

*Exxon Valdez* Oil Spill  
Long-Term Monitoring Program “Gulf Watch Alaska” Final Report

A High Temporal and Spatial Resolution Study to Validate the Juvenile Herring Condition  
Monitoring Project

Restoration Project 13120111-M  
Final Report

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May 2020

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## Restoration Project 13120111-M Final Report

**Study History:** The work described in this report is part of the *Exxon Valdez* Oil Spill Trustee Council's Herring Research and Monitoring Program in Prince William Sound, Alaska. An overarching goal was to monitor the overwinter success of juvenile Pacific herring (*Clupea pallasii*) in Prince William Sound in an effort to understand why recruitment has remained at low levels. The herring population in Prince William Sound has only been fished commercially twice since it crashed in 1993. It is clear that recovery of the population will require recruitment of strong year classes, yet that has yet to occur. Previous work under the Sound Ecosystem Assessment Program (Project 98320) funded by the *Exxon Valdez* Trustee Council in the mid- to late-1990's indicated that winter was a distinct bottleneck to the survival of juvenile herring. As a result, the Herring Research and Monitoring Program focused much of its initial effort at understanding the processes regulating winter survival of juvenile herring. A conclusion that developed out of the Sound Ecosystem Assessment Program was that the energy content of juvenile herring prior to winter determined their success over winter. One study that contributed to this conclusion was a controlled examination of the starvation process in age-0 herring. This study revealed that survival by age-0 herring during a winter fast was associated with energy densities similar to those observed among wild herring in early spring. This result led to the idea that food was a limiting resource in winter. As part of the Herring Research and Monitoring Program, an Overwinter Model was developed (Restoration Project 10100132-C). This model predicted overwinter survival of a cohort of age-0 herring based on the idea that herring fasted in winter and lost energy at a predictable rate. However, the model was limited by the assumptions that fish were not foraging, and energy loss rates were constant and independent of fish length. The study reported here was initiated to better understand the implications of differing energy allocation strategies in fall for large and small juvenile herring in the context of overwinter survival. Thus, we explored how fish lose energy over winter and the extent of foraging during this time. Age-0 herring were sampled monthly over the course of 11 months to examine energy management strategies during winter.

**Abstract:** At high latitudes, cohort survival of forage fish during their first winter is critical to subsequent recruitment. An overwintering model (OWM) was developed to predict the survival of age-0 Pacific herring (*Clupea pallasii*) in Prince William Sound (PWS), Alaska. The OWM is based on data collected between 2007 and 2010 and predicts the distribution of energy densities of age-0 herring in March using a distribution observed in November. Here, we empirically test this model with field data and resolve the impacts of size, foraging, and energy reserves on the overwintering success of the 2011 cohort of age-0 herring in PWS. The OWM underestimated energy densities of smaller herring and overestimated energy densities of larger herring in March. These discrepancies are derived from differential foraging by different sized fish during winter. In early winter, large herring (>76 mm fork length) relied on stored energy and spent little time foraging, while small herring relied on foraging to meet their metabolic needs. Presumably, increased foraging by smaller sized herring led to increased predation risk. By winter's end, large herring were starving more than small herring because their absolute

requirement for food was three times greater. Due to their smaller required ration, small herring had a survival advantage in late winter when food supplies began increasing. These observations can be used to refine the OWM and improve its predictive skill. In addition, shifting survival advantages for different sized age-0 herring might explain the diversity of spawn times reported for Gulf of Alaska herring.

**Key words:** Bioenergetics, *Clupea pallasii*, Diet, Gulf of Alaska, Overwinter model, Pacific herring, Prince William Sound, Proximate composition, Recruitment, Ribonucleic acid/ deoxyribonucleic acid ratio, Stable isotopes, Survival, Whole body energy density.

**Project Data:** Project data include the sample collection information for juvenile herring from Simpson Bay in Prince William Sound (PWS), Alaska and additional samples from Cordova Harbor, Windy Bay and Alice Cove (all in the PWS region). At collection, samples were randomly allocated to either Prince William Sound Science Center or NOAA's Auke Bay Laboratories for further analysis. Data collected and retained by Prince William Sound Science Center included sampling date, location name, sampling location latitude and longitude, sampling gear, sample identification number, length, weight, energy density, and stable isotope ratios for carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ). Data collected and retained by Auke Bay Laboratories included sampling date, location name, sampling latitude and longitude, sampling gear, sample identification number, length, weight stomach content composition, percent lipid, percent protein, percent ash, and percent moisture content. In addition, both agencies retained quality assurance data for all analyses.

Data collected for the Herring Research and Monitoring Program projects that contributed to this report are available through the Alaska Ocean Observing System (AOOS) Gulf of Alaska data portal:

<http://portal.aos.org/gulf-of-alaska#metadata/4e73652c-858f-4a2a-9d0d-de53b62a27db/project>

<http://portal.aos.org/gulf-of-alaska#metadata/b7bb940a-391b-48fb-bcdd-11e5013d8c6c/project>

The Alaska Ocean Observing System data custodian is Carol Janzen, Alaska Ocean Observing System, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703, [janzen@aos.org](mailto:janzen@aos.org). The data may also be found through the DataONE earth and environmental data archive at <https://search.dataone.org/#data> and by selecting the Gulf of Alaska Data Portal under the Member Node filter.

### **Citation:**

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# A High Temporal and Spatial Resolution Study to Validate the Juvenile Herring Condition Monitoring Project

## EXECUTIVE SUMMARY

This project examines monthly changes in the energy content, diet, growth, body composition and ration size of age-0 Pacific herring (*Clupea pallasii*) from Simpson Bay, Prince William Sound, Alaska between August 2011 and June 2012. The project developed out of the need to understand the patterns of energy loss in age-0 herring in order to refine a model developed to predict over-winter survival (OWM). Previous studies of overwintering in juvenile herring conducted during the Sound Ecosystem Assessment (SEA) Program and the Herring Monitoring and Research Program have only examined herring at the beginning and end of winter (i.e., November and March). Those studies have shown that herring lose energy over winter, that winter mortality is size selective, and that small herring (length  $\leq 76$ ) allocate energy to growth while larger (length  $> 76$  mm) herring store energy prior to winter. Differences in the way that small and large herring provision themselves prior to winter is thought to account for size selective mortality during winter. However, it is unclear exactly how size influences overwintering behavior. The purpose of this study was to add temporal and spatial detail to these previous studies by collecting monthly observations of growth, nutritional condition and foraging behavior of small and large age-0 herring during the winter months.

Age-0 herring were collected monthly in the vicinity of Simpson Bay in order to characterize changes in their energy content, growth, diet, and consumption rates. In addition, we examined their proximate compositions to understand the mechanisms underlying the expected changes in energy content. Simpson Bay was chosen for continuity with historical observations made in previous studies and its accessibility from Cordova. We identified winter as the period when growth, measured using RNA/DNA, was minimized. Energy changes were documented using calorimetry, and proximate analysis was used to determine the contributions of lipid and protein to energy lost during the over winter period. Collection of the energy data allowed us to apply the OWM to determine if survival differed between small and large herring. Finally, we combined data describing energy, size, and stomach content analysis to model consumption rates using a bioenergetic model.

Results of our analyses indicated that small fish had nearly 50% more mortality than large fish and relied on foraging throughout the winter. Small fish gave up relatively little energy during the over-winter period, presumably by foraging throughout the winter. In contrast, large fish expended more energy and contributed greater amounts of lipid and protein to energy loss than small fish. Large fish were able to delay foraging until later in winter, but their expenditures of lipid and protein meant that they were nearer to starvation at the end of winter than the few remaining small fish. Ration sizes for large fish were nearly threefold those of small fish in late winter when food resources are minimal.

Small fish encounter significant impediments to their winter survival. When winter begins their small size imposes a greater risk of predation mortality and limits their ability to store energy. Consequently, their survival probability is minimal because they have few energy stores and their need to forage throughout winter exposes them to predation. Larger fish have a higher probability of survival than smaller fish at the start winter because their large size minimizes

predation risk, their energy stores reduce starvation risk to zero, and they spend less time foraging. However, in late winter, when food supplies are most scarce, large fish with depleted energy reserves must also forage to survive. While their size offers less predation risk than that of foraging small fish, the large fish must spend more time foraging owing to their larger rations. Thus, the differences in survival probability between small and large fish observed in early winter decrease by late winter.

## INTRODUCTION

Pacific herring (*Clupea pallasii*, hereafter herring) are a key component of the marine food web that characterizes the Prince William Sound (PWS) region of the Gulf of Alaska given the species role in energy transfer from lower to higher trophic levels. Herring are known to rely on a diversity of zooplankton species, which can vary throughout the life stages of herring as well as the annual cycle (Norcross et al. 2001). In turn, herring often constitute a major proportion of the diet consumed by higher trophic level predators such as other fishes, seabirds and marine mammals (e.g., Jodice et al. 2006, Womble and Sigler 2006, Sturdevant et al. 2012), indicating a role for the species in the maintenance of marine predator populations. In addition to their ecological importance, herring populations in PWS historically supported commercial fisheries and were of critical importance to local economies. However, herring populations were severely reduced following the grounding of the oil tanker, *Exxon Valdez*, on Bligh Reef in March of 1989 and the subsequent spill that covered over 2000 km of regional coastline. By 1993, PWS herring stocks were below minimum thresholds for the fishery (Thorne and Thomas 2008). However, the fishery reopened during the late 1990's, but has since been closed since 2000. To date, the herring population of PWS, Alaska remains an injured resource from the *Exxon Valdez* oil spill, as the population biomass has remained at ~20,000 mt since 1998 and continues below a level that would allow a commercial catch (Muradian et al. 2017).

Several hypotheses have been offered as to why PWS herring initially declined, in addition to why the population has not yet fully recovered since the late 1980's. Regarding the latter, research efforts have focused on factors that influence herring recruitment such as altered oceanographic conditions (both physical and biological), disease, and increased predation or competition. Additionally, juvenile over-winter energetics that influence survival and recruitment is also considered a potential limiting factor, and has therefore been the focus of past and ongoing research (e.g., Norcross et al. 2001, Gorman et al. 2018). Herring vary seasonally in their energetic content (Vollenweider et al. 2011, Gorman et al. 2018). Consistent observations from early studies by the Sound Ecosystem Assessment (SEA) Program, and more recent studies as part of the Herring Research and Monitoring (HRM) Program, demonstrated a decline in whole body energy density (Norcross et al. 2001, Gorman et al. 2018) and lipid stores (Heintz et al. 2018, Sewall et al. 2019) of age-0 herring between November and March at sites throughout PWS, in addition to observations of low winter survival (Brown and Norcross 2000, Norcross et al. 2001).

Previous attempts to characterize the overwintering process relied on the idea that food is a limiting resource in winter. Seasonal zooplankton sampling in PWS revealed a decrease in both the biomass and number of potential prey during winter (Foy and Paul 1999, Cooney et al. 2001, McKinstry and Campbell 2018). This prompted Paul and Paul (1998) to conduct laboratory studies on young herring to estimate energy loss rates during starvation. The energy density of

survivors at the end of the experiment was similar to that of herring collected from Resurrection Bay, Alaska in spring, supporting the idea that energy loss during winter was a critical component of early life history (Norcross et al. 2001) and that cohort survival probabilities could be predicted from their energy densities.

The idea that juvenile herring lose energy over winter was used as a basis for a model to predict over-winter survival of age-0 herring cohorts in PWS (Kline 2013). The underlying concept of this overwinter model (OWM) was to decrease the frequency distribution of energy densities observed in November by a daily energy loss rate to predict the frequency distribution of energy densities in March. Resulting energy densities below a critical value are assigned a probability of survival and the proportion of fish surviving from the November distribution can be estimated. The model distributions combined data collected over the winters of 2007 to 2010 and estimated a substantial overwinter mortality at 52% and daily energy loss at 19 J/g per day, which is 4 J/g per day less than observed in laboratory starvation studies (Paul and Paul 1998). In addition, field observations and model predictions included fish with energy densities below the critical values for survival observed in the laboratory studies. The difference in daily energy density decrease observed in the lab, and that predicted by the model, was attributed to herring foraging during winter. Foraging was also hypothesized to account for the ability of fish to maintain energy densities below the critical levels reported in the starvation study, which is in direct conflict with the underlying assumption of the OWM that fish are starving through the winter.

One way of testing the OWM's assumption of starvation would be to examine fish protein and lipid content, in addition to their energy content to assess the extent of starvation in the population. This is due to the fact that the starvation process in fish proceeds in three stages. Initially, both protein and lipid are catabolized to meet metabolic needs in the first few days of anorexia. As anorexia continues, lipid becomes the primary energy source. Eventually, lipid catabolism drives lipid levels to a critical level and fish begin catabolizing protein (Bar and Volkoff 2012). Consequently, a starving fish has minimal amounts of lipid and reduced amounts of protein in their tissues compared with those fish that have foraged.

Any analysis of the relationship between winter energy loss and survival requires an understanding of the role of body size in the relationship. At high latitudes, autumnal energy allocation strategies are driven by fish size and depend on the selective pressures of predation and starvation (Post and Parkinson 2001). Small fish are susceptible to predation by a wide range of gape limited predators and selection favors individuals that channel surplus energy into growth. Once fish become sufficiently large the risk of predation is outweighed by the risk of winter starvation and selection favors individuals that store energy in the form of lipid (Biro et al. 2005, Siddon et al. 2013, Sewall et al. 2019). Examination of the trade-offs between growth and energy storage in herring from PWS over seven years indicated that the critical size for shifting energy allocation strategies occurs at 76 mm (Sewall et al. 2019).

The risk of starvation increases during winter, when prey resources decline to a minimum in January (McKinstry and Campbell 2018) or February (Cooney et al. 2001). At some point prey density declines to a level at which a predator's energy intake is exceeded by its metabolic demands. The prey resource density at which energy intake and need are exactly balanced has been termed the critical resource density (CRD) (Bystrom et al. 2006). The CRD increases exponentially with size so that the difference between winter prey densities and CRD is greatest for larger fish. Larger fish are more able to rely on stored energy to meet these deficits than

smaller fish because larger fish store more energy prior to winter (Sewall et al. 2019). At the same time, smaller fish maintain a significant risk of predation-related mortality but are better able to maintain their body composition because of the allometry with CRD. Winter mortality may therefore depend on where fish exist along a continuum of risks with predation mortality on one end and starvation on the other.

The purpose of this study was to investigate the generally poorly understood overwinter nutritional ecology of marine fish and inform the OWM model by developing a more comprehensive understanding the trade-offs between size, foraging, and energy loss during winter for PWS herring. Here, we test OWM model predictions and expand on previous studies by sampling monthly between September and June near Simpson Bay, PWS, Alaska. Simpson Bay was chosen for continuity with historical observations made in previous studies. For this study, winter is defined as the period when growth is minimized as indicated by monthly estimates of RNA/DNA ratios (Sewall et al. 2019). We interpret these minima as an indication of the period in which energy intake is insufficient for fish to maintain surpluses or grow. The study examines predictions made by the OWM model, energy loss as a function of size, and how size influences winter foraging. In particular, we examine the idea that large fish are more prone to starvation when prey are sufficient to support only small fish by relating overwinter losses of lipid and protein to fish size. This analysis is based on observations that during the final stage of starvation fish catabolize protein to meet their energy needs because lipid reserves have been depleted (McCue 2010). We use bioenergetic modeling to estimate monthly ration sizes necessary for fish to maintain their mass over winter and use isotopic analysis to determine if fish alter their foraging behavior in winter in an effort to minimize the difference between energy intake and CRD. Finally, we collected samples from multiple sites in Simpson Bay to determine if our analyses can be generalized over broader spatial scales.

## **OBJECTIVES**

1. Use seasonal measures of RNA/DNA to identify the period when growth is minimized for both size groups.
2. Measure seasonal changes in energy density to document energy loss as a function of size.
3. Apply the OWM to contrast the survival of small and large fish overwinter.
4. Use carbon and nitrogen stable isotope ratios to determine if fish fed over winter.
5. Model the daily rations required for small and large fish to identify the extent to which foraging is important for small and large fish.

## **METHODS**

### **Field sampling**

Juvenile herring, principally age-0, were collected monthly by cast net and gill net during 23 separate sampling events between August 2011 and June 2012 within the vicinity of Simpson Bay, PWS, Alaska (Table 1, Figures 1a, b and 2). Whereas most of the samples were collected

using a cast net, including gill net samples in our analyses increased the study's temporal scope. Collected herring were randomly divided and packaged for three analyses: (1) diseases (not reported here); (2) whole body energy density (WBED) derived from dry/wet mass and carbon (C)/nitrogen (N) atomic ratios, as well as stable carbon ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ,  $\delta^{15}\text{N}$ ) isotope values of herring samples processed at Prince William Sound Science Center (PWSSC, Cordova, Alaska) and the Alaska Stable Isotope Facility at the University of Alaska Fairbanks (Fairbanks, Alaska); and (3) RNA/DNA ratios, proximate and diet composition of herring samples processed at NOAA Auke Bay Laboratories (ABL, Juneau, Alaska). Samples were frozen in the field and transported to PWSSC or ABL for preparation and/or analysis. Because of close proximity, samples obtained from Simpson Bay proper were aggregated into two repeatedly sampled sites: (1) West Arm and (2) East Arm (Figure 1b). West Arm, the most frequently sampled site during the observation period, is also where juvenile herring have been successfully sampled at other times (see Gorman et al. 2018). In addition, samples were collected from Alice Cove, Windy Bay and Cordova Harbor (Table 1). These latter three locations were subsequently removed from any further analysis because sampling in these locations was only sporadic.

### **Sample processing for energy density and isotopic analysis**

In the laboratory, frozen juvenile herring were thawed and wet mass (mg) was obtained using an analytical balance (Mettler). Length of each fish was measured to the nearest mm. Fish were oven-dried (60 °C) and the final dry mass (mg) recorded. Dried herring were ground to a fine powder using a ball mill. Approximately 0.1–0.2 mg from each powdered herring was loaded into a tin capsule and subsequently sent to the Alaska Stable Isotope Facility where C and N mass spectrometric analyses were performed. Resultant data for juvenile herring included %C, %N,  $^{13}\text{C}/^{12}\text{C}$ , and  $^{15}\text{N}/^{14}\text{N}$  with the heavy to light isotope ratios reported using delta notation,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, calculated using the following equation:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = ([\text{R}_{\text{sample}}/\text{R}_{\text{standard}}]-1) \times 1000$ , where  $\text{R}_{\text{sample}}$  is the ratio of the heavy to light isotope for either  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ , and  $\text{R}_{\text{standard}}$  is the heavy to light isotope ratios for international standards using Vienna PeeDee Belemnite for carbon and atmospheric  $\text{N}_2$  (Air) for nitrogen.

Table 1. Sampling events, dates, locations, and the number of small ( $\leq 76$  mm) and large ( $> 76$  mm) age-0 herring captured using cast net and gill net gear, and processed for further analysis at either Prince William Sound Science Center (PWSSC) or NOAA's Auke Bay Laboratory (ABL), as part of the juvenile herring intensives study in Prince William Sound, Alaska (2011-2012).

Event	Year	Month	Day	Location1	Location2	Latitude	Longitude	PWSSC Cast Total	PWSSC Cast Small	PWSSC Cast Large	PWSSC Gill Total	PWSSC Gill Small	PWSSC Gill Large	ABL Cast Total	ABL Cast Small	ABL Cast Large	ABL Gill Total	ABL Gill Small	ABL Gill Large	ABL Cast & Gill Total	ABL Cast & Gill Small	ABL Cast & Gill Large	Notes
1	2011	8	27	Sirnpson Bay							10												Not used in analysis
2	2011	9	11	Sirnpson Bay	East Arm	60.63818	-145.8698							20	18	3							
3	2011	9	11	Sirnpson Bay	West Arm	60.6791	-145.8794	35	33	2				20	19	1							
4	2011	9	12	Sirnpson Bay	Alice Cove	60.61263	-145.9858	49						20									Not used in analysis
5	2011	10	10	Condova Harbor		60.54694	-145.7649	35															Not used in analysis
6	2011	10	13	Sirnpson Bay	West Arm	60.6793	-145.8799	50	50	0				20	20	0							
7	2011	11	14	Windy Bay		60.56933	-146.1315	2															Not used in analysis
8	2011	11	14	Condova Harbor		60.54694	-145.7649	45															Not used in analysis
9	2011	11	15	Sirnpson Bay	West Arm	60.67692	-145.8585	91	87	4				10	10	0							
10	2011	11	15	Sirnpson Bay	West Arm	60.6609	-145.8798	28			25	0	25					10	0	10			
11	2011	11	16	Sirnpson Bay	East Arm	60.64258	-145.8391	33	33	0				20	20	0							
12	2011	12	13	Sirnpson Bay	West Arm	60.67783	-145.8787	62	62	0				20	20	0							
13	2012	1	11	Sirnpson Bay	West Arm	60.67775	-145.8787	51	51	0				20	20	0							
14	2012	2	17	Sirnpson Bay	West Arm	60.67783	-145.8792	17	12	5				16	16	0							
15	2012	3	15	Sirnpson Bay	West Arm	60.67783	-145.88				8	0	8										
16	2012	3	16	Sirnpson Bay	East Arm	60.64383	-145.8452	1	1	0													
17	2012	3	16	Sirnpson Bay	West Arm	60.6715	-145.8658				1	0	1										
18	2012	3	18	Sirnpson Bay	West Arm	60.6771	-145.8572	36	6	30				10	3	7							
19	2012	3	18	Sirnpson Bay	West Arm	60.6767	-145.8619											10	0	10			
20	2012	4	20	Sirnpson Bay	West Arm	60.68033	-145.8805	145	72	73													
21	2012	5	17	Sirnpson Bay	West Arm	60.68017	-145.8805				30	11	19							20	5	15	
22	2012	6	16	Sirnpson Bay	West Arm	60.67127	-145.8894	35	1	34				20	0	10							
23	2012	6	16	Sirnpson Bay	West Arm	60.67133	-145.8805				5	0	5										

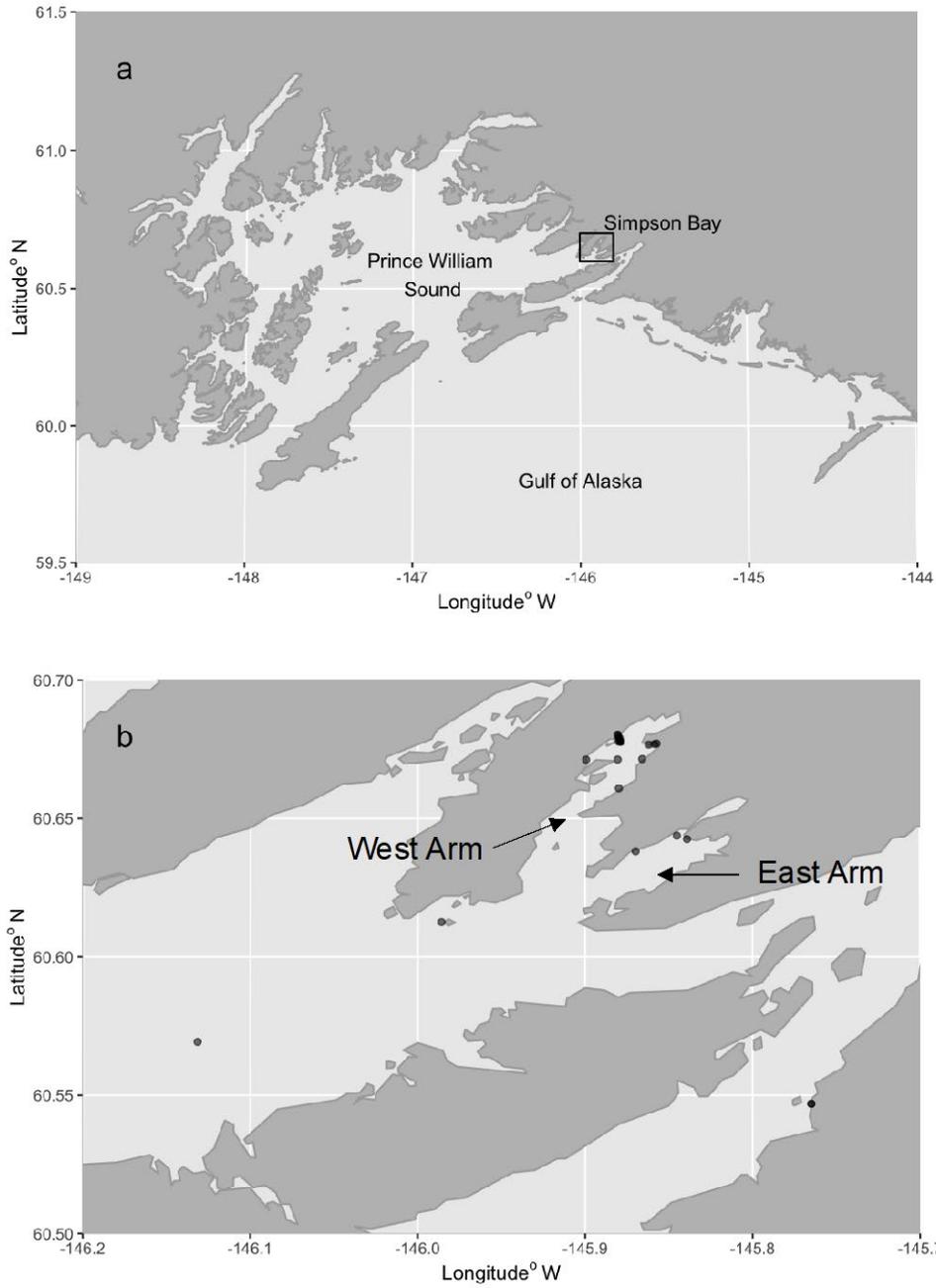


Figure 1. (a) Simpson Bay study site located in eastern Prince William Sound, Alaska. (b) All sampling locations between September 2011 and June 2012.

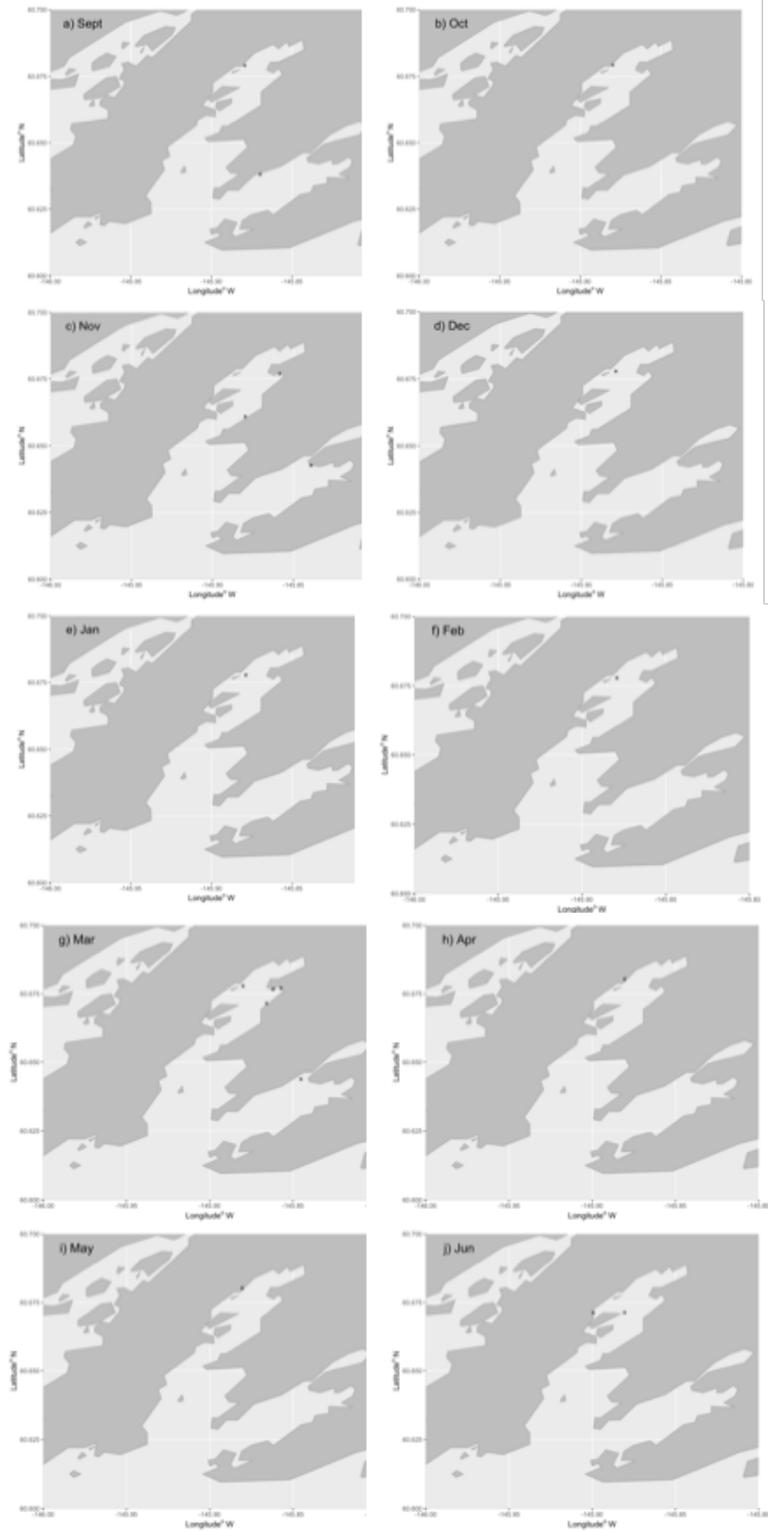


Figure 2. (a-j) Monthly sampling locations in Simpson Bay, Prince William Sound, Alaska for data used in analysis.

Percent C and N data were used to ascertain C/N atomic ratios. The ratios of dry to wet mass and C to N atoms were used to determine WBED based on relationships derived from Paul et al. (2001) and refined by Kline (2013) using the following equation: WBED (kJ/g wet mass) =  $-2.90242 + 32.585 \times (\text{dry/wet mass ratio}) + 0.103514 \times \text{C/N atom ratio}$  (see also Kline and Campbell 2010). Raw  $\delta^{13}\text{C}$  data were mathematically corrected for lipid content using the method of McConnaughey and McRoy (1979), see also Kline and Campbell (2010), which allows for comparisons of fish with differing fat contents due to the fact that lipids typically have lower  $^{13}\text{C}/^{12}\text{C}$  ratios than protein or carbohydrate. Lipid-corrected values of  $\delta^{13}\text{C}$  are hereafter reported as  $\delta^{13}\text{C}'$ . See Table 2 for a summary of samples processed for energy density and isotopic analysis. A semi-micro calorimeter (model 6725, Parr Instrument Company, Moline, IL) was used to perform bomb calorimetry on a subset (~10%) of dried herring samples analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotopes to ground-truth energy density estimates from dry/wet and C/N ratios. It was expected that estimates of WBED derived from dry/wet and C/N ratios would tightly correlate with energy density estimates derived from bomb calorimetry as has been shown for similar juvenile herring datasets (see Gorman et al. 2018).

### **Sample processing for RNA/DNA, diet, and proximate analysis**

Frozen juvenile herring were sent to ABL for length and weight measurements, diet assessment, and chemical analysis (see Table 2 for summary). Individual fish were measured for length and wet mass. While still frozen, a 10-15 mg biopsy of white dorsal muscle was removed for RNA/DNA analysis, which represented <1% of total fish body mass. Fish stomachs were removed, and the contents were weighed and stored in formalin. The stomach was then replaced with the frozen carcass.

We used RNA/DNA ratios as a relative growth rate index of individual fish. This ratio has previously been used as an index of juvenile herring growth (e.g., Bernreuther et al. 2013), with higher RNA/DNA indicating a higher rate of protein synthesis (Buckley 1984). Herring down regulate RNA/DNA ratios in winter (Sewall et al. 2019), we use it only as a relative index of growth rate. The RNA and DNA concentrations in 10–15 mg of dorsal white muscle tissue were determined using the fluorometric dye-binding assay of Caldarone et al. (2001) as adapted by Sreenivasan (2011). Ratios of RNA/DNA in known quantities of standard materials, calf liver 18s + 28s ribosomal RNA and calf thymus DNA (Sigma), were used for quality control to verify RNA/DNA analysis.

The lipid content of herring was used as a measure of stored energy. After muscle plugs and stomach contents were removed, individual juvenile herring were homogenized with a mortar and pestle. A 1.5 g sample of the homogenate was used to assess the mass of lipid as a percentage of wet tissue mass following procedures described in Vollenweider et al. (2011). Briefly, lipids were extracted using a modified Folch method (Folch et al. 1957) with a Dionex 200 Accelerated Solvent Extractor, followed by drying and weighing lipid extracts. National Institute for Standards and Technology (NIST) reference materials were used for quality control to verify lipid analysis. The remaining homogenates were analyzed for moisture, ash and protein. Ash weight was only determined for homogenates with sufficient mass remaining.

Table 2. Number of small and large age-0 herring processed for body composition at NOAA Auke Bay Labs and their monthly average  $\pm$  1 SD length, weight, lipid, protein, moisture and ash. Analysis for ash was only done when sufficient material remained following other assays. Only 1 large fish was processed for lipid in September.

	Number Processed	Length (mm)	Weight (g)	% Lipid	% Protein	% Moisture	% Ash
<b>SMALL <math>\leq</math> 76 mm</b>							
Sept 11-12	37	51.6 $\pm$ 8.9	1.3 $\pm$ 0.8	2.3 $\pm$ 0.5	15.3 $\pm$ 0.7	80.2 $\pm$ 1.0	2.9 $\pm$ 0.3
Oct 13	20	51.1 $\pm$ 4.1	1.3 $\pm$ 0.3	2.5 $\pm$ 0.6	14.2 $\pm$ 0.4	79.5 $\pm$ 0.8	
Nov 15-16	30	57.9 $\pm$ 3.3	1.6 $\pm$ 0.3	4.0 $\pm$ 0.8	14.8 $\pm$ 0.4	77.4 $\pm$ 1.0	
Dec 13	20	55.0 $\pm$ 3.4	1.4 $\pm$ 0.2	3.0 $\pm$ 0.5	14.7 $\pm$ 0.7	79.7 $\pm$ 1.3	
Jan 11	19	55.1 $\pm$ 2.5	1.4 $\pm$ 0.2	2.4 $\pm$ 0.6	14.5 $\pm$ 0.7	80.2 $\pm$ 0.9	
Feb 17	16	59.9 $\pm$ 5.7	1.7 $\pm$ 0.5	2.0 $\pm$ 0.3	13.9 $\pm$ 0.7	81.3 $\pm$ 0.9	
Mar 15-18	3	69.0 $\pm$ 6.6	2.7 $\pm$ 0.5	1.2 $\pm$ 0.2	14.5 $\pm$ 0.8	81.2 $\pm$ 1.1	3.8 $\pm$ NA
Apr 20	5						
May 17	0	57.2 $\pm$ 14.7	2.3 $\pm$ 1.3	4.7 $\pm$ 1.7	15.8 $\pm$ 0.5	78.1 $\pm$ 1.6	2.7 $\pm$ 0.1
<b>LARGE 77- 115 mm</b>							
Sept 11-12	3	86.0 $\pm$ 5.2	5.9 $\pm$ 1.0	3.7 $\pm$ NA	16.3 $\pm$ 0.7	76.9 $\pm$ 1.6	3.1 $\pm$ 0.1
Oct 13	0						
Nov 15-16	10	97.1 $\pm$ 5.2	7.7 $\pm$ 1.9	7.0 $\pm$ 1.6	16.4 $\pm$ 0.6	74.7 $\pm$ 1.0	3.0 $\pm$ 0.2
Dec 13	0						
Jan 11	0						
Feb 17	0						
Mar 15-18	17	93.7 $\pm$ 6.5	6.5 $\pm$ 1.4	1.9 $\pm$ 1.0	14.1 $\pm$ 0.5	81.1 $\pm$ 1.4	3.9 $\pm$ 0.2
Apr 20	0						
May 17	15	94.6 $\pm$ 11.2	8.6 $\pm$ 3.0	4.6 $\pm$ 2.7	15.6 $\pm$ 0.5	78.2 $\pm$ 2.2	2.7 $\pm$ 0.2

Moisture and ash content were determined using a Leco TGA-601 thermogravimetric analyzer. Approximately 2.5 g of wet homogenate was heated from ambient to 135 °C over 6 minutes and then held until repeated measurements of the mass varied by less than 5%. Afterwards, the temperature was increased to 600 °C and held until repeated measurements stabilized. Moisture content was estimated as the difference between the initial wet mass and the stable dry mass at 135 °C. Ash content was estimated as the stabilized mass observed at 600 °C. Quality assurance for moisture and ash analyses included blank, duplicated samples to assess cleanliness and precision. A National Institutes of Status and Trends standard reference material (SRM 1546) was used to assess accuracy of moisture and ash. If the initial sample mass was less than 2.5 g, ash weight was not determined.

Protein content was estimated from the total nitrogen content observed in a 0.1 g sample of dried homogenate. Samples were dried in the thermogravimetric analyzer and collected prior to ashing. Nitrogen content of the sample was measured with a LECO FP 528 Nitrogen Analyzer following methods by Dumas et al. (1971) in which the homogenate was combusted at 850 °C and the expelled nitrogen measured by thermal conductivity. Total mass of protein was estimated by multiplying the total nitrogen content by 6.25 (Jones 1931). The instrument was calibrated daily using ethylenediaminetetraacetic acid (EDTA). Quality assurance samples included with each

batch of 17 samples included a blank reference consisting of pure cane sugar, and a NIST standard reference material (SRM1546). In addition, all analyses were duplicated to ensure the coefficients of variation for estimated nitrogen content was less than 15%.

Stomach contents of herring were analyzed to assess diet influences on herring growth and energy storage, and as an indicator of foraging activity. Stomach contents were removed from frozen herring individually and weighed prior to chemical analysis of herring (see above). Following removal of contents, empty stomachs were returned to fish carcasses for tissue homogenization and lipid analysis.

### **Data analysis**

For statistical analyses many response variables were examined relative to size. For the purposes of presenting results, “small” fish represent those  $\leq 76$  mm fork length and fish  $\leq 115$  mm were considered “large”. This designation between small and large fish is the size at which age-0 herring in PWS transition energy allocation strategies (Sewall et al. 2019). Fish below that 76 mm threshold allocated greater amounts of energy towards growth in November. In contrast fish greater than 76 mm allocated progressively more energy towards storage during this time. Fish greater than 115 mm may include age-1 individuals and therefore excluded from this study.

#### *RNA/DNA*

The goal of the RNA/DNA analysis by month was to determine the period in which growth was minimized. Sewall et al. (2019) noted a nonlinear relationship between length and RNA/DNA in November and March in a previous study. Consequently, we examined monthly RNA/DNA values through ANCOVA with length as a covariate nested within size (small or large) and month as a fixed main factor. Tukey’s method was used to conduct pairwise comparisons of RNA/DNA values between months in order identify months with the overall lowest values.

#### *Energy density*

Parameters from the OWM model were used to predict the March energy density frequency distribution based on November observations, following Kline’s method (2013). The predicted March distribution was compared with the observed distribution in March using quantile-quantile plots (Sokal and Rohlf 1995). Energy densities were rounded to the nearest tenth of a kJ and a cumulative percentage of fish represented by each energy density between 0.9 kJ/g and 4.8 kJ/g was calculated for observed and predicted distributions. The observed and predicted cumulative percentages for each energy density were plotted and compared to a line with slope equal to 1, the expectation if both distributions were equivalent. Overwinter survival was estimated by dividing the number of fish in the predicted March distribution by the number in the November distribution.

Temporal variation in energy density was examined by ANCOVA. Exploratory plots were first produced to characterize monthly data on the length and energy density of the juveniles for the entire time series (September to June). To formalize these relationships, least-squares general linear models were used to explain variation in energy density among the months from November to March. Explanatory variables included main effects for fish length (fork length), month, and an interaction term for length and month. A backwards regression selection process was used to remove non-significant ( $p > 0.05$ ) explanatory variables from the full model. The

final model included only significant parameters. Pairwise comparisons were then conducted using Tukey's method to identify months that differed.

Spatial variation in energy density was examined by comparing the energy densities of fish collected during the same month from different sampling sites (Figure 2). Using a similar approach to the temporal analysis, least-squares general linear models were used with explanatory variables including main effects for fish length (fork length), site, and an interaction between these variables. A backwards regression selection process was used to remove non-significant ( $p > 0.05$ ) explanatory variables from the full model. The final model included only significant parameters. Pairwise comparisons were then conducted using Tukey's method following the analysis to identify sites that differed.

#### *Size and starvation risk*

We evaluated the relationship between size and starvation potential by estimating total energy lost as a function of length and portioning that loss into contributions from lipid and protein. We assumed fish that contributed a greater proportion of protein to their overall energy loss were those approaching starvation. We relied on the relationship between energy and length to construct energy budgets for herring in each month between November and March following Hurst and Conover (2003). The total energy derived from lipid for each fish was estimated as the product of the percent lipid, wet mass, and the caloric equivalent for lipid (36.43 kJ/g lipid, Groot et al. 1995). Similar quantities were estimated for protein using a caloric equivalent of 23.84 kJ/g (Groot et al. 1995). Total energy content for each fish was estimated from the sum of the energy derived from lipid and protein. An ANCOVA was used to describe the relationship between length and energy content for each month. Factors included fish length, month and their interaction. Data were transformed by natural logs and models were fit for total energy content, energy derived from lipid and energy derived from protein. The total energy content of the fish was compared across months using pairwise contrasts using Bonferroni corrections. The model coefficients were used to generate 95% confidence intervals for the estimates of total energy, and point estimates for lipid and protein energy for fish ranging between 40 mm to 110 mm fork length for each of the months. Lengths whose 95% confidence intervals did not overlap between months were assumed to have experienced statistically significant energy loss. The percent contribution of protein to that total energy loss was calculated as the change in protein energy between the initial and final months divided by the change in total energy content over the same interval. A similar analysis was conducted for lipid.

#### *Diets and consumption rates*

The diets of fish retained for proximate analysis were examined to identify prey, estimate the total mass consumed, and estimate dietary energy density for each fish. Stomach contents were removed from frozen fish, prey items were identified to the lowest taxon practical and counted. Counts were multiplied by published values for their fresh wet mass to estimate the total mass contribution of each prey item to the total fresh mass consumed. These mass contributions were multiplied by published values of prey energy densities to estimate the energy density of each fish's diet. Prey masses, energy densities and aggregated prey categories were taken from Foy and Norcross (1999) to provide comparability with previous work.

We modeled the consumption rates of fish from different size classes to determine how size influenced foraging over winter. We used FishBioenergetics v 4.0 (Deslauriers et al. 2017) to

estimate of the proportion ( $p$ ) of the maximum possible ration ( $C_{max}$ ) consumed each month and total mass consumed during winter, based on the assumption that fish lengths were constant over winter (Sewall et al. 2019). Values for  $C_{max}$  depend on fish size and water temperature (Deslauriers et al. 2017). Parameters associated with consumption, respiration, specific dynamic action, and egestion were those described previously by Rudstam (1988) for age-0 Atlantic herring (*Clupea harengus*). The activity coefficient was held constant at 1.0, which allowed for comparing different size groups by assuming they move the same amount. Estimates were derived for the intervals spanning November to December, December to January, January to February, and February to March for fish of lengths 45, 55, 65, 75, 85, 95 and 105 mm.

Each model run consisted of a specific time interval for a fish with a given length and required five additional variables: temperature, herring energy density, dietary energy density, initial weight and final weight. Herring energy densities were modeled for each day of the winter by estimating the energy density loss rate for all fish over each time interval. Loss rates were determined from  $ED_f = ED_i e^{-rt}$  where  $ED_f$  is the average energy density at the end of the interval and  $ED_i$  is the average energy density at the beginning of the interval,  $r$  is the loss rate, and  $t$  is the number of days in the interval. Energy density for the intervening days was estimated for each day so that the  $ED_i$  and  $ED_f$  were equal to the values observed on the mean date of capture in each month.

The initial and final weights each month for fish from each length category were predicted from an ANCOVA using the observed lengths and wet masses of fish collected each month. The ANCOVA used months as the main fixed factor, length as a covariate and included their interaction. Comparisons of lengths between months were used to examine our assumption that length is constant over winter. Lengths and weights were transformed using natural logarithms. Temperatures were taken from the tide gauging station in Cordova, Alaska. Estimates of  $p$  and the total mass consumed were compared by ANOVA with size class, month, and their interaction as the main effects. The model considered fish of 45, 55, and 65 mm as “small” and fish 85, 95 and 105 mm to be “large”. Fish 75 mm were excluded from the analysis because they are approximately equal in size to fish at the threshold between small and large fish.

#### *Stable isotope variability*

Similar to WBED, exploratory plots were produced to characterize monthly data on the size of juvenile herring (fish length) and  $\delta^{13}C'$  or  $\delta^{15}N$  values of the fish tissue homogenate for the entire time series. Least-squares general linear models were used to explain variation in  $\delta^{13}C'$  or  $\delta^{15}N$  values between the months of November to March. Explanatory parameters for both  $\delta^{13}C'$  or  $\delta^{15}N$  analyses included main effects for fish length (fork length), winter month of collection, and an interaction between fish length and winter month of collection. A backwards regression selection process was used to remove non-significant ( $p > 0.05$ ) explanatory variables from the full model. The final model included only significant parameters.

Exploratory plots were produced to characterize the monthly  $\delta^{13}C'$  or  $\delta^{15}N$  isotopic area occupied by juvenile herring during the entire time series in an effort to determine if foraging behavior changed as a function of size. To formalize these relationships, stable isotope Bayesian ellipse areas were calculated for each month using the SIBER package (Jackson et al. 2011) in the R language environment (R Core Team 2019). Within a Bayesian framework, SIBER functions model probability distributions for stable isotope ellipse area by incorporating sources of

variability such as that within the originally derived means of monthly groups. Bayesian models ran for 2 million iterations with a burn-in of 50,000 and was thinned by 15, resulting in 130,000 posterior draws.

## **RESULTS**

### **Months and catch**

Catches were higher during warmer months. Both large and small fish were caught in cast nets between September 2011 and June 2012 (Table 1). Initially, small fish were caught in large numbers, but became relatively scarce in February and were outnumbered by large fish beginning in March. In contrast, large fish were relatively rare prior to winter, absent from cast nets in January and nearly absent in February. Gill nets were used less often than cast nets and caught greater numbers of large fish whenever they were deployed. In order to maximize sample sizes for large fish both gears were combined for later analyses, consistent with the approach used in the OWM.

### **RNA/DNA**

RNA/DNA values indicated that growth was minimized between November and March, leading us to conclude that these months defined winter. The interaction terms involving size class and month could not be examined due to the absence of large fish in some months. However, RNA/DNA values were significantly affected by length within size classes, size class, and month of sampling ( $F > 3.87$ ,  $p < 0.036$ ). The mean RNA/DNA values by month decreased from a high of 15.8 in May to 5.24 in March. No difference was detected among the monthly averages observed in November, December, January, February and March in either size class (Figure 3). However, there was some evidence of an increase in March for small fish. RNA/DNA ratios increased again in May and June. No April samples were available for analysis.

### **Energy density**

The OWM predicted reduced survival for small ( $\leq 76$  mm) herring relative to that of large ( $>76$  mm) herring. Small fish had an estimated 39.1% survival compared to 60.7% for the large fish. However, the energy density distributions predicted from the OWM model differed from the distribution observed in March (Figure 4). The OWM model underestimated the energy densities of the large fish ( $>76$  mm) in March and overestimated the energy densities of the small. For example, the lowest energy density observed for large fish ( $>76$  mm) in March was 2.7 kJ/g, but the model predicted energy densities would decline to a minimum of 2.5 kJ/g indicating energy loss rates for large fish were less than those used by the model. Conversely, the lowest energy density observed among small fish was 3.0 kJ/g but the model predicted a minimum of 2.4 kJ/g in March indicating energy loss rates used by the model exceeded those experienced by small fish. The model predicted an average energy density of 3.2 kJ/g compared with an observed value of 3.1 kJ/g for the large fish in March. Small fish averaged 3.3 kJ/g in March (Table 3) compared with a predicted average of 2.9 kJ/g. When both large and small fish frequency distributions were combined the fit between the observed and predicted distributions was closer, but the model still overestimated the proportion of fish with low energy densities and underestimated the proportion with high energy densities (Figure 4). Examination of Table 3 indicates the decrease in energy density averaged 12 J/g per day during the 124-day period for

small fish and 19 J/g per day for large fish between November and March compared to 19 J/g per day used in the model.

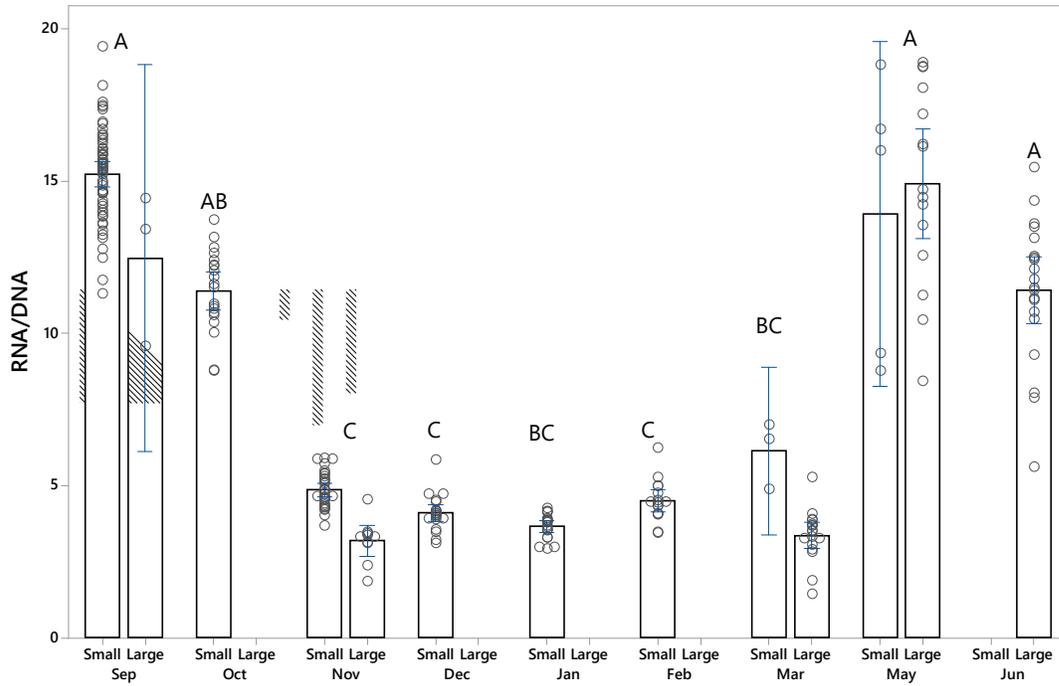


Figure 3. Average ( $\pm$  95% confidence interval) RNA/DNA ratios for small ( $\leq 76$  mm) and large ( $>76$  mm – 115 mm) Pacific herring during different months of sampling. Small fish are shown in open bars, large fish are shown as shaded bars, missing bars indicate no fish were caught during that period. Letters above bars show statistically similar months ( $p > 0.05$ ) based on pairwise comparisons, note the analysis used length as a covariate and the figure shows averages for size categories open circles show observed data values.

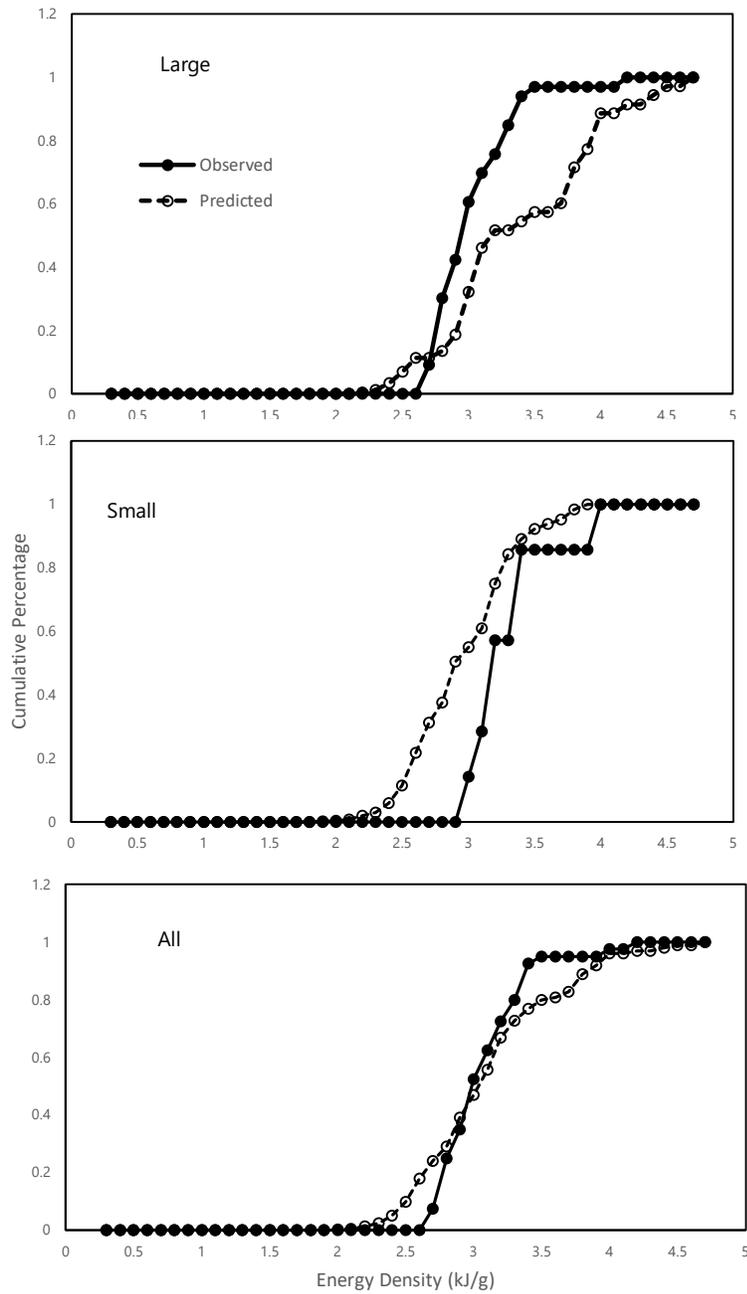


Figure 4. Cumulative frequency distributions for the observed (closed symbols) energy densities and those predicted (open symbols) by the OWM for large age-0 Pacific herring (length > 76 mm) (top panel), small age-0 herring (length < 76 mm), and all fish combined.

Table 3. Number of small and large juvenile herring processed at Prince William Sound Science Center and their monthly average length, weight, whole body energy density (WBED, kJ/g wet mass), stable carbon ( $\delta^{13}\text{C}$ ), and stable nitrogen ( $\delta^{15}\text{N}$ ) isotope values  $\pm 1$  SD.

	Number Processed	Fork Length (mm)	Weight (g)	WBED (kJ/g wet mass)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
<b>SMALL <math>\leq 76</math> mm</b>						
Sept 11-12	33	52.9 $\pm$ 8.5	1.3 $\pm$ 0.8	3.6 $\pm$ 0.5	-18.2 $\pm$ 0.2	10.9 $\pm$ 0.2
Oct 13	50	51.4 $\pm$ 3.6	1.2 $\pm$ 0.3	3.9 $\pm$ 0.4	-18.0 $\pm$ 0.3	11.7 $\pm$ 0.2
Nov 15-16	124	58.0 $\pm$ 4.5	1.6 $\pm$ 0.5	4.8 $\pm$ 0.5	-18.4 $\pm$ 0.3	12.2 $\pm$ 0.3
Dec 13	62	57.4 $\pm$ 4.5	1.5 $\pm$ 0.4	3.6 $\pm$ 0.5	-18.0 $\pm$ 0.3	12.5 $\pm$ 0.3
Jan 11	51	57.2 $\pm$ 2.5	1.3 $\pm$ 0.2	3.3 $\pm$ 0.3	-17.9 $\pm$ 0.3	12.4 $\pm$ 0.2
Feb 17	12	59.6 $\pm$ 3.2	1.4 $\pm$ 0.3	3.2 $\pm$ 0.3	-18.2 $\pm$ 0.3	12.1 $\pm$ 0.2
Mar 15-18	7	63.9 $\pm$ 6.0	2.0 $\pm$ 0.8	3.3 $\pm$ 0.4	-18.5 $\pm$ 0.5	12.5 $\pm$ 0.4
Apr 20	71	64.6 $\pm$ 5.0	2.4 $\pm$ 0.6	4.0 $\pm$ 0.3	-18.2 $\pm$ 0.2	12.0 $\pm$ 0.2
May 17	11	67.2 $\pm$ 4.8	2.7 $\pm$ 0.6	4.7 $\pm$ 0.5	-18.0 $\pm$ 0.2	12.4 $\pm$ 0.1
Jun 16	1	73 $\pm$ NA	3.0 $\pm$ NA	3.9 $\pm$ NA	-18.0 $\pm$ NA	12.2 $\pm$ NA
<b>LARGE <math>&gt; 76</math> mm</b>						
Sept 11-12	2	87.5 $\pm$ 7.8	6.1 $\pm$ 2.2	4.5 $\pm$ 0.2	-18.9 $\pm$ 0.7	11.4 $\pm$ 0.2
Oct 13	0	NA	NA	NA	NA	NA
Nov 15-16	50	91.3 $\pm$ 7.2	6.9 $\pm$ 2.0	5.5 $\pm$ 0.8	-19.7 $\pm$ 0.7	12.1 $\pm$ 0.5
Dec 13	0	NA	NA	NA	NA	NA
Jan 11	0	NA	NA	NA	NA	NA
Feb 17	5	82.2 $\pm$ 1.8	3.9 $\pm$ 0.4	3.5 $\pm$ 0.4	-19.1 $\pm$ 0.6	12.3 $\pm$ 0.2
Mar 15-18	33	88.4 $\pm$ 6.1	5.2 $\pm$ 1.2	3.1 $\pm$ 0.3	-19.6 $\pm$ 0.6	12.1 $\pm$ 0.3
Apr 20	72	89.6 $\pm$ 7.0	6.2 $\pm$ 1.5	3.9 $\pm$ 0.4	-19.0 $\pm$ 0.5	11.8 $\pm$ 0.3
May 17	19	94 $\pm$ 3.9	7.5 $\pm$ 1.0	4.5 $\pm$ 0.6	-18.5 $\pm$ 0.2	12.2 $\pm$ 0.2
Jun 16	27	94.0 $\pm$ 10.1	8.1 $\pm$ 2.8	4.9 $\pm$ 0.7	-18.6 $\pm$ 0.3	12.2 $\pm$ 0.3

Exploratory plots suggested a positive relationship between energy density and fish length across the winter months, particularly for November (Figure 5). The full least-squares linear model that included main effects for fish length (fork length), winter month of collection (November to March), and an interaction between fish length and month indicated that the overall model was significant ( $p < 0.001$ ) and explained a large proportion of the variation in WBED ( $R^2 = 0.78$ ). The interaction between fish length and month of collection was not significant ( $p > 0.05$ ) and was removed from subsequent model fitting. The final model included main effects for fish length and winter month of collection, which were all significant (overall model  $p < 0.001$ ,  $R^2 = 0.77$ ). Average energy densities were greatest in November and declined through March (Table 3) with the greatest decline being between November and December for small fish (Figure 6). Between November and December small fish lost an average of 1.2 kJ/g or about 9.6 J/g per day (Table 3).

There was evidence for spatial variation in energy density in November; the only month in which sufficient numbers of fish were sampled from multiple sites. Two of the sites were in western

Simpson Bay and the other site was located in eastern Simpson Bay (Figure 2c). Length was found to differ significantly among the sites ( $F_{3,212} = 94.17, p < 0.001$ ). The analysis of energy density therefore included site and length as a covariate and their interaction. All three terms contributed significantly to the model ( $p < 0.021$ ). Pairwise comparisons among the sites indicated that the lowest energy density was observed at one of the western Simpson Bay sites, the other two were indistinguishable.

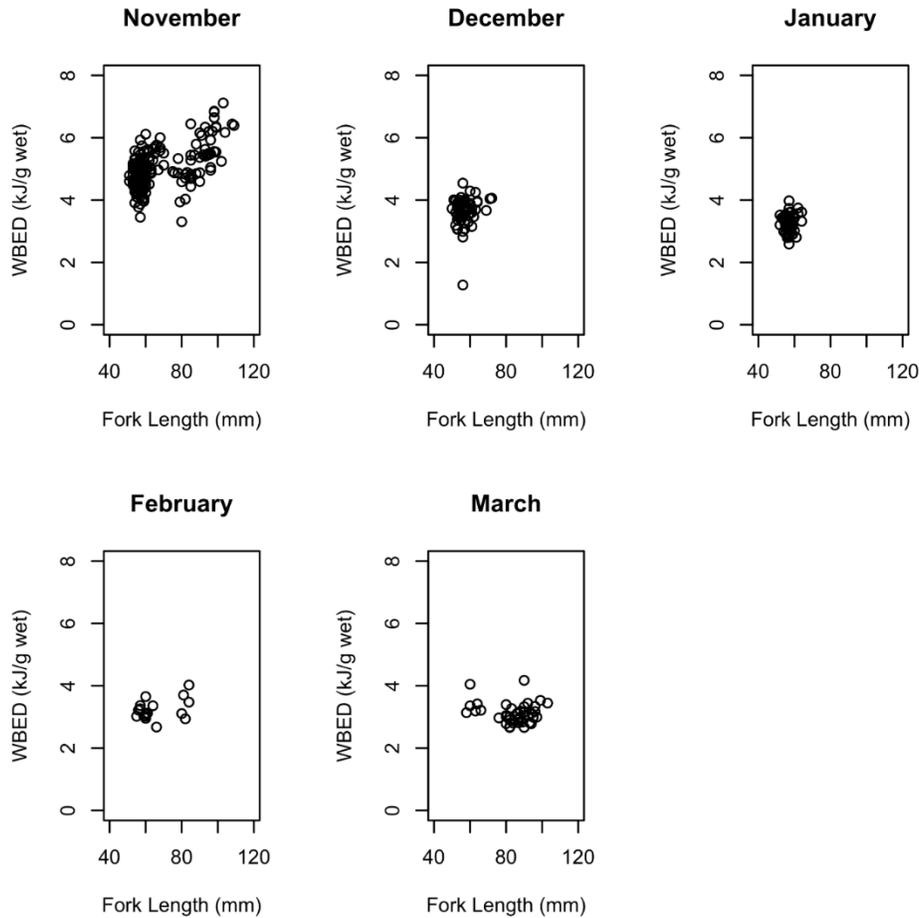


Figure 5. Energy density (kJ/g wet mass) of age-0 Pacific herring as a function of length for each sampling month.

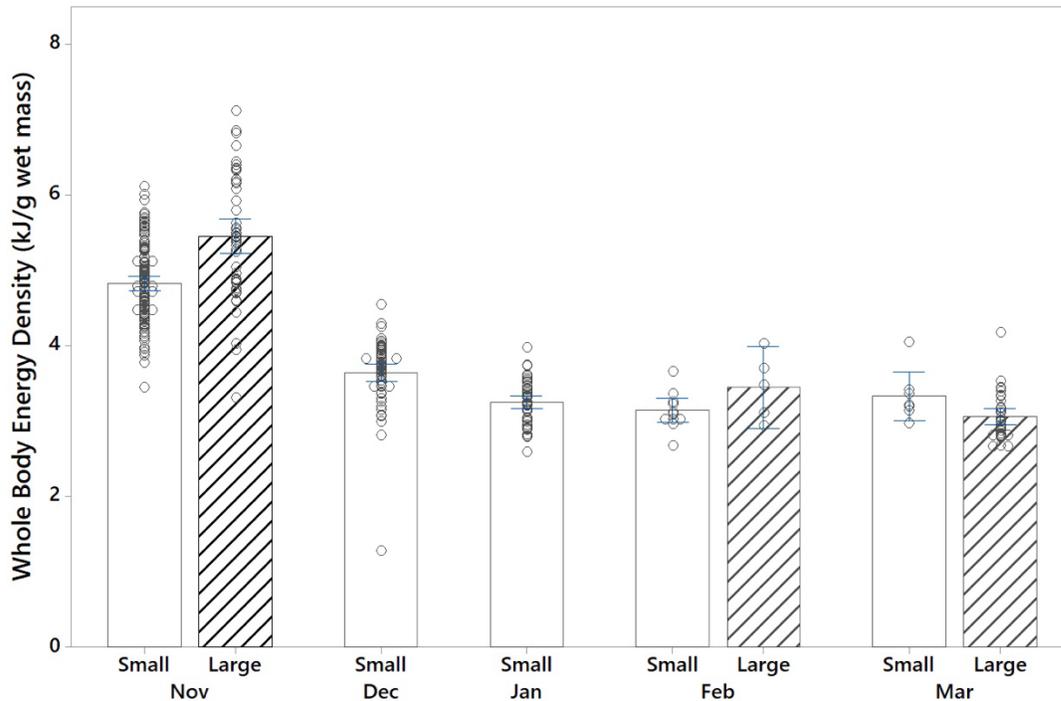


Figure 6. Energy densities of age-0 Pacific herring sampled during winter of 2011-2012. Values are group means  $\pm$ 95% confidence intervals.

### Size and starvation risk

Fish size influenced the magnitude and sources of energy used to forestall starvation as demonstrated by the non-linear relationships between total energy and size (Figure 7). The ANCOVA for total energy revealed a significant interaction between length and month ( $F_{4,103} = 3.42$ ,  $p = 0.011$ ) indicating the slopes relating total energy and length changed over winter (Figure 7). Pairwise contrasts revealed detectable differences in the total energy content of fish between November and February ( $t = 8.07$ ,  $p < 0.001$ ) and November and March ( $t = 4.4$ ,  $p < 0.001$ ). No large fish were caught in February preventing an evaluation of the impact of size on energy loss. A comparison of the 95% confidence intervals for the relationship between total energy and length for November and March indicates large fish lost disproportionately more energy than small fish (Figure 7 lower right panel). The confidence intervals for total energy content overlapped for fish less than 54 mm in both November and March indicating that their energy loss was not detectable. From that size onward fish lost progressively more energy. Fish between 55 mm and 76 mm lost an average 29% of their energy over winter compared with an average 39% for fish between 77 and 110 mm (Figure 7 lower right panel).

Analysis of the contribution of protein to total energy loss indicated the greatest contributions were from large fish. The ANCOVA for protein energy revealed a significant interaction between month and length ( $F_{4,112} = 5.41$ ,  $p < 0.001$ ) demonstrating that large fish lost disproportionately more protein energy than small fish over winter (Figure 7). Fish less than 77 mm fish lost an average of 4.6% of their protein energy compared with 18% for fish between

77mm and 110 mm. Consequently, protein losses represented an average of 10% of the total energy loss for fish between 55 and 76 mm and 28% for fish between 77 and 110 mm.

Conversely, the contribution of lipid energy to total energy loss was greatest for small fish. The ANCOVA for lipid energy found no interaction between month and length ( $F_{4,114} = .71, p = 0.590$ ) indicating that all fish lost the same proportion of lipid over winter. Re-analysis of the model without the interaction term revealed an effect of month ( $F_{4,114} = 69.11, p < 0.001$ ) and pairwise contrasts showed that there was no difference in lipid energy between November and December and December and January ( $t > 2.69, p > 0.084$ ). All other contrasts were significant ( $t < 3.28, p < 0.014$ ). Fish lost an average 75% of their energy between November and March. Length also influenced lipid energy ( $F_{1,114} = 528, p < 0.001$ ) (Figure 7). Losses of lipid among small fish represented an average 80% of the total energy loss for small fish (55 mm and 76 mm) and 70% for large fish (77-110 mm).

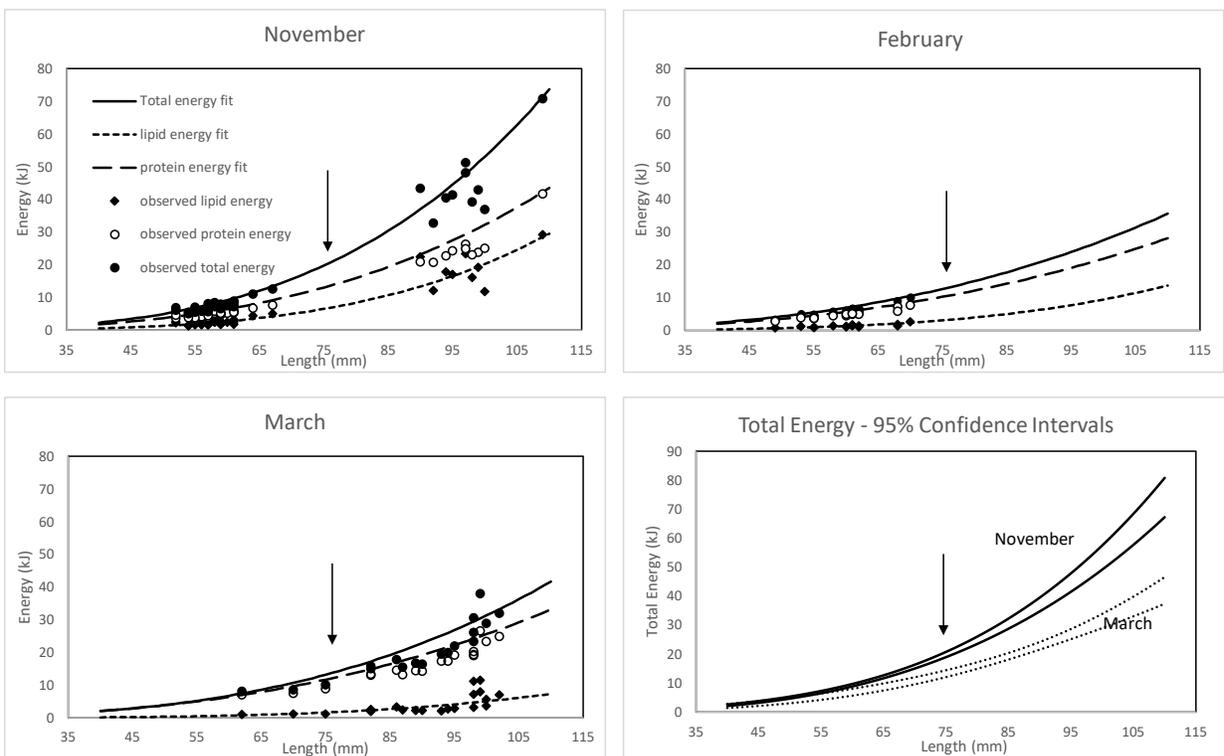


Figure 7. Relationships between length and energy for age-0 Pacific herring during winter 2011 – 2012. Panels labeled November, February and March show the fitted relationships between length and total energy (black line), energy found as protein (long dashes) and energy found as lipid (short dashes) and observed values (points). Areas to left of downward arrows show small fish areas to the right show large fish. The panel in the lower right shows the upper and lower bounds of 95% confidence intervals for fitted line describing total energy content as a function of length for fish sampled in November and March. Areas where bounds do not overlap indicate fish sizes with detectable energy loss.

## Diets and consumption rates

The stomach contents of 107 fish collected between November 2011 and March 2012 were examined and only one was found empty. Copepods were important contributors to the overall mass of the stomach contents. Beginning in November *Psudeocalanus* sp. and *Metridia pacifica* accounted for 50% of the stomach content weight, but in December calanoids made up only about 20% of the diet mass, while *Oikopleura* accounted for another 63%. Later in winter calanoids remained important. In January 68% of the mass was made up of small and large calanoids. In February large and small calanoids along with *Calanus marshallae* accounted for nearly 89% of the diet mass. By March diets shifted to fish eggs (52% of the mass) and barnacle cyprids (28% of the mass), calanoid copepods comprised only about 11% of the mass.

Dietary energy density decreased during the first half of the winter and differed little between small and large fish in November and March (Figure 8 top panel). For small fish dietary energy density decreased from November to February and increased in March (Figure 8 top panel). The decreasing trend in energy density was mirrored by water temperatures (Figure 8 bottom panel). Average daily water temperatures declined steadily from 8 °C in early November until reaching an average 3 °C in mid-January. Temperatures remained low until they began increasing in late March.

Fish consumed proportionally more of  $C_{max}$  as winter progressed in order to maintain their mass (Table 4, Figure 9). The weight of a fish at a given length did not change as winter progressed ( $F_{4,381} = 1.08$ ,  $p = 0.365$ ). Least square estimates of the predicted weights for different sized fish in each month are given in Table 4. Comparison of the proportion of  $C_{max}$  consumed between large and small fish revealed a significant interaction between size class and month ( $F_{3,16} = 14.26$ ,  $p < 0.001$ ) because the proportion of  $C_{max}$  consumed by large fish was less than that of small fish between November and December, while the reverse was true in the period between February and March (Figure 9). Size and sampling period were also significant factors ( $F > 12.98$ ,  $p < 0.001$ ). Overall, large fish consumed a lower proportion of  $C_{max}$  than small fish ( $t = 3.06$ ,  $p = 0.002$ ). Averaged over winter, the proportion of  $C_{max}$  consumed declined from 16.8% for 45 mm fish to 14.8% for 105 mm fish. However, consumption rates increased as winter progressed ( $t > 6.51$ ,  $p < 0.001$ ). The proportion of  $C_{max}$  consumed for all fish increased from 2% to 25.7% between November and March (Figure 9).

The absolute mass of food consumed by herring was determined by the interaction between the month they were collected and size class ( $F_{3,16} = 11.8$ ,  $p = 0.035$ ). Between November and December large fish needed a lower amount of food than small fish (Table 4). However, by the end of winter the reverse was true. In total, small fish required a total of 3.0 to 6.0 g of food between November and March. In contrast, large fish required 10.0 to 14.5 g. A fish at the transition size of 76 mm required approximately 8.0 grams of food over winter (Table 4).

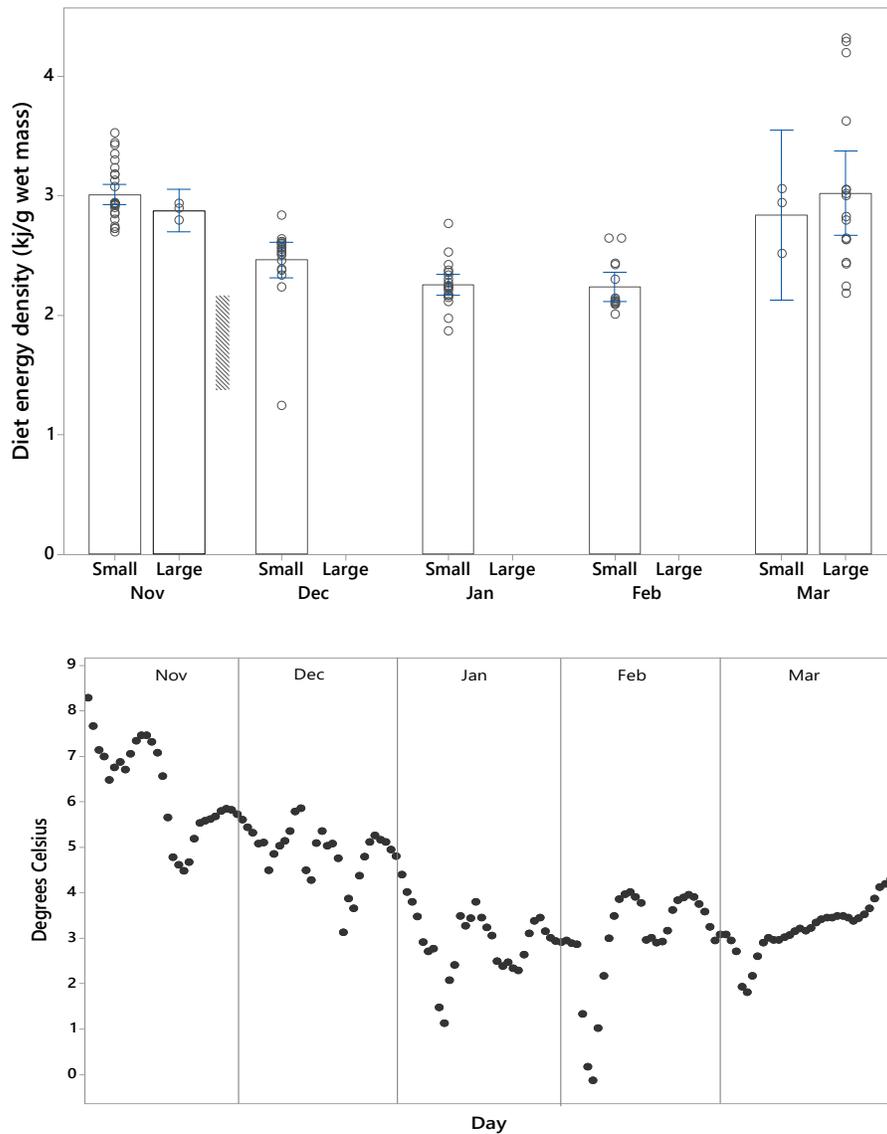


Figure 8. Top Panel: Mean ( $\pm 95\%$  confidence intervals) for energy densities of diets consumed by small ( $\leq 76$  mm) and large ( $> 76$  mm) age-0 Pacific herring during winter 2011-2012. No fish were captured in months with missing bars, open circles show observed data values. Bottom Panel: Temperatures at the Cordova tide gauge station during winter 2011 – 2012.

Table 4. Bioenergetic model estimates of consumption rates for different size herring by sampling period in Simpson Bay during the winter of 2011-2012. Estimates show the proportion of Cmax consumed ( $p$ ) and the total mass of food required during each month ( $\Sigma$ ) in grams. Estimates of  $W_0$  (g), fish weight at the start of a period, and  $W_f$  (g), weight at the end, are derived from length weight regressions constructed from monthly samples.

Size (mm)	November - December				December - January				January - February				February - March			
	$W_{t_0}$	$W_{t_f}$	$p$	$\Sigma$	$W_{t_0}$	$W_{t_f}$	$p$	$\Sigma$	$W_{t_0}$	$W_{t_f}$	$p$	$\Sigma$	$W_{t_0}$	$W_{t_f}$	$p$	$\Sigma$
45	0.81	0.82	0.060	0.36	0.82	0.82	0.144	0.71	0.82	0.75	0.232	0.97	0.75	0.81	0.239	0.90
55	1.44	1.47	0.058	0.53	1.47	1.44	0.128	0.98	1.44	1.33	0.230	1.46	1.33	1.43	0.246	1.42
65	2.30	2.29	0.033	0.43	2.29	2.30	0.137	1.46	2.30	2.13	0.227	2.05	2.13	2.29	0.253	2.06
75	3.45	3.44	0.030	0.53	3.44	3.45	0.132	1.90	3.45	3.19	0.224	2.73	3.19	3.43	0.259	2.85
85	4.91	4.89	0.011	0.25	4.89	4.91	0.127	2.38	4.91	4.54	0.221	3.51	4.54	4.88	0.264	3.79
95	6.72	6.70	0.000	0.00	6.70	6.73	0.122	2.88	6.73	6.21	0.218	4.37	6.21	6.68	0.270	4.88
105	8.92	9.13	0.008	0.29	9.13	8.93	0.097	2.85	8.93	8.24	0.214	5.31	8.24	8.87	0.274	6.14

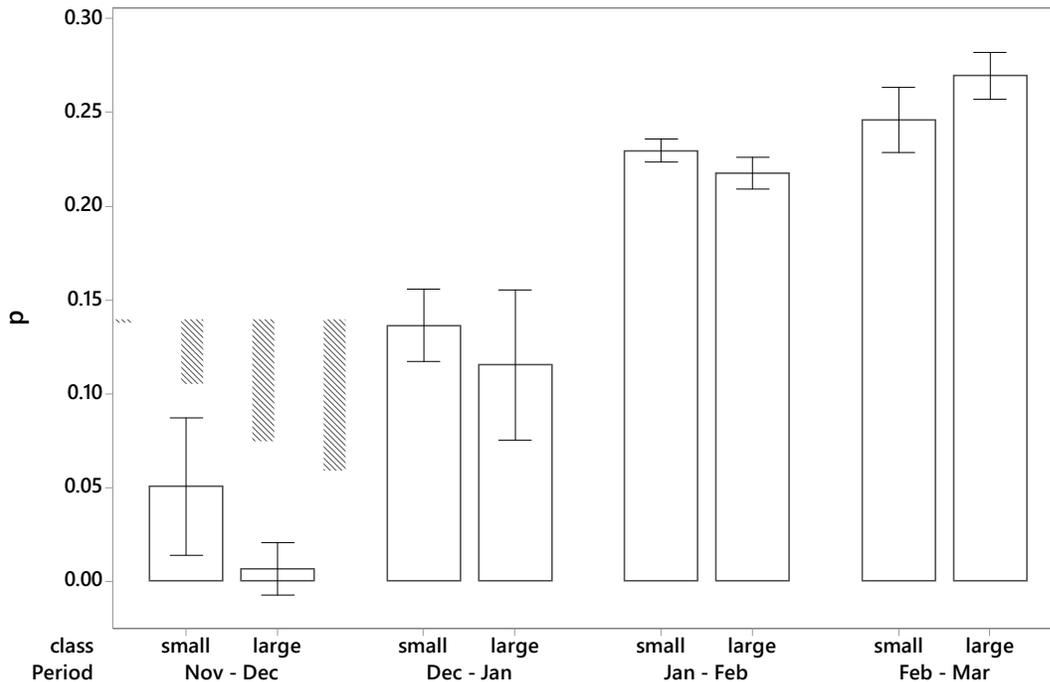


Figure 9. The average proportion of Cmax ( $p$ ) consumed during different periods of the winter by large ( $>76$  mm) and small ( $\leq 76$  mm) age-0 Pacific herring based on modeling consumption. Open circles show modeled data values.

### Stable isotope variability

Exploratory plots of monthly relationships between the body size of juvenile herring and  $\delta^{13}\text{C}'$  or  $\delta^{15}\text{N}$  values of tissue suggested that a negative relationship may exist between fish size and  $\delta^{13}\text{C}'$  values, particularly during some winter months such as November, February, and March (Figure 10). Similarly, exploratory plots suggested a possible negative relationship between fish size and  $\delta^{15}\text{N}$  values during November and March (Figure 11). The full least-squares liner model for explaining  $\delta^{13}\text{C}'$  variability that included main effects for fork length, winter month of collection, and an interaction between fish length and winter month of collection was significant ( $p < 0.001$ ), and explained a large proportion of the variation in  $\delta^{13}\text{C}'$  ( $R^2 = 0.70$ ); however, all parameters for the interaction between fish length and winter month of collection were not significant ( $p > 0.05$ ). Thus, the interaction term was removed in subsequent model fitting. The final model for  $\delta^{13}\text{C}'$  variability included main effects for fork length and winter month of collection. All parameters for these terms were significant with the exception of month for January and February (overall model  $p < 0.001$ ,  $R^2 = 0.70$ ).

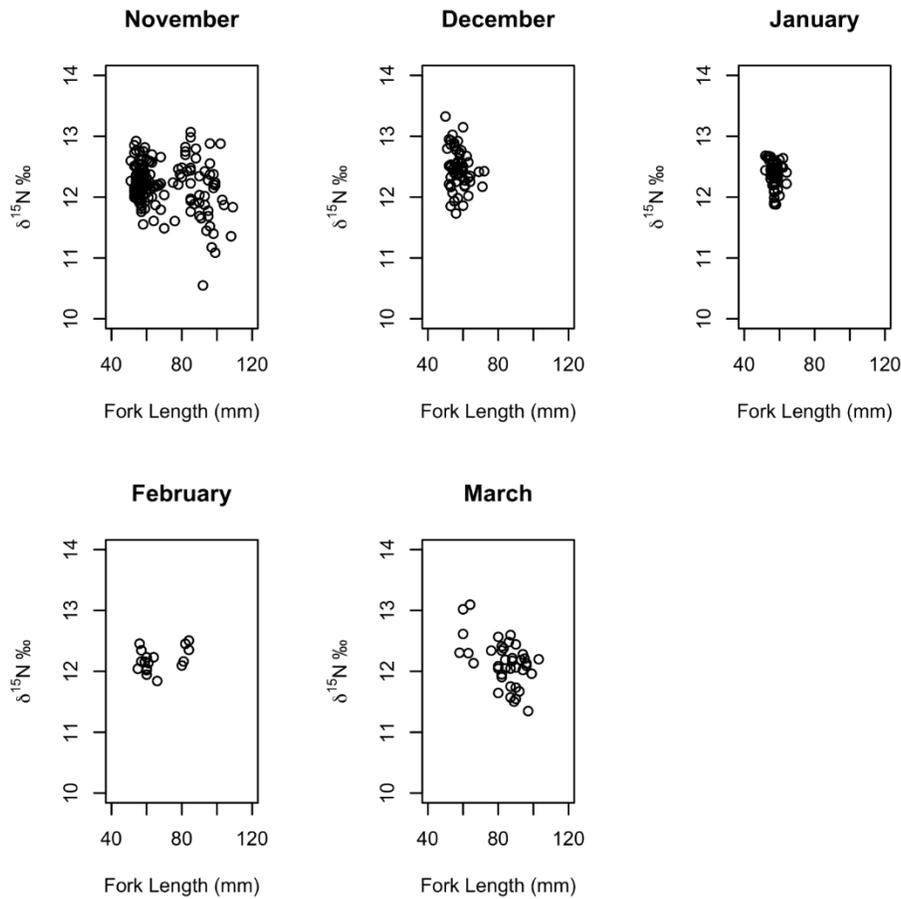


Figure 10. Carbon stable isotope value of age-0 Pacific herring as a function of length for each month.

The full least-squares liner model for explaining  $\delta^{15}\text{N}$  variability that included main effects for fork length, winter month of collection, and an interaction between fish length and winter month of collection was significant ( $p < 0.001$ ), but only explained a small proportion of the variation in  $\delta^{15}\text{N}$  ( $R^2 = 0.18$ ); however, all parameters for the interaction between fish length and winter month of collection were not significant ( $p > 0.05$ ). Thus, the interaction term was removed in subsequent model fitting. The final model for  $\delta^{15}\text{N}$  variability included main effects for fork length and winter month of collection. All parameters for these terms were significant with the exception of month for January (overall model  $p < 0.001$ ,  $R^2 = 0.15$ ).

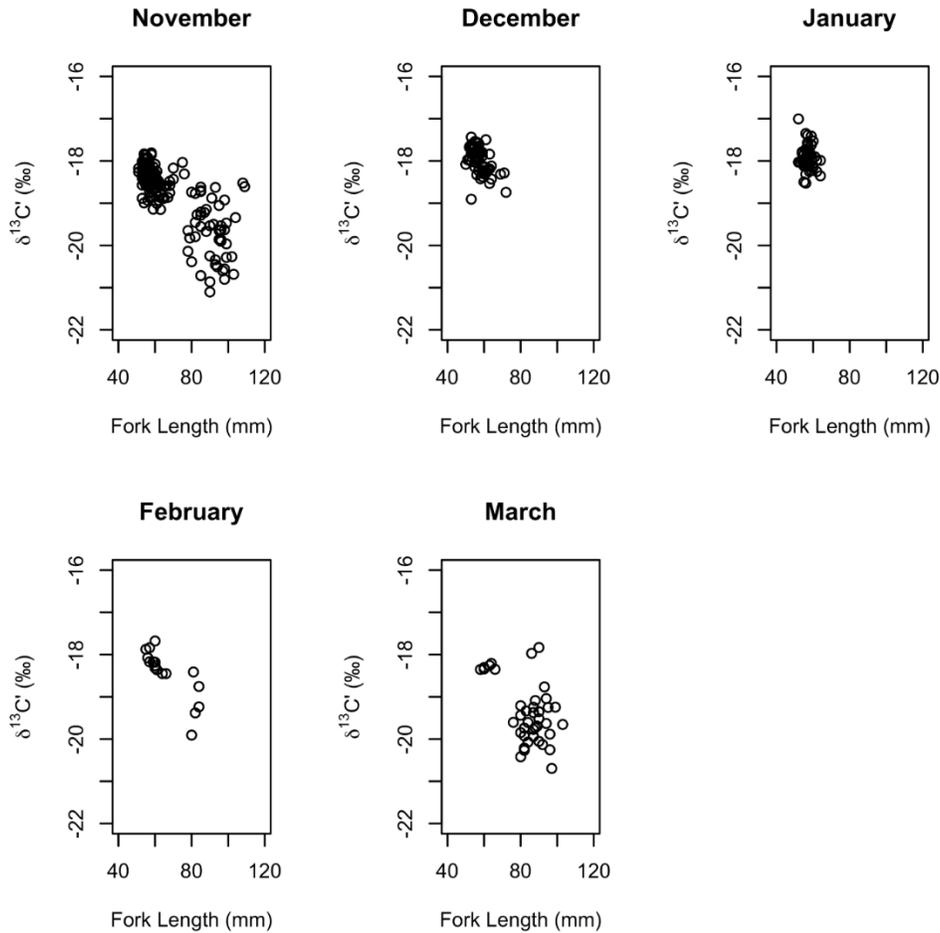
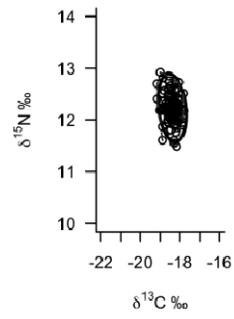


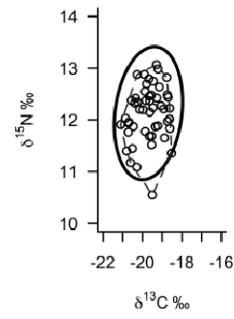
Figure 11. Nitrogen stable isotope value of age-0 Pacific herring as a function of length for each month.

Exploratory plots of monthly  $\delta^{13}C'$  or  $\delta^{15}N$  isotopic area occupied by juvenile herring during the entire time series indicated that the foraging niche occupied by large fish compressed during winter, while that of small fish remained more constant (Figure 12). Bayesian ellipse area analysis indicated that monthly relationships with isotopic area was generally the same for each size class for February and March, while in November larger fish had a significantly larger isotopic area than smaller fish (Figure 13).

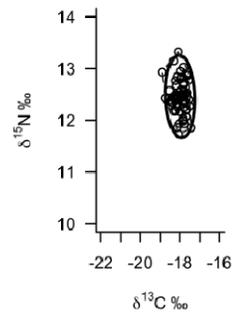
**Nov Small Fish**



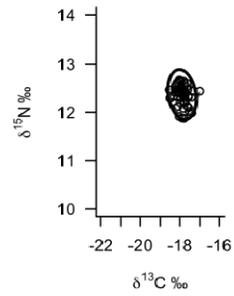
**Nov Large Fish**



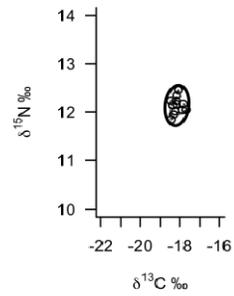
**Dec Small Fish**



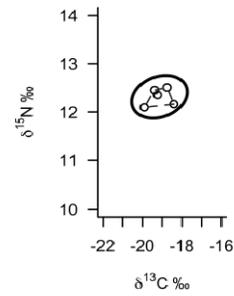
**Jan Small Fish**



**Feb Small Fish**



**Feb Large Fish**



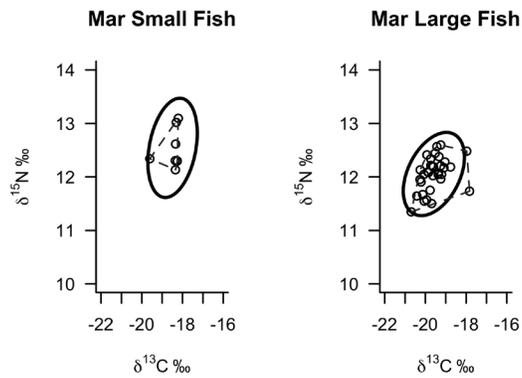


Figure 12. Carbon and nitrogen stable isotope biplots with convex hull and Bayesian ellipse areas superimposed for each size class of age-0 Pacific herring.

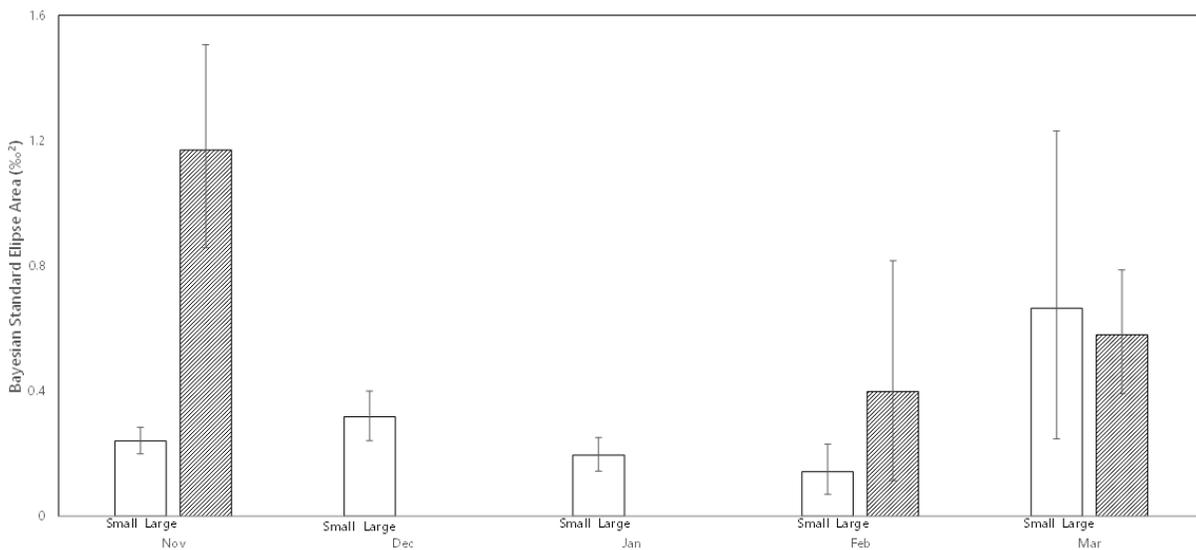


Figure 13. Average ( $\pm 95$  credible interval) Bayesian ellipse areas for each month and size class of age-0 Pacific herring.

## DISCUSSION

Observations of size-related differences in wintering behavior indicate that food supply is an important component to age-0 herring survival. Theory posits that size-dependent mortality during winter can develop because small fish incur a greater risk of starvation owing to their minimal energy stores and high energy depletion rates (Schultz and Conover 1999, Biro et al. 2005). Our observations of winter foraging combined with energy loss demonstrate that food supplies were insufficient to maintain fish condition during winter. Estimates of survival derived from the OWM and reductions in the catch of small fish support the idea that winter mortality of age-0 herring is size dependent (Sewall et al. 2019), and that small fish face a lower probability of surviving winter than large fish. However, examination of the proximate composition of the

small and large fish suggest more nuance to their relative survival probabilities. By winter's end large fish had lost more protein and lipid than surviving small fish. Starvation is thought to occur in stages with the final stage characterized by lipid depletion and active protein catabolism to meet metabolic need (McCue 2010). The greater contributions of protein to the total energy loss by large fish indicates that they more closely resembled starving fish at the end of winter than the surviving small fish. This is consistent with their greater absolute need for food relative to small fish and the scarcity of food in late winter (McKinstry and Campbell 2018). Thus, the difference in survival probabilities observed between small and large fish is greatest in early winter when small fish are actively foraging and exposed to predators while large fish are able to rely on their lipid reserves and avoid exposure. By late winter, starvation risk motivates both small and large fish to forage (Sogard and Olla 1997, Sewall et al. 2019), and predation risk for large fish begins to catch up to that of small fish, narrowing the difference in survival probabilities.

The importance of winter foraging to age-0 herring has direct bearing on the accuracy of the OWM. The model overestimated energy loss for small fish and underestimated energy loss for large fish. The result of these biases will be overestimation of winter mortality for small fish and underestimation for large fish. The OWM predicted that energy densities in March would include values lower than were observed. Moreover, the observed daily decrease in energy density was lower than the value used in the model. The relatively low daily decrease in energy density was driven by that of the small fish. Their energy densities decreased by about 12 kJ/g per day between December and March. In contrast, the average reduction for large fish exactly matched the value used in the model, 19 kJ/g per day. Thus size, which is not a feature of the model, influences the shape and magnitude of the energy density decrease function, as does temperature (Bernreuther et al. 2013). Size related differences in energy density decreases indicate that the model will be of limited value unless the initial energy density distribution indexes the unbiased distribution of energy densities in fall and size-related energy loss rates are incorporated.

The ability to forage also has an influence of the survival function employed by the OWM. Kline (2013) observed fish with energy densities below the critical value for mortality (3.2 kJ/g, Paul and Paul 1998) and hypothesized that they may have been able to maintain those levels if they are foraging. Observations of energy densities and lipid levels that are below critical levels identified in laboratory studies have been reported for herring elsewhere (Gorman et al. 2018, Sewall et al. 2019). Variability in energy density is typically driven by lipid content (Anthony et al. 2000) and when lipid levels fall to low levels fish begin foraging or taking greater risks to forage (Metcalf and Thorpe 1992, Sogard and Olla 1997, Biro et al. 2005). Sewall et al. (2019) indicated that foraging increased in herring when lipid levels dropped to 1.3% of wet mass. In March, we observed an average lipid content of 1.2% for the small fish and estimated their daily consumption rates at approximately 25% of the maximum between February and March. These data support the hypothesis that extremely sub-critical nutritional levels can be maintained if there is exogenous energy available to forestall further energy loss and may require re-evaluation of the critical levels employed by the OWM. Alternatively, it is possible that laboratory studies bias these critical levels to a higher lipid level.

Additionally, observations of the contribution of protein to overall energy loss demonstrated that starvation risk for larger fish developed as winter progressed. At the end of winter, protein losses had accounted for nearly 28% of the overall energy lost by large fish versus 10% for the small fish. In Atlantic cod (*Gadus morhua*) 16 weeks of starvation led to a 15% reduction in muscle

protein mass and diminished swimming endurance, sprint and maximum swimming speeds (Martinez et al. 2003, Martinez et al. 2004). Large herring in PWS lost approximately 18% of their protein compared with about 4.6% for small fish over the 18 weeks between November and March. Starvation impacts on the swimming ability of juvenile herring were therefore likely most extreme for large fish. However, Sewall et al. (2020 in prep) failed to detect a difference in the critical swimming speeds of juvenile herring that had starved for 93 days when compared to those on limited or full rations. Lipid energy losses were independent of length and represented 75% of their levels in November. This suggests a limit to lipid energy loss and suggests that approximately 25% of lipid energy likely represents the minimum mass required to maintain cell integrity in juvenile herring.

While the OWM provides insight into the overwintering process, it provides little information on the mechanisms of mortality. In fall, small fish avoid the selective pressure of size-selective predation by growing rapidly (Biro et al. 2005), while larger fish store energy to forestall starvation in winter. The reduced RNA/DNA ratios we observed in winter were inconsistent with those of growing fish (Sewall et al. 2019, Sewall et al. 2020 in prep) and there was no evidence of growth between November and March. Small fish therefore continued foraging in response to their limited energy storage capacity (Sewall et al. 2019) and potential for rapid energy loss (Schultz and Conover 1999) rather than attempting to outgrow predation risk. Foraging time became an important mediator of predation risk. As winter progresses, starvation risk increases because small fish must find and consume progressively larger rations but food supplies remain minimal (McKinstry and Campbell 2018). Hence their foraging time and associated predation risk increase throughout winter until food supplies return to levels that can afford the opportunity for growth or at least reduced foraging effort. In contrast, predation risk for larger fish is low at the onset of winter because they can rely on energy reserves and minimize exposure to predators. We observed little evidence of foraging by large fish early in winter. However, in late winter their energy reserves were depleted, and they required much greater ration sizes than the small fish. These observations support the idea that size-selective predation in winter is manifested by increasing exposure to predators wherein small fish respond to increased starvation risk earlier in the season than their larger conspecifics.

In addition to the potential disadvantage of decreased swimming ability, large fish required larger ration sizes in late winter, which can impose greater searching times. Differential foraging activity in winter is demonstrated by the size and month effects in the stable isotope models (Figures 10-13). Importantly, stable isotope values of fish muscle tissue reflect assimilated forage over the past one to two months in juvenile Atlantic cod (Ankjærø et al. 2012), and controlled studies of adult Pacific herring showed muscle tissue to have a relatively fast isotopic turnover rate for  $\delta^{15}\text{N}$  between two and three months (Miller 2006). Thus, we interpret our stable isotope data to reflect prey consumption over the previous one to two months. Limited prey densities in late winter are well documented in PWS (Cooney et al. 2001, McKinstry and Campbell 2018). The decreased isotopic niche area for large fish indicated a potential decrease in the variety of available forage between fall and late winter that generally corresponds with decreased zooplankton numbers (McKinstry and Campbell 2018) and biomass (Cooney et al. 2001). In contrast the isotopic niche area occupied by small herring did not change appreciably between November through February, but increased in March. The CRD - prey density that allows for a prey intake rate equal to metabolic demand - increases exponentially with size (Bystrom et al. 2006). Consequently, as resource density decreased in winter the difference

between the energy gained by foraging and the metabolic need got larger, particularly for the largest fish leading to increased starvation risk. Large fish likely spent more time searching for food than the smaller fish and consequently had greater exposure to potential predators. This results in a decreasing difference in survival probabilities between small and large fish as winter progresses.

In contrast to the large fish, small fish were better able to meet their energy needs in late winter because their absolute need for energy was smaller than that of larger fish. The disparate losses of protein between small and large fish demonstrate that these issues were more important to large fish. In March, diets included fish eggs, a high energy and easily digested prey that likely accounted for the increase in dietary energy density, as well as the increased isotopic niche area for small fish in March (Figure 14). The availability of fish eggs was likely a greater benefit to small fish because they needed to ingest fewer to exceed their CRD. Herring have previously been shown to consume eggs in late winter (Foy and Paul 1999), which coincides with the availability of eggs spawned by many abundant and fecund fish species in the northern Gulf of Alaska (Doyle and Mier 2012). The importance of this disparity in CRD was demonstrated by small Steelhead Salmon (*Oncorhynchus mykiss*) that grew better at low ration sizes than larger fish when held under identical winter conditions (Connolly and Petersen 2003). Matching between fish egg production and the lower CRD's for smaller individuals in late winter potentially created conditions that improved survival probabilities of small fish as indicated by upticks in their protein content and RNA/DNA and maintenance of their energy density between February and March. In contrast large fish continued to lose energy between February and March indicating a continued loss of energy. These data suggest that winter ended sooner for small fish as the difference between each group's survival probabilities narrowed. This narrowing of survival probabilities in late winter provides a potential selective mechanism for maintaining the presence of small juveniles at winter's onset.

## CONCLUSIONS

The ability of age-0 Pacific herring to survive winter depends on their ability to provision themselves prior to winter and the foraging conditions they encounter as winter proceeds. Accurately modeling winter survival therefore requires an accounting of size-related differences in the rates of energy loss, re-evaluation of the critical values for survival, and unbiased sampling of the population at winter's start.

Small fish encounter significant impediments to their winter survival. Their small size imposes a greater risk of predation mortality and limits their ability to store energy. Consequently, their survival depends on their ability to forage throughout winter while evading predation. Larger fish experience higher survival than small fish at the start of winter because their large size minimizes predation risk and their energy stores reduce starvation risk to zero. However, in late winter, when food supplies are most scarce, large fish with depleted energy reserves must also forage to survive. While their size offers less predation risk than that of foraging small fish, the large fish must spend more time foraging. Thus, the differences in survival probability between small and large fish observed in early winter become narrower in late winter. These data suggest the need to quantify the relative proportions of small and large fish at the beginning of winter and examine the implications of this narrowing of size-related survival probabilities for population structure.

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