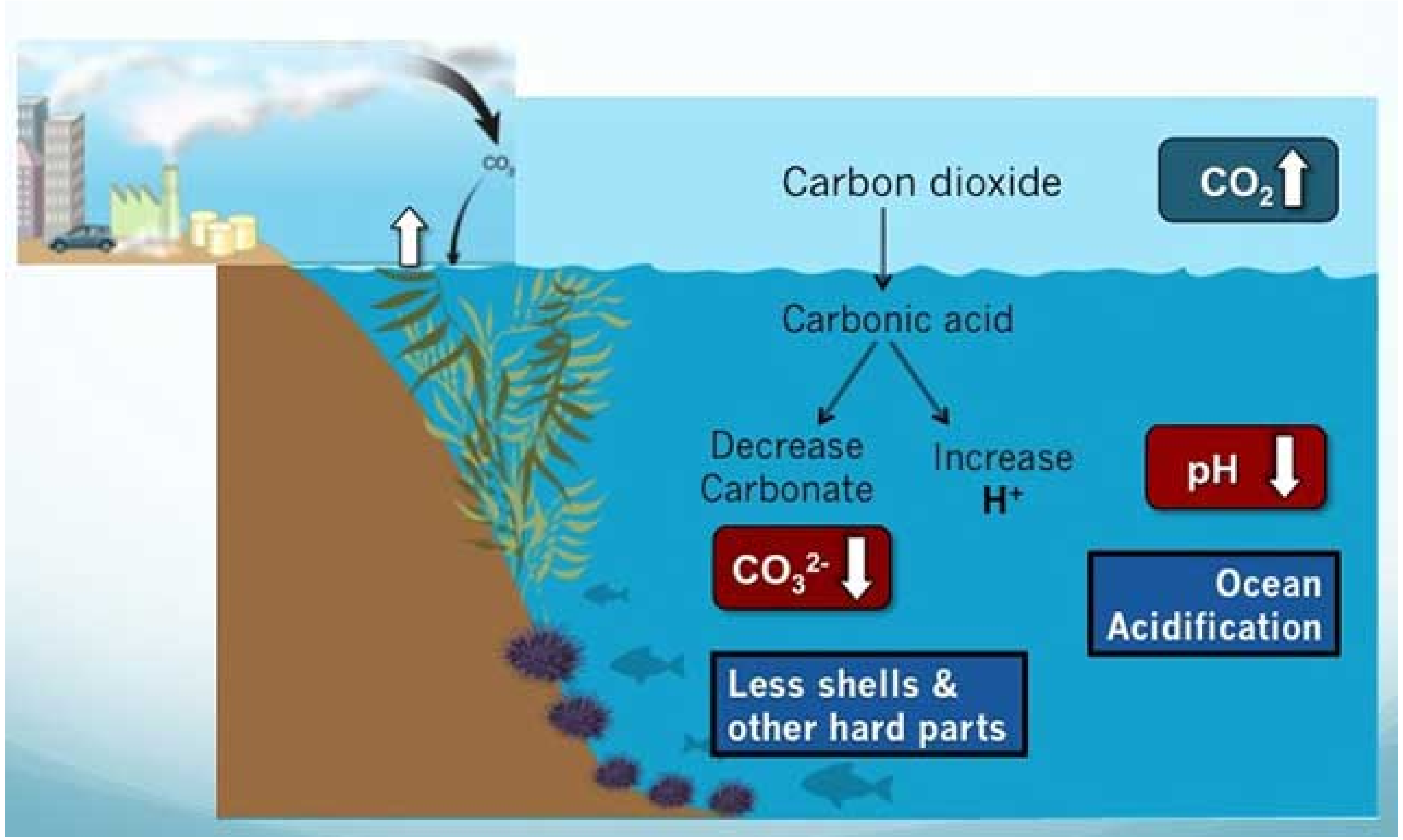
Ocean Acidification

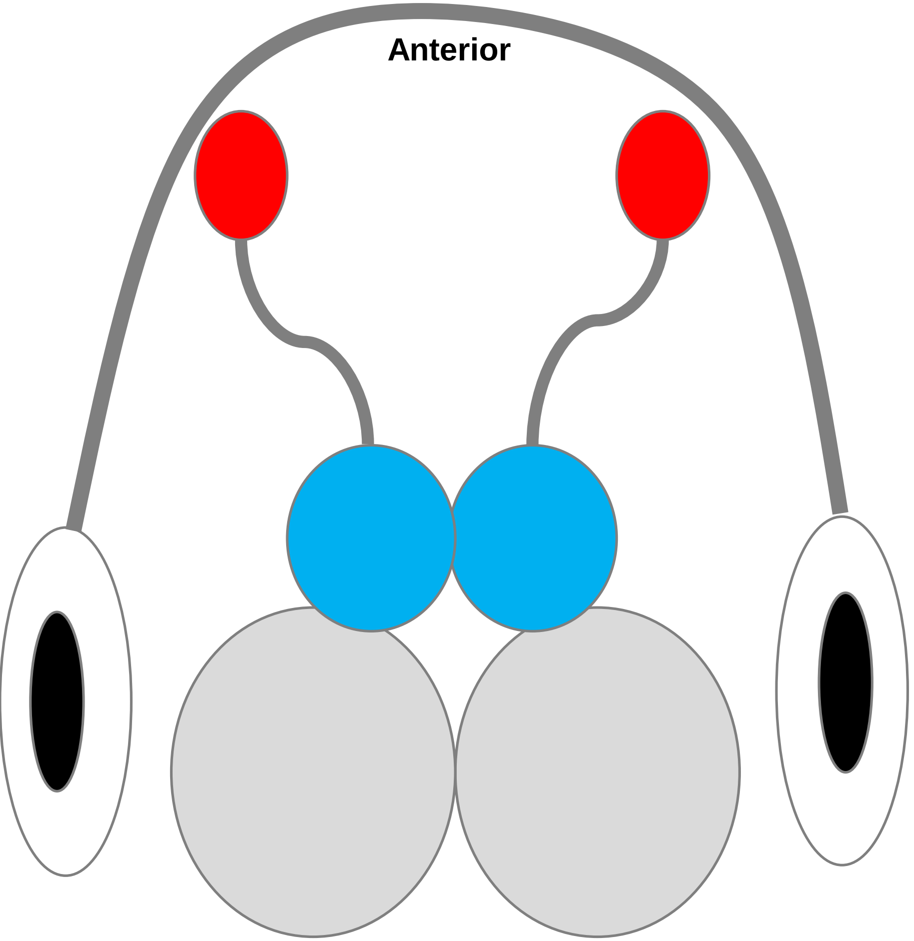


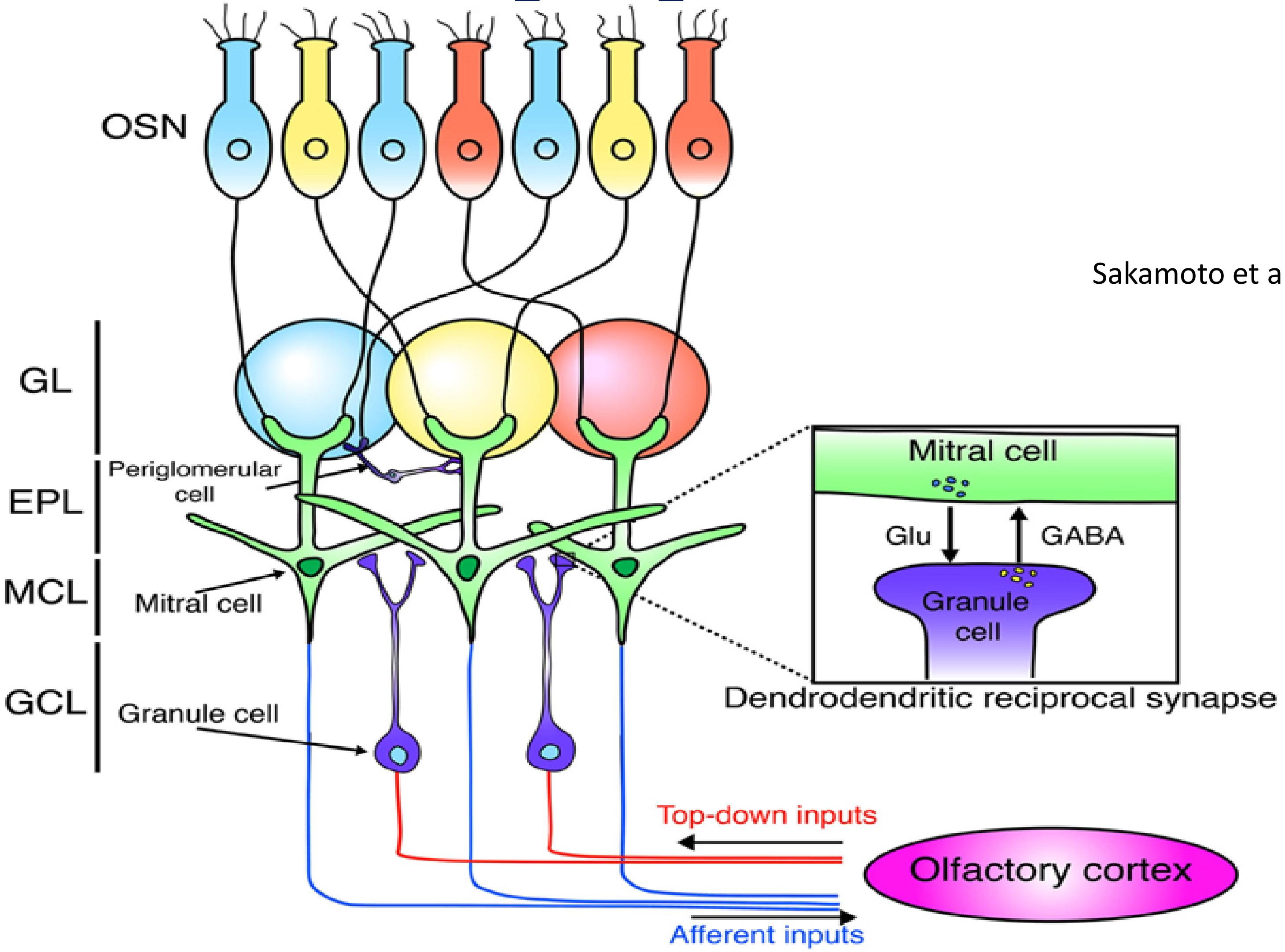
Data: Mauna Loa (ftp://aftp.cmdl.noaa.gov/products/trends/co2/co2_mm_mlo.txt) ALOHA (http://hahana.soest.hawaii.edu/hot/products/HOT_surface_CO2.txt)
Ref: J.E. Dore et al, 2009. Physical and biogeochemical modulation of ocean acidification in the central North Pacific. *Proc Natl Acad Sci USA* 106:12235-12240.



From Marine Science today

Vertebrate olfactory system





Sakamoto et al., 2014

Coho salmon

- Anadromous
- Ecologically and economically important fish species
- Olfaction plays a central role in survival, navigation and reproduction.

Project aim

- **Specific aim: Characterize the effects of predicted increases in CO_2 levels relevant to Washington waters on olfactory function in juvenile coho salmon.**

  - **Sub-aim 1: Determine if predicted increases in CO_2 levels impair olfactory-mediated responses in juvenile coho salmon.**

  - **Sub-aim 2: Determine if predicted increases in CO_2 levels alter olfactory neuronal signaling in juvenile coho salmon.**

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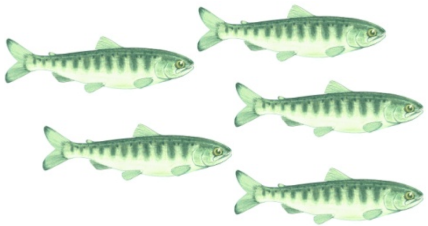
Experimental paradigm

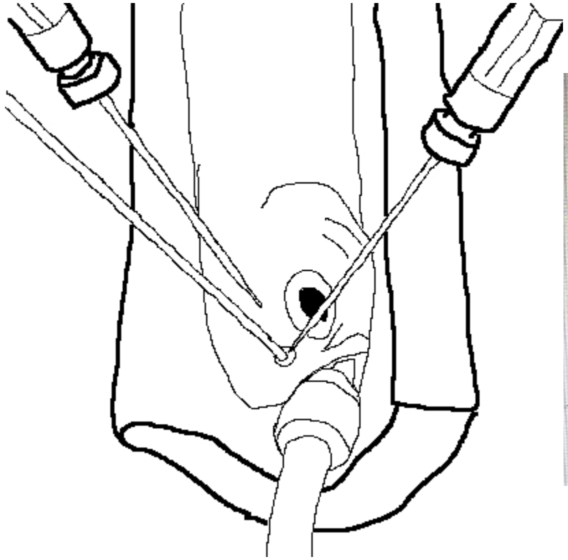
Two-week exposure

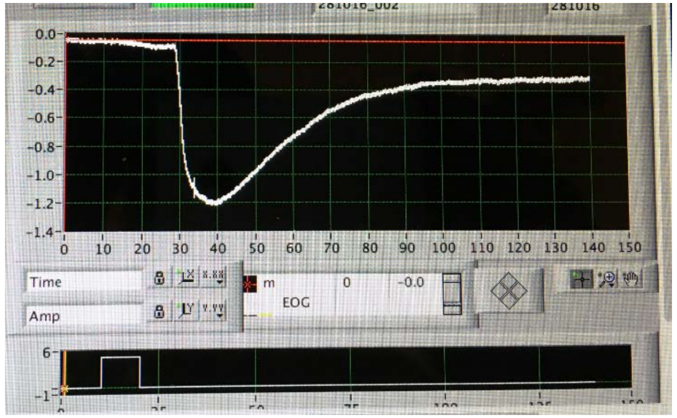


Control: pH 7.8 (~800µatm)
Medium: pH 7.5 (~1600µatm)
High: pH 7.2 (~3200µatm)

Behavioral response to odorants

EOG/EEG analysis on odorant responses



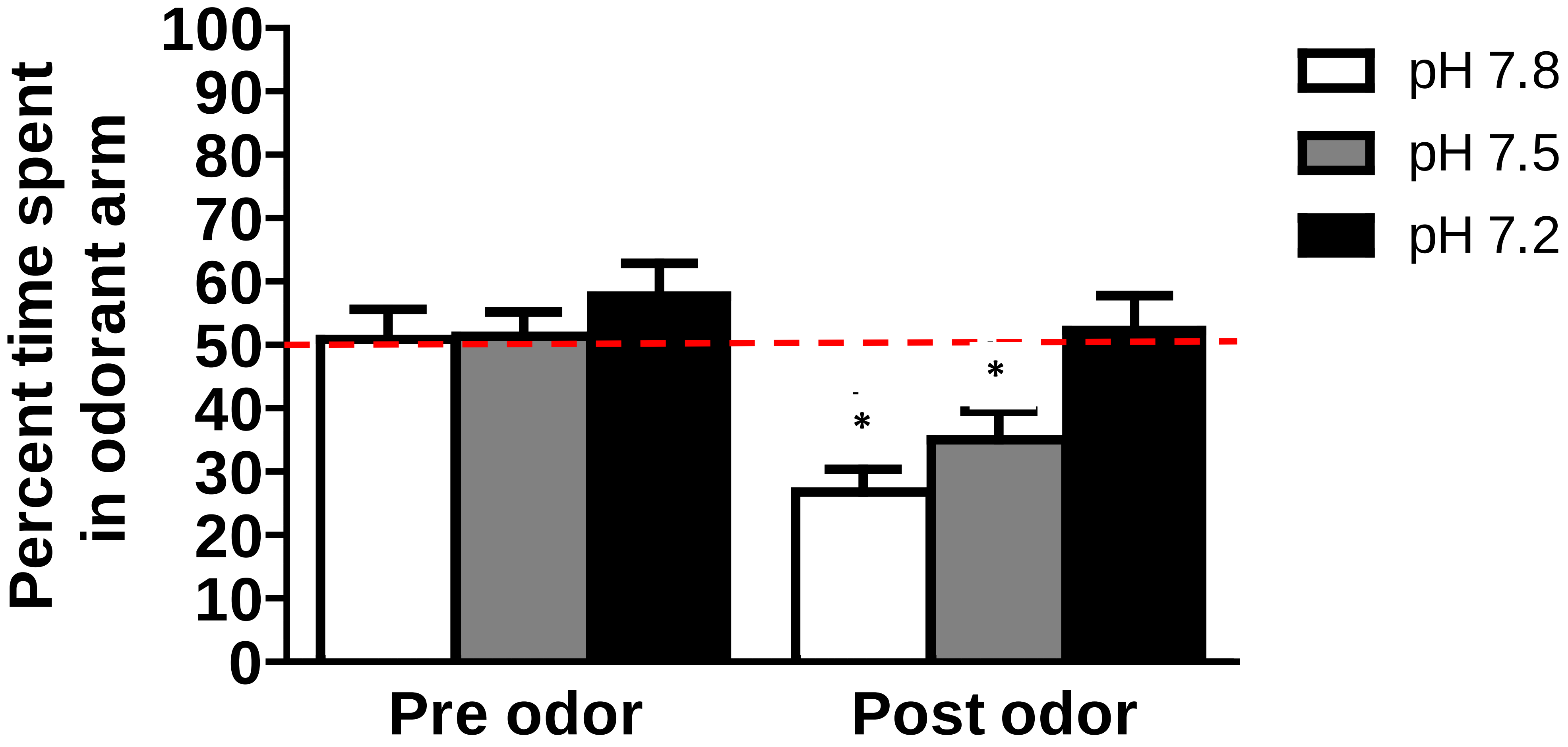


Experimental odorants

1. Behavior: Salmon- Skin extract (alarm cue)

1. EOG/EEG: 10^{-2}M L-serine
   10^{-2}M L-alanine
   Skin extract

Elevated CO_2 altered an olfactory driven behavior in coho salmon



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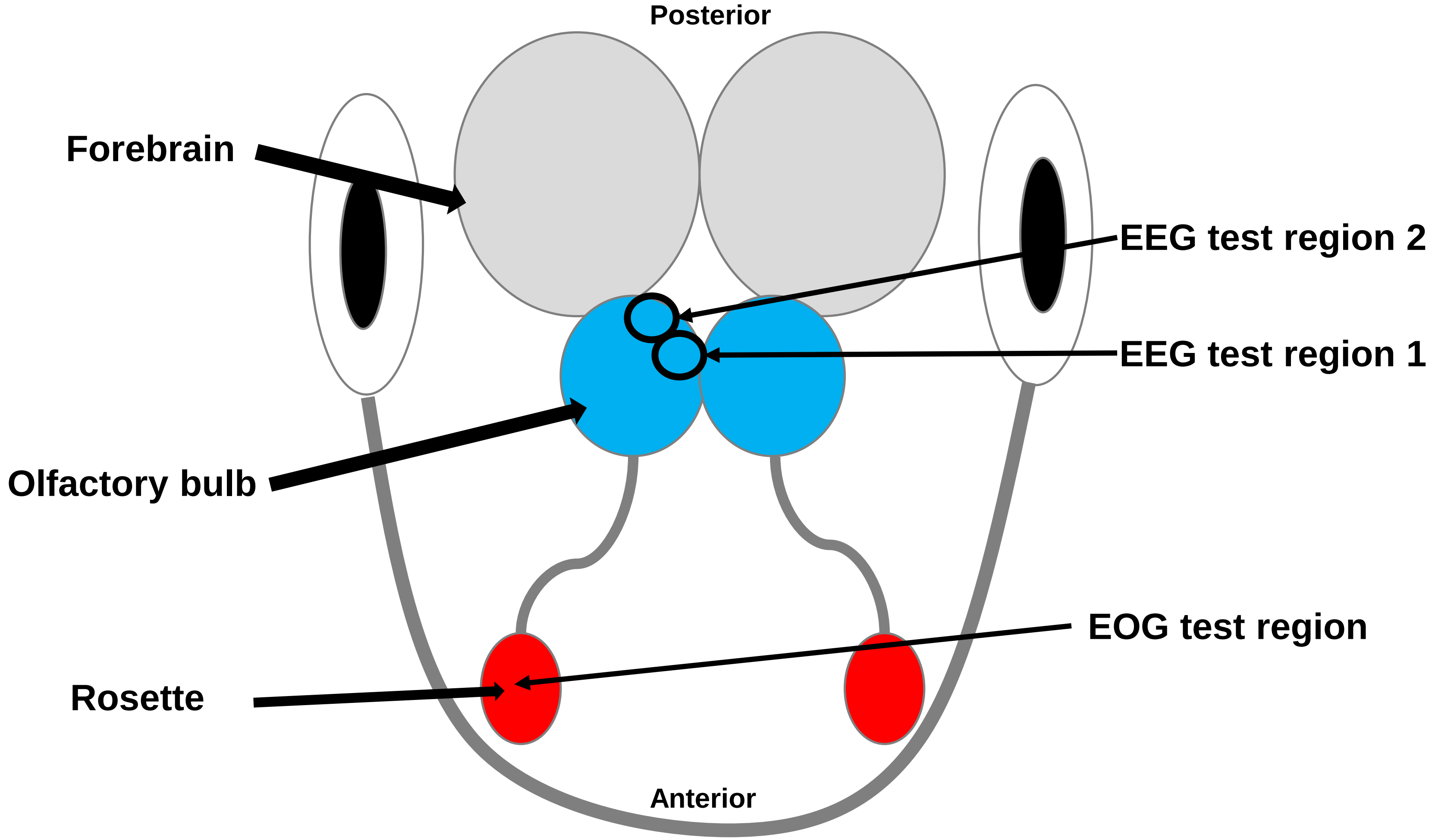
Top view of salmon olfactory system and electrophysiology test sites



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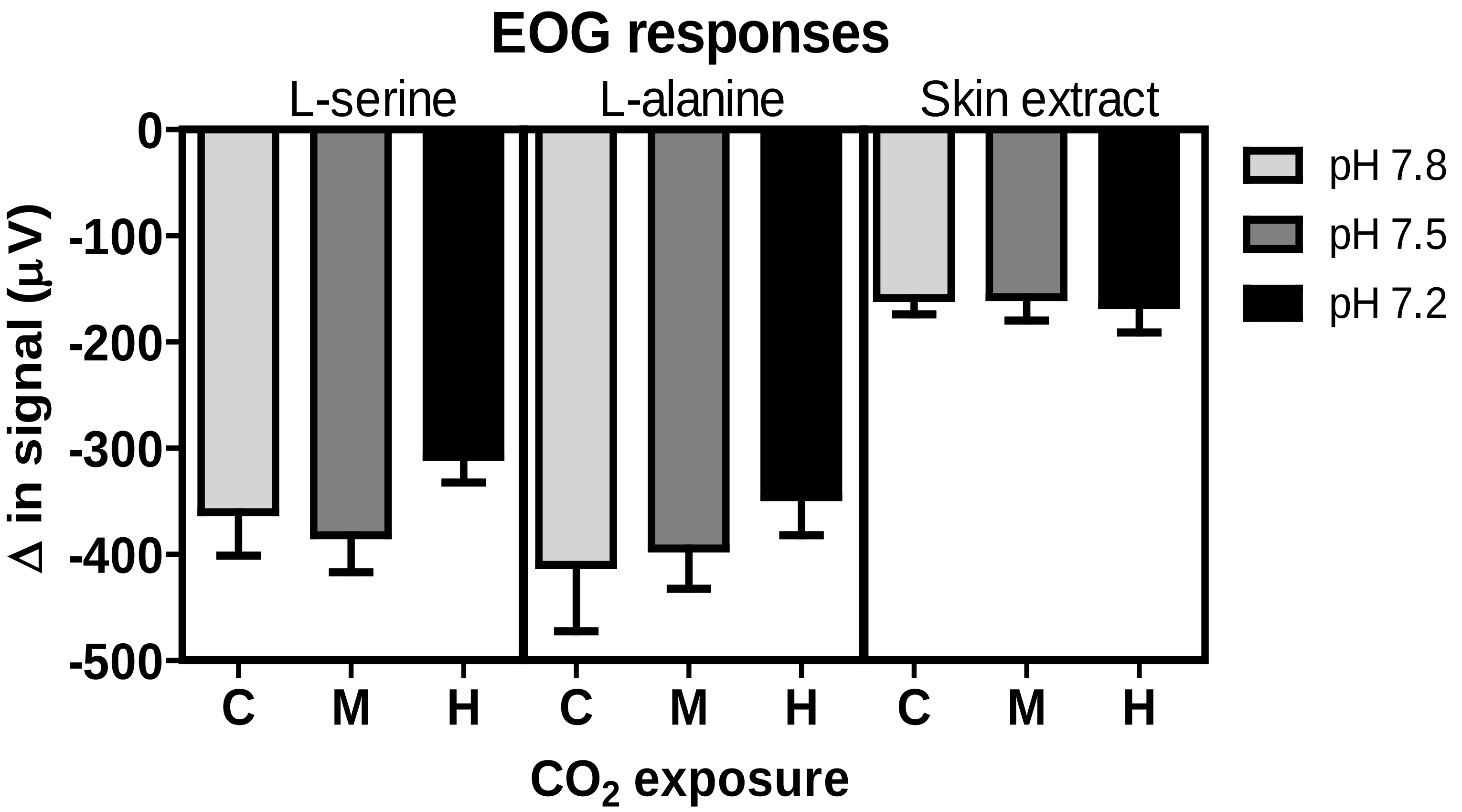
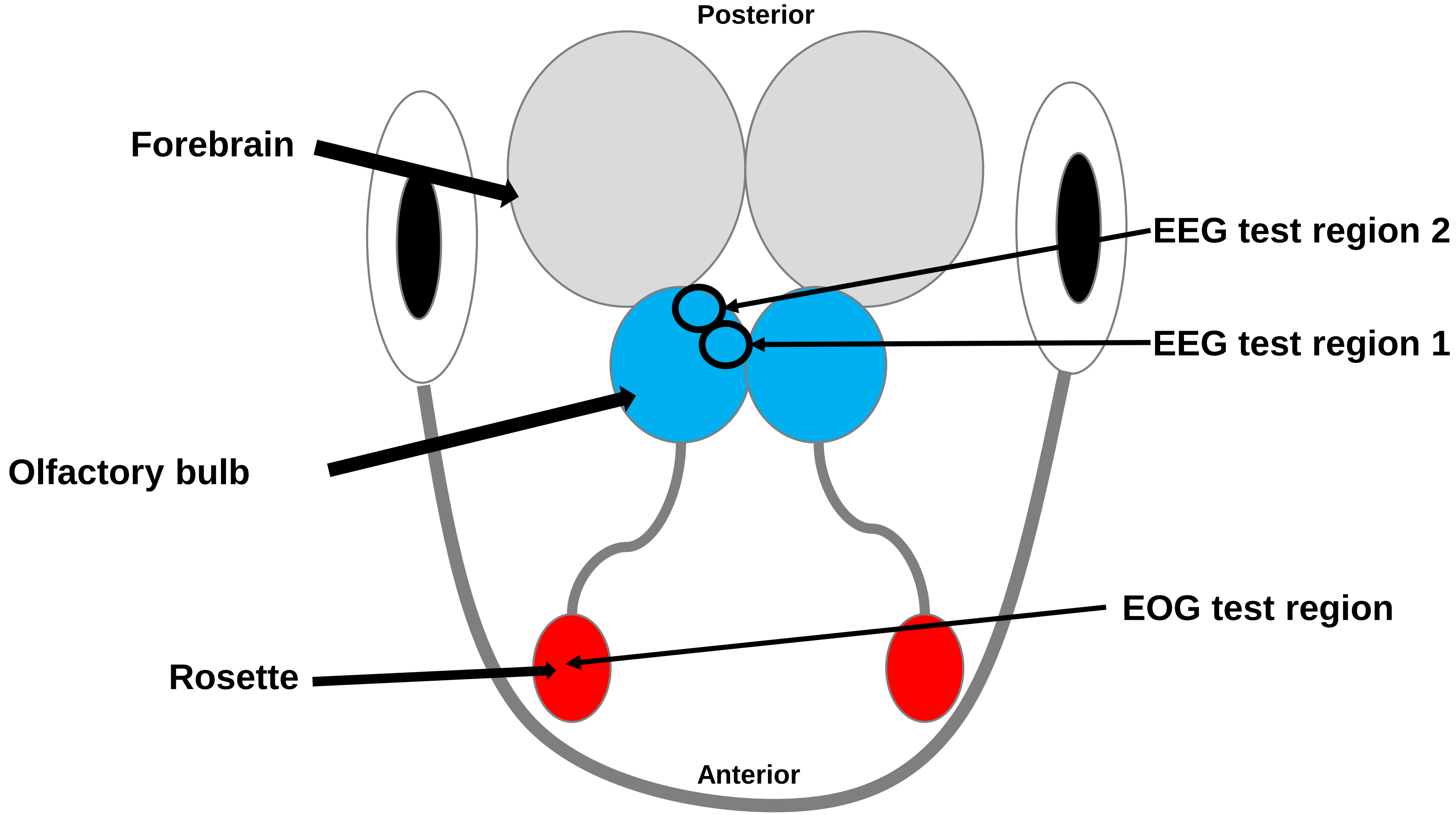
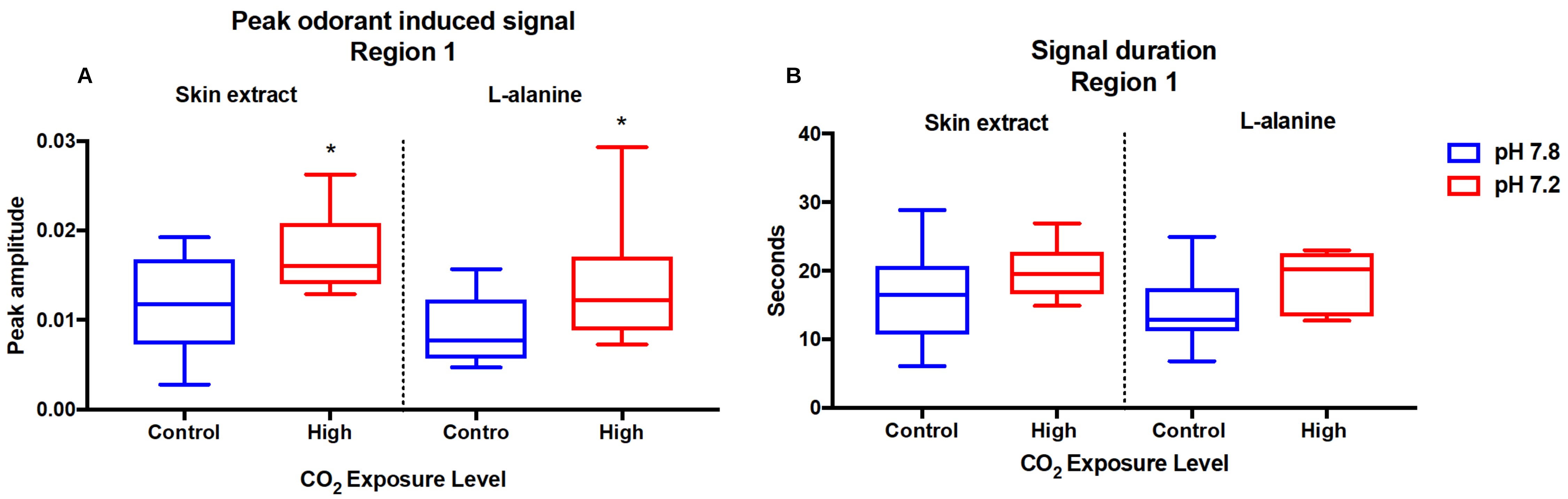
Elevated CO_2 did not disrupt coho salmon neuron signaling in the rosettes



Top view of salmon olfactory system and electrophysiology test sites



Elevated CO_2 altered neuronal signaling in the olfactory bulbs



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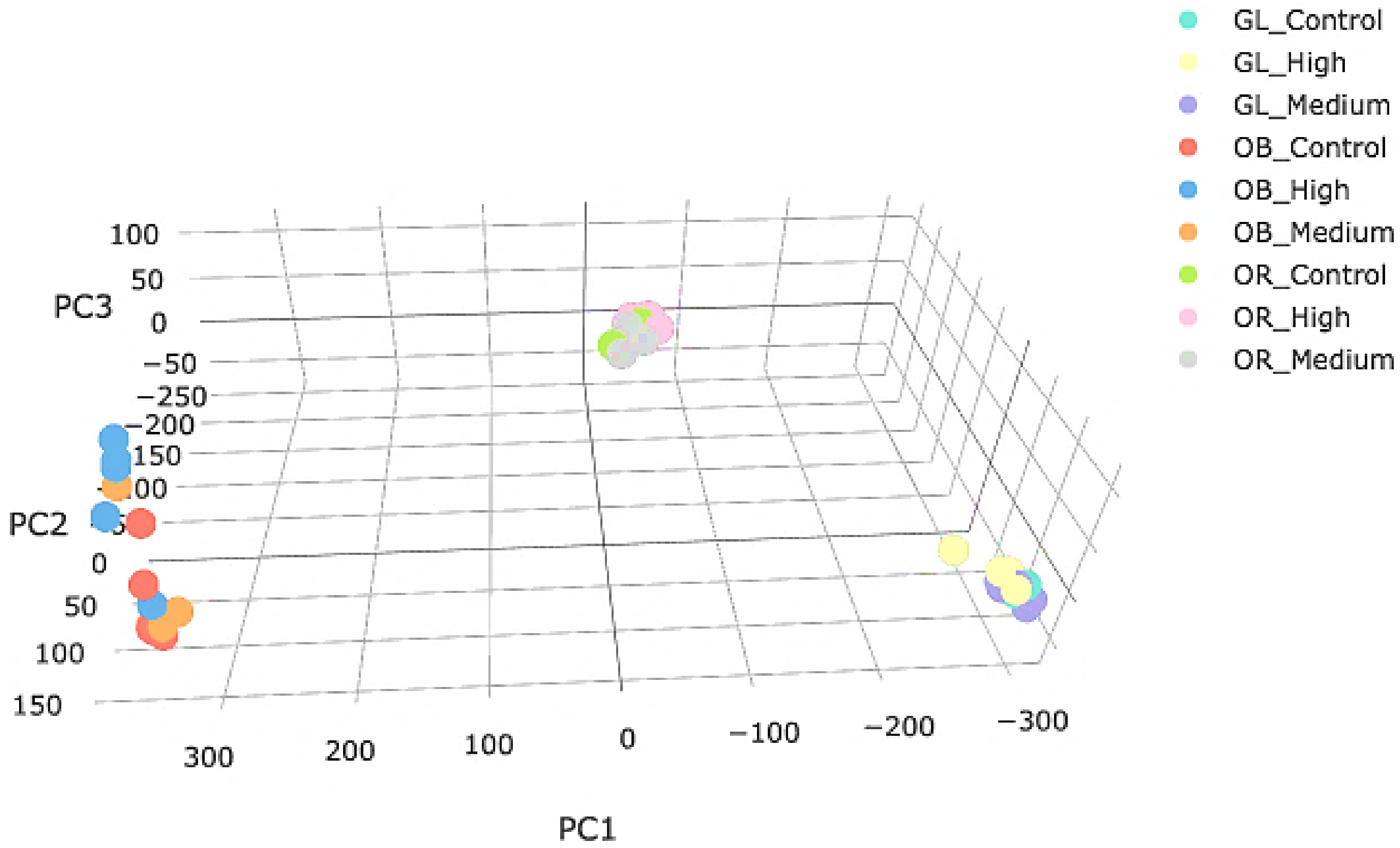
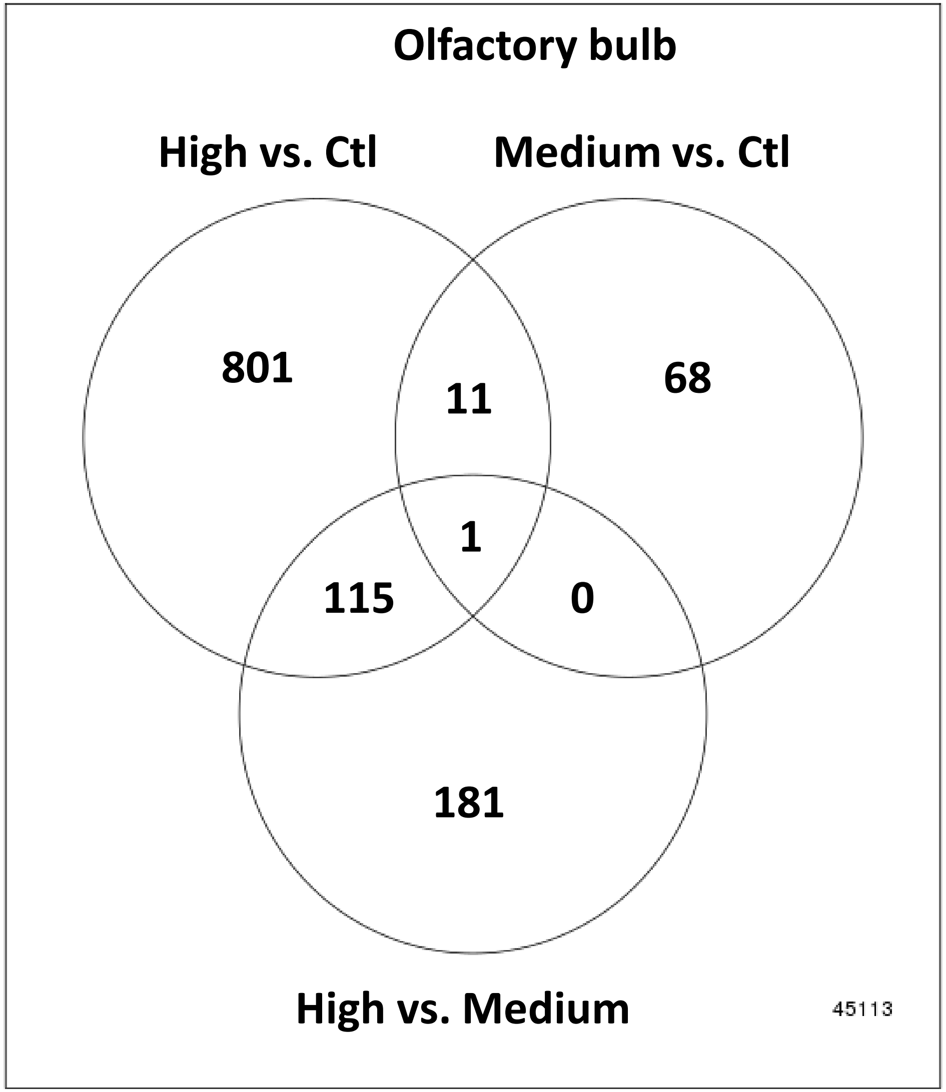
Analysis of gene expression within the gills, rosettes and olfactory bulbs



RNA-Seq analysis of CO_2 effects on olfactory rosettes and olfactory bulbs



Changes in gene expression in control vs. high CO_2 olfactory bulbs

ENTREZID	GENENAME	SYMBOL	log fold change	Hypothesized function
106568477	complexin 4	cplx4	4.490533842	Both an inhibitor and a facilitator of synaptic vesicle fusion and neurotransmitter release
106574477	excitatory amino acid transporter 5-like	slc1a7	4.125884632	A sodium- and potassium-dependent glutamate transporter
106588157	potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2-like	hcn	3.966737036	GABA-b linked
106613596	excitatory amino acid transporter 5-like	slc1a7	3.901196459	Glutamate uptake
106562041	guanine nucleotide-binding protein subunit alpha-14-like	gna14	3.303694213	Modulators or transducers in various transmembrane signaling systems.
106561698	solute carrier organic anion transporter family member 1C1-like	slco1c1	3.136496308	Regulates uptake of thyroid hormones in brain
106574723	gamma-aminobutyric acid type B receptor subunit 2-like	gabbr2	2.644787506	GABA beta subunit 2- mediates coupling to G-proteins
106577203	potassium voltage-gated channel subfamily H member 1-like	kcnh7	2.465837224	Modulation of neural firing
106605091	guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3-like	gnb3	2.402243063	Integrate signals between receptors and effector proteins
106587671	guanine nucleotide-binding protein subunit beta-5-like	gnb5	2.359161613	Involved in the termination of the signaling initiated by the G protein coupled receptors
106572933	voltage-dependent L-type calcium channel subunit alpha-1D-like	cacna1d	2.282059336	Calcium influx, neuron excitation
106592065	neuronal acetylcholine receptor subunit alpha-3	chrna3	2.226879883	Neural excitation. Receptor family related to GABA a RECEPTORS
106580796	solute carrier family 6 member 4	slc6a4	2.050101723	Serotonin reuptake, Serotonin increases synaptic activity in olfactory bulb glomeruli
106611384	synaptosomal-associated protein 25-B-like	snap25	1.882814946	Synaptic transmitter uptake and release. GABA and glutamate associated
106572937	voltage-dependent L-type calcium channel subunit alpha-1F-like	cacna1f	1.879567818	Mediates the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes.
106572934	voltage-dependent L-type calcium channel subunit alpha-1D-like	cacna1d	1.806896225	Mediates the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes.
106605869	gamma-aminobutyric acid type B receptor subunit 2-like	gabbr2	1.772909967	GABA beta subunit 2- mediates coupling to G-proteins
106577267	neuronal pentraxin-1-like	np1	1.726930504	Involved in excitatory synapse remodeling.
106578273	vesicular glutamate transporter 1-like	vglut1	1.625190857	Excitatory glutamate transport
106572936	voltage-dependent L-type calcium channel subunit alpha-1S-like	cacna1s	1.622496056	Skeletal muscle associated calcium transporter
106564793	sodium/calcium exchanger 1-like	slc8a1	1.534701876	Exports Ca to repolarize cell
106566781	solute carrier family 26 member 6-like	slc26a6	1.458399237	A protein involved in transporting chloride, oxalate, sulfate and bicarbonate
106569207	solute carrier family 12 member 7-like	kcc1	1.368291678	Exportation of Cl- needed for GABA signaling
106582421	sodium channel subunit beta-1-like	scn1b	1.215528748	Signal propogation in neurons and neuronal growth
106564801	potassium voltage-gated channel subfamily H member 1-like	kcnh1	1.124457553	Involved in neural excitation and neurotransmitter release
106562494	guanine nucleotide-binding protein subunit beta-5-like	gnb5	1.104305787	Involved in the termination of the signaling initiated by the G protein coupled receptors
106607984	solute carrier family 22 member 16-like	slc22a16	1.072500486	L-carnatine transport a precursor to acetylcholine
106612651	sodium-dependent serotonin transporter-like	slc6a4	1.049560641	Terminates the action of serotonin and recycles it in a sodium-dependent manner
106561149	solute carrier organic anion transporter family member 3A1-like	slc21a11	1.018675617	Organic anion transporter
106603743	glutamate receptor ionotropic, kainate 4-like	grik4	1.012385533	Excitatory receptor
106583542	sodium- and chloride-dependent GABA transporter 2-like	slc6a13	0.971746727	GABA uptake
106573780	solute carrier organic anion transporter family member 3A1-like	slc21a11	0.859871022	Organic anion transporter
106610602	solute carrier family 4 member 1 adaptor protein	slc4a1ap	-0.253944956	Bicarbonate transport
106561537	solute carrier family 27 member 4	slc27a4	-0.340968729	Role in fatty acid uptake
106578986	glutamate receptor ionotropic, delta-1-like	grid1	-0.409369941	Mediate most of the fast excitatory synaptic transmission in the central nervous system and play key roles in synaptic plasticity
106604348	glutamate receptor 1-like	grm1	-0.44906311	Glutamate receptor that functions by activating phospholipase C
106584763	potassium voltage-gated channel subfamily C member 1-like	kcnc1	-0.601248818	Plays a role in the rapid repolarization of fast-firing brain neurons, forms complex with KCNC2
106570824	neuroligin-3-like	nlgn3	-0.608511265	Members of this family may be involved in the formation and remodeling of central nervous system synapses
106585781	solute carrier family 2 member 6	slc2a6	-1.210017821	Glucose transport
106603834	solute carrier family 22 member 5-like	slc22a5	-1.251537509	L-carnatine transport - a precursor to acetylcholine
106613200	short transient receptor potential channel 2-like	trpc2	-1.429904475	Receptor-activated non-selective calcium permeant cation channel
106600164	aldehyde dehydrogenase family 9 member A1-like	aldh9a1	-5.87785611	A protein involved in the dehydrogenation of gamma-aminobutyraldehyde to GABA
106579379	tubby protein homolog	---	4.090225821	Related to control of neural differentiation /maintanence
106566029	tubby-related protein 1-like	---	4.076974342	Related to control of neural differentiation
106586510	acetylserotonin O-methyltransferase	asmt	4.053361562	Production of melatonin. Sleep cycle related. Next step enzyme after AANAT
106607367	serotonin N-acetyltransferase-like	aanat	4.020461837	Production of melatonin. Sleep cycle related
106572384	sodium-coupled neutral amino acid transporter 3-like	slc38a3	2.165144325	Role in glutamate/GABA transport, associated with circadian rhythm as well maybe

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Changes in gene expression in control vs. high CO_2 olfactory bulbs

***All are putative functions**

- GABA-B beta subunit 2- mediates coupling to G-proteins
- Exportation of Cl- needed for GABA signaling
- GABA uptake
- Synaptic transmitter uptake and release. GABA and glutamate associated
- GABA-b linked
- Bicarbonate transport
- Neural excitation and neurotransmitter release
- Glutamate/GABA transport, associated with circadian rhythm

- Calcium influx, neuron excitation
- Mediate fast excitatory synaptic transmission in the central nervous system and plays key roles in synaptic plasticity
- Organic anion transporter
- Both an inhibitor and a facilitator of synaptic vesicle fusion and neurotransmitter release
- Involved in the dehydrogenation of gamma-aminobutyraldehyde to GABA

Summation of the results

- Juvenile coho salmon exposed to a high CO_2 level experienced a disruption of olfactory driven behaviors.

- Exposure to the high CO_2 level did not alter odorant induced signaling in the olfactory rosettes but did induce significant changes in signaling within the olfactory bulbs.

- RNA-seq analysis revealed significant changes in expression of many genes involved in neuronal signaling and signal modulation within the olfactory bulbs from coho exposed to the high CO_2 level compared to control coho.

Acknowledgments

- **Gallagher lab:**
  Richard Ramsden

- **NOAA collaborators:**
  Meg Chadsey
  David Baldwin
  Frank Sommers
  Darran May
  Danielle Perez

- **Funding:**
  Washington Sea Grant
  Washington Ocean Acidification Center

- **All the fish used in the study!**

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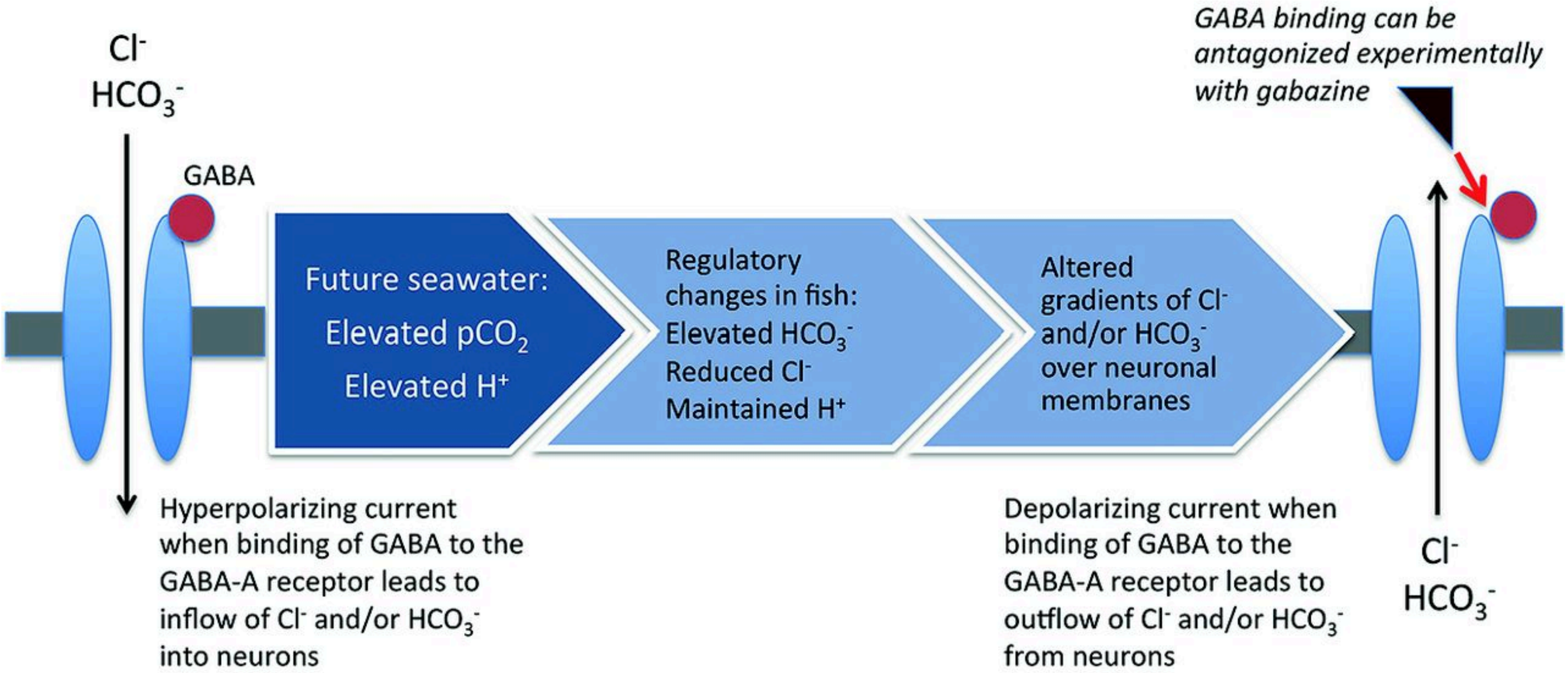
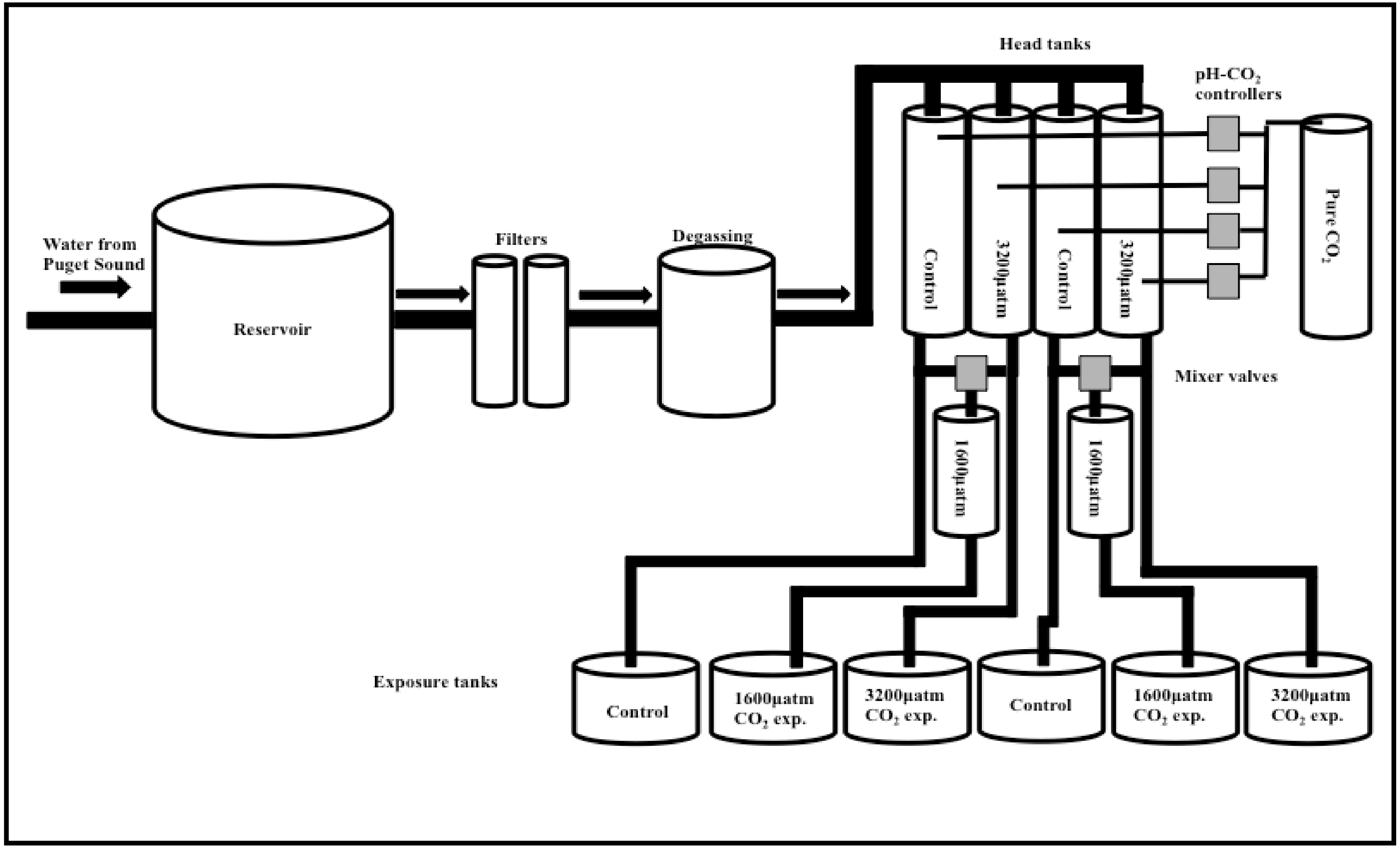
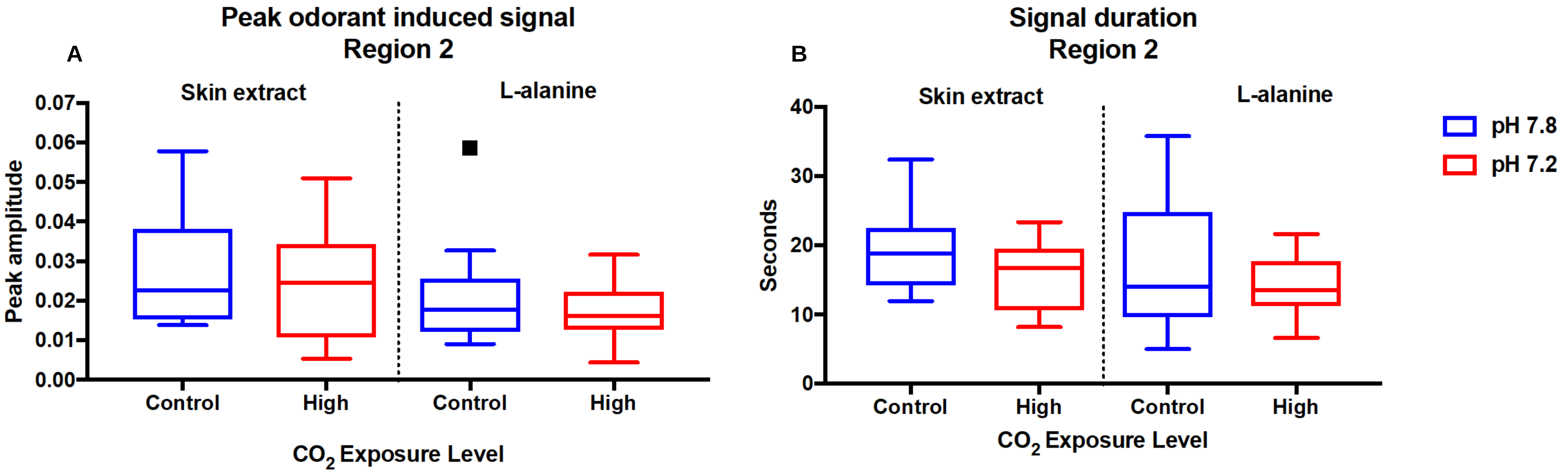


Nilsson et al., 2012

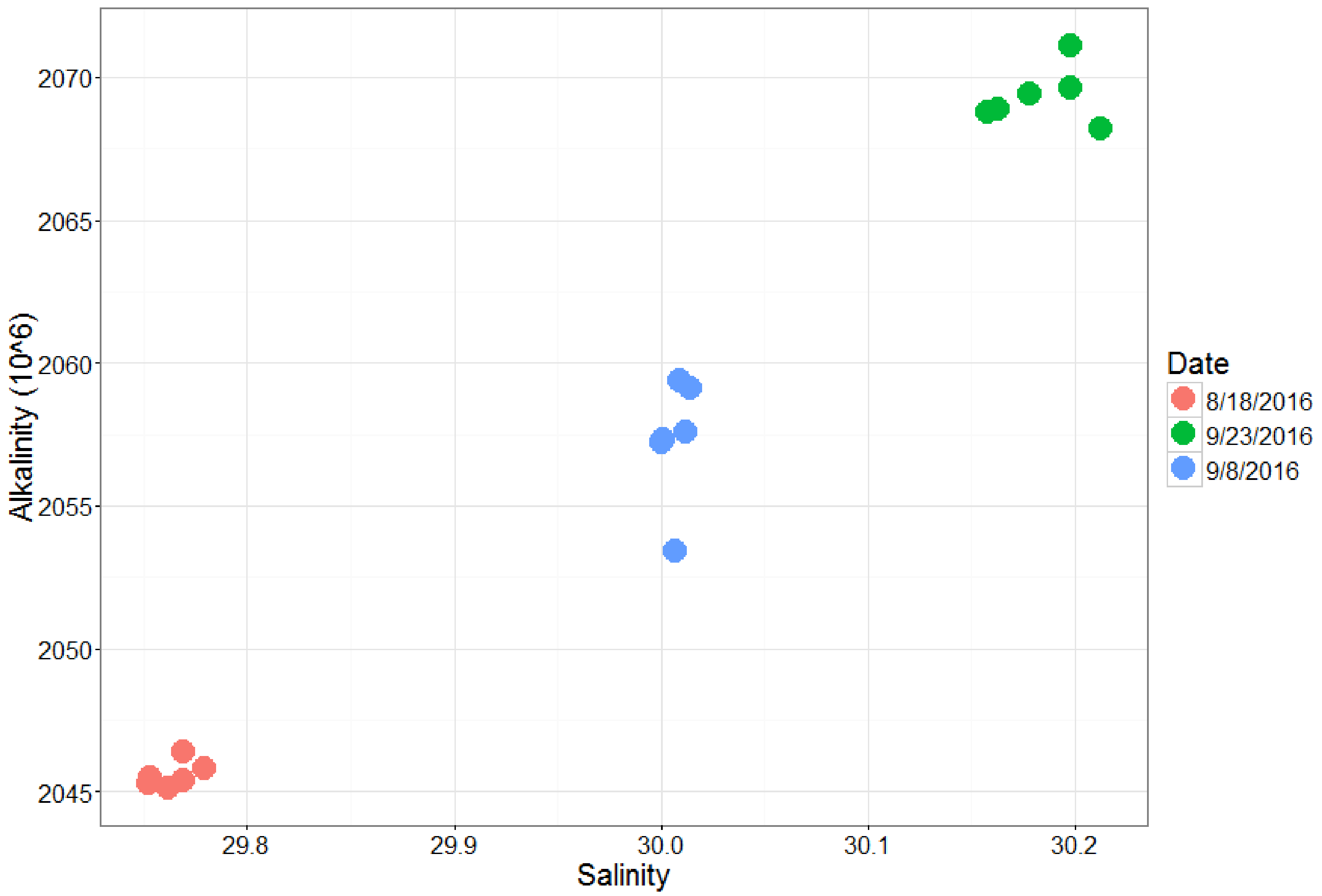
Exposure system



Elevated CO_2 altered neuronal signaling in the olfactory bulbs



Exposure chemistry



Results

Exposure chemistry