Exxon Valdez Oil Spill
Restoration Project Final Report

Prince William Sound Food Webs: Structure and Change

Restoration Project 01393
Final Report

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Study History: Project 393 commenced as project 99393 in April 2000 and is ending as project 01393 in April 2002. Years 1 and 2 focused on supplementing the isotopic database to resolve Ecopath model validation data gaps. The results of this project goal were presented in the 2001 Annual Report. A portion of these results were published as two Wakefield symposium volumes. The second goal was a retrospective isotopic analysis based on the periostracum of *Mytilus californianus*. Preliminary results reported in the 2001 annual report suggested modifications to sampling methods. These changes were carried out during the final project year, which are reported herein.

Abstract: Project 393 was funded to address gaps in our knowledge of Prince William Sound ecological processes based on recent stable isotope. In particular, stable isotope research has shown that the advective regime connecting the northern Gulf of Alaska (GOA) with Prince William Sound (PWS) fluctuates in its effect upon nutritional processes in Fishes on an annual basis. This project: (1) conducted a retrospective analysis carbon-13 since EVOS, and (2) addressed Ecopath model validation data gaps. These analyses may enable a better understanding of the ecological role of “regime shift” processes conjectured to be impeding the natural restoration of populations in PWS affected by the EVOS.

Key Words: Ecosystem, Food Webs, Prince William Sound, Stable Isotopes

Project Data: Description of data - Data consist of natural carbon and nitrogen stable isotope abundance measurements expressed in intentionally recognized delta units. Format - Data are being published in the scientific manuscript in the form of tables and figures. Custodian - Contact Dr. Thomas C. Kline, Jr., Prince William Sound Science Center, P. O. Box 705, Cordova, AK 99754. e-mail: tkline@pwscc.gen.ak.us. Availability – in the open scientific literature or contact Dr. Kline for specific data, which can be e-mailed.

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Published Results


Executive Summary

Recent research has shown that the oceanographic conditions connecting the northern Gulf of Alaska with Prince William Sound may affect nutritional processes in fishes. Accordingly, food webs are subject to changes in carbon flow occurring between Gulf of Alaska and Prince William Sound. This project sought to (a) conduct retrospective analysis of Gulf of Alaska production shifts since the oil spill, and (b) address Ecopath model validation data gaps. Under direction of Chief Scientist objective (b) was deleted during the second project year. Nevertheless, two manuscripts derived from that aspect.
of this study were published during 2001, re-prints of which are included as appendices. The report narrative, however, addresses objective (a).

**Introduction**

Shifts in carbon flow, which were driven by variations in the physical environment, represent fundamental changes in the way the PWS ecosystem supports commercially important species and is a probable mechanism explaining regime shifts. PWS fish populations may thus depend upon physical processes that regulate the availability of their macro-zooplankton forage. Stable isotope analysis (SIA) suggested that a portion of variability of macro-zooplankton may be ascribed to a varying subsidy of oceanic sources that are transported by currents into PWS from the adjacent Gulf of Alaska (Kline 1999).

The SIA approach, which uses stable isotope ratios of carbon and nitrogen, is unique in its ability to integrate time and spatial scales at mesoscale levels. The tracer aspect of the SIA is analogous to artificial tracer experiments without the burden of needing to generate signals or experimental artifacts. Because SIA provided the evidence suggesting that the Gulf carbon subsidy of PWS pelagic production appears to vary among years (Kline 2001), it is a useful GEM parameter for resolving how oceanographic processes affect fisheries recruitment over longer time scales. For example, SIA suggested long-term shifts from retrospective analysis of bowhead whale baleen (Figure 1; Schell 2001). Long-term systematic shifts among a collection of baleen from several whales provide evidence for change although the nature of that change is not yet understood (Schell, 2001). Furthermore, the shifts observed in baleen exceed those predicted by the Seuss effect (which was expected to be notable, given the multi-decade nature of the data series in the baleen study) and thus reflect local environmental changes (Schell 2001). Investigation into the nature of isotopic change was therefore needed. There is also substantial seasonal isotopic variability (Figure 2; Kline 1999). Seasonal affects accounted for up to 40% of the variability in SIA of net plankton from one growth period. It is not known whether this pattern exists every year. The large intra-annual (seasonal) and inter-annual (e.g., Kline 2001, Schell 2001) may also provide clues for the observed long-term shifts. However, at present there is no long-term context for the Gulf of Alaska and PWS as provided by whale baleen for the Bering Sea.

The presently available limited temporal extent of the SIA database for PWS suggested a need for expansion through retrospective analysis. Shelled mollusks secrete a protein outer covering while they grow. In the case of bivalves the oldest material is located on the umbone, typically the pointed end of the shell, while the youngest is at the lip, where the mantle edge is in contact with the shell. The mantle secretes the protein layer, periostracum, along with CaCO$_3$-containing parts of the shell. Winter growth checks enable aging the shell and the layer of periostracum above it. Mytilid bivalves have a particularly thick periostracum layer that spontaneously separates from the shell as it dries. Periostracum samples can thus be aged from from animals collected live. The objective of this project was thus to perform SIA on the periostracum of *Mytilus*
*californianus* and assess its potential paleoindicator for the Northern Gulf of Alaska. For the purposes of this study it was assumed that once laid down, periostracum is metabolically inert, thus preserving changes that took place at the time it was laid down. Evidence that this assumption comes from the fact that once damaged, periostracum is not replaced (in mussels), effecting ‘bald spots.’
Figure 1. Inter-decadal shift in $^{15}$N/$^{14}$N and $^{13}$C/$^{12}$C content (vertical axis) of bowhead whale baleen ascribed to feeding in the Bering Sea; from Schell (2001).
Figure 2. Seasonal shifts in the in $^15\text{N}/^14\text{N}$ (A) and $^{13}\text{C}/^{12}\text{C}$ (B (raw data) & C (lipid-normalized)) content (vertical axis) of bulk net zooplankton collected during March to June 1995 (Julian Day = Day of the Year) in the northern Gulf of Alaska and Prince William Sound, from Kline (1999). Panels B and C reflect increase in correlation resulting from correction for lipid content.
Methods

Sea-mussels, Mytilus californianus, were collected at Middleton Island in September 1997 with help from Cordova fishermen. They provided the Principal Investigator with the knowledge and opportunity to acquire the Mytilus californianus samples that are providing an approach to retrospective analysis in the present study. Middleton Island’s location in the Alaska Current provides an “upstream perspective” on the EVOS area since samples from there reflect changes in plankton upstream before interaction with PWS-origin carbon is possible. Mytilus californianus were collected from a rocky reef exposed at low tide (~ 0.5m MLLW) from the southeast shore of Middleton Island on 16 September 1997. From a collection measuring approximately 50 L in volume (three large buckets), 50 Mytilus californianus with mostly intact periostracum (showing little wear) and showing little to no signs of bio-fouling were “high-graded” and saved frozen (-20 °C). Stable isotopic analysis (SIA) was made on the outer protein layer (periostracum) on the shells and body tissues of Mytilus californianus of varying ages from this sub-collection.

Periostracum was initially sampled at the annulus level for isotopic analyses (Kline 2001). Growth checks were scored using a scalpel. Mussels were placed in a freeze-drier. Freeze-drying caused the periostracum to separate from the calcareous portion of the mussel shell. Flakes of periostracum that were peeling off the shell were forcepsed from each annulus and placed in a vial. Each periostracum sample (~ 2 mg) was sub-sampled according to the need of the then newly installed Finnegan Delta Plus stable isotope ratio mass spectrometer at the UAF Stable Isotope Facility. The new machine facilitated making several SIA from each annulus. However, the SD of the data was large (Kline 2001), suggesting the need for a more refined periostracum sampling technique. Developing and implementing micro periostracum sampling thus became the goal of project 393 during 2001-2. The problem consisted of collecting, sequentially, a series of small pieces of periostracum from along the long-axis of the shell. The difficulty lay in controlling the spontaneous peeling or flaking of periostracum material. Several methods were attempted – the rubber cement band technique, however, was successful and is described below.

Whole (soft tissues intact) mussel specimens were stored frozen (-20 °C) until preparation commenced. A scalpel was used to make two parallel incisions, approximately 10 mm apart, along the major axis of a shell valve while still frozen. Incisions ran from the narrow or beak end to the edge of the valve most distal to the beak. The incisions were re-traced several times to ensure that the resulting grooves were as deep as possible. A few additional narrow incisions were made within the two main tracks to facilitate sample collection. The mussels were placed in a freeze dryer (Labconco Shelf Drier) at –30 °C , which was operated for ~ 1-2 hours following evacuation. Freeze-drying was observed during this period to mitigate unwanted spontaneous separation of the periostracum from the valve. Mussels were removed from the freeze-dryer at the first
signs of separation or peeling. Rubber cement was has the property of self-adhesion in preference to the object it is attached to, minimizing contamination of the object. Approximately 10 to 20% of the material in a sample would have to be contaminant to significantly affect and isotopic determination, with less effect occurring with smaller differences in the isotopic composition between the contaminant and sample. A layer of rubber cement (Stanford) was then applied between the two incisions and allowed to air dry, which took ~30-40 minutes. Mussels were then placed back into freeze dryer set at −30 °C and re-evacuated. The temperature setting of the freeze drier was gradually increased to +10 °C over a three day period. During this time, rubber cement was applied twice daily, re-evacuating each time, until temperature of freeze dryer reached about +10 degrees. The freeze-drying process was completed with the freeze dryer set at 20 °C for 24 hours. Mussels were then removed from the freeze-drier. The rubber cement was gently lifted from the mussel while a scalpel was run underneath to ensure a clean break of the attached and intact periostracum strip. The strip, rubber cement side down, was tacked onto a styrofoam block. The block facilitated holding the periostracum while ~1-3 mm long sections were removed while being examined under a stereo microscope (Wild) set at low power. A scalpel and fine-tip forceps were used to handle the small samples, which ranged in length from 1.3mm-2.8mm, average 2.25mm. The samples, which were ~0.3 mg dry mass each, were collected from the valve’s edge or lip towards the beak and placed into separate vials. The beginning and ending position of each sampled relative to the valve lip was measured with a dial micrometer. The soft tissues of each mussel were saved in a vial after being removed from the shell and ground to a fine powder.

Annuli positions were assessed on mussel valves after removal of the periostracum and dried soft tissues. A growth check was ascertained to be an annulus and not a ‘false check’ if it formed a continuous perimeter line. Annuli generally co-occurred with a change in slope of the valve surface that caused a shadow when examined under direct oblique sunlight (< 30 ° elevation) using a low power stereo microscope (Wild). The distance of each annulus was measured using a dial micrometer to the nearest 0.1 mm.

Samples were sent to the UAF Stable Isotope Facility for analysis using a Finnegan Delta Plus stable isotope ratio mass spectrometers equipped with Conflo II and III front-ends. Stable nitrogen and carbon isotope rations, $^{15}$N/$^{14}$N and $^{13}$C/$^{12}$C are reported using conventional delta notation reflecting the natural abundance deviation from international standards, air N$_2$ and VPDB.

Isotope data analysis was facilitated on a Macintosh G4 micro-computer and the Statview (SAS, Inc.) analysis software. This software was used to plot the data according to mean sample position for each mussel. The mean integer value for each isotope among all samples was used as a y-axis reference value for plotting annuli positions with isotope data, which were placed on the x axis. Seasonal isotopic analysis was facilitated by converting sample positions (spatial) to fractional growth-year (implied temporal) such that the starting annulus position had a value of 0 while the ending position was 1. Data
plotted according to fractional growth year were limited to those years for which a series of data (generally ≥ 3 observations) were available. Cubic spline fits to the data were used. The resulting plots were assessed qualitatively.

Effects of calcium carbonate contamination on $^{13}\text{C}/^{12}\text{C}$ values were estimated to increase values systematically by 1.3 based on the difference of acid and non-acid cleaned periostracum from a pool of five mussels. Samples were not acid cleaned to avoid $^{15}\text{N}/^{14}\text{N}$ artifacts.

**Results**

The three mussels prepared for SIA using the rubber cement band technique are summarized in Table 1. The Stable isotope data versus valve position and annuli position for mussels A, B and C are shown in Figure 3. These figures include the integer values nearest the mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were +8 and −18, respectively, in conjunction with the indicated annuli positions.

Table 1. Summary comparing whole soft tissues with periostracum mean stable isotope values.

<table>
<thead>
<tr>
<th>Mussel ID</th>
<th>Length of Shell (mm)</th>
<th>Date processed</th>
<th>Total length of periostracum sample (mm)</th>
<th>Sample Increment (mm)</th>
<th>Number samples taken</th>
<th>Number of data points</th>
<th>Soft tissues N-15</th>
<th>Soft tissues C-13 (raw data)</th>
<th>Periostracum N-15</th>
<th>Periostracum N-15 SD</th>
<th>Periostracum C-13</th>
<th>Periostracum C-13 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>115</td>
<td>Oct-01</td>
<td>66</td>
<td>2</td>
<td>34</td>
<td>31</td>
<td>8.8</td>
<td>-20.2</td>
<td>7.8</td>
<td>0.4</td>
<td>-18.2</td>
<td>1.0</td>
</tr>
<tr>
<td>B</td>
<td>104</td>
<td>Oct-01</td>
<td>72</td>
<td>3</td>
<td>25</td>
<td>21</td>
<td>9.1</td>
<td>-18.7</td>
<td>8.1</td>
<td>0.5</td>
<td>-17.9</td>
<td>0.7</td>
</tr>
<tr>
<td>C</td>
<td>117</td>
<td>Oct-01</td>
<td>111</td>
<td>3</td>
<td>38</td>
<td>34</td>
<td>9.2</td>
<td>-19.0</td>
<td>7.7</td>
<td>0.4</td>
<td>-17.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure 3. δ^{15}N and δ^{13}C values of *Mytilus californianus* periostracum samples, for individual animals A to C, in relation to the lip of the shell, commencing with the mantle edge at position 0. GCK positions are locations of growth checks interpreted to be annuli. Actual calendar years can be estimated starting with 1997, the year of sampling, between position 0 and the first annulus tick mark.
All three mussels had downward trending (towards origin) isotope values just prior to sampling – position 0 and adjacent samples. The $\delta^{13}C$ values for more recent years was above $-18$.

The last three samples of each mussel had $\delta^{15}N$ less than +8. This was similar to the overall increase in $^{13}C$ content during the 1992 to 1997 period in the mussels sampled. There was no systematic shift in $\delta^{15}N$ across the years. There were, however, year-to-year shifts on the order of 1‰ that is similar to the isotopic gradient observed between PWS and the GOA and year-to-year differences observed in GOA stations (Kline 1999).

The fractional growth year plots, Figure 4, suggest large inter-annual differences in whether isotopic signatures increased, decreased or stayed the same. $\delta^{15}N$ values seem to start each growth period with values near +8 then diverge from that while $\delta^{13}C$ appear start with a more divergent range. Early divergent is consistent with the observed variations of GOA carbon during SEA since it was in May 1996 that $\delta^{13}C$ were very high. May is probably near the beginning of the growth period for Mytilus as growth rate is likely to be strongly influenced by seasonal warming such that spring growth is more likely to be slower per unit time than summer growth.

Further interpretation of inter-annual variation will require more sampling. This would be consistent with the bowhead whale baleen studies - over a decade of research was incorporated into Figure 1. Nonetheless, the inter-annual and seasonal isotopic variability suggest several levels of complexity in the ecological processes occurring in the Gulf.

Recent observations as part of on-going GLOBEC research appear to corroborate year-to-year inconsistencies in seasonal isotopic patterns observed in mussel periostracum (Kline unpublished). During 2001, $\delta^{15}N$ stared low in March and April then increased in July and August whereas in 2002 it was uniformly high from May to October. During 2001, $\delta^{13}C$ stared high in March, decreased to very low values in April then increased in July and August whereas in 2002 it gradually from a high a in May to low values in October. The results thus far suggest greater levels of inter-annual variability then previous observed. Further research into the causes of isotopic shifts may reveal how ecosystem shifts take place.
Mytilus californianus - A

![Graph showing Delta N-15 and Delta C-13 against Fractional growth-year for three different samples labeled 1, 2, and 3.](image_url)
Mytilus californianus – B

Delta N-15

Delta C-13

fractional growth-year

fractional growth-year
Figure 4. Fractional growth-year isotopic patterns. $\delta^{15}$N and $\delta^{13}$C values of *Mytilus californianus* periostracum samples, for individual animals A to C, in relation to apparent annual growth patterns. Values near 0 reflect early growth while values near 1, reflect growth just prior to an annulus. 1, 2, 3 of *Mytilus* ‘A’ corresponded to 1990, 1989, and 1988; 1, 2, 3, 4 of *Mytilus* ‘B’ corresponded to 1989, 1987, and 1986, while 1, 2, 3, 4, and 5 of *Mytilus* ‘C’ corresponded to 1985, 1984, 1983, 1982, and 1981.
Literature cited


Bidigare, R.R. and 15 other authors. 1997 Consistent fractionation of $^{13}$C in nature and in the laboratory: Growth-rate effects in some haptophyte algae. Global Biogeochemical Cycles. 11:267-278.


