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

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Ocean Acidification

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.
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 CO₂ altered olfactory driven behavior in coho salmon

![Bar graph showing percent time spent in odorant arm before and after odor exposure for different pH conditions. The graph indicates a significant change in behavior for pH 7.2 after exposure.]
Top view of salmon olfactory system and electrophysiology test sites

- Forebrain
- Olfactory bulb
- Rosette
- EEG test region 1
- EEG test region 2
- EOG test region

Posterior

Anterior
Elevated CO$_2$ did not disrupt coho salmon neuron signaling in the rosettes.
Top view of salmon olfactory system and electrophysiology test sites

- Forebrain
- Olfactory bulb
- Rosette
- EEG test region 1
- EEG test region 2
- EOG test region
Elevated CO$_2$ altered neuronal signaling in the olfactory bulbs
Analysis of gene expression within the gills, rosettes and olfactory bulbs.
RNA-Seq analysis of CO$_2$ effects on olfactory rosettes and olfactory bulbs

Olfactory bulb

High vs. Ctl

- 801
- 115
- 181

Medium vs. Ctl

- 11
- 1
- 0

High vs. Medium

- 68
<table>
<thead>
<tr>
<th>ENTREZID</th>
<th>GENENAME</th>
<th>SYMBOL</th>
<th>log fold change</th>
<th>Hypothesized function</th>
</tr>
</thead>
<tbody>
<tr>
<td>10056877</td>
<td>complex 4</td>
<td>cpl4</td>
<td>0.608511265</td>
<td>both an inhibitor and a facilitator of synaptic vesicle fusion and neurotransmitter release</td>
</tr>
</tbody>
</table>
| 100538157 | potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2-like | hcn | 1.018675617 | glutamate receptor 1 
| 100532096 | excitatory amino acid transporter 5-like | slc1a7 | 3.012169497 | GABA uptake |
| 100502691 | guanine nucleotide-binding protein subunit alpha-14-like | gna14 | 3.038664213 | Modulators or transducers in various transmembrane signaling systems. |
| 100516098 | solute carrier organic anion transporter family member 1C1-like | slo1c1 | 3.136496308 | 4.076974342 | 3.136496308 |
| 100574423 | gamma-aminobutyric acid type B receptor subunit 2-like | gabrb2 | 1.012385533 | solute carrier family 26 member 6 |
| 100572093 | voltage-dependent L-type calcium channel subunit alpha-1D-like | cacna1d | 2.359161613 | aldehyde dehydrogenase family 9 member A1 |
| 100550991 | guanine nucleotide-binding protein subunit beta-5-like | gna1b | 4.020461837 | 2.359161613 | 0.859871022 |
| 100579667 | guanine nucleotide-binding protein subunit beta-5-like | gna1b | 2.02430363 | Integrate signals between receptors and effector proteins |
| 100572993 | voltage-dependent L-type calcium channel subunit alpha-1D-like | cacna1d | 2.82058363 | Calcium influx, neuron excitation |
| 100593085 | neuronal acetylcholine receptor subunit alpha-3 | chm3 | 2.23687983 | Neural excitation. Receptor family related to GABA and RECEPTORS |
| 100586704 | solute carrier family 6 member 4 | slc6a4 | 2.09210723 | Serotonin regulates synaptic activity in olfactory bulb glomeruli |
| 100511889 | synaptic-somatostatin-associated protein 25-B-like | snyp25 | 1.88215846 | Synaptic transmitter uptake and release. GABA and glutamate associated |
| 100572997 | voltage-dependent L-type calcium channel subunit alpha-1D-like | cacna1d | 1.87957824 | Mediates the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes. |
| 100530689 | gamma-aminobutyric acid type B receptor subunit 2-like | gabrb2 | 1.77200697 | 1.77200697 |
| 100577287 | neuronal pentraxin-1-like | np1 | 1.72509305 | Involved in excitatory synapse remodeling. |
| 100578223 | vesicular glutamate transporter 1-like | vglut1 | 1.622496056 | Excitatory glutamate transporter |
| 100572996 | voltage-dependent L-type calcium channel subunit alpha-15-like | cacna1a5 | 1.52449251 | Solute carrier family 22 member 5 |
| 100566793 | sodium/calcium exchanger 1-like | sloca3 | 1.458399247 | A protein involved in transporting chloride, oxalate, sulfate and bicarbonate |
| 100586027 | solute carrier family 12 member 7-like | slc12a7 | 1.368215846 | Export of Cl⁻ and Na⁺ into sweat and salivary glands |
| 100538425 | sodium channel subunit beta-1-like | scn1b | 1.15576177 | Sodium channel subunit beta-1-like |
| 100564801 | potassium voltage-gated channel subfamily H member 1-like | kcnh1 | 1.14457554 | Involved in neural excitation and neurotransmitter release |
| 100562494 | guanine nucleotide-binding protein subunit beta-5-like | gna1b | 1.14048578 | Involved in the termination of the signaling initiated by the G protein coupled receptors |
| 100570984 | solute carrier family 22 member 16-like | slc22a16 | 1.07256445 | L-carnitine transporter a precursor to acetylcholine |
| 100532651 | sodium-dependent serotonin transporter-like | slc6a9 | 1.049560641 | Terminates the action of serotonin and recyclizes it in a sodium-dependent manner |
| 100561149 | solute carrier organic anion transporter family member 3A1-like | slc2a11 | 1.01870517 | Organic anion transporter |
| 100507347 | glutaamate receptor ionotropic, kainate 4-like | grik4 | 1.012385533 | Excitatory receptor |
| 100583542 | sodium- and chloride-dependent GABA transporter 2-like | sloa13 | 0.97176727 | GABA uptake |
| 100578390 | solute carrier organic anion transporter family member 3A1-like | slc2a11 | 0.89870102 | Organic anion transporter |
| 100601022 | solute carrier family 4 member 1 adaptor protein | slc4a1ap | 0.724949516 | Bicarbonate transporter |
| 100515337 | solute carrier family 27 member 4 | slc27a4 | 0.74987283 | Role in fatty acid uptake |
| 100578896 | glutaamate receptor ionotropic, delta-1-like | gnr4 | 0.842228494 | Mediate most of the fast excitatory synaptic transmission in the central nervous system and play key roles in synaptic plasticity. |
| 100504348 | glutaamate receptor 1-like | grm1 | 0.84936331 | Glutamate receptor that functions by activating phosphatase C |
| 100584763 | potassium voltage-gated channel subfamily C member 1-like | kcnj1 | 0.821498818 | Plays a role in the rapid repolarization of fast-firing brain neurons, forms complex with KCNJ2 |
| 100571004 | neurotrophin-3-like | np3 | 0.685192487 | Members of this family may be involved in the formation and remodeling of central nervous system synapses |
| 100585781 | solute carrier family 2 member 6 | slc2e6 | 1.02017821 | Glucose transporter |
| 100500384 | solute carrier family 22 member 6 | slc22a6 | 1.12531705 | Glucose transporter - a precursor to acetylcholine |
| 100511300 | short transient receptor potential channel 2-like | trpc2 | 1.29926479 | Receptor-activated non-selective calcium permanent cation channel |
| 100500164 | aldehyde dehydrogenase family 9 member A1-like | aldh9a1 | 0.87569161 | Protein involved in the dehydrogenation of gamma-aminobutyraldehyde to GABA |
| 100571979 | tubby-related protein 3-like | tubb3 | 0.46828381 | Related to control of neural differentiation /maintenance|
| 100566629 | tubby-related protein 3-like | tubb3 | 0.746974344 | Related to control of neural differentiation |
| 100585010 | acyl-CoA thiolase | cmt | 0.53831654 | Production of melatonin. Sleep cycle related. Next step enzyme after AANAT |
| 100507867 | serotin-9-acetyltransferase-like | aat | 0.40645837 | Production of melatonin. Sleep cycle related |
| 100573284 | sodium-coupled neutral amino acid transporter 3-like | slc3a3 | 2.10514423 | Role in glutamate/GABA transport, associated with circadian rhythm as well maybe |

Changes in gene expression in control vs. high CO2 olfactory bulbs.
Changes in gene expression in control vs. high CO₂ olfactory bulbs

- 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

*All are putative functions

- 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 CO₂ level experienced a disruption of olfactory driven behaviors.

• Exposure to the high CO₂ 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 CO₂ level compared to control coho.
Acknowledgments

• **Gallagher lab:**
  Richard Ramsden

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  Danielle Perez

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  Washington Sea Grant
  Washington Ocean Acidification Center

• **All the fish used in the study!**
Exposure system
Elevated CO\textsubscript{2} altered neuronal signaling in the olfactory bulbs

**Peak odorant induced signal**

- **Region 2**
  - Skin extract
  - L-alanine

**Signal duration**

- **Region 2**
  - Skin extract
  - L-alanine

**CO\textsubscript{2} Exposure Level**

- Control
- High

**A**

**Peak amplitude**

- pH 7.8
- pH 7.2

- Skin extract
- L-alanine

**B**

**Seconds**

- pH 7.8
- pH 7.2

- Skin extract
- L-alanine
Exposure chemistry

The image shows a scatter plot with two axes: Alkalinity (10^6) on the y-axis and Salinity on the x-axis. The plot includes data points for different dates:
- Red dots represent 8/18/2016
- Green dots represent 9/23/2016
- Blue dots represent 9/8/2016

The spread of the data points suggests variations in alkalinity and salinity over time.
Results
Exposure chemistry