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

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Speaker
Chase Williams, Evan Gallagher, Andrew Dittman, Paul McElhany, Shallin Busch, Theo Bammler, and James MacDonald

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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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Theo Bammler
James MacDonald

NOAA
Andrew Dittman: PI
Paul McElhany
Shallin Busch
Michael Maher
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 an olfactory driven behavior in coho salmon

Percent time spent in odorant arm

- Pre odor
- Post odor

- pH 7.8
- pH 7.5
- pH 7.2

* Significant difference
Top view of salmon olfactory system and electrophysiology test sites

- Forebrain
- Olfactory bulb
- Rosette
- Posterior
- Anterior
- EEG test region 1
- EEG test region 2
- EOG test region
Elevated CO₂ did not disrupt coho salmon neuron signaling in the rosettes

![Graph showing EOG responses to different CO₂ exposures and pH levels for L-serine, L-alanine, and skin extract](image-url)
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 CO₂ effects on olfactory rosettes and olfactory bulbs

Olfactory bulb

High vs. Ctl

Medium vs. Ctl

High vs. Medium

801
11
68
115
1
0
181
<table>
<thead>
<tr>
<th>ENTREZID</th>
<th>GENENAME</th>
<th>SYMBOL</th>
<th>log fold change</th>
<th>Hypothetical function</th>
</tr>
</thead>
<tbody>
<tr>
<td>100365787</td>
<td>complex 4</td>
<td>Cplk4</td>
<td>-0.44906311</td>
<td>Plays a role in the rapid neuropilation of fast-firing brain neurons, forms complex with KCNE2</td>
</tr>
<tr>
<td>100378986</td>
<td>glutaemate receptor ionotropic, delta-1-like</td>
<td>Gld1</td>
<td>-0.40389964</td>
<td>Mediate most of the fast excitatory synaptic transmission in the central nervous system and play key roles in synaptic plasticity</td>
</tr>
<tr>
<td>100304348</td>
<td>glutamate receptor 1-like</td>
<td>Gm1</td>
<td>-0.44906311</td>
<td>Glutamate receptor that functions by activating phospholipase C</td>
</tr>
<tr>
<td>100348763</td>
<td>potassium-voltage-gated channel subfamily C member 1-like</td>
<td>Kcnc1</td>
<td>-0.00124881</td>
<td>Plays a role in the formation and remodeling of central nervous system synapses</td>
</tr>
<tr>
<td>100307804</td>
<td>neuropeptide-Y</td>
<td>Npy3</td>
<td>-0.00811265</td>
<td>Members of this family may be involved in the formation and remodeling of central nervous system synapses</td>
</tr>
<tr>
<td>100358781</td>
<td>solute carrier family 2 member 6</td>
<td>Slc2a6</td>
<td>-1.21003782</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>100300834</td>
<td>solute carrier family 22 member 6</td>
<td>Slc22a6</td>
<td>-1.02553755</td>
<td>Co-transporter of glucose and galactose</td>
</tr>
<tr>
<td>100330400</td>
<td>short transient receptor potential channel 2-like</td>
<td>Tpr2</td>
<td>-1.42990479</td>
<td>Receptor-activated non-selective cation permeant cation channel</td>
</tr>
<tr>
<td>100001664</td>
<td>aldehyde dehydrogenase family 9 member A1-like</td>
<td>Aldh6a1</td>
<td>-3.87785611</td>
<td>Protein involved in the dehydrogenation of aldehydes and ketones from GABAergic interneurons to GABAergic interneurons</td>
</tr>
<tr>
<td>100077959</td>
<td>tubby</td>
<td>Tubb</td>
<td>0.00922583</td>
<td>Related to control of neural differentiation</td>
</tr>
<tr>
<td>100665209</td>
<td>tubby-related protein 1-like</td>
<td>Tubr1</td>
<td>4.07697434</td>
<td>Related to control of neural differentiation</td>
</tr>
<tr>
<td>100350310</td>
<td>acyl-CoA-thioesterase</td>
<td>Acsm3</td>
<td>4.03831564</td>
<td>Production of melatonin. Sleep cycle related. Next sleep stage after.AANAT</td>
</tr>
<tr>
<td>100307687</td>
<td>serotonin-9-acetyltransferase-like</td>
<td>Satel</td>
<td>4.02443873</td>
<td>Production of melatonin. Sleep cycle related. Next sleep stage after. AANAT</td>
</tr>
<tr>
<td>100372944</td>
<td>sodium-coupled neutral amino acid transporter 3-like</td>
<td>Slc3a13</td>
<td>2.16514429</td>
<td>Role in glutamate/GABA transport, associated with circadian rhythm as well as sleep cycles.</td>
</tr>
</tbody>
</table>
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

• NOAA collaborators:
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  David Baldwin
  Frank Sommers
  Darran May
  Danielle Perez

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  Washington Sea Grant
  Washington Ocean Acidification Center

• All the fish used in the study!
Nilsson et al., 2012
Elevated CO$_2$ altered neuronal signaling in the olfactory bulbs
Exposure chemistry

![Exposure chemistry graph]

- Alkalinity (10^-6)
- Salinity
- Date:
  - 8/18/2016
  - 9/23/2016
  - 9/8/2016
Results

Exposure chemistry