Biomarker assessment in Arctic cod, *Boreogadus saida*, from the Chukchi Sea: results from the COMIDA program.

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ABSTRACT

With the growing interest in oil exportation in the Arctic Ocean, there is a crucial need to carry out baseline mapping of the types and numbers of species in these locations prior to drilling as well as determining the baseline health status of representative organisms using biomarker endpoints that are known to respond to oil exposure. This will enable more accurate assessments of potential impacts of future oil usage on these arctic ecosystems. As part of the COMIDA project, we analyzed three common biomarkers for exposure and effects of petroleum polyaromatic hydrocarbons (PAHs) in Arctic cod, *Boreogadus saida*. A total of 24 Arctic cod from 6 sites (length; 7.2-14 cm) were collected during the 2010 sampling cruise in the Chukchi Sea. Parallel sampling of particles and sediments for organic contaminant analysis was also carried out. Fish were dissected and tissues collected for various assays onboard with additional tissues frozen and stored in liquid nitrogen until processing in the laboratory. To assess exposure to PAHs levels of gene expression and enzymatic activity of cytochrome P4501A (CYP1A1) and the common antioxidant enzyme, glutathione-*S*-transferase (GST), were measured in fish liver tissue. To assess the effects of PAHs, DNA damage was measured using the Comet assay for liver samples. Results of toxicological data will be compared with hydrocarbon burdens measured in corresponding fish muscle tissue, sediment and water PAH and heavy metal levels. Results from the various fish species and comparisons among different size classes will be compared to chemical body burdens, environmental levels and toxicological responses to determine their sensitivity to PAHs as well as the critical timing of exposure and toxicity levels throughout different life stages.

INTRODUCTION

The increasing interest for oil exportation in the Arctic Ocean has prompted the development of the Chukchi Sea Offshore Monitoring in Drilling Areas (COMIDA) Program to establish baseline data and help identify future impacts from oil production. As the toxicology part of this program, we are evaluating the baseline health status of target organisms including Arctic cod, *Boreogadus saida,* by analyzing common biomarkers for exposure and effects of petroleum PAHs. Exposure to PAHs has been shown increase gene, protein, and enzymatic activity levels of oxidative stress and antioxidant enzymes including CYP1A1, GST, CAT (catalase), and SOD (superoxide dismutase) as well as cause DNA damage in Arctic cod (Nahrgang *et al.* 2009, 2010; Jonsson *et al.* 2010). However, little is known about the natural levels of these biomarkers in Arctic cod within the Arctic Ocean. Therefore, in this study we seek to provide necessary baseline PAH exposure and effects biomarker levels in Arctic cod capable of being used to identify future impacts.

STUDY LOCATIONS

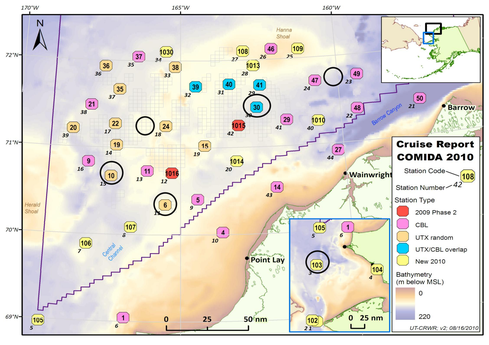


Fig 1. Station map for locations occupied during COMIDA 2010 cruise in the Chukchi Sea, Alaska. Sites where Arctic cod were collected are circled in black.

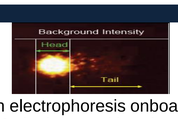
Table 1: Arctic cod collection information

Station Code	Date	Latitude	Longitude	Number of Cod Collected	Mean Size ±STD (cm) (Size Range)
103	7-26	67°40.223'	-168°57.467'	4	10.3±0.216 (10-10.5)
6	7-30	70°20.400'	-165°22.900'	3	11.2±0.458 (10.8-11.7)
10	7-31	70°41.670'	-167°07.000'	5	8.98±1.31 (7.8-11.1)
22-24	8-10	71°16.289'	-166°02.553'	4	8.625±0.754 (7.9-9.5)
47-49	8-4	71°44.750'	-160°01.300'	5	11.9±2.356 (8-14)
30	8-5	71°27.780'	-162°37.150'	3	9.4±2.762 (7.2-12.5)

Collection of Arctic cod:
LGL fisheries employed demersal nets for benthic trawling and a subset of fish was provided to us for analysis.

METHODS

- **Dissection and collection of liver/muscle tissue:**
  - Conducted onboard and stored in liquid N_2
- **Preparation of cellular fractions from liver tissue:**
  - Homogenization using standard centrifugation method to separate microsomal and cytosolic fractions as described in Sullivan *et al.* (2007).
- **Enzymatic Assays:** following Nahrang *et al.* (2010)
  - Determined total protein concentrations using a bicinchoninic acid assay (Pierce BCA kit).
  - Measured cytosolic glutathione-*S*-transferase activity using a kinetic spectrophotometric assay (455nm).
  - Measure microsomal cytochrome p4501A1 activity using an ethoxyresorufin-*O*-deethylase (EROD) fluorometric kinetic assay (yet to be conducted).



- **DNA Integrity/Damage:**
  - Prepared slides and ran electrophoresis onboard.
  - Examined extent of DNA single strand breaks (SSB) using the Comet assay (Mitchelmore & Chipman 1998) by quantifying:
    1. **% Tail DNA=**tail intensity/total intensity in head & tail
    2. **Tail Length** = distance of DNA migration from head.
    3. **Olive Moment =** (tail length)*(fraction total DNA in tail)
- **Molecular:**
  - Examined gene expression using real-time reverse transcription PCR with SYBR green as described in Nahrgang *et al.* (2009, 2010).
  - Measured relative mRNA expression of: cyp1a1, gst, and β-actin (reference control)

GST ENZYME ASSAY RESULTS

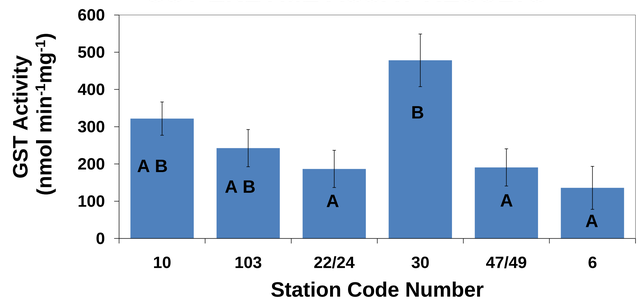


Fig 2. Glutathione-S-transferase activity (nmol $min^{-1}mg^{-1}$ total protein) in the liver of Arctic cod (mean ± SE, n=2-5). Different letters signify significant differences (p value <0.05) using one-way ANOVA with Tukey's test.

COMET ASSAY RESULTS

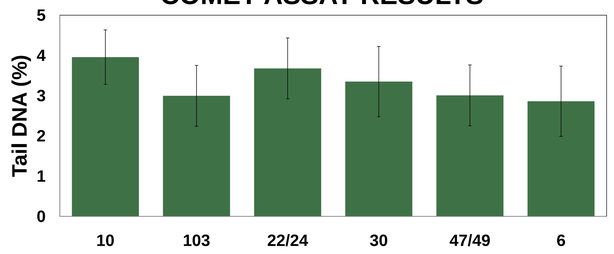


Fig. 3. DNA SSB measured as percent tail DNA in comet tails of liver cells from Arctic cod (mean ± SE, n=3-5)

Table 2: DNA damage in Arctic cod liver tissue by location (mean ± SE, n=3-5)

Station Code	Tail Length	Olive Tail Moment
10	4.10 ± 0.64	0.204 ± 0.037
103	2.19 ± 0.71	0.105 ± 0.041
22/24	3.92 ± 0.71	0.159 ± 0.041
30	3.62 ± 0.82	0.141 ± 0.047
47/49	2.54 ± 0.71	0.125 ± 0.041
6	2.39 ± 0.82	0.116 ± 0.047

RESULTS

- Cytosolic GST activity was significantly higher in livers of Arctic cod from station code 30 than in Arctic cod from station codes 22/24, 47/49, and 6 (respectively: p values= 0.0378, 0.0413, 0.0178).
- There was no significant differences in liver cell DNA damage (% Tail DNA, Tail Length, or Olive Tail Moment) between Arctic cod from the 6 sites (using one-way ANOVA with Tukey's test for comparison of means).
- There were no significant differences in liver microsomal gene expression (relative mRNA levels) of cytochrome p4501a1 between Arctic cod from the 6 sites (using one-way ANOVA with Tukey's test for comparison of means).
- There were no significant differences in liver cytosolic gene expression (relative mRNA levels) of glutathione-s-transferase between Arctic cod from the 6 sites (using one-way ANOVA with Tukey's test for comparison of means).

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GENE EXPRESSION RESULTS

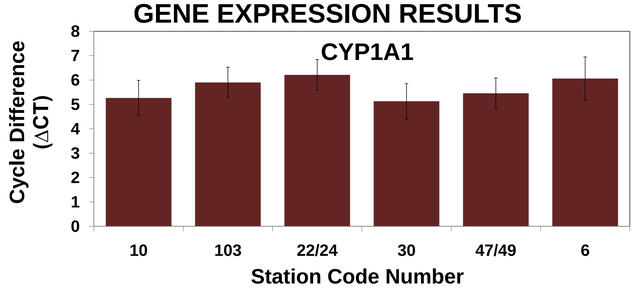

Fig. 4. Relative microsomal cyp1a1 mRNA expression in livers of Arctic cod (mean ± SE, n=3-5).

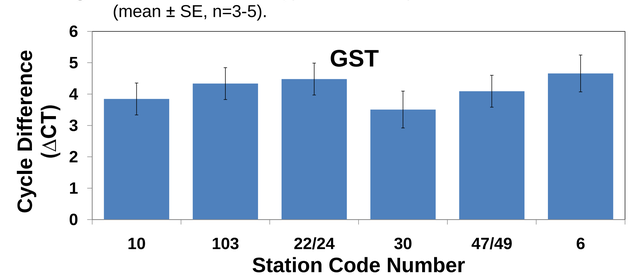

Fig. 5. Relative cytosolic gst mRNA expression in livers of Arctic cod (mean ± SE, n=3-5).

DISCUSSION AND CONCLUSIONS

- GST activity levels were similar to those measured by Nahrgang *et al.* (subm.) in Arctic cod (their values ranged from 200-600 nmol $min^{-1}mg^{-1}$protein (Nahrgang *et al.* 2010)).
- GST activity levels were highest at station 30, which was significantly higher than at three other stations. Slightly higher levels of liver cytosolic gst gene expression at station 30 were observed, although not statistically significant. Station 30 was located near Shell's historic Burger prospect well, which is estimated to contain large amounts of natural gas and oil. In the 2009 COMIDA cruise, overall surface sediment PAH and n-Alkanes were higher at site 30 than any of the other re-occupied stations from which we analyzed cod samples, which could help to explain these biomarker levels.
- DNA damage in liver cells was low. While no studies have examined DNA damage by COMET assay in Arctic cod liver tissue, Nahrgang *et al.* (2010) have shown blood cells in control, lab cultured Arctic cod to have 9-9.5% tail DNA. Other research using fathead minnows resulted in control fish with 2-4% tail DNA (Bearr *et al.* 2010), which is similar to our observations.
- As lower ΔCT measurements equate to higher gene expression, the mRNA expression levels of gst among stations appears to follow the same trend as GST enzyme activity. Further analysis is necessary to determine if statistical correlation exists.

FUTURE WORK

- Conduct EROD assay to determine CYP1A1 enzyme activity and correlate to cyp1a1 mRNA.
- Determine relationship between levels of biomarkers measured and size of cod.
- Relate levels of biomarkers to PAH levels measured in corresponding samples' muscle tissue.
- Determine if relationship exists between PAH levels measured in muscle tissue and PAH levels measured in water samples and surface sediment extracted from the corresponding stations.
- Conduct the same biomarker analyses on Bering flounder samples collected during the 2010 COMIDA cruise.
- Compare toxicity data measured with that of heavy metal data collected from corresponding sites.
- If possible, repeat sampling in different seasons and with larger sample sizes.

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