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Growth, condition, and swimming performance of juvenile Pacific herring with winter feeding rations

Fletcher Sewall, Brenda Norcross, and Ron Heintz

Abstract: Juvenile fish winter mortality, whether through starvation, predation, or disease, depends in part on feeding history. Assessing mortality risk thus requires metrics that can distinguish well-fed from poorly fed individuals. To investigate the effects of winter feeding and spring re-feeding after winter fasting on young-of-the-year Pacific herring (*Clupea pallasii*), captive herring were maintained on different feeding rations for 20 weeks under ambient winter and spring conditions and evaluated for differences in size, gut mass, RNA/DNA ratio, body composition, and swimming performance. Lipid and moisture levels were inversely related indicators of feeding history, differing most between full-ration and fasted herring. Fasted herring that were re-fed in spring had evidence of compensatory growth without impacting swimming performance. Minimal growth and reduced gut mass observed even among fully fed herring suggest limits to winter feeding benefits. Metabolically processing stored fat rather than foraging and incurring greater predation risk may thus be an advantageous strategy regardless of winter food availability. Mortalities due to starvation and possibly disease were highest among small herring across rations, supporting the importance of size-dependent winter mortality.

Résumé : La mortalité hivernale des poissons juvéniles, par privation de nourriture, prédation ou maladie, dépend en partie de l'historique d'alimentation. Ainsi, l'évaluation du risque de mortalité nécessite la mesure de paramètres pouvant distinguer les individus bien nourris et mal nourris. Afin d'examiner les effets de l'alimentation hivernale et de la reprise de l'alimentation au printemps après le jeûne hivernal sur de jeunes harengs du Pacifique (*Clupea pallasii*) de l'année, différentes rations ont été données à des harengs captifs pendant 20 semaines dans des conditions ambiantes hivernales et printanières, et les différences de taille, de masse du tube digestif, de rapport ARN/ADN, d'embonpoint et de performance de nage ont été évaluées. Les teneurs en lipides et l'humidité pondérale sont des indicateurs inversement reliés de l'historique d'alimentation, leurs différences étant les plus grandes entre les harengs nourris de rations complètes et ceux ayant jeûné. Les harengs ayant jeûné en hiver qui ont ensuite été nourris au printemps présentaient des signes de croissance compensatoire sans incidence sur la performance de nage. Une croissance très limitée et une baisse de la masse du tube digestif observées même chez les harengs nourris de rations complètes indiqueraient qu'il y a des limites aux avantages de l'alimentation hivernale. Le traitement métabolique de graisses emmagasinées plutôt que la quête de nourriture engendrant un plus grand risque de prédation pourrait donc constituer une stratégie avantageuse, peu importe la disponibilité de nourriture en hiver. La mortalité causée par la privation de nourriture et, possiblement, par la maladie est la plus grande chez les petits harengs, peu importe la ration, ce qui souligne l'importance de la mortalité hivernale dépendante de la taille. [Traduit par la Rédaction]

Introduction

For high-latitude juvenile fishes, winter feeding is commonly believed to be insufficient to achieve metabolic demands, resulting in caloric deficits and corresponding declines in condition, though winter starvation mortality is rarely demonstrated (Hurst 2007). In addition to the hypothesized risk of starvation, poor condition may increase mortality indirectly through decreased swimming performance (Martinez et al. 2003) that compromises foraging ability and predator avoidance. Predation and starvation risk may be higher among smaller fish than larger fish due to their high metabolic rates and susceptibility to predation. Juvenile fish may compensate for winter mass loss by increasing allocation of food energy towards growth and condition with the return of abundant food in spring, but with potential physiological costs and trade-offs in swimming performance (reviewed in Ali et al. 2003).

Responses to food scarcity can be complex, involving trade-offs among structural maintenance, growth, energy storage, behavior, and other factors. Modeling these responses is challenging due to incomplete understanding of the processes involved. A variety of indicators have been used to quantify fish growth and condition, such as length–weight ratios, organ masses, RNA/DNA ratios, and proximate composition. The sensitivity of these indices to fasting, and thus their usefulness as indicators of mortality risk, varies across life stages and species (Weber et al. 2003).

Pacific herring (*Clupea pallasii*; hereinafter herring) is a useful model organism for investigating these issues due to its ecologically important role as a trophic intermediary between zooplankton and higher-level predators such as fishes, seabirds, and marine mammals. Understanding the biology and population dynamics of this key planktivore is vital to understanding the functioning of many northern marine ecosystems. In particular,

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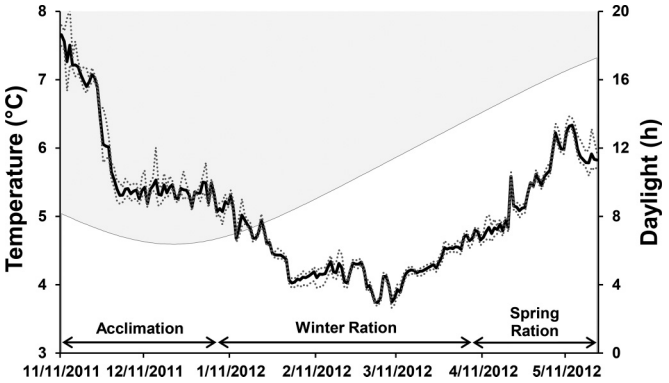
F. Sewall. Auke Bay Laboratories, Alaska Fisheries Science Center, NMFS, NOAA, 17109 Point Lena Loop Rd., Juneau, AK 99801, USA.
B. Norcross. College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, P.O. Box 757220, Fairbanks, AK 99775, USA.
R. Heintz. Sitka Sound Science Center, 834 Lincoln Street, Sitka, AK 99835, USA.

Corresponding author: Fletcher Sewall (email: fletcher.sewall@noaa.gov).

Fig. 1. Daily mean ambient seawater temperatures (°C) and daylight hours (unshaded) for tanks in the laboratory feeding study of young-of-the-year Pacific herring in Auke Bay, Southeast Alaska, throughout the study period, November 2011 to May 2012. Solid black line indicates daily mean temperature averaged across five representative tanks. Dotted gray lines indicate the daily minimum and maximum individual tank means. Periods of acclimation, winter ration, and spring ration treatments are indicated above dates.



management of herring stocks would benefit from improved understanding of factors affecting recruitment, many of which are associated with first winter mortality (Norcross et al. 2001).

Few studies have established criteria for discriminating juvenile herring that are healthy from those at high risk of winter mortality. Previous work has described the energetic response of juvenile herring to fasting under controlled conditions (Paul and Paul 1998), though no published work has simultaneously considered multiple condition and performance indices. Swimming performance of juvenile herring declines in response to oil exposure (Kennedy and Farrell 2006), but the effects of herring nutritional status and condition on swimming performance are unknown. Such investigations with captive herring can be constrained by unplanned disease effects, as juvenile herring can experience outbreaks of naturally occurring diseases during capture and captivity, possibly related to handling and confinement stress (Hershberger et al. 2006). Unplanned disease effects, including energetic costs and mortality (Hershberger et al. 2006; Gregg et al. 2011), may act in concert with factors such as feeding history to affect herring health and mortality risk.

The goal of this study was to investigate how young-of-the-year (YOY) herring growth, condition, and swimming performance respond to varied winter and spring feeding conditions. This research strives to provide a framework to assess the likelihood of first winter survival of wild juvenile herring and consequently improve the ability to predict year-class strength.

Materials and methods

Field collection and laboratory conditions

YOY herring were captured on 27 October 2011 by beach seine in Auke Bay, Southeast Alaska, adjacent to the National Marine Fisheries Service, Auke Bay Laboratories (animal research was approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee, approval No. 322986-2). Herring were maintained in duplicate 189-L oval fiberglass tanks with sand-filtered seawater flowing through at 5–6 $L \cdot min^{-1}$ at ambient Auke Bay temperature, which ranged from a daily mean high of 7.7 °C in early November to a low of 3.7 °C in early March (Fig. 1). Herring tanks were exposed to ambient sunlight on a natural photoperiod, which ranged from a minimum of 6.4 h of daylight in mid-December to 17.3 h at the study end in mid-May. The timing of the study thus enabled observation of herring under simulated winter and spring conditions. Herring loading densities were 78–92 fish per tank.

Table 1. Study design for laboratory feeding experiment with number of individuals analyzed (*n*) by ration level at the end of each treatment period for young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Acclimation (Nov.–Jan.)		Winter (Jan.–Apr.)		Spring (Apr.–May)	
Tank	Ration	*n*	Ration	*n*	Ration	*n*
1			Full*	5	Full	5
2			Full*	5	Full	5
3	Full	2	Reduced	5	Reduced	5
4					Reduced re-fed†	5
5	Full	2	Reduced	5	Reduced	5
6					Reduced re-fed†	5
7	Full	2	—‡		—‡	
8	Full	2	—‡		—‡	
9	Full	2	Fasted	5	Fasted	5
10					Fasted re-fed†	5
11	Full	4	Fasted	5	Fasted	3
12					Fasted re-fed†	5
Total		14		30		48

*Separate full ration tanks established by transplanting from all tanks in January after acclimation period sampling.

†Re-fed tanks established in spring after April winter sampling using transplants from tank 3 to 4, 5 to 6, 9 to 10, and 11 to 12.

‡Two full ration tanks discontinued after January acclimation period sampling.

During a 10-week acclimation period through the first week of January, all herring were fed daily on weekdays in excess of satiation (~15.0 g per tank) to establish experimental baseline fish condition and feeding behavior before manipulation. Feed consisted of commercially available frozen euphausiid meal (*Euphausia superba*) stored at −20 °C. Ration levels were then manipulated during the study treatment periods that followed, with all herring fed a given ration constituting a treatment group. In the first treatment period, from early January (study week one) through early April (week 14; 93 days), two replicate tanks of herring per treatment were provided one of three winter rations to examine responses to contrasting feeding levels: (*i*) full ration (fed daily to satiation); (*ii*) reduced ration (fed to satiation twice weekly); and (*iii*) fasted (Table 1). In the second treatment period, from early April through May (week 20; 42 days), in addition to continuing all three treatments, the latter two treatment groups were subdivided and half of each distributed to two replicate tanks each (four total) that were re-fed at full ration to create two new spring re-feeding treatments: (*i*) re-fed full ration following reduced ration, and (*ii*) re-fed full ration following fasting. The January to April–May period of food deprivation for fasted herring approximated the conditions potentially experienced by some herring populations in the North Pacific during winter (Norcross et al. 2001). The return of food and the increase in temperatures and daylight hours during the re-feeding period likewise simulated natural conditions during spring.

Sampling

Subsamples of herring were collected by random grabs with an aquarium fish net. To determine the average size of herring near the start of captivity ("start acclimation" in Results), 40 herring from one tank were anaesthetized by brief immersion in tricaine methanesulfonate (MS-222) in seawater (0.3 $g \cdot L^{-1}$), measured for fork length and wet body mass, and returned live to the tank. Herring from subsequent sampling periods (Table 1) were euthanized by prolonged immersion in MS-222 in seawater and immediately frozen for later chemical analysis:

1. 6 January: subsamples of two to four herring from six replicate tanks were collected after the acclimation period on full ration (end acclimation);
2. 6 April: subsamples of five herring from two replicate tanks per ration treatment were collected after the winter ration period (end winter);
3. 21–22 May: subsamples of five herring from two replicate tanks per ration were collected after the spring re-feeding period (end spring).

Chemical analyses were conducted on 120 fish in total: 92 euthanized herring from all five treatments and 28 natural mortalities from only the full-ration and fasted treatments. Natural mortalities were collected opportunistically within 24 h of death on several dates in April and May; eight mortalities were collected from full-ration tanks and 20 from fasted tanks. Mortalities from April and May were pooled within each treatment to boost sample sizes for analysis. Four fish (one reduced ration fish collected in April, one fasted fish in May, and two fasted mortalities in April) were excluded from analysis because data were incomplete or failed quality control checks.

Growth

Growth of herring was inferred from changes in mean length and weight by treatment and from individual measurements of nucleic acid ratios (RNA/DNA). The RNA/DNA ratio, which provides an index for protein synthesis rates over the preceding few days (Buckley 1984; Buckley et al. 1999), was determined using a fluorometric dye-binding assay developed by Caldarone et al. (2001) and modified by Sreenivasan (2011). To ensure comparability of RNA/DNA ratios among sample processing batches and to facilitate comparisons with other studies, we standardized RNA/DNA ratios across batches (Caldarone et al. 2006) by multiplying each sample RNA/DNA value by an average reference material DNA to RNA calibration slope ratio of 3.84 divided by the individual batch calibration slope ratio.

Gut condition

The effects of feeding history on herring gut condition were assessed by monitoring changes in relative gut mass, which is the dry mass of the gastrointestinal (GI) tract as a percentage of body dry mass. GI tracts were excised from esophagus to vent, stomach contents and feces discarded, and gall bladder, liver, and fat deposits removed to the extent practical and returned to the body carcass. GI tracts were dried and weighed to within 0.1 mg, then returned to the body before homogenizing tissues in preparation for whole-body composition analysis.

Body condition

To evaluate the effect of feeding history on condition, we homogenized individual herring with mortar and pestle, then chemically analyzed for moisture, lipid, and protein content following procedures described in Vollenweider et al. (2011*b*). Moisture data were used to express protein content of dried samples on a wet mass basis and lipid content of wet samples on a dry mass basis.

Swimming performance

To assess the effect of feeding history on swimming performance, we conducted swimming trials based on modifications to procedures developed by Beamish (1978). Herring randomly selected from each of the three winter treatments were subjected to critical swimming speed testing in groups of five together at a time in a flume swimming chamber during the April sampling event and from each of the five spring treatments during the May sampling at the study's end. Critical swimming speed, which is the maximum speed maintained for a defined time interval, is an index of prolonged swim speed that is intermediate between burst and sustained swim speeds and defined as the speed individuals can maintain for 20 s to 200 min before fatiguing (Beamish 1978). Following a swim chamber acclimation period of 5 min with slow water flow (3 cm·s^{-1}), swim trials began at a flow speed of 14 cm·s^{-1} for 5 min, followed by speed increases of 7 cm·s^{-1} every 5 min until herring became fatigued. Time to fatigue was defined as the time when an individual remained against the back of the swim chamber for more than 10 s (Melzner et al. 2009). Individual fish were immediately removed upon fatiguing, euthanized, and frozen for later body composition analysis.

Critical swimming speeds (cm·s^{-1}) for individual fish were calculated using Brett's (1964) formula:

$$U_{\text{crit}} = u_i + (t_i / t_{ii} \times u_{ii})$$

where u_i is the maximum speed (cm·s^{-1}) maintained for a complete time interval, u_{ii} is the speed increment used (7 cm·s^{-1}), t_i is the time (min) fish swam at fatigue speed, and t_{ii} the time interval used (5 min). Speeds (in cm·s^{-1}) were divided by fork length (cm) to express speed in terms of body lengths per second (BL·s^{-1}).

Mortality and disease

To assess the effects of feeding levels on herring mortality, tanks were checked daily for dead fish, which were removed and their lengths recorded. Statistically comparing mortalities among treatments required several steps. First, daily mortality (M) by tank was calculated as the number of dead fish divided by the number alive the previous day, minus any sample removals. Cumulative percent mortality (A) across a treatment period was then estimated by tank as

$$A = 1 - e^{(-\overline{M} \times t)}$$

where $\overline{M}$ is the average daily mortality over t days in the treatment period. Cumulative percent mortalities were then averaged across replicate tanks to statistically compare treatments. Graphically representing day-to-day increases in cumulative percent mortalities required a modified approach in which counts of dead fish were first summed across both replicate tanks by treatment on a given day. Deaths were then expressed as a percentage of summed fish alive the previous day, minus any sample removals, and added to the percent mortality from previous days to yield cumulative mortality as of that day.

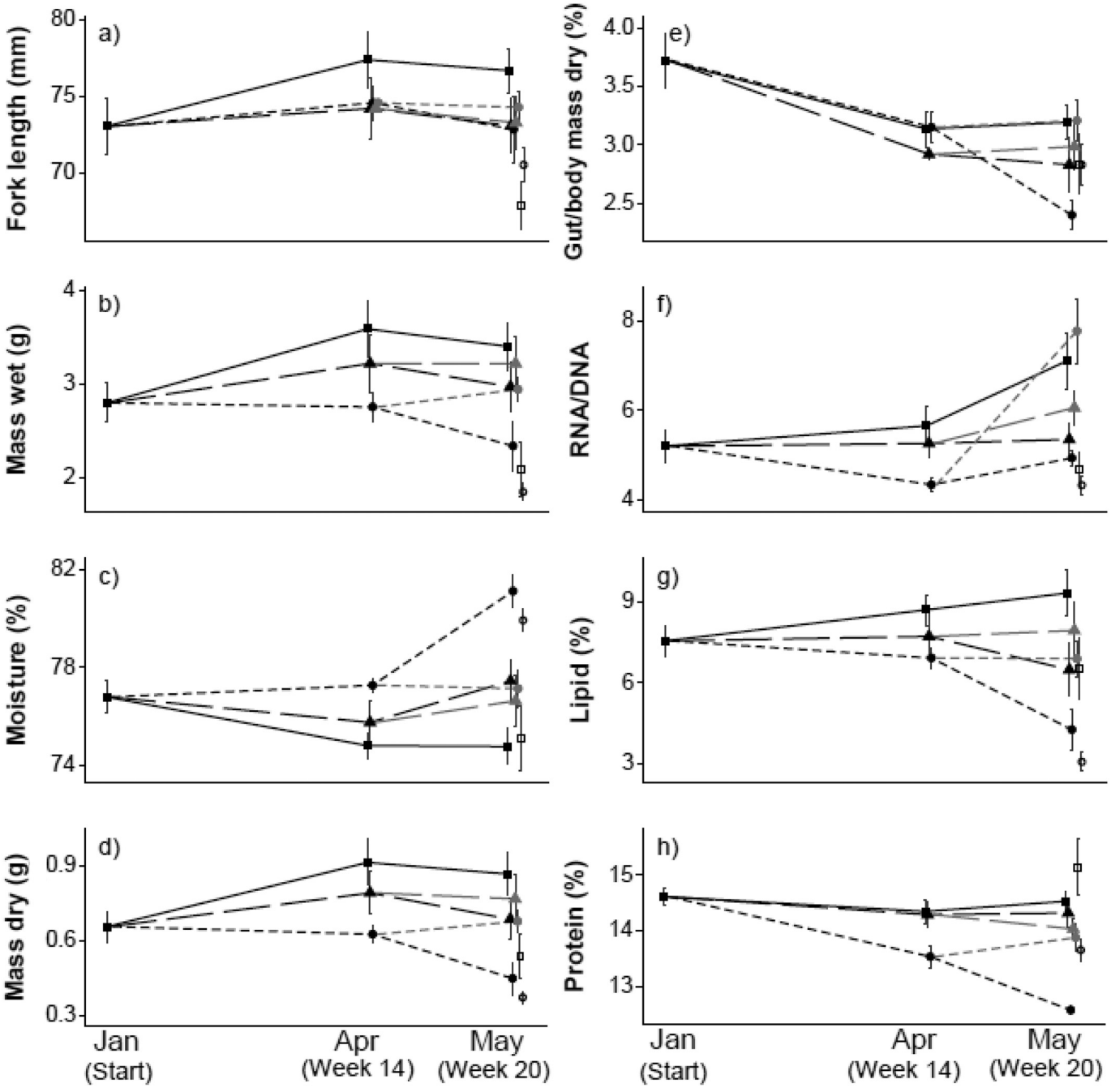
To evaluate whether mortality was length-dependent, we compared median fork lengths between live and dead herring by treatment in spring. For these comparisons, lengths of sampled herring and all remaining live herring in April within treatments were pooled with lengths of herring euthanized for sampling in May. Likewise, lengths of dead herring in April and May were pooled due to low sample numbers.

Owing to apparent signs of disease such as bleeding around the head, blood and tissue samples from recently dead herring were examined for evidence of pathogens and associated diseases (T. Meyers, Alaska Department of Fish and Game pathology laboratory, Juneau, Alaska, USA, personal communication), including heart tissue cultures for ichthyophoniasis, kidney and spleen cell cultures for viral hemorrhagic septicemia (VHS), and blood smears for viral erythrocytic necrosis (VEN). Samples for pathology were taken from mortalities collected opportunistically among fish fed full rations during acclimation (30 November through 16 December) and from fish pooled across all rations at the study end (30–31 May). Only a small fraction (~17%) of dead fish were subjected to disease testing due to logistical constraints, and no live fish were sacrificed for testing to avoid compromising other analyses.

Statistical analyses

Treatments were compared over time and among treatments for differences in mean fork length, wet mass, percent moisture, dry mass, relative gut mass, RNA/DNA, wet mass percent lipid, wet mass percent protein, and critical swim speed. Comparisons

Fig. 2. Morphometric and biochemical indices (mean ± 1 SE) for young-of-the-year Pacific herring from Auke Bay, Southeast Alaska, in the laboratory feeding study, shown at different ration levels: full (black squares, black solid line), reduced (black triangles, black dashed line), fasted (black circles), reduced then re-fed (gray triangles, gray dashed line), fasted then re-fed (gray circles), and mortalities from full (white squares) and fasted (white circles) treatments that occurred on various dates during April and May, shown pooled in May for clarity. Panels show (*a*) fork length, (*b*) body mass on a wet tissue basis, (*c*) moisture content as a percentage of body mass, (*d*) body mass on a dry tissue mass basis, (*e*) dry gut mass as a percentage of dry body mass, (*f*) RNA/DNA ratio, (*g*) lipid mass as a percentage of wet body mass, and (*h*) protein mass as a percentage of wet body mass.



in May included dry mass percent lipid and protein to check for agreement with comparisons on a wet basis. Statistical significance (α = 0.05) of differences was determined using (ANOVA) and post hoc Tukey pairwise comparisons, unless otherwise specified. Assumptions of equal variances and normal distribution of residuals from ANOVA model fits were checked using Bartlett's test (Bartlett 1937) and Anderson–Darling test (Anderson and Darling 1954), respectively. Groups with unequal variances were compared using Welch's ANOVA (Welch 1951; Day and Quinn 1989) employing weighted least squares and Games–Howell pairwise comparisons (Games and Howell 1976; Day and Quinn 1989). Groups with non-normal data were analyzed using Kruskal–Wallis tests (Kruskal and Wallis 1952) and post hoc Dunn pairwise comparisons (Dunn 1964). Previous work showed growth and condition

Table 2. Full-ration herring morphometric and biochemical indices (mean ± 1 SD or median [95% CI in brackets] for non-normal data) by sampling event (month) during the laboratory feeding study (November–May) of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Sampling event (month)				ANOVA (F) or Kruskal–Wallis (H)		
	Start acclimation (Nov.*)	End acclimation (Jan.)	End winter (Apr.)	End spring (May)	R^2	F or H	p
n	40	14	10	10	—	—	—
Fork length (mm)	69.5±9.0b	73.1±6.8ab	77.4±5.8a	76.7±4.5ab	15.4	4.26	0.008
Mass wet (g)	2.44±0.92b	2.80±0.79ab	3.59±0.95a	3.40±0.81a	21.7	6.45	0.001
Moisture (%)†	—	76.8±2.5	74.8±1.6	74.8±2.3	10.6	1.83	0.177
Mass dry (g)‡	—	0.63 [0.48, 0.81]	0.85 [0.65, 1.14]	0.74 [0.68, 1.06]	—	5.61	0.061
Gut/body mass dry (%)	—	3.72±0.88	3.14±0.48	3.19±0.47	15.6	2.87	0.072
RNA/DNA	—	5.21±1.33b	5.66±1.43ab	7.10±2.01a	21.9	4.35	0.022
Lipid wet (%)†	—	7.56±2.20	8.72±1.80	9.34±2.74	6.53	1.08	0.351
Protein wet (%)	—	14.6±0.6	14.4±0.7	14.5±0.6	3.45	0.55	0.580

Note: Values with shared letters did not differ in Tukey's pairwise comparisons (or Dunn's for non-normal data) among sampling events, with each index tested separately. Letters are shown only when some values differed. R^2 = coefficient of determination for ANOVA, F = ANOVA test statistic, H = Kruskal–Wallis test statistic, p = significance value.

*No chemical analyses conducted on fish collected in November.

†ANOVA and Tukey post hoc tests based on residuals from regressions versus length. Means (±1 SD) not adjusted for length are shown.

‡Medians, Kruskal–Wallis test, and Dunn pairwise comparisons of ranked data using Bonferroni-adjusted significance values.

Table 3. Reduced ration herring morphometric and biochemical indices (mean ± 1 SD or median [95% CI in brackets] for non-normal data) by sampling event (month) from January through May during the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Sampling event (month)			ANOVA (F) or Kruskal–Wallis (H)		
	Start acclimation (Jan.)	End acclimation (Apr.)	End spring (May)	R^2	F or H	p
n	14	9	10	—	—	—
Fork length (mm)	73.1±6.8	74.2±5.9	73.1±5.7	0.72	0.11	0.897
Mass wet (g)	2.80±0.79	3.22±0.94	2.97±0.82	4.43	0.70	0.507
Moisture (%)*	76.8±2.5	75.7±2.7	77.4±2.8	5.71	0.91	0.414
Mass dry (g)	0.66±0.23	0.79±0.26	0.69±0.26	5.45	0.86	0.432
Gut/body mass dry (%)†	3.57 [3.10, 4.09]a	2.92 [2.81, 3.03]ab	2.67 [2.34, 3.33]b	—	9.83	0.007
RNA/DNA	5.21±1.33	5.27±0.95	5.36±1.08	0.30	0.05	0.956
Lipid wet (%)*	7.56±2.20	7.73±2.49	6.49±3.12	4.33	0.68	0.515
Protein wet (%)	14.6±0.6	14.3±0.7	14.3±0.8	5.19	0.82	0.449

Note: All fish were fed full ration during acclimation until the January sampling event. Values with shared letters did not differ in Tukey's pairwise comparisons (or Dunn's for non-normal data) among sampling events, with each index tested separately. Letters are shown only when some values differed. R^2 = coefficient of determination for ANOVA, F = ANOVA test statistic, H = Kruskal–Wallis test statistic, p = significance value.

*ANOVA and Tukey post hoc tests based on residuals from regressions versus length. Means (±1 SD) not adjusted for length are shown.

†Medians, Kruskal–Wallis test, and Dunn pairwise comparisons of ranked data using Bonferroni-adjusted significance values.

indices can vary with herring length (Sewall et al. 2019), so significant relationships between fork length and each body composition index and between fork length and swimming speed were identified using simple linear regressions applied to all samples combined, unless otherwise specified. Regression residuals were then used to compare variables that were length-dependent among groups. This removed effects of the covariate length and facilitated use of non-parametric tests when ANOVA assumptions were violated.

Linear discriminant analysis (LDA; Ripley et al. 2019) was performed to find the morphometric and body composition indices that best classified individual herring membership among all seven groups (five treatment groups and two mortality groups) at the study end in May. To make measurement scales comparable for LDA, we first standardized data for each variable by subtracting the mean and dividing by the standard deviation. Model accuracy in classifying samples was estimated as the bootstrapped mean accuracy from randomly resampling the training data (~80% of data) and test data (~20% of data) 1000 times.

Differences in cumulative mortalities among treatments were determined using ANOVA and post hoc Tukey pairwise comparisons. To evaluate whether mortality was size-dependent, we compared lengths of live and sampled herring versus mortalities by ration during spring using Mann–Whitney rank-based tests due to skew in the length data.

Results

Growth and condition

Full ration

Fully fed herring had only modest growth through winter (Fig. 2). Herring mean fork length and wet mass showed statistically significant increases from the start of the acclimation period in November

Table 4. Fasted herring morphometric and biochemical indices (mean ± 1 SD or median [95% CI in brackets] for non-normal data) by sampling event (month) from January through May during the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Sampling event (month)			ANOVA (F) or Kruskal–Wallis (H)		
	Start acclimation (Jan.)	End acclimation (Apr.)	End spring (May)	R^2	F or H	p
n	14	10	7	—	—	—
Fork length (mm)	73.1±6.8	74.6±3.5	72.9±5.6	1.91	0.27	0.763
Mass wet (g)	2.80±0.79	2.75±0.48	2.33±0.70	7.60	1.15	0.331
Moisture (%)*	76.5 [75.9, 78.2]b	77.0 [76.6, 77.9]b	81.4 [80.6, 82.2]a	—	13.4	0.001
Mass dry (g)	0.66±0.23	0.63±0.15	0.45±0.17	17.8	3.04	0.064
Gut/body mass dry (%)†	3.72±0.88a	3.15±0.42a	2.40±0.33b	40.6	15.71	<0.001
RNA/DNA†	5.21±1.33	4.35±0.52	4.94±0.44	14.2	4.25	0.031
Lipid wet (%)	7.56±2.20a	6.92±1.21a	4.25±2.03b	34.4	7.33	0.003
Protein wet (%)†	14.6±0.6a	13.5±0.6b	12.6±0.2c	71.8	72.10	<0.001

Note: All fish were fed full ration during acclimation until the January sampling event. Values with shared letters did not differ in Tukey's pairwise comparisons (or Dunn's for non-normal data, Games–Howell comparisons for unequal variances) among sampling events, with each index tested separately. Letters are shown only when some values differed. R^2 = coefficient of determination for ANOVA, F = ANOVA test statistic, H = Kruskal–Wallis test statistic, p = significance value.

*Medians, Kruskal–Wallis test, and Dunn pairwise comparisons of ranked data using Bonferroni-adjusted significance values.

†Welch's ANOVA and Games–Howell pairwise comparisons.

Table 5. April herring morphometric and biochemical indices (mean ± 1 SD or median [95% CI in brackets] for non-normal data) and pairwise comparisons by ration level following the winter ration period during the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Ration level			ANOVA (F) or Kruskal–Wallis (H)		
	Full	Reduced	Fasted	R^2	F or H	p
n	10	9	10	—	—	—
Fork length (mm)	77.4±5.8	74.2±5.9	74.6±3.5	7.9	1.12	0.341
Mass wet (g)	3.59±0.95	3.22±0.94	2.75±0.48	17.0	2.66	0.089
Moisture (%)*,†	74.8±1.6b	75.7±2.7ab	77.3±0.9a	22.1	8.04	0.004
Mass dry (g)*	0.91±0.29a	0.79±0.26ab	0.63±0.11b	22.7	4.95	0.023
Gut/body mass dry (%)*	3.14±0.48	2.92±0.12	3.15±0.42	7.60	2.09	0.161
RNA/DNA‡	5.61 [4.75, 6.08]a	5.05 [4.59, 6.33]ab	4.40 [4.03, 4.70]b	—	9.37	0.009
Lipid wet (%)†	8.72±1.80	7.73±2.49	6.92±1.21	10.8	1.57	0.228
Protein wet (%)‡	14.6 [13.9, 14.8]	14.6 [13.3, 14.8]	13.4 [13.1, 14.0]	—	6.86	0.032

Note: Values with shared letters did not differ in Tukey's pairwise comparisons (or Dunn's for non-normal data, Games–Howell comparisons for unequal variances) among sampling events, with each index tested separately. Letters are shown only when some values differed. R^2 = coefficient of determination for ANOVA, F = ANOVA test statistic, H = Kruskal–Wallis test statistic, p = significance value.

*Welch's ANOVA and Games–Howell pairwise comparisons.

†ANOVA and Tukey post hoc tests based on residuals from regressions versus length. Means (±1 SD) not adjusted for length are shown.

‡Medians, Kruskal–Wallis test, and Dunn pairwise comparisons of ranked data using Bonferroni-adjusted significance values.

(FL = 69.5 mm, wet mass = 2.44 g; Table 2) through April (FL = 77.4 mm, wet mass = 3.59 g). Relative gut mass had a marginally nonsignificant decrease from 3.72% in January to 3.14% in April, with a slight rebound to 3.19% in May, despite being fully fed through winter. In agreement with the minimal size increases observed during the study, mean RNA/DNA levels were similarly low in January (5.21) and April (5.66) and did not significantly increase until May (7.10; Table 2).

Reduced ration

Herring on a reduced ration diet from January through May did not differ significantly over time on most measures, with the exception of relative gut mass, which was significantly lower in May than at the end of full-ration acclimation in January (Table 3).

Fasted

Herring that fasted through winter largely maintained body size in length and wet mass, though body composition and gut mass changed (Table 4). Wet mass loss was mitigated by gains in water mass, as indicated by significantly higher median moisture levels from January (76.5%) to May (81.4%). Nominal decreases in mean wet and dry mass by May might not have been statistically significant due to low sample sizes of fasted fish in May. Median relative gut mass significantly decreased from January (3.72%) through May (2.40%), indicating preferential loss of gut tissue. The observed decline in dry mass was reflected in significant losses in the two main constituents; lipid declined to 4.25% of wet mass by May, while protein declined to 12.6%.

Differences among treatments

Morphometric and biochemical indices generally differed most between the full-ration and fasted treatments over time, with other treatments intermediate (Fig. 2). Herring fed at full ration during winter from January through April were not significantly larger in mean length (77.4 mm, $p = 0.341$) or wet mass (3.59 g, $p = 0.089$) than reduced ration or fasted herring (Table 5). Differences were not significant at the end of the study end, although full-ration herring

Table 6. May herring morphometric and biochemical indices (mean ± 1 SD or median [95% CI in brackets] for non-normal data) and pairwise comparisons by ration level following the spring re-feeding period during the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Ration level						ANOVA (F) or Kruskal–Wallis (H)		
	Full	Reduced	Fasted	Reduced re-fed	Fasted re-fed	Full mort	Fasted mort	R^2	F or H	p
n	10	10	7	10	10	8	18	—	—	—
Fork length (mm)	76.7±4.5a	73.1±5.7ab	72.9±5.6ab	73.3±5.5ab	74.3±3.2ab	67.9±4.4b	70.6±4.6b	23.2	3.33	0.006
Mass wet (g)*	3.09 [2.80, 4.04]a	2.89 [2.60, 3.44]ab	2.11 [1.84, 3.14]abc	2.97 [2.69, 3.43]ab	3.05 [2.51, 3.27]ab	1.97 [1.30, 3.00]bc	1.90 [1.52, 2.09]c	—	36.3	<0.001
Moisture (%)†	74.8±2.3c	77.4±2.8abc	81.1±1.7a	76.6±3.2bc	77.1±2.4bc	75.1±3.8c	79.9±1.9ab	43.2	8.37	<0.001
Mass dry (g)*	0.74 [0.68, 1.06]a	0.63 [0.51, 0.90]ab	0.38 [0.32, 0.59]bc	0.64 [0.55, 0.89]ab	0.71 [0.54, 0.82]ab	0.51 [0.26, 0.83]abc	0.36 [0.31, 0.42]c	—	36.2	<0.001
Gut/body mass dry (%)	3.19±0.47	2.83±0.76	2.40±0.33	2.99±0.62	3.21±0.57	2.84±0.71	2.83±0.74	12.3	1.55	0.177
RNA/DNA*	6.86 [5.40, 9.26]ab	5.23 [4.61, 5.82]abc	4.99 [4.48, 5.35]abc	5.99 [4.96, 6.94]ab	7.78 [5.80, 8.38]a	4.71 [3.62, 5.17]bc	4.49 [3.77, 4.89]c	—	34.7	<0.001
Lipid wet (%)†	9.34±2.74a	6.49±3.12abc	4.25±2.03bc	7.95±3.29ab	6.89±2.04abc	6.50±3.24ab	3.05±1.50c	38.0	6.73	<0.001
Lipid dry (%)†	36.5±7.5a	27.6±10.3a	21.9±8.4ab	32.9±9.6a	29.6±6.4a	24.9±10.5a	14.7±5.7b	39.4	7.15	<0.001
Protein wet (%)‡	14.5±0.6a	14.3±0.8ab	12.6±0.2c	14.0±0.6ab	13.9±0.8ab	15.1±1.4ab	13.7±0.8b	41.5	29.8	<0.001
Protein dry (%)‡	58.1±6.1ab	64.4±8.5ab	67.1±5.4ab	61.0±8.2ab	61.3±6.9ab	61.4±6.4a	68.3±3.9b	22.1	3.11	0.010

Note: Values for mortalities represent full-ration (Full mort) and fasted (Fasted mort) herring that died during spring (April–May). Values with shared letters did not differ in Tukey's pairwise comparisons (or Dunn's for non-normal data, Games–Howell comparisons for unequal variances) among sampling events, with each index tested separately. Letters are shown only when some values differed. R^2 = coefficient of determination for ANOVA, F = ANOVA test statistic, H = Kruskal–Wallis test statistic, p = significance value.

*Medians, Kruskal–Wallis test, and Dunn pairwise comparisons of ranked data using Bonferroni-adjusted significance values.

†ANOVA and Tukey post hoc tests based on residuals from regressions versus length. Means (±1 SD) not adjusted for length are shown.

‡Welch's ANOVA and Games–Howell pairwise comparisons.

Table 7. Coefficients of linear discriminants (LDs) from analysis of standardized data for all morphometric and biochemical indices at the end (May) of the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Linear discriminants		
	LD1	LD2	LD3
Fork length (mm)	−0.439	−0.496	0.686
Mass wet (g)	0.405	0.947	1.981
Moisture (%)	7.529	−2.589	−4.096
Mass dry (g)	0.384	−0.639	−3.510
Gut/body mass dry (%)	0.159	0.493	0.185
RNA/DNA	0.601	−0.023	1.058
Lipid wet (%)	7.054	−1.594	−2.526
Protein wet (%)	1.670	0.090	−1.359
Separation achieved (%)	73.4	14.4	5.5

Note: Top three discriminants explaining ~93% of between-group variability (Separation achieved (%)) are shown.

again were longest and heaviest and fasted herring were smallest among sampled treatment groups (Table 6). In May, surviving full-ration herring were significantly larger than mortalities from the full-ration and fasted treatments, the two treatments from which mortalities were analyzed. Full-ration herring exceeded fasted herring in mean or median dry body mass, RNA/DNA, lipid level, and protein, with differences typically increasing from April (Table 5) to May (Table 6). In contrast, moisture content was ~6% lower in the full-ration treatment than in the fasted treatment.

Moisture and lipid content had contrasting relationships to size, as moisture decreased with length for all treatments pooled in April (R^2 = 12.2%, F = 3.75, p = 0.063) and May (R^2 = 9.9%, F = 7.76, p = 0.007), while lipid increased with length in April (R^2 = 12.6%, F = 3.90, p = 0.059) and May (R^2 = 19.4%, F = 17.1, $p < 0.001$). Regardless of length, moisture was strongly inversely related to lipid content of wet tissue (R^2 = 84.8%, F = 396.4, $p < 0.001$) and dry tissue (R^2 = 73.2%, F = 194.2, $p < 0.001$) across all herring in May. Lipid levels among full-ration herring were more than twice those of fasted herring and more than three times those of fasted herring mortalities in May (Table 6). Lipids were largely similar among other treatments, though full-ration mortalities had more than twice the lipid content of fasted mortalities. Lipid content calculated on a dry mass basis in May increased with length (R^2 = 21.4%, F = 19.3, $p < 0.001$) and had similar contrasts among rations to lipid on a wet mass basis. Mean dry mass lipid significantly differed by ration overall after accounting for length, though the difference between full-ration and fasted herring was marginally nonsignificant (p = 0.090) in pairwise comparisons.

Protein as a percentage of wet body mass was less variable than lipid but had a significant 2% difference between full-ration and fasted herring. Protein content on a dry mass basis decreased with length in May (R^2 = 11.5%, F = 9.19, p = 0.003) and had the reverse pattern among treatments as protein on a wet mass basis. After accounting for length, mean dry mass protein significantly differed by ration overall, with lower levels among full-ration herring than among fasted herring, though only full-ration mortalities and fasted mortalities significantly differed (p = 0.044). Increased percentage of protein and decreased lipid on a dry mass basis indicate conservation of protein mass relative to lipid mass when body tissues were metabolized by fasted herring.

Moisture and lipid indices were the most useful for distinguishing herring from different treatments, as indicated by the large moisture and lipid coefficients in LDA functions (Table 7). LDA showed the clearest differences between full-ration and fasted fish and between mortalities and non-mortalities (Fig. 3). The LDA

Fig. 3. Linear discriminant analysis scores with centroids and 95% CI bars for discriminants LD1 and LD2 derived from all standardized morphometric and biochemical data at the end (May) of the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska. Points represent individual herring by ration level: full (black squares), reduced (black triangles), fasted (black circles), reduced then re-fed (gray triangles), fasted then re-fed (gray circles), and mortalities from full (white squares) and fasted (white circles) treatments.

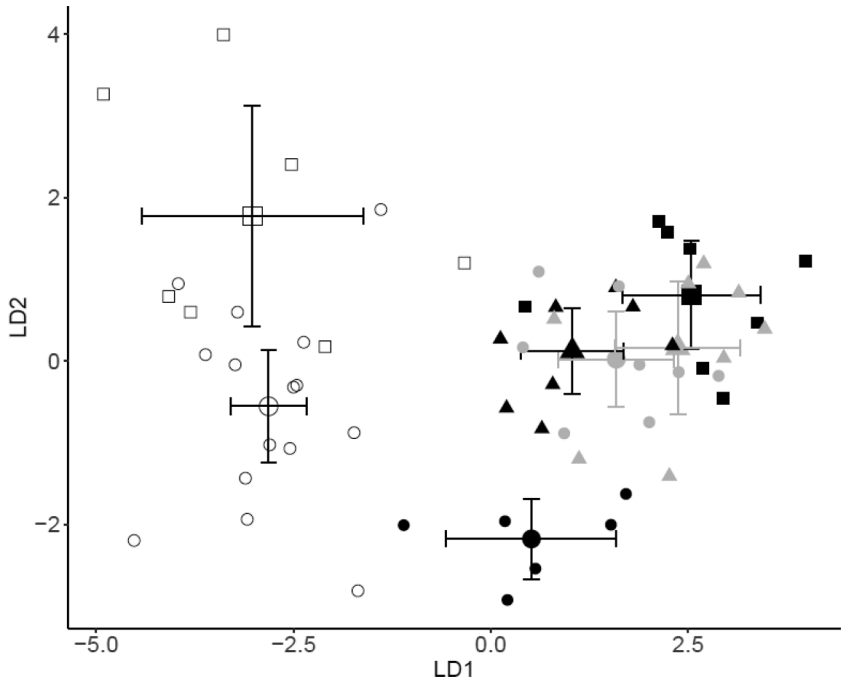
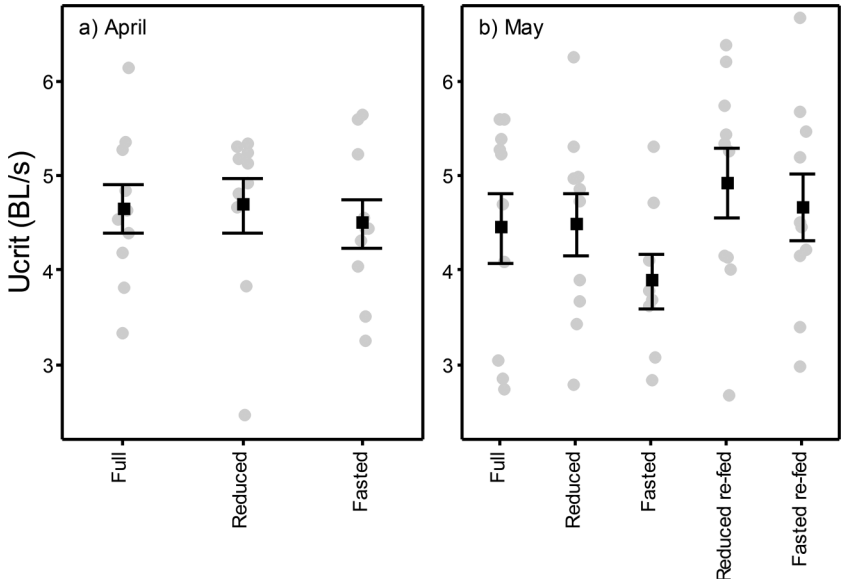


Fig. 4. Critical swimming speeds (U_{crit}; body lengths (BL) per second) of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska, in the laboratory feeding study, shown by ration level after (*a*) the end of winter ration treatments in April and (*b*) the end of spring re-feeding treatments in May. Individual speeds (gray circles), means (black squares), and SE bars shown. Sample sizes: $n = 8$ for fasted treatment in May; $n = 10$ for all other treatments.



model classified fish with ~54% accuracy, better than a null model classifying evenly across groups (for seven groups, 100/7 = 14%).

Re-feeding

Herring re-fed in spring following a winter period of reduced ration or fasting had evidence, though not statistically significant, of greater average length, wet mass, relative gut mass, RNA/DNA, lipid, and protein content and lower moisture in May compared with those not re-fed (Fig. 2; Table 7). Re-fed herring thus had less separation in the LDA plot from full-ration herring than did herring not re-fed (Fig. 3). Indices for re-fed herring in May were similar to those of reduced ration and fasted herring before they began re-feeding in April. Similar wet mass, lipid, and protein levels from

Fig. 5. Cumulative daily mortality (% initial *n*) in the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska, by ration level for (*a*) winter ration period (January–April; three ration levels) and (*b*) spring re-feeding period (April–May; five ration levels). Lines indicate sums across replicate tanks by ration level: full (black solid line), reduced (black dashed line), fasted (black dotted line), reduced then re-fed (gray dashed line), and fasted then re-fed (gray dotted line).

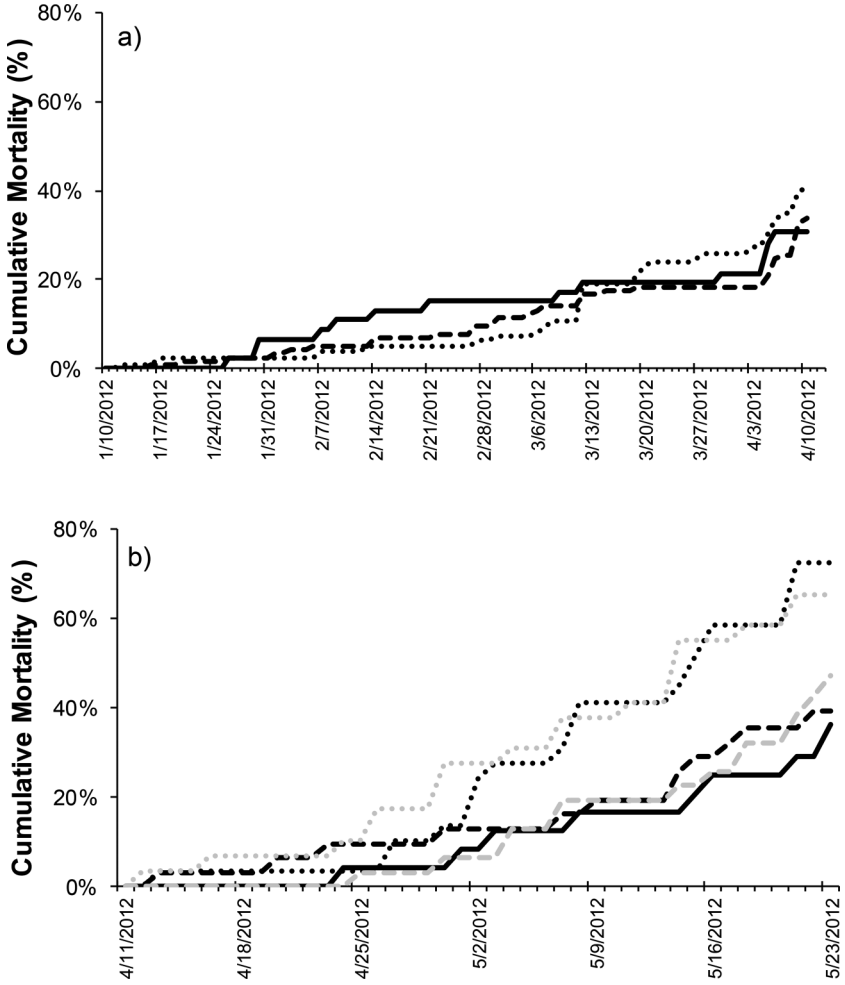


Table 8. Cumulative percent mortality (mean ± 1 SD) across replicate tanks (*n*), adjusted for sampling and other removals, during the winter ration period (January–April; 93 days) and spring re-feeding period (April–May; 42 days) in the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Winter (Jan.–Apr.)					Spring (Apr.–May)				
Ration level	*n*	No. of fish start	No. of fish removed	No. of fish died	Mortality (%)	*n*	No. of fish start	No. of fish removed	No. of fish died	Mortality (%)
Full	2	46	10	12	24.6±13.3	2	24	10	8	35.5±1.1b
Reduced	2	123	26	35	33.4±5.2	2	31	10	12	37.5±1.7ab
Fasted	2	133	29	43	36.6±0.8	2	29	8	21	69.6±2.6a
Reduced re-fed*	—	—	—	—	—	2	31	10	14	44.4±16.8ab
Fasted re-fed*	—	—	—	—	—	2	29	10	19	62.0±7.5ab

Note: Values with shared letters did not differ in Tukey's pairwise comparisons among ration levels within a period, with winter and spring periods tested separately. Letters are shown only when some values differed.

*Re-fed treatments established after spring (April) sampling.

April to May indicate that re-fed herring had stopped catabolizing body tissues, but had not significantly increased energy stores or size. However, RNA/DNA among fasted fish re-fed in spring increased to surpass that of the full-ration treatment.

Swimming performance

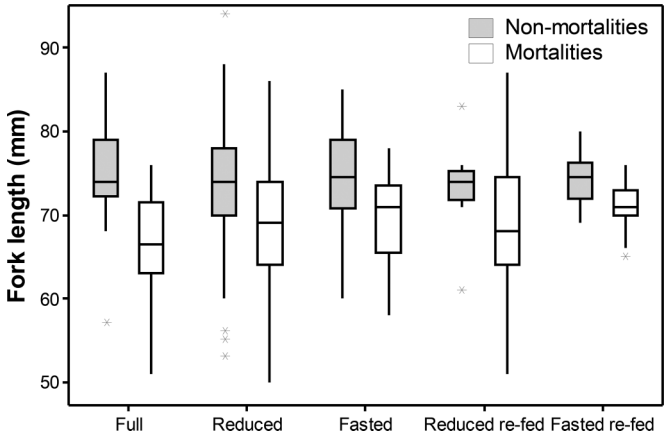
Critical swimming speeds ($BL{\cdot}s^{-1}$) did not significantly differ among treatments in April following the winter ration period (ANOVA; $F_{[2,27]} = 0.14$, $R^2 = 1.03\%$, $p = 0.870$) or in May following spring re-feeding (ANOVA; $F_{[4,43]} = 1.11$, $R^2 = 9.43\%$, $p = 0.360$), although the fasted herring were slowest among treatments within April and May, while the re-fed herring were fastest among treatments within May (Fig. 4). Critical swimming speed increased linearly with length for herring among all rations pooled in the April swim test ($R^2 = 17.8\%$, $F = 6.06$, $p = 0.020$) and with April and May tests pooled ($R^2 = 5.8\%$, $F = 4.69$, $p = 0.033$),

Table 9. Median length and range (mm) of live and sampled fish (non-mortalities) versus fish that died (mortalities) by ration level during April and May in the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska.

	Non-mortalities		Mortalities		Mann–Whitney	
Ration level	*n*	Length (range) (mm)	*n*	Length (range) (mm)	*W*	*p*
Full	44	74 (57–87)	10	66.5 (51–76)	116	<0.001
Reduced	94	74 (53–94)	26	69 (50–86)	1 024.5	<0.001
Fasted	90	74.5 (60–85)	33	71 (58–78)	1 327.5	<0.001
Reduced re-fed*	10	74 (61–83)	13	68 (51–87)	133.5	0.171
Fasted re-fed*	10	74.5 (69–80)	19	71 (65–76)	236.5	0.027
All pooled	248	74 (53–94)	101	70 (50–87)	11 706.5	<0.001

Note: Values include the end of "winter" and all of "spring" treatment periods. *W* = Mann–Whitney test statistic, *p* = significance value adjusted for ties.
*Re-fed treatments established after April sampling.

Fig. 6. Fork length (mm) of live and sampled fish (non-mortalities) and fish that died (mortalities) by ration level during spring (April–May) in the laboratory feeding study of young-of-the-year Pacific herring from Auke Bay, Southeast Alaska. Medians, interquartile ranges (IQR), whiskers (≤1.5 IQR), and outliers (*, >1.5 IQR) are shown.



though not in May (R^2 = 2.29%, F = 1.08, p = 0.304). Regardless, herring did not significantly differ by ration based on residuals from swim-speed length regressions or absolute swimming speed ($cm \cdot s^{-1}$).

Mortalities

Cumulative mortalities during the winter ration and spring re-feeding periods were highest in the fasted treatment and lowest in the full-ration treatment. Mortalities in the reduced and re-fed treatments were intermediate between those of the full ration and fasted treatments (Fig. 5), similar to the patterns seen in the morphometric and biochemical indices. Cumulative mortality differences needed to be large to be statistically significant due to comparisons being based on means of only two tanks per treatment. The differences were not significant during the period from the end of acclimation in January through the end of winter ration treatments in April (ANOVA; $F_{[3,2]}$ = 1.14, R^2 = 43.3%, p = 0.427). During the spring re-feeding period (April–May), mortalities significantly differed among treatments (ANOVA; $F_{[4,5]}$ = 6.58, R^2 = 84.0%, p = 0.032), with the highest mortality (70%) in the fasted treatment (Tukey's post hoc test; Table 8) and lowest (36%) in the full-ration treatment. Resuming feeding at full ration in spring had little effect on mortality of herring previously on restricted winter rations, as cumulative mortalities among the re-fed treatments appeared similar to their fasted and reduced ration cohorts that were not re-fed.

Mortality appeared to be size-dependent, as herring that died were of smaller average size than live or euthanized herring regardless of ration (Fig. 6). Based on spring (April–May) data in which all rations were represented, median fork lengths of herring mortalities (70 mm) were significantly smaller than those of non-mortalities (74 mm) across rations (Table 9). Within rations, median lengths of mortalities were significantly smaller than non-mortalities by 3.5–7 mm in all cases, with the exception of reduced ration herring that were re-fed in spring, and mortalities typically included the smallest fish in each treatment.

Herring had signs of disease such as bleeding around the head throughout the study. Pathology laboratory examination of dead herring detected no evidence of ichthyophoniasis, but found 70% prevalence of VHS infection and 83% prevalence of VEN in November–December and 77% prevalence of VEN in May. While infection prevalence is not necessarily an accurate predictor of mortalities because not all infected fish die (Kocan et al. 2001), the pathology results indicated high prevalence of potentially lethal infections.

Discussion

Winter feeding challenges and benefits

Herring growth was modest over the course of the study, likely due to the relatively cold temperatures and short daylight during the winter months (Stokesbury et al. 1999; Foy and Paul 1999), even with abundant food provided. Relatively small decreases in temperature can substantially slow growth, as fully fed captive YOY herring from Puget Sound gained only one-third the mean mass when reared for 10 weeks at 6.5 versus 8.5 °C (~1 versus ~3 g gain; Sreenivasan 2011). In comparison, water temperatures in the present study in Southeast Alaska were below 5 °C during winter (January–April) and likely contributed to growth slowing to levels difficult to detect. Growth and metabolism may have also slowed in response to short daylight hours, a winter adaptation seen in other high-latitude visual feeders (perch (*Perca fluviatilis*); Karås 1990). In addition to low temperatures and short daylight, growth is further constrained for wild herring by winter declines in zooplankton prey availability (McKinstry and Campbell 2018) and herring stomach fullness (Foy and Norcross 1999). However, stomach fullness in late winter is greater among lean herring (Sewall et al. 2019), and high proportions of herring feed in March before the return of high zooplankton biomass (Foy and Paul 1999). This suggests that winter feeding or fasting is not entirely a reflection of prey availability. Instead, whether a fish feeds or fasts reflects trade-offs in costs and benefits to foraging. Foraging costs include high predation risk due to compromised antipredator schooling behaviors (Sogard and Olla 1997). Meanwhile, foraging

benefits appear constrained in winter, as feeding may support only limited growth, as indicated in this study by minimal size gains among herring with abundant food. Only when a herring has exhausted its winter fat stores may the balance shift in favor of foraging to avoid starvation. An overwintering strategy of metabolically consuming stored fat thus may be preferred to foraging and increasing predation risk, regardless of zooplankton prey availability.

Food quality can affect the growth and condition of herring at early life stages (Foy and Norcross 1999; Malzahn et al. 2007) and should be considered when interpreting responses to feeding, especially under artificial conditions. Growth of some species can be negatively affected by high fluoride levels found in euphausiid exoskeletons (Yoshitomi et al. 2006), though others appear unaffected (Julshamn et al. 2004). Food quality was not suspected to constrain growth in this study, as the Antarctic euphausiids provided as food are lipid-rich (Cho et al. 1999), and various species of euphausiids are energy-dense prey for Prince William Sound herring (Foy and Norcross 1999). Euphausiid diets are commonly used in captive Pacific herring studies (Kocan et al. 1999; Kennedy and Farrell 2008) and promote feeding and growth of other northern marine species (Olsen et al. 2006; Tibbetts et al. 2011). However, growth limitation due to an exclusively euphausiid-based diet cannot be ruled out.

Winter zooplankton prey availability may offer limited benefits to herring if assimilation efficiency is reduced by gut atrophy. Relative gut mass apparently declined through winter across all treatments including fully fed herring. Changes in gut mass in response to seasonally variable food occur in wild Dolly Varden (*Salvelinus malma*; Armstrong and Bond 2013) to reduce energy spent on gut maintenance. Reductions in gut mass and length also have been observed in response to fasting in laboratory studies in at least 11 fish species (reviewed in Zaldúa and Naya 2014). However, while gut mass losses were greatest among fasted herring in the present study, gut atrophy among fish provided excess food suggests influence by other environmental cues such as limited daylight hours or low temperatures. If gut atrophy commonly occurs in overwintering herring populations, winter feeding benefits could be limited even when conditions support high zooplankton prey abundance.

Resumption of growth in spring with the return of abundant food should be critically important, though low temperatures persisting after winter may constrain its benefits to survival. Herring that resumed feeding in spring after winter fasting had signs of compensatory growth, increasing their median RNA/DNA levels by 77% from April to May, moving them up from the lowest RNA/DNA among all rations in April to the highest among rations in May. This renewed growth effort enabled previously fasted herring to achieve sizes only slightly smaller than their continuously fed counterparts through the study's end. Additional time beyond that provided in this study for re-feeding at full ration might have allowed full compensation in size, although growth deficits after fasting for several weeks can be insurmountable even at relatively warm temperatures (e.g., 28 °C for channel catfish (*Ictalurus punctatus*); Gaylord and Gatlin 2000). The efficiency of translating RNA into protein-based growth declines with temperature (Buckley et al. 1999), low temperatures persisting after winter, as observed during April–May (daily mean 5.4 °C), may thus limit compensatory growth in spring.

Compensatory growth effort in spring did not appear to negatively affect swimming performance, as re-fed fasted herring had higher RNA/DNA levels and critical swimming speeds than continuously fully fed herring. Re-fed fasted herring may have supported high growth without sacrificing physiological capacity for swimming by increasing consumption to levels comparable to or exceeding those of continuously fed cohorts, as reflected by their slightly larger relative gut mass following spring re-feeding. Temporarily increasing consumption is a common response to feeding deficits (Won and Borski 2013) and is how re-fed fasted juvenile walleye pollock (*Gadus chalcogrammus*) are likewise able to achieve compensatory growth without impacting critical swimming speed (Sogard and Olla 2002). Not all species can use this strategy, as re-fed fasted sablefish (*Anoplopoma fimbria*) apparently cannot eat more than continuously fed counterparts and so achieve compensatory growth at the expense of swimming performance (Sogard and Olla 2002). Supporting swimming performance concurrently with growth likely reflects the urgent need of fasted herring to forage efficiently while avoiding size-based predation. In contrast, continuously fed herring had high fat stores and submaximal RNA/DNA and swimming speed, suggesting they were not allocating all available energy consumed towards growth. This agrees with previous findings that YOY herring will shift energy allocation away from growth in favor of storing lipids as long as they attain a critical size (Sewall et al. 2019). Spring re-feeding thus appeared to promote swimming performance after winter and may also allow healthy fish to rapidly resume growth and lipid storage. However, the observed minimal gains in size, lipid levels, and high mortality suggest limited survival benefits to spring re-feeding after winter fasting. These challenges to improving fish size, condition, and survival in spring when low winter temperatures persist underscore the importance of achieving large size (Beamish and Mahnken 2001) and lipid stores (Paul and Paul 1998) in the critical period before winter.

Lipid and moisture variables were the most informative for inferring feeding and condition of wild herring. Given the strong influence of lipid and moisture in the LDA, their utility for distinguishing poorly fed and well-fed wild herring appears similar to that of the LDA functions, and their biological interpretation is more straightforward. Lipid and moisture were strongly inversely related, as has been reported elsewhere across several species (Anthony et al. 2000; Wuenschel et al. 2019), indicating that either variable alone may be adequate for distinguishing wild fish. If the results had indicated instead that several measured variables made similar contributions to the LDA functions, thus making the effect of any specific variable ambiguous, then applying LDA functions derived from lab fish could be especially useful for assessing wild herring with measurements on those variables. Developing LDA functions with nondiseased fed and fasted herring and comparing the results with this study would enable their appropriate application to wild fish of known disease status.

Size-dependent mortality by starvation and disease

Mortalities were unexpectedly high throughout the study. For example, mortalities were anticipated to be under ~5% in a 3-month period (Gregg et al. 2011) among full-ration herring, with starvation risk minimized, but ~25% died during the 3-month winter period and another ~36% in the 1.5-month spring period, with higher mortalities at other rations. It is possible disease caused much of the mortality among full-ration herring, while herring on restricted rations may have experienced interactive effects of disease and starvation. Attributing deaths to starvation versus disease is difficult because disease exposure was not a controlled variable in this study.

Smaller herring appeared to be at higher risk of death than larger herring, regardless of winter feeding history. Starvation was likely size-dependent due to higher mass-specific metabolic rates (Slotte 1999) and lower lipid stores (Sewall et al. 2019) among smaller herring that caused them to deplete their stored energy more rapidly than larger individuals. Energetic costs of disease (Vollenweider 2011*a*) may have compounded that effect and been the primary reason fasted herring in this study lost total body energy from January to May at twice the daily rate for captive herring reported previously (~46 $J \cdot day^{-1}$, based on mean lipid and protein losses, versus 23 $J \cdot day^{-1}$ in Paul and Paul 1998), despite water temperatures 1–2 °C colder. Disease may also have

effectively raised the minimum survival threshold level of lipid. Lipid levels of fish that died were well above the minimum survival threshold lipid of 1.28% established in fasting disease-free herring and higher than the mean lipid level of 2.15% among wild Prince William Sound herring winter survivors in March (Sewall et al. 2019). It is less clear why size-dependent mortality also occurred among fully fed herring. If higher mortality among smaller herring was due to VHS or VEN, it could reflect a relationship between body size and viral pathogen dose, though the mechanism is unclear (P. Hershberger, US Geological Survey, Marrowstone field station, Nordland, Washington, USA, 2019, personal communication). Increased mean size of Prince William Sound herring collected before and after winter is believed to be due to loss of small herring to predation and starvation (Foy and Paul 1999; Paul and Paul 1998). If smaller herring are also more vulnerable to disease, this reinforces the notion that growing beyond a critical size before winter (Sewall et al. 2019) is essential for reducing herring mortality risk.

Conclusions

In summary, herring growth was minimal during winter months even with abundant food available, supporting the importance of growth before winter to promote survival (Norcross et al. 2001; Sewall et al. 2019). Spring re-feeding following winter food limitation may stop body mass loss and even promote compensatory growth and swimming performance but may not reduce mortality compounded by disease. Winter feeding benefits to herring growth and condition may be limited not only by cold temperatures but also by gut atrophy, causing lower assimilation efficiency even among fully fed fish. These constraints suggest why a fat-burning strategy is favored rather than incurring greater predation risk by foraging during winter, regardless of food availability, and why maximizing fat stores before winter is critical. Further, ocean conditions that promote zooplankton prey availability may offer only limited benefits to herring if they are inconsistent or poorly matched to herring needs. If high zooplankton availability occurs too early in the spring, herring may have not yet recovered gut mass lost in winter for efficient assimilation and temperatures may be too low to promote growth. Alternatively, herring may not benefit if spring zooplankton peak too late to counteract effects of limited winter feeding compounded by disease. Reduced disease effects can occur at warmer temperatures (Gregg et al. 2011; Hershberger et al. 2013) but may be contingent on herring obtaining sufficient energy from food to meet increased metabolic demands. Timing and magnitude of peak zooplankton prey biomass in spring responds to ocean temperature (Batten et al. 2018); thus, recent warming trends in the North Pacific could promote a mismatch among prey availability, herring digestive capacity, and energetic demands that negatively impacts herring survival.

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