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Estuarine Distribution, Nutritional and Thiaminase Content of Eulachon (*Thaleichthys pacificus*) in Southeast Alaska, With Implications for Steller Sea Lions

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ABSTRACT

Eulachon (*Thaleichthys pacificus)* are small, pelagic fish whose spawning habits and marine distribution are not well understood. Eulachon are a nutrient-rich, seasonally important source of food for predators, including Steller sea lions (*Eumetopias jubatus*) in southeast Alaska. Echo integrated-trawl surveys were conducted from 3 April to 6 May 2006 to estimate eulachon population structure and biomass, identify fish movement, location, and acoustic targets. The presence of Steller sea lions and other marine predators attracted to pre-spawning aggregations of eulachon were used to fine-tune the timing and location of our survey sites. We identified the distribution of pre-spawning adults, sub-adult, and juvenile eulachon in five estuaries of northern southeast Alaska during the 2006 spring spawning season and analyzed their nutritional and thiaminase content. Mean eulachon weight and nutritional energy varied between sites, from a low of 10.68 g body weight $\pm$ 2.93 g with a total energy of 94.4 kJ/fish in Berners Bay, to a high of 33.22 g body weight $\pm$ 5.99 g with a total energy of 291.9 kJ/fish in Lutak Inlet. Of the five estuaries surveyed, juvenile and sub-adult eulachon were found only in Taku Inlet, Berners Bay, and Chilkat Inlet and were concentrated at a distinct depth of 50-80 m throughout the estuaries. Adult eulachon were found in three of the five estuaries: Lutak Inlet, Taku Inlet, and Berners Bay with no fish identified with our fishing gear or acoustics in Taiya Inlet. Eulachon school biomass and the duration these schools spend in the estuaries correlated with sea lion abundance. Sea lion numbers were directly proportional to eulachon biomass. Spawning eulachon move quickly into their freshwater spawning river, with estuary size directly proportional to the time spent in the estuary. Thiaminase, a potentially harmful enzyme, was found in eulachon at all five sites, with a mean activity range of 5.0-7.0 $\mathrm{umols} \cdot \mathrm{g}^{-1} \cdot \mathrm{min}^{-1}$. The ingestion of thiaminase laced eulachon by Steller sea lions does not appear to affect the health of

sea lions in our study area because they were not ingested long enough for the thiaminase to take effect. The role that thiaminase plays in the health of marine predators is not well understood and warrants further study and could play a role in the junk food theory that was used to explain Steller sea lion declines in other areas especially in populations with low dietary diversity.

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INTRODUCTION

Eulachon *Thaleichthys pacificus* (Richardson 1836), are a small, anadromous, energy-dense and nutritious forage species that are widespread in southeast Alaska (Carlson et al.[1], Csepp et al. 2011). Adult eulachon display little size variation throughout their broad latitudinal range and relatively little genetic diversity among populations (McLean et al. 1999, McLean and Taylor 2001). There appears to be two distinct geographical populations in the Gulf of Alaska: a northern one from Yakutat to Cook Inlet and a southern one that encompasses all of southeast Alaska inside waters (Flannery et al. 2009). Eulachon are planktivorous, feeding primarily on copepods and euphausids in deeper nearshore water when not spawning (Hay and McCarter 2000).

Eulachon are found from northern California to Norton Sound in southwestern Alaska (Wilson et al. 2006). They spawn in the lower reaches of the mainland and a few island rivers (Blackburn et al. 1981, Hay et al. 1997). Little is known about their marine distribution (Willson et al. 2006) except that adults concentrate outside their spawning rivers in shallow water estuaries (Marston et al. 2002) just prior to spawning. In southeast Alaska they loosely aggregate in nearby waterways near the seafloor during the remainder of the year (Csepp et al. 2011). Even less is known of their offshore distribution with some eulachon showing up in areas with the right oceanographic conditions (Emmet and Brodeur 2000). Concentrations of eulachon are

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[1] Carlson, H. R., R. E. Haight, and K. J. Krieger. unpubl rep. Species composition and relative abundance of demersal marine life in waters of southeastern Alaska, 1969−77. Alaska Fisheries Science Center, Auke Bay Laboratories, 17109 Point Lena Loop Rd., Juneau, Alaska 99801.

found around Vancouver Island (Hay et al. 1997, Hay and McCarter 2000), and inconstant

abundance have been found in the western Gulf of Alaska (Anderson and Piatt 1999).

Eulachon spawn in early spring, and are lipid-rich (Kuhnlein 2000, Iverson et al. 2002),

making them a valuable energy source for marine predators at a time of year when there are few

food sources. Seasonally abundant spawning aggregations of energy-rich forage fish are critical

resources for the survival and reproductive success of many marine predators including Steller

sea lions (*Eumetopias jubatus*) (Iverson et al. 2002). Variation in prey consumption indicates that

Steller sea lions forage on seasonally abundant and densely aggregated prey (Gende et al. 2001,

Sinclair and Zeppelin 2002, Marston et al. 2002, Womble and Sigler 2006, Sigler et al. 2009),

especially those that aggregate during spawning or seasonal migrations. A study of Steller sea

lion diets from the summer months found no eulachon present, which indicates eulachon are a

seasonally important prey during eulachon spawning season in spring (Sigler et al. 2004, Sigler

et al. 2009).

In a study of 18 fish species in the Gulf of Alaska, eulachon had the highest lipid and

greatest energy content, ranging from 12% - 28% lipid and 9 - 13 kJ/g, wet mass (Payne et al.

1999, Anthony et al. 2000, Vollenweider et al. 2011). Smaller, younger eulachon as well as

adults with depleted energy stores in spawning condition had lower lipid and energy content,

similar to two species of myctophids at 14% - 22% lipid, and 10 - 10 kJ/g, and sablefish

(*Anoplopoma fimbria*) at 18% - 20% lipid, and 10 - 11 kJ/g. The remaining 14 fish species were

significantly lower in lipid, and energy content compared to eulachon, including forage fish such

as Pacific herring (*Clupea pallasii*) at 6 - 20% lipid and 7 - 8 kJ/g, Pacific sand lance

(*Ammodytes hexapterus)* at 3 - 6% lipid and 4 - 6 kJ/g, and capelin (*Mallotus villosus*) at 2 - 12%

lipid and 4 - 7 kJ/g (Payne et al. 1999, Anthony et al. 2000, Vollenweider et al. 2011). Of the 18

fish species studied in the Gulf of Alaska, eulachon was one of the species with relatively low

protein content at 12% - 14% than other forage species like capelin at 13% - 15% and sand lance

at 16% - 18% (Payne et al. 1999, Vollenweider et al. 2011). Eulachon have also been reported to

contain high levels of vitamin A and E, calcium, iron, and zinc (Kuhnlein et al. 1996).

Eulachon and rainbow smelt (*Osmerus mordax*) are members of the Osmeridae family; a

family known to contain thiaminase (Tillitt et al. 2005, Honeyfield et al. 2012). Thiaminase has

not been previously reported in eulachon. Thiaminase is an anti-nutrient that destroys thiamine,

also known as vitamin B1. The enzymatic degradation of thiamine by thiaminase I (enzyme

number 2.5.1.2; Webb 1992) deactivates thiamine by acting as a catalyst for a base substitution

reaction where the thiazole portion of a thiamine molecule is exchanged for one of several

potential nucleophiles resulting in a non-functional molecule (Fujita 1954, Lienhard 1970,

Honeyfield et al. 2012).

Thiamine is a water-soluble vitamin required by transketolase for the conversion of

glucose to important end products and for enzymes in the Krebs cycle that converts glucose to

adenosine triphosphate (ATP) for metabolic energy. Low levels of thiamine have a variety of

secondary effects, which include immune dysfunction, reduced growth, reduced predator

avoidance, reduced vision, alterations in genes and gene expression (Brown et al. 2005a, Brown

et al. 2005b, Carvalho et al. 2009, Fitzsimons et al. 2009, Ottinger et al. 2012). Thiamine

deficiency has been diagnosed in captive gray seals (*Halichoerus grypus*), California sea lions

(*Zalophus californianus*), an Atlantic bottlenose dolphin (*Tursiops truncatus*), and has been induced in harp seals (*Pagophilus groenlandicus*) (Fowler 1978, Dierauf 1990). Harp seals showed signs of altered behavior within 40 - 60 days; refusing to eat, becoming inactive, unresponsive to touch, noise, or light and were breathing irregularly. Within 2 - 3 days of the onset of these preliminary signs severe tremors, fore-flipper spasms, head shaking, muscle quivering occurred followed by death (Geraci 1970). Therefore, documenting the presence of thiaminase in eulachon and the length of time and the exclusivity Steller sea lions feed on eulachon is important to their health.

Our study goals were to track the estuarine distribution of eulachon and to measure the energy, lipid, protein, ash, vitamins A and E content, and thiaminase activity in eulachon from five estuaries in southeast Alaska. Our specific objectives were to characterize eulachon estuarine aggregations by 1) estimating the biomass, timing, and location of aggregations, 2) characterizing the population age structure and distribution within each estuary, 3) characterizing pre-spawning distribution within a large geographic area with numerous pre-spawning locations, 4) measuring the nutritional content of eulachon, 5) determining the presence or absence of thiaminase, and 6) characterize Steller sea lion activity during eulachon runs within a large geographic area.

METHODS

Field Operations – Study Area

We chose five estuaries to study within northern southeast Alaska that have historically supported year-round populations of Steller sea lions and seasonal spawning runs of eulachon.

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The study area spanned a 200-km section from Taku Inlet in Stephens Passage to Taiya Inlet in Lynn Canal which included four inlets and one bay (Fig. 1). Our five study locations, from south to north were Taku Inlet (58°11.303´N, 134° 05.891´W), Berners Bay (58°44.554´N, 134°58.959´W), Chilkat Inlet (59°06.178´N, 135°22.048´W), Lutak Inlet (59°17.190´N, 135°26.368´W), and Taiya Inlet (59°26.718´N, 135°21.051´W) (Fig. 1). Surveys were initially conducted from south to north between 3 April and 6 May 2006 in an effort to follow the eulachon spawning season.

Field Operations – Hydroacoustic Methods

Hydroacoustic data were collected in whole estuary standard box surveys and smaller school specific focal box surveys that were classified into five categories by location (Fig. 2). The five categories were Taku Inlet, the largest estuary surveyed at 96 km long with a total area of 49.04 km^2 and two school-specific, smaller focal surveys of 10.75 and 20.2 km long (n = 15 separate surveys). Chilkat Inlet survey was 87.8 km long with a total area of 35.6 km^2 (n = 2 surveys). Berners Bay survey was 76 km long with a total area of 26.8 km^2 and two overlapping school-specific smaller focal surveys of 18 and 16.5 km long (n = 7 surveys). Lutak Inlet survey was 60.2 km long with a total area of 15.8 km^2 (n = 4 surveys), plus a periodically used sea lion haulout at Low Point (n = 2 surveys) just outside Lutak Inlet in Chilkoot Inlet. Taiya Inlet survey was 27.8 km long with a total area of 7.4 km^2 (n = 2) (Fig. 2). Eulachon biomass at each location was calculated by multiplying total surface area (km^2) by eulachon density (weight per area).

Hydroacoustic data were collected using a Kongsberg Simrad Fisheries EK 60 split-beam system with two frequencies: 120 kHz 7º and 38 kHz 12º transducers set in a tow body. The tow

body was towed at roughly 12 - 16.7 km·hr^{-1} at a depth of 2 m. Both transducers were calibrated using a multi-frequency tungsten carbide calibration sphere and conductivity, temperature, and depth (CTD) data for environmental parameters were collected just prior to and at the same location of the calibration. A standard parallel box survey was laid out for each of the five locations with survey replicates conducted at each location. Aerial surveys and opportunistic observations by survey and fishing vessels of foraging sea lions were used to coordinate acoustic surveys on schools of pre-spawning eulachon. Smaller focal hydroacoustic surveys were conducted when acoustic data showed schooling fish sign during the larger acoustic surveys. Midwater trawling or gillnetting were used to collect aquatic fauna for species identification, to verify acoustic data, obtain length, and weight data for relative biomass estimates, and to collect samples for nutritional analysis. Dipnets and fish traps were also used to obtain additional samples for nutritional analyses.

RAW acoustic data were collected as nautical area backscatter coefficient (NASC), which was monitored and saved in real-time with Kongsberg Simrad software on a laptop, copied to an external hard drive, and entered into Echoview® Software Pty. Ltd. analytical software to delineate the seafloor, erroneous data, to identify species and schools of interest, and to export NASC values. Two frequencies were chosen to better identify species with 120 kHz frequency used for weak less reflective (Rayleigh) backscatters like eulachon without gas bladders and 38 kHz for strong more reflective (Geometric) backscatters like walleye pollock (*Gadus chalcogrammus*) with gas bladders. A Kongsberg Simrad general purpose transceiver (GPT) actively produced an echo and then integrated and summed the returning echoes from fauna caught within the acoustic beams during surveys. Acoustic data were categorized by

species on an echogram, integrated for 0.183-km length and 10-m depth intervals, and corrected for transducer calibration using Echoview® software. Frequency threshold was lowered to -76 dB from its standard -70 dB in Echoview® to better identify eulachon, which are less acoustically reflective because they do not have swim bladders (Gauthier and Horne 2004), without introducing extra noise. According to Simmonds and MacLennan 2005, the output of acoustic scattering or NASC is proportional to species and size. To convert NASC to fish density in numbers, acoustic reflectivity, quantified as target strength (TS) for single fish, was needed. Target strength for single fish depends on length in centimeters. The equation used in this study to determine TS was

$$TS = 20\log L_F + b,$$

where L_F equals fork length and the constant (b) used for eulachon in brackish estuarine water b is -81 (Sigler et al. 2004). The b constant was adjusted between saltwater and freshwater values to best match published TS values for 120 kHz (Gauthier and Horne 2004). Target strength (TS) was then transformed to backscattering cross-section (σbs) where

$$\sigma bs = 4\pi 10 TS/10.$$

Eulachon density in numbers was computed by dividing NASC by σbs. Eulachon density in weight equals density in number multiplied by average weight (Simmonds and MacLennan 2005). Eulachon biomass was then calculated for each location by multiplying the surface area of each location (km^2) by eulachon density in weight.

NASC values attributed to eulachon were used to show when and where eulachon moved into an area, and showed when numbers peaked and dropped. After species were identified on the echogram we used acoustic data as an ethological tool to follow the movement of eulachon

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within an estuary and thus determine the pre-spawning behavior and the juvenile and sub-adult

distribution. We determined distribution by live viewing acoustic data, and followed the survey

on Nobeltec™ navigational software, which provided real-time global positioning system

reference. We marked the edges of the schools seen throughout each survey on the navigational

software map and labeled edge marks by species. Related marks were connected to construct

polygons around the school to plot the school's location, shape, and size in real time per day for

each estuary. This allowed us to document GPS data in real time on a map of the surveyed

location, enabling us to track daily and weekly movements of eulachon and other species

throughout and between estuaries on maps of their specific locations during the time of each

survey.

Field Operations – Fishing Methods

Fish samples were collected for nutritional analysis, to identify species with acoustic

sign, and to obtain biological information (e.g., length, weight, age and sex) to estimate biomass.

At the two southern most sites (Taku Inlet and Berners Bay), we used the NOAA ship *John N.*

Cobb to fish with a 164 Nordic rope trawl with a 7 m-high and 17 m-wide net opening and a

32-mm mesh codend liner. In Berners Bay, we also collected riverine eulachon samples from

fish traps operated by the U. S. Forest Service. In the northern estuaries of Chilkat, Lutak, and

Taiya inlets, we sampled fish using gillnet and local recreational dipnet catches. The demersal

gillnet was deployed from the FV *Nanasse* to sample from the sea floor up into the water

column. The gillnet was 27.5 m-long and 2.4 m-deep with five 5.5 m panels (13, 19, 25, 38, and

51 mm of stretched mesh) oriented vertically along its length. We used standard recreational

dipnets, which have a 31-cm deep bag made of 5 mm mesh with a 31-cm diameter mouth

attached to a telescoping pole. In Lutak Inlet, we also had the opportunity to collect dipnet

samples and visual observations from the bridge over the Chilkoot River (< 100 m upstream

from Lutak Inlet) when fish density was very high. Fish used for thiamine analysis were

collected in the Ferebee, Chilkat, Chilkoot, and Taiya rivers using recreational fishing methods

by the Southeast Alaska Watershed Coalition and ADF&G, Haines, Alaska, throughout May

2015. Fish were collected and frozen whole in the field. Lengths and weights were recorded for

whole fish in the laboratory prior to thiaminase analyses, gender identification, and for

determination of maturity stage.

Field Operations – Steller Sea Lion Enumeration

Steller sea lions were located, counted, and special attention given to identifying feeding

behavior in order to locate schooling eulachon. Sea lion activity was noted during all fishing and

acoustic sampling surveys and recorded using marine mammal sampling forms. Data recorded

included count, behavior (e.g., feeding, transiting, and milling), orientation (bearing), and

distance to boat. Sea lion counts from the acoustic survey vessel were made by photographing

larger (>10) aggregations with a Canon D5 digital single-lens reflex (DSLR) camera with a

28 - 300 mm image stabilizing (IS) lens. Sea lions were marked on images in Adobe Photoshop

software and animals in the image were enumerated; counts were made twice for accuracy.

When sea lion aggregations were small (< 10), enumeration was done by two observers using

Pentax 10×42 binoculars, with two to three counts collected per aggregation and averaged.

Aerial surveys were conducted at approximately 5 - 7-day intervals between 1,000 and

1,400 hours using a fixed-wing single engine Cessna 206 aircraft, flown at an altitude of 316 m

and a speed of 183 - 210 km·h⁻¹. Aerial surveys were conducted in Taku Inlet from 2 April to 27 April 2006, and in Berners Bay and Lutak Inlet from 2 April to 12 May 2006. Transects were flown perpendicular to the shoreline, but when sea lions were present, we deviated from this pattern to better enumerate the sea lions. Sea lions throughout the bay were enumerated and locations were relayed to the acoustic survey vessel. Digital photographic images of sea lions were taken from the aircraft using a Nikon D1X digital camera with an 80 - 300 mm telephoto lens. Small aggregations of sea lions (< 10) were counted by the observer while larger aggregations were photographed. The majority of photos were taken when sea lion groups were at the surface; however, when aggregations were active (e.g., diving and transiting), the plane circled until all members of the group were at the surface and could be photographed. Counts from aerial surveys represent the observed number of sea lions in the study area, with an unknown proportion of sea lions not at the surface and thus unavailable to be counted or photographed.

Date, time, GPS location, frame numbers, and estimated numbers were recorded for all sea lions that were encountered at all locations. Digital photographic images were stored on compact flash media cards and then downloaded to a computer. The clearest digital image was imported into the geographic information system software ArcView®, and each sea lion was marked on the image and counted. Marks were checked and counted twice by an experienced counter. The variability of the total sea lion counts for each estuary was not measured, as only one survey was flown each day.

Laboratory Operations – Eulachon Physical and Chemical Analyses

Fish length (fork length) was recorded when collected and immediately frozen whole at -20 °C, held for 10 - 20 days, and then transferred to -80 °C. In the laboratory, fish were weighted prior to analysis for nutritional content. We classified juveniles as those from 4.6 - 8.9 cm long, sub-adults as 9.0 - 15.0 cm, and pre-spawning adults > 15.1 cm (fork length). Adult lengths < 16 cm came from fish that were collected, measured, and dissected for chemical analysis and verified as pre-spawning adults.

Individual fish were homogenized while frozen and aliquots of the total body homogenate were randomly selected for the analysis with every seventeenth fish sampled was analyzed twice. A 0.5 - 1.0 g aliquot of homogenate was used for lipid analysis. Lipid was extracted using a solution of 2:1 *v:v* chloroform:methonal in an accelerated solvent extractor with two cycles on a temperature/pressure regime of 120 °C and 8.27 million Pa. The resultant filtrate was washed twice with a 0.5% KCl solution and 1:1 *v:v* methonal:deionized water to remove the co-extractables. Percent lipid was calculated gravimetrically.

Moisture content was determined gravimetrically by drying ~ 4 g of sample homogenate in a 75 °C oven until a constant weight was attained. After percent moisture was calculated, dried samples were ground to a fine powder and nitrogen content was analyzed using a nitrogen analyzer. Percent protein was calculated from the nitrogen content using a conversion factor of 6.25 (Craig et al. 1978). Total-body lipid and protein contents were converted from relative percentages to masses on a wet-weight basis and then multiplied by their corresponding conversion factors to calculate total-body energy content.

Energy content in thermochemical kJ was calculated as the sum of caloric contribution by total body lipid and protein, using the accepted conversion factors of 9.50 kcal·g⁻¹ for lipid and 5.65 kcal·g⁻¹ for protein (Watt and Merrill 1963):

$$(1) \quad 9.50 \text{ kcal/g x \% lipid} + 5.65 \text{ kcal/g x \% protein}$$

$$(2) \quad 1 \text{ kcal} = 4.1840 \text{ thermochemical kJ.}$$

Vitamin analysis was conducted according to methods at the University of Connecticut's Department of Nutritional Sciences. Whole body fish homogenates were stored frozen at –70°C until analyzed. Subsamples of homogenate were weighed, homogenized in phosphate buffer and hexane-extracted. Vitamin A1 (retinol) and vitamin E (α-tocopherol) were determined by reversed-phase isocratic high-performance liquid chromatography (HPLC). Vitamins were separated on a Waters C18 Resolve Column (15 cm × 3.9 mm; Millipore, Milford, MA) protected with an Upchurch C18 guard column (Upchurch Scientific, Oak Harbor, WA). A mobile phase of methanol/water (98/2) at 1 mL/min was used to elute the vitamins. Quantitation for both vitamins were at 300 nm (Catignani 1986).

Laboratory Operations – Thiaminase I Analysis

Thiaminase activity was measured using the colorimetric thiaminase I assay of Honeyfield et al. (2010). Reaction volumes were scaled down to quantify changes in optical density of 4NTP on a microplate spectrophotometer (BioTek®, PowerWave 340) rather than a standard UV-vis spectrophotometer. Samples of approximately 0.3 g from ground, whole, frozen

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fish were first homogenized in five volumes of chilled buffer. The sample was then vortexed, centrifuged, and the supernatants transferred into 2-ml Pierce centrifuge columns (~30 µm pore size, Thermo Fisher Scientific Inc.) to remove any residual particulate matter. Then 6 µl of processed, filtered supernatant was placed in each of four replicate wells of the microplate (NUNC, 96 well, Thermo Fisher Scientific Inc.) containing 194 µl of cocktail A (with thiamine) and 4 wells containing 194 µl cocktail B (no thiamine). The wells without thiamine served as assay controls for each sample. Plates were placed into a pre-heated (37°C) plate reader, the microplate was shaken at medium speed for 10 s, and absorbance was read at 411 nm. After initial absorbance was recorded, absorbance was measured at 1-min intervals over 1 hour. Rate of 4NTP degradation was calculated and reported as µmol of thiamine inactivated per gram of tissue per minute. All buffers and reagents were prepared immediately prior to use.

RESULTS

Eulachon Abundance and Distribution

We estimated the biomass of pre-spawning eulachon in Berners Bay at 332 metric tons (t) during 2006 which could be one of the lowest in recent years (pers. comm. with USFS and ADF&G, Juneau, AK). We did not identify any pre-spawning eulachon acoustic sign in Chilkat or Taiya inlets, but found what could be a relatively high run of 3,180 (t) in Lutak Inlet and a relatively average run in Taku Inlet of 1,785 (t) (Table 1).

Pre-spawning eulachon did not spend much time lingering in the estuary, with time spent proportional to estuary size. Taku Inlet was the only study site where we observed adult eulachon schools lingering in the estuary, in this case it was a few dense schools in the middle and inner

parts of the inlet for a few days. Pre-spawning runs varied in duration with Lutak Inlet having

one of the shortest with a rapid increase to a maximum biomass day then a rapid 3-day decrease

in biomass. Taku Inlet had the longest lasting pre-spawn run with a less rapid increase than

Lutak increase to a mid-level biomass plateau lasting days then a doubling of biomass in a day to

a high level plateau lasting 3 days before a rapid one day decrease (Fig. 3). Since we had only

one boat to survey five estuaries in a large geographic area we were unable to spend the time at

each estuary to determine the precise beginning and end of each run.

In addition to pre-spawning eulachon, we observed other life stages of eulachon and

aquatic fauna in our study estuaries. One of our more interesting observations was a dense

consistent layer of osmerids between 50 m and 80 m found throughout three of the five estuaries

(Fig. 4) based on our acoustic sampling. This "osmerid layer" was approximately 80% juvenile

and sub-adult eulachon with some capelin mixed in, with the remaining percentage comprised of

crustaceans (mainly euphausids) based on our physical sampling. When this layer was present, it

was seen throughout the estuary at a consistent depth at each estuary. The presence, size, and

density of this layer appears to be related to the size and shape of the estuary. Taku Inlet had the

largest and densest layer, Chilkat Inlet next, Berners Bay a close third, with Lutak and Taiya

showing no osmerid layer. Lutak and Taiya inlets are both fed by small river systems into small,

fairly shallow inlets with simple straight landscapes. Our trawl data shows a variety of species

like capelin, euphausids (*Euphausiacea*), Pacific herring, walleye pollock, northern lampfish

(*Stenobrachius leucopsarus*) and Pacific glass shrimp (*Pasiphaea pacifica*) inhabiting most

estuaries and are also found in specific layers. Capelin were found in the osmerid layer in low

numbers, with most adults found tightly aggregated along the shoreline near the river mouth.

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Juvenile walleye pollock were found at all locations, from 80 m to the bottom and were mainly located in the outer portions of the estuary where it was $\geq$ 80 m which was below the osmerid layer and outside the estuary where the osmerid layer was found.

Length Frequency

Length frequency of pre-spawning eulachon in Taku and Lutak inlets and Berners Bay were variable showing distinct life stage patterns of juveniles, sub-adults, and adults (Fig. 5). Berners Bay had an average length of 11.5 cm (Table 1) with a range of 7.9 - 15.7 cm (Fig. 5). The average fish length in Taku Inlet was 11.9 cm (Table 1) with a range of 4.6 - 20.5 cm (Fig. 5). Lutak Inlet had the largest average size of 17.4 cm (Table 1) with a range of 15.5 - 19.3 cm, which were all adults (Fig. 5). The average adult size in all locations was 17.4 cm (Fig. 6), SD = 1.1, n = 249 with 8.8 % of the adult fish < 16.0 cm (Fig. 6). The smallest adult fish in our study was found in Taku Inlet at 14.6 cm, we also found a 15.5 cm gravid female in Lutak Inlet, which falls below the normal size range ($\geq$ 16 cm) (Clark et al. 2007) for an adult. All fish < 16 cm were determined as gravid adults though dissection prior to chemical analysis of collected specimens.

Eulachon Nutritional Content

There were little significant differences in total nutrition between locations. Eulachon from all estuaries combined had an average of 11.96% protein, 15.04% lipid content (wet mass oil), 72.51% moisture, 1.57% ash, 184.58 µg/100g vitamin A and 7267.54 µg/100g vitamin E (Fig. 7). Mean weight and total energy available per fish from Berners Bay were, 10.68g ± 2.93 g and 94.4 kJ, Taku Inlet 16.55g ± 1.16 g and 145.4 kJ, and from Lutak Inlet 33.22g ± 5.99 g and

291.9 kJ (Table 1). Nutritional content of eulachon varied to some extent among and within estuaries with a lipid content range of 10.04% - 19.76% and an average of 15.04%, with considerable overlap among estuaries (Fig. 7). Protein content varied less than lipid with a range of 10.21 - 13.90% and an average of 11.96%. Combining these data with trawl and acoustic analysis we get total energies available by location of 2.9 billion kJ for Berners Bay, 15.7 billion kJ for Taku Inlet and 27.9 billion kJ for Lutak Inlet (Table 1).

The mean vitamin A content of eulachon was 195.3±11.1 µg/100 g and vitamin E content was 7,249 ± 213 µg/100 g. Neither vitamin varied by collection month or maturation status (pre-spawner vs. spawner, mature vs. spent) (ANOVA, P > 0.05). Nor did vitamin content vary with fish length $R^2 < 0.01\%$ or lipid content $R^2 \leq 9.5\%$ (regression), both vitamins are lipid-soluble.

Thiaminase Content

Eulachon thiaminase activity was highly variable for all fish sampled from the four sites, with a range of 1.05 - 17.92 umols·g^{-1}·min^{-1}. Mean thiaminase activity (umols·g^{-1}·min^{-1}), however, was not significantly different (ANOVA, P = 0.81) among eulachon from Ferebee River (5.67), Chilkat River (6.48), Chilkoot River (5.54), or Taiya River (7.04) (Figs. 1 and 2) with an overall mean activity range of 5.0 - 7.0 umols·g^{-1}·min^{-1}.

Steller Sea Lion Abundance and Activity

Taku Inlet sea lion aerial counts started on 2 April 2006 with < 10 animals, growing to 40 on 10 April, then increased rapidly to 160 animals by 14 April (Fig. 3). By 15 April, sea lion numbers had rapidly decreased to 55 on 19 April and stayed consistent until 24 April, when

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numbers started to slowly decline to a low of < 10 by the end of April. Berners Bay aerial counts

started on 2 April 2006 with 15 sea lions counted with a gradual increase to a maximum of 40

animals counted on 13 April followed by a few decreases to 0 and increases to 10 to 30 between

1 May and the end of the aerial survey on 15 May (Fig. 3). On 24 April we enumerated only 10

sea lions but on April 26 we counted the peak number of 158 with a drop to 70 on 27 April and

to < 10 by 30 April (Fig. 3). Lutak Inlet sea lion counts started on 7 April 2006 with no animals

found until 28 April when the numbers increased precipitously to 400 on 3 May followed by an

equally steep decrease to 50 animals counted on 7 May and decreased to 0 on 15 May. Vessel

counts for Berners Bay were from 6 to 30 April 2006 with counts varying up and down from 15

to 40 animals between 6 and 21April. The sea lion counts from vessels from 3 to 15 April, 2006

counted < 10 animals until 10 April when 20 animals were counted with a maximum of 50 sea

lions on 14 April and numbers declining to < 10 on 15 April (Fig. 3).

In general it took a day or few for sea lion numbers to catch up to eulachon biomass in all

locations. Comparing sea lion numbers to pre-spawning eulachon biomass in Lutak Inlet, on

28 April the eulachon biomass was at its peak but < 10 sea lions were counted. Then on 1 May

the count was up to 300 sea lions but the eulachon numbers were dropping fast and on 3 May the

sea lion count reached its maximum of more than 800 animals but the eulachon numbers were

falling fast until both bottomed out on 5 May (Fig. 3).

In summary, Steller sea lion numbers were relatively low in Berners Bay and Chilkat and

Taiya Inlets, average in Taku Inlet, and unusually high in Lutak Inlet (Fig. 3). Acoustic tracking

of eulachon demonstrated that sea lions move into the inlets soon after the eulachon start

congregating, but do not arrive en masse until after eulachon numbers have peaked (Fig. 3). Taku Inlet had a more gradual increase and decrease in eulachon biomass then peaked and leveled off for a few days, providing sea lions more time to forage. We also observed a few dense schools of pre-spawning (adult) eulachon lingering within Taku Inlet, which also enabled sea lions more time to forage.

DISCUSSION

We examined three ecologically connected variables: location and movement of eulachon, their nutritional composition, and movement of Steller sea lions with respect to eulachon spawning in four Lynn Canal and one Stephens Passage estuaries. Because eulachon spawn in bays and inlets earlier than most other fish species in southeast Alaska and have a high energy content, eulachon can be an ecologically important species for southeast Alaska's marine predators by providing much needed energy at a crucial time of the year. Our study provided additional data on eulachon for Berners Bay, and new data for four additional estuaries. From these data, we can infer some much needed information about the marine distribution and life history of eulachon. Eulachon ethology and morphology make this forage species hard to study in the marine environment. They are hard to catch by net because of their dispersed marine shoaling behavior associated with the seafloor when not in their pre-spawning inlets or bays (Csepp et al. 2011). They are also hard to detect using hydroacoustics because they are weak acoustic reflectors that are in association with the sea floor in a heterogeneous species layer (Csepp et al. 2011) of equally weak acoustic reflectors (Gauthier and Horne 2004).

We used length frequencies to determine life stage of fish that were not dissected for nutritional analysis. With the length frequencies we observed and an age at maturity of 3 to 4 years (Clark et al. 2007), we were able to estimate the marine distribution of juvenile and sub-adult eulachon in the estuaries within our study area. According to Clark et al. 2007, 16.0 cm is the minimum size for spawning eulachon with size increasing with latitude, although, we found 8.8% of our adults were < 16.0 cm, with our smallest adult at 14.6 cm. We can say with certainty that both length and weight increase with age (Spangler 2002) and that size differs between rivers and years (Barraclough 1964, Spangler 2002).

Other studies have found that eulachon spawning locations are inconsistent and spawning occurs in some locations during some years but not others, making regular appearances in some areas but rarely seen in others (Minckley et al. 1986, Hinrichsen 1998, Willson et al. 2006). Berners Bay is a classic example of this with its fairly regular pre-spawning aggregations and variable fluctuations in biomass with a high year seen in 2002 and low in 2003 (Sigler et al. 2004) and during our study in 2006. The low biomass seen in Chilkat Inlet, Berners Bay, and Taiya Inlet, and a very high biomass in Lutak indicate a correlation between these four northern estuaries and the possible sharing of a spawning stock. On the other hand, Taku Inlet with its typical biomass could suggest a separate breeding stock from the four northern estuaries or just another example of the erratic spawning nature of eulachon.

Eulachon Distribution

Through eulachon length frequencies (Fig. 5), life stage structure, and the presence of a distinct osmerid (and euphausid) layer (Fig. 4) found in Berners Bay, Chilkat, and Taku Inlets,

we were able to describe eulachon estuarine distribution by life history stage. Surveys of other similar inlets south of our study area within southeast Alaska's inside waters also show a similar layer throughout the year (Csepp et al. 2011). We propose from these data that estuaries that have the right size, shape, and bathymetry can create the right conditions where immature eulachon could stay for the first 1 to 2 years, using these estuaries as nursery grounds. This is in agreement with Hay and McCarter (2000), who found that larvae are immediately carried into the estuary and may use it as a nursery area. Smaller Inlets could offer less area for outgoing juveniles to aggregate in and have more tidal and river currents pushing small fish out of the estuary more rapidly. Note in Figure 2 the small size and linear shape of Taiya and Lutak inlets, which have no osmerid layer to the larger size and more serpentine or bottleneck shape of Chilkat and Taku Inlets and Berners Bay which harbor an osmerid layer. We do not know how long eulachon stay in their natal estuary, where they go or how far they travel when they leave or weather they return to it to spawn. We have, however, regularly found adults outside their spawning bays and inlets in the main waterways of southeast Alaska in a heterogeneous, biotic layer associated with the sea floor (Csepp et al. 2011).

Spawning

Eulachon are known to spawn unpredictably, by how many fish show up (biomass), by where they choose to spawn (location) and by when they chose to spawn (timing). For example, eulachon usually spawn once in a lifetime making them semelparous (monocyclic) (Clarke et al. 2007) but some are known to spawn twice, making a select few iteroparous (polycyclic). The frequency of iteroparity and the variability among and between populations is still unresolved (Hart and McHugh 1944, Barraclough 1964, Blackburn et al. 1981, Hay and McCarter 2000,).

Most eulachon spawning rivers have only one spawn per year but some have two (Langer et al. 1977, Bishop et al. 1989, Betts 1994). There are several examples of this with one found in our study area; Chilkat River has its regular run in May, and a sporadic one in February (Bishop et al. 1989, Betts 1994). The timing when eulachon spawn does not show a latitudinal relationship, early in the south to later in the north (Hay and McCarter, 2000), making attempts at timing a run difficult. For example, Chilkat River run can begin late April and peak by early May, but in other years it peaks around 23 May (Bishop et al. 1989). On Twentymile River the run can start from 17 April to 4 May (Spangler 2002), and in Berners Bay it can start from early April to early May (Marston et al. 2002, Sigler et al. 2004). Eulachon not only change their run timing, they even fail to show up in their regular spawning rivers in some years like the Cowlitz (Hinrichsen, 1998) and the Sacramento (Minckley et al. 1986). We observed in the fish traps in the Lace and Antler Rivers that the runs into Berners Bay and the recreational fishery in Chilkat River that runs into Chilkat Inlet showed up but in such low numbers they could not be caught or seen with our acoustics in the estuary and apparently moved over to nearby Lutak Inlet as per the unusually high biomass seen in 2006. Hay and McCarter (2000) also noted this straying behavior. Berners Bay spawning runs were usually very reliable from year to year (Marston et al. 2002). Berners Bay, however, appears to have regular fluctuations in run biomass as seen by us and Sigler et al. 2004. The Sigler et al. (2004) study resembles ours in that eulachon runs in Berners Bay cycled between high and low years, which could be indicative of good and bad brood years or just verify the erratic nature of eulachon spawning locations. Our study combined with Sigler's et al. (2004) clearly shows the unpredictable nature of eulachon spawning in Berners Bay. For future studies, high and low spawning years could help identify brood stock if the spawning population returns to the same river to spawn. In addition, when studying a larger

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geographic area with many spawning locations attention is needed in determining separate spawning populations.

Steller Sea Lion Abundance

Factors that appear to influence sea lion presence and numerical response are eulachon biomass, estuary schooling density, duration, and location in the water column, average fish size, and fish behavior. In our study, sea lion numbers are directly proportional to eulachon biomass, and schooling duration. Sea lion numbers grew from a few to several hundred in 2 - 3 days, soon after eulachon biomass increased. This suggests that sea lions may be using some form of communication regarding the presence of eulachon to animals across a larger area. We know that sea lions use haulouts throughout the area with a large, centrally located haulout at Benjamin Island in Lynn Canal. Even though sea lion numbers are directly proportional to eulachon biomass, sea lion arrival is a bit off sync from eulachon peak biomass, with sea lions showing up en masse 2 - 3 days after eulachon biomass has peaked (Fig. 3). In other words, there is a 2 - 3 day delay for sea lions throughout an area to react to a forage species schooling event; Lutak Inlet was a typical example of this. With the small size of Lutak Inlet the eulachon pre-spawning event had a shorter duration compared to larger inlets. Taku Inlet had a smoother biomass increase, building up to a plateau that lasted 5 to 6 days, with sea lion numbers slowly and steadily increasing to a peak of 160 animals at the end of the biomass plateau (Fig. 3). One thing similar between large and small inlets is that once the eulachon pre-spawning biomass starts declining it declines quickly as do the sea lion numbers (Fig. 3). During a previous study Berners Bay sea lions peaked at 949 in 2003, but the peak count in 2002 was only 120 (Sigler et al. 2004), suggesting that there may have been a reduced abundance of eulachon in 2002 when

using the sea lion count alone. Sea lion activity in Berners Bay in 2006 was also a low year with a maximum of only 156 animals.

Our survey vessel and aerial counts were comparable, in that they showed similar timing to the increases and decreases in sea lion numbers in association with eulachon biomass. Our vessel counts however, were greater than our aerial counts for all locations except Taku Inlet (Fig. 3). There may be several reasons for the different in vessel and aerial counts. First, vessel remain in the area over a much longer time period, thus providing a greater likelihood of observing and sea lions in a given area. In contrast, aerial surveys, occur over a short time period in a given study area. In addition, the size and shape of the bay and inlets, which are small enough and shaped so the acoustic survey vessel could see the entire waterway. Taku Inlet, on the other hand, was too large and serpentine in shape for viewing all sea lion activity from the vessel.

Eulachon Energetics and Nutrition

Our data and previous studies demonstrate that eulachon are a lipid-rich forage species with an energy density higher than other forage species used by Steller sea lion such as herring, capelin, cod, pollock, or flounder (Perez 1994). The fish tested in this study represent data for estuary and river captured fish. Eulachon in our study and those previously collected from the Gulf of Alaska contained 18 - 20% lipid, which is higher than other fishes like sand lance at 3% - 6%, or capelin at 2% - 10% lipid (Payne et al. 1999). Similar to previous studies, protein content was slightly lower for eulachon at 12% - 13%, when compared with 13% - 15% for capelin and 16% - 18% for sand lance (Payne et al. 1999). Fish energetics, particularly lipid and

consequently energy content, are known to cycle seasonally in temperate environments in response to seasonal pulses in energy availability and reproductive cycles (Vollenweider et al. 2011, Robards et al. 1999, Paul and Paul 1998).

Eulachon in our study tested within published values for protein, lipid, moisture, ash and energy content (Kuhnlein 2000, Clark et al. 2007); however, our numbers were just a bit low, possibly due to our lower average sizes than previously sample fish. Our catches had a high percentage of juvenile and sub-adult fish, which were found in the pelagic, osmerid layer seen in three of the five surveyed locations (Fig. 4). The use of the 164 Nordic rope trawl in our study with its 32 mm mesh codend was better designed to catch smaller fish found throughout the water column than the standard commercial shrimp trawl used in other studies.

Eulachon also contain high levels of vitamins A and E (Fig. 7) (Kuhnlein et al. 1996) compared to similar fish species. Data describing vitamin content of cold-water marine fish are limited. Vitamin E content of eulachon whole-body homogenates (7,249) was found to be intermediate to vitamin content in muscle (1,700 - 2,100 µg/100 g) and lipid extract (11,800 - 14,700 µg/100 mg) of Atlantic herring (*Clupea harengus harengus*), a similarly oily fish (Engelhardt et al. 1975). Similarly, vitamin E content of eulachon was intermediate to walleye pollock fillets (490 µg/100 g) (USDA 2017) and carcass lipids (10,300 - 17,200 µg/100 g) (Wu and Bechtel 2009). In another gadid, Pacific cod (*Gadus macrocephalus*), vitamin E content (540 µg/100 g) was 13 times less than eulachon (USDA 2017). Vitamin A content of eulachon was over 6 times greater than reported in Pacific herring (32 µg/100 g), Atlantic herring (28 µg/100 g), and cisco fillets (*Coregonus* sp.) (30 µg/100 g) (USDA 2017). Unlike Atlantic herring in

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which vitamin E content was greater in ripe fish than undeveloped or spent fish, vitamins in eulachon were consistent between reproductive states.

Thiaminase Content

This is the first report showing that eulachon are thiaminase-positive and contain sufficient enzyme activity to result in thiamine deficiency under certain conditions. The mean eulachon thiaminase activity range was 5.0 - 7.0 umols·g^{-1}·min^{-1}. Thiaminase activity greater than 2.5 umols·g^{-1}·min^{-1} has been shown to reduce the thiamine status of the consumer (Fujita 1954, Lienhard 1970, Honeyfield et al. 2012). Prey species with thiaminase activity < 1.5 umols·g^{-1}·min^{-1} have not been associated with thiamine deficiency. The negative effects of thiaminase are directly proportional to the duration and exclusivity of its ingestion. For example, harp seals fed prey fish high in thiaminase showed signs of altered behavior within 40 - 60 days: refusing to eat; becoming inactive; unresponsive to touch, noise, or light, and were breathing irregularly. Within 2 - 3 days of the onset of these preliminary signs, severe tremors, foreflipper spasms, head-shaking, muscle quivering, and then death occurred (Geraci 1970). Eulachon, however, are only available to sea lions for 7 - 14 days during pre-spawning, which is not long enough to affect their health even if they feed on them exclusively. Lake trout consumption of rainbow smelt has been linked to low egg thiamine in the Laurentian Great Lakes (Fitzsimons and Brown 1996). Low egg thiamine has been shown to cause fry mortality in lake trout and other salmonid species (Honeyfield et al. 2005, Wolgamood et al. 2005).

Steller Sea Lion Nutrition

The benefits of eulachon as a prey item for sea lions are associated with the timing of the availability of the resource and their nutritional value. The time of year when eulachon spawn is a very energy demanding time for sea lions, with increased nutritional energy needed for the breeding season (Winship et al. 2002). During April and May, pregnant females are in their third trimester, when most of the fetal mass is accumulated (Frazer and Hugget 1974). According to Calkins and Pitcher (1982), 61% of female sea lions are still nursing a pup in May and lactating females require 70% more calories than lone females (Gittleman and Thompson 1988, Winship et al. 2002). Pregnant females will soon give birth, after which time they fast for 11- 14 days before foraging (Gentry 1970, Sandegreen 1970) and need to accumulate energy stores for both gestation and lactation demands. The primary energetic demands for adult male sea lions is their extended period of fasting during breeding season, which can last up to 68 days (Gentry 1970, Sandegreen 1970, Gisiner 1985). During this time, breeding males focus on maintaining their breeding territory and breeding with females. Thus, adequate energy reserves prior to the breeding season are likely key to a male's ability to maintain a territory and ultimately to breeding success. The demands put on sea lions at this time of year is why the timing of eulachon spawning is so critical for successful sea lion breeding and survival of pups and adults (Sigler et al. 2009).

The high energy density (Table 1) and percent lipid content of eulachon, in addition to its other nutritional values (Fig. 7), and the timing of spawning make eulachon an essential energy source for Steller sea lions at an energetically demanding time of year. Eulachon protein content is lower than other forage fish at 12% - 13%, but they contain high levels of vitamins A and E

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(Fig. 7), calcium, iron, and zinc (Kuhnlein et al. 1996) compared with other common forage fish species. Large eulachon contain 14.5% lipid, which is similar to northern lampfish, making eulachon and lampfish two of the most energy-dense fish tested (Anthony et al. 2000, Vollenweider et al. 2011). However, lampfish are generally too deep and dispersed for sea lions to obtain in any quantity and regularity, as are eulachon when not pre-spawning. With the lack of eulachon in Berners Bay during our study, sea lions were likely feeding on other species present like capelin, herring, and walleye pollock.

Relating vitamin content of fish to requirements of marine mammals is difficult, as few studies have quantified this. Serum vitamin E and vitamin A have been measured in free-ranging gray seals (*Halichoerus grypus*) (Schweigert et al. 1987, 1990). In captivity, vitamin levels have been measured in northern fur seals (*Callorhinus ursinus*), California sea lions (*Zalophus californianus*), and gray (*Halichoerus grypus*) and harbor seals (*Phoca vitulina*) (Mazzaro 1994). Diets low in vitamin E for 18 months have been found to cause electrolyte imbalance and abnormal molting patterns in phocid seals (Engelhardt and Geraci 1978). Vitamins A and E have many health benefits in mammals. Vitamin A is essential for reproduction, bone and muscle growth, cell maintenance, and vision (Ross 1999, Biswas et al. 2000). Vitamin E is known principally as an anti-oxidant (Azzi and Stocker 2000) and is essential for reproduction and lactation (Baldi et al 2000). Vitamin E deficiency can result in muscle degeneration, steatitis, liver necrosis, and anemia (Geraci 1981).

CONCLUSION

One of our observations during this study was the identification of a distinct juvenile and sub-adult eulachon layer in certain estuaries (Fig. 4), adding much needed data to eulachon estuarine distribution and life history. With a species composition of 80% juvenile and sub-adult eulachon in this layer, we propose that estuaries with the right characteristics with spawning rivers associated with them are possibly used as nursery grounds for eulachon. Our echo-integrated trawl surveys also show a correlation between the location of juvenile pollock found at > 80 m and the osmerid layer found at < 80 m (Fig. 4), suggesting that depths of < 80 m could be protection from feeding pollock.

A second observation from our results was that sea lion abundance was correlated with prey biomass and schooling duration, and not with nutritional content of eulachon (Table 1, and Fig. 3). Even though individual pre-spawning eulachon were energy and lipid-rich, the sheer abundance of fish outweighs the differences in fish condition, attracting more sea lions where more fish are present.

Our results could also indicate (although a bit more uncertainly) the possibility of two separate eulachon spawning populations shared between our five sites: one being shared between the four northern estuaries and the other population spawning in Taku Inlet, the southernmost estuary.

Our study confirmed that eulachon are nutrient-rich and for the first time we showed that eulachon contain the anti-nutrient thiaminase, similar to other osmerids that have been tested. It is known that sea lions feed on dense schools of spawning forage species when available which could be detrimental to their survival if the forage species is fed on exclusively and long enough and has an anti-nutrient present. However, the short duration (7 - 14 days) that sea lions in our study area fed solely on eulachon would not be of sufficient duration (40 - 60 days) to lead to thiamine deficiency. Even species like Steller sea lions which have a varied diet could be susceptible considering the 50/50 chance of a forage species containing an anti-nutrient. The presence of an anti-nutrient in a forage species could shed some light onto why changes in prey species, which happen in regime shifts, affect predator population numbers, considering that 51% of marine fish examined contained the anti-nutrient thiaminase (Deutsch and Hasler 1943, Neilands 1946, Ceh et al. 1964, Greig and Gnaedinger 1971, Fowler 1978, National Research Council 1982, National Research Council 1983, Dierauf 1990, Lichtenberger 2011). A simple change from one prey species (or genus) to another with any decrease in the dietary variety for an extended period of time could affect the survival of the predator. Such dietary changes could switch a predator's diet from a nutritious species to a less nutritious species or one with anti-nutrient activity. Merrick et al. (1997) also found a strong correlation between diet diversity and sea lion decline; as diet diversity decreased, the sea lion population decreased no matter the nutritional value of the forage species. Special attention should be given to any nutritional study of predatory species that does not have a variable diet, especially when the predatory species is in decline. Trites and Donnelly (2003), in their review of Steller sea lion decline in Alaska, hypothesized that the decline was caused by nutritional stress from eating "junk food" species with poor nutritional value. Anti-nutrients like thiaminase should also be added to the junk food

species nutritional content list in that seemingly nutritious species could be considered junk food when they contain anti-nutrients and when such prey species are ingested too long and with too much exclusivity. Much more study needs to be done on anti-nutrients and their effects on predators and how widespread they are in forage species.

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Table 1. -- Data from estuaries where biological values were collected to get average weight (g), fork length (cm), energy content/fish (with standard errors), and hydroacoustic NASC values were used to get total energy (kJ)/area, and biomass metric tons (t)/area.

Area	Average weight (g)	Average fork length (cm)	Energy (kJ/fish)	Total energy (billion kJ/area)	Biomass (t)
Taku	16.55 ± 1.16	11.9 ± 0.07	145.4 ± 3.10	15.7	1,785
Berners	10.68 ± 2.93	11.5 ± 0.08	94.4 ± 1.13	2.9	332
Lutak	33.22 ± 5.99	17.4 ± 0.42	291.9 ± 14.88	27.9	3,180
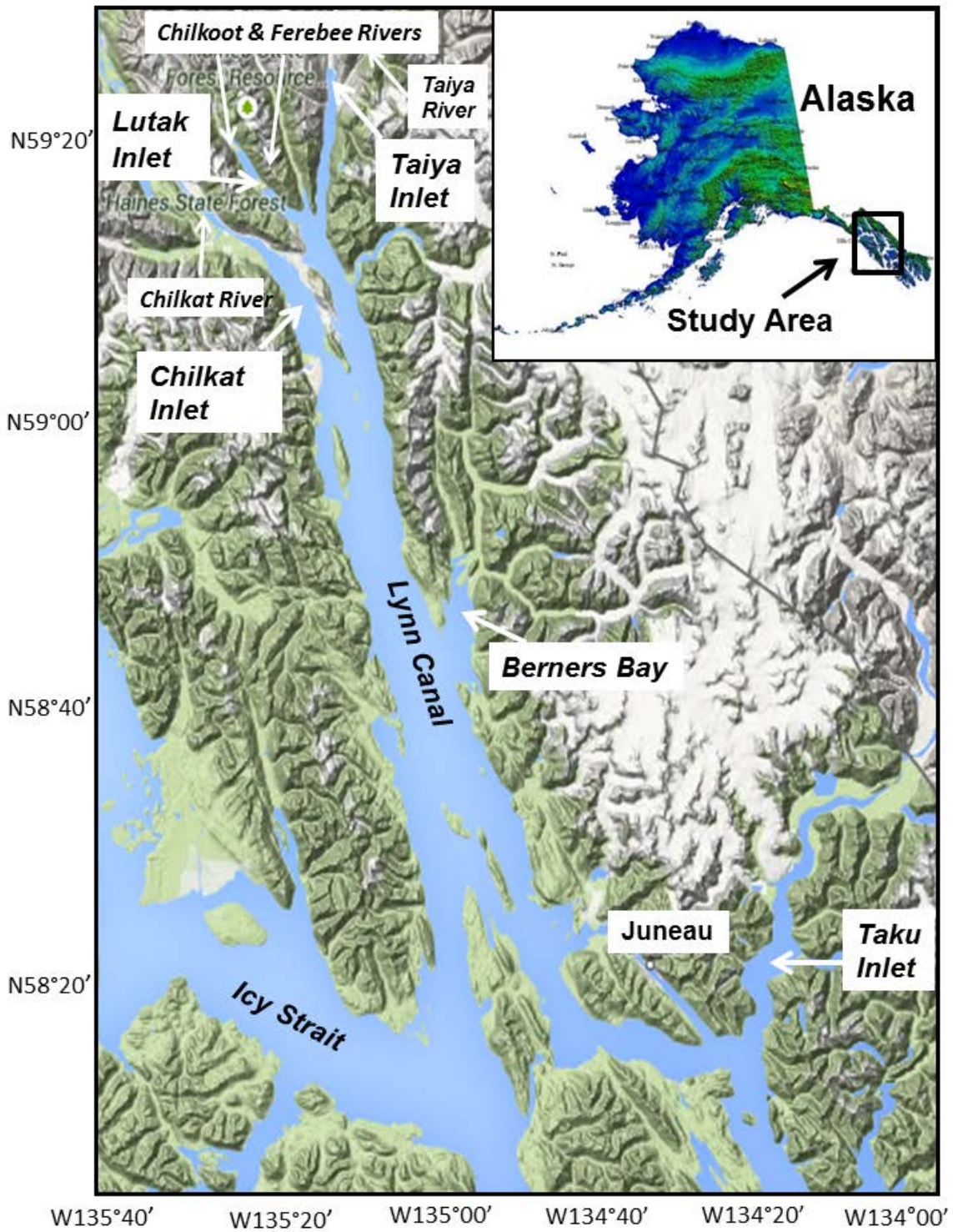
Figure 1. -- Map of southeast Alaska showing the five study sites surveyed for eulachon aggregations and associated foraging Steller sea lions.
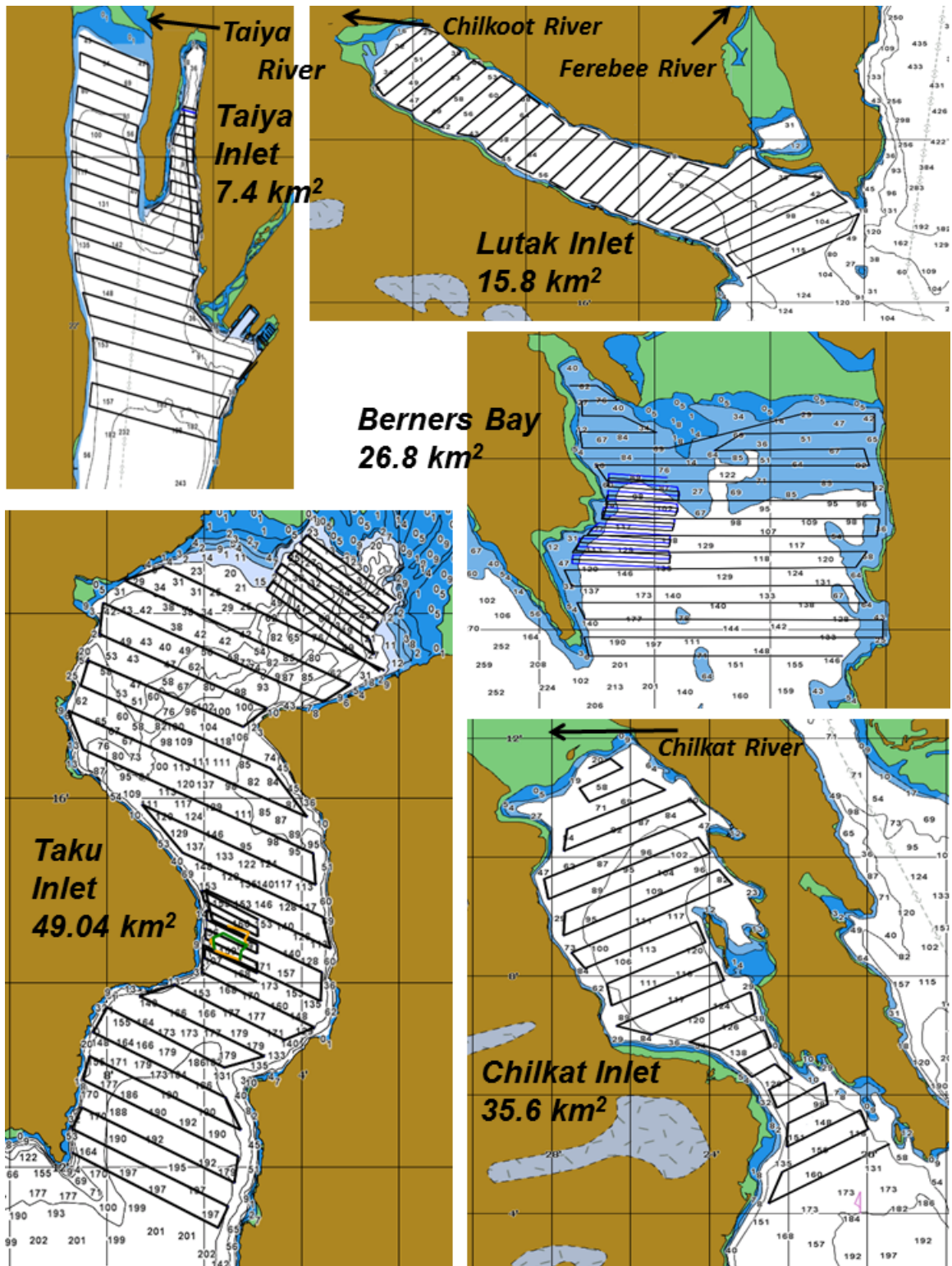
Figure 2. -- Maps of the inlets and bay in our study, to compare size and shape of each estuary and to show the rivers that were sampled for thiaminase. The black lines within each map are the hydroacoustic survey track lines.
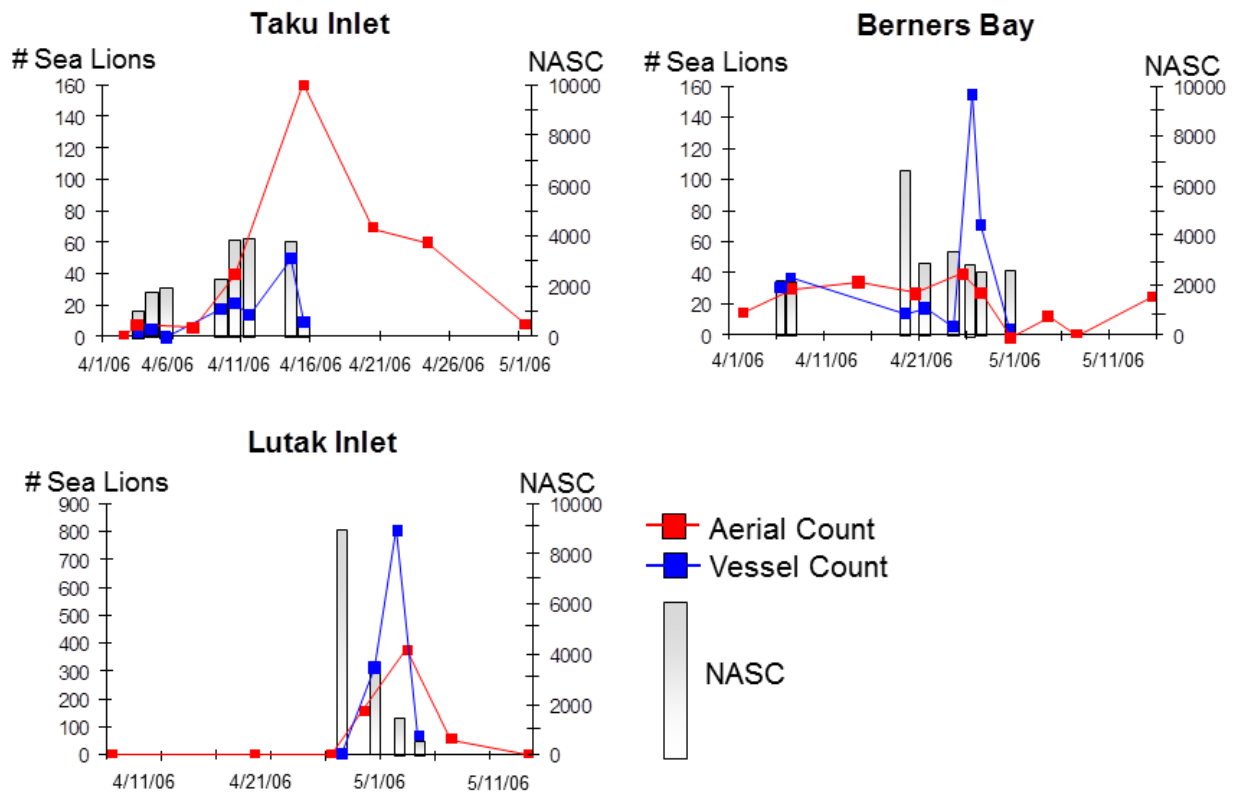
Figure 3. -- Aerial and vessel counts of Steller sea lions and nautical area scattering coefficient (NASC) values from pre-spawning adult eulachon by day and location.
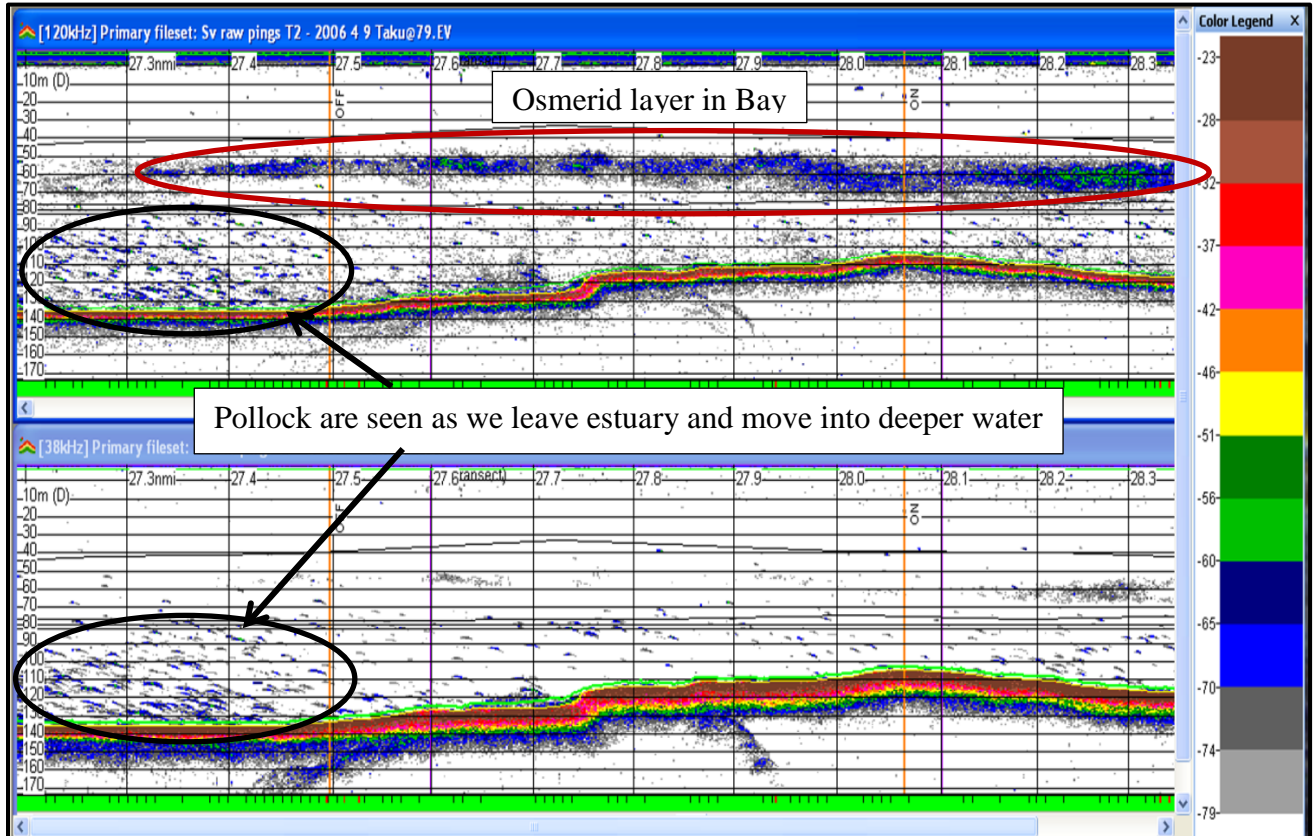
Figure 4. -- Two hydroacoustic echograms visually showing RAW nautical area scattering coefficient (NASC) values of the same (spatially and temporally) osmerid layer collected with 120 kHz on the top and 38 kHz on the bottom as seen in Berners Bay, and Chilkat and Taku inlets. Note how the osmerid layer disappears when walleye pollock are present.
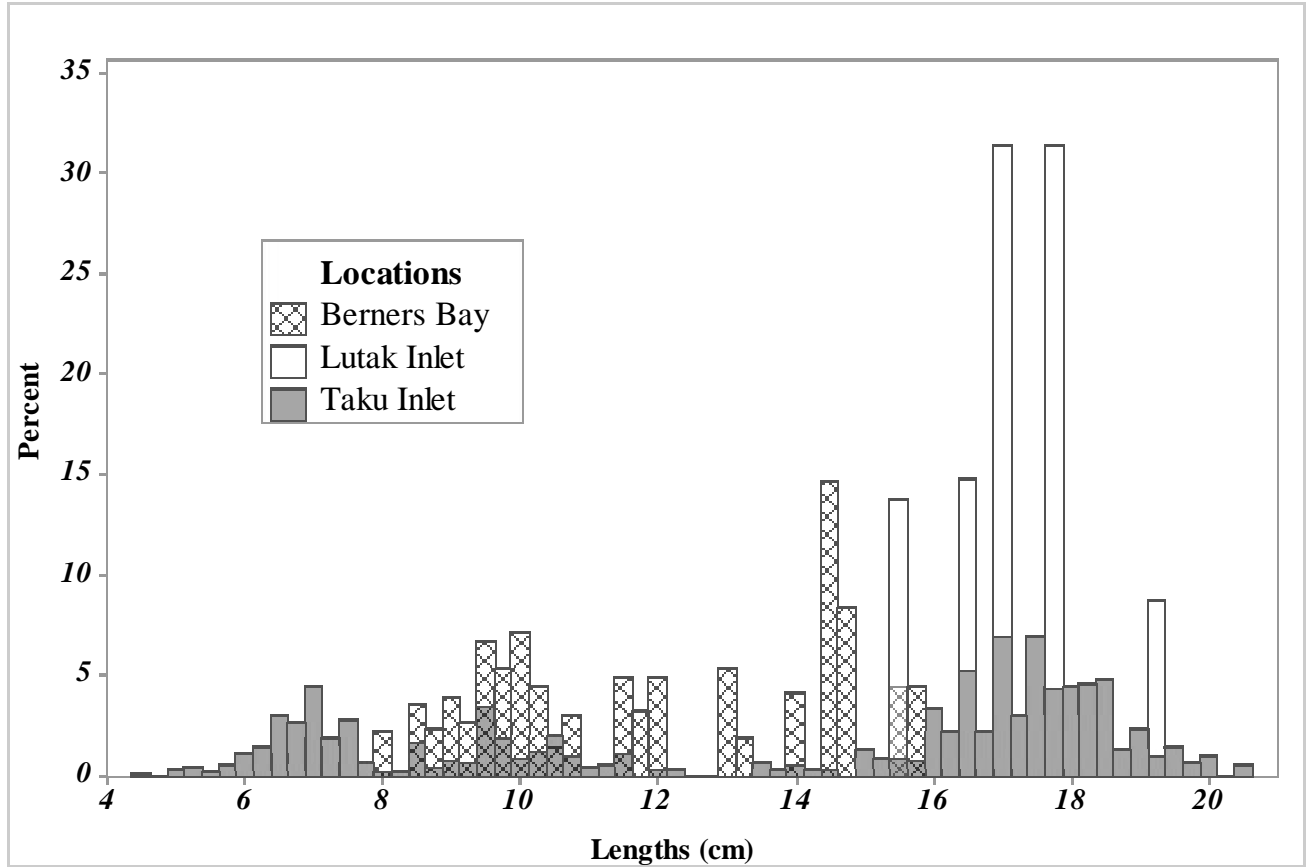
Figure 5. -- Length frequencies of all life stages of eulachon sampled in Berners Bay, Taku Inlet, and Lutak Inlet; juvenile = 4.6-8.9 cm, sub-adult = 9-15 cm, pre-spawning adults ≥ 15.1 cm.
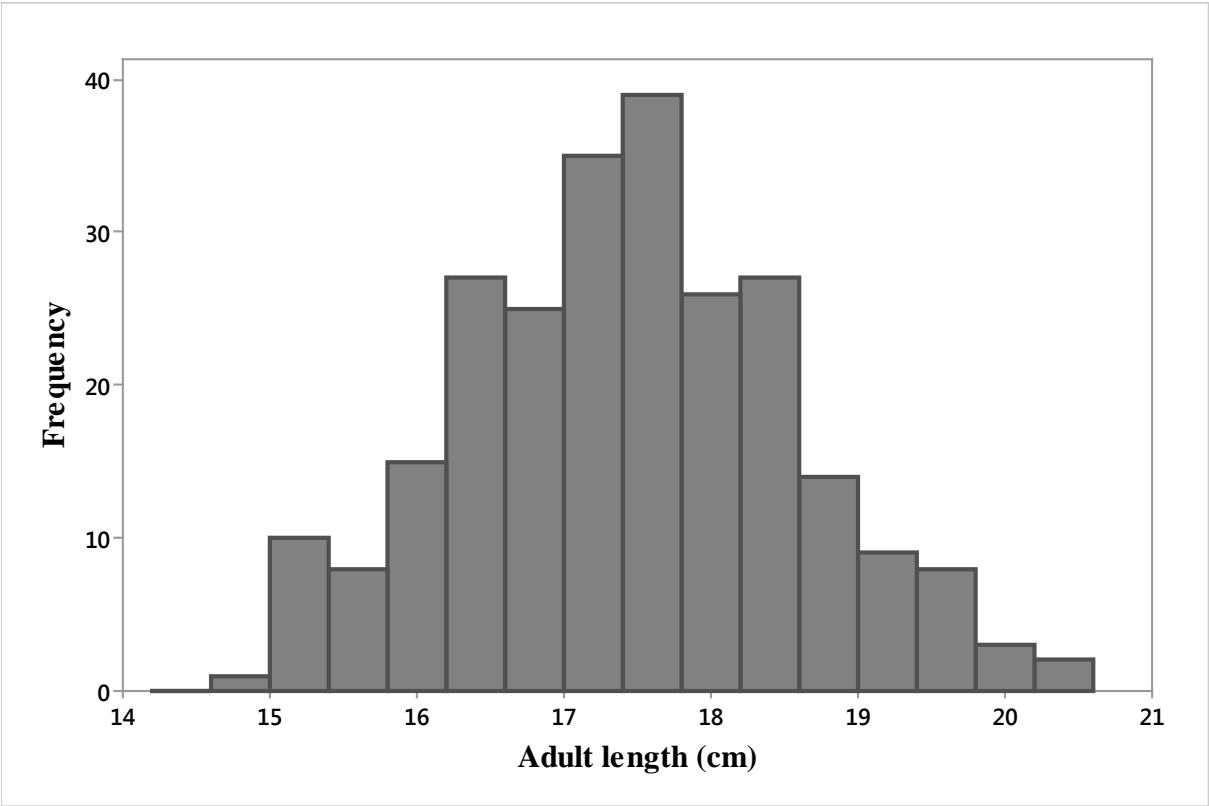
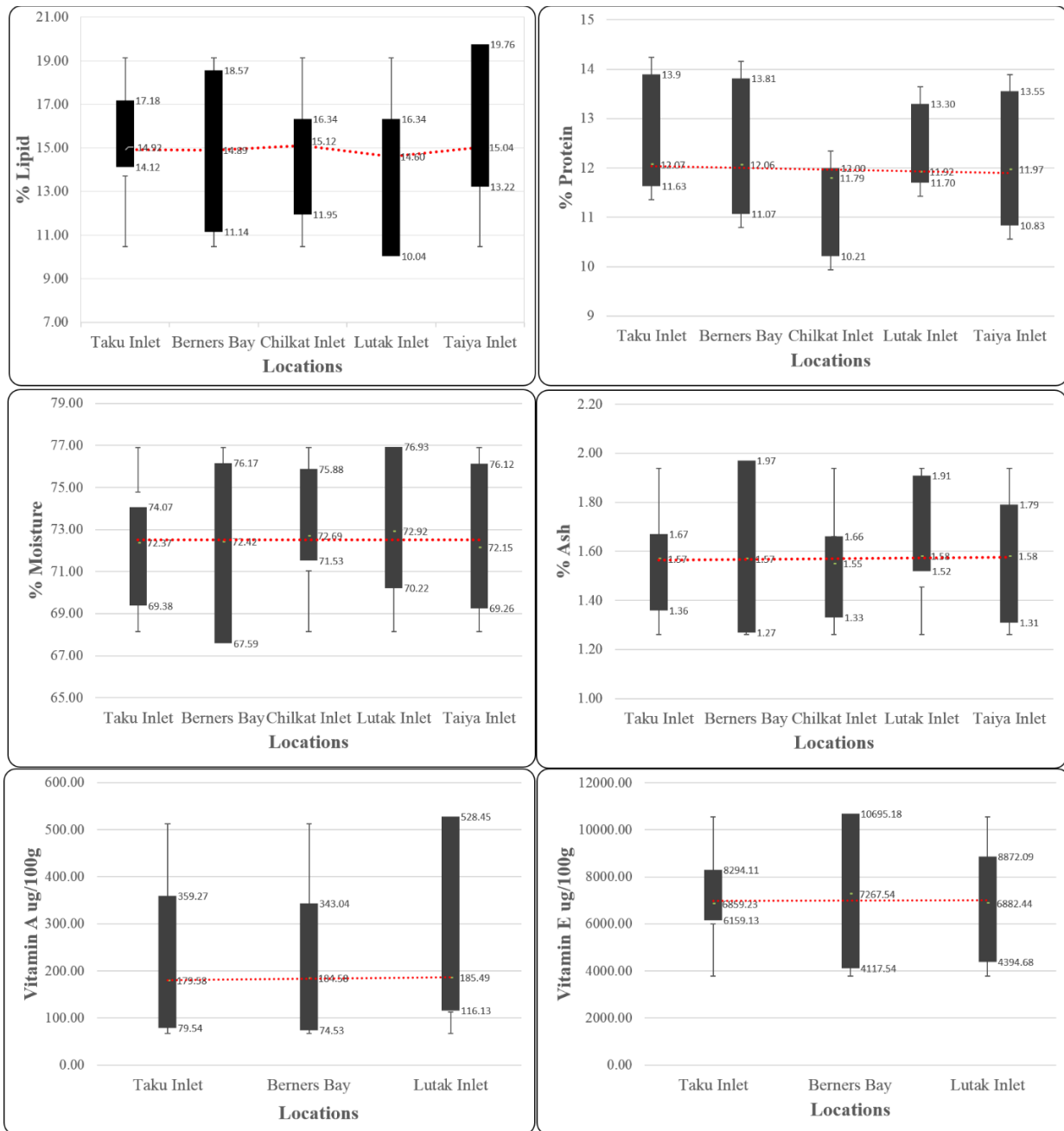Figure 6. -- Length frequencies for adult fish sampled, averaged for all five locations.

Figure 7. -- Nutritional (% lipid, protein, moisture and ash) and vitamins A and E (µg/100g) contents for eulachon by location, showing range with black bar, mean with red dotted line, and standard deviation with whiskers.

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