# The role of temperature on overwinter survival, condition metrics and lipid loss in juvenile polar cod (*Boreogadus saida*): a laboratory experiment

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**Highlights**

- Both larger body sizes and colder culture temperatures significantly prolonged polar cod winter survival duration
- During simulated Arctic winters, polar cod lost lipid mass at twice the rate that they lost wet mass
- Mortality of polar cod occurs at a Fulton's K of 0.44, a HSI of 0.67, and lipids per WWT of 12.4 mg. $g^{-1}$
- Lipid storage in polar cod from a warm year and a cold year indicate that poor winter starvation resistance may occur following warm summers
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ABSTRACT

In the Arctic, winter warming and loss of sea ice pose largely unknown risks to keystone species and the marine ecosystem that they support. Young-of-the-year juvenile polar cod, *Boreogadus saida*, are an energy-rich forage fish that accumulate high levels of lipid in the summer but retain a relatively small body size during the winter. To address winter bioenergetics and survival, we held age-0 juveniles under simulated winter conditions (food deprived, 24-hr darkness) at a range of four constant temperatures (-1, 1, 3, 5 ℃). Our goals were to 1) determine how age-0 polar cod utilize lipid energy in muscle and liver across variable temperatures and durations of food deprivation, 2) understand temperature- and size-dependent impacts on survival and 3) provide energy loss models using multiple condition metrics that are commonly used in fisheries science (lipids, morphometric ratios, body weight). These data have relevance to projecting winter outcomes for polar cod sampled pre-winter, when fish are more easily sampled in the field. As expected, in the absence of food, juvenile polar cod better conserved lipids and survived longer at colder temperatures. There was no negative impact of cold extremes on this pattern; for example, 50% mortality was at 170 days when polar cod were held at -1 ℃, compared to only 94 days when they were held at 5 ℃. During the first 28 days of simulated winter, polar cod preferentially catabolized triacylglycerols from muscle tissue, then depleted this storage lipid class in their muscle and liver until starvation. Mortality occurred when whole-body lipid concentrations fell below 12.4 mg. $g^{-1}$ wet weight. Temperature-dependent declines in morphometric condition (hepatosomatic index and Fulton's K) and lipid content were parameterized and developed into temperature-dependent condition loss models. Applying a laboratory-based lipid loss model to field-collected polar cod demonstrated that winter survival is highly sensitive to small changes in temperature between -1 and 1 °C when fish are in good condition at the end of the preceding summer. Alternatively, fish in poor summer condition cannot survive winter relying exclusively on stored energy reserves, and will be required to forage throughout the winter. Collectively, these results suggest that lipid-based indices offer a sensitive means of predicting overwintering success for polar cod experiencing climate-driven changes in summer and winter habitats in the Arctic.

**Keywords**: *Boreogadus saida*, Lipids, Energetic condition, Winter, Temperature, Fulton's K, Hepatosomatic index

## 1. **Introduction**

Although studying the overwintering ecology of fish is logistically challenging in cold and ice-covered marine systems (Berge et al., 2020b), improved understanding of species-specific winter survival trajectories is crucial to projecting population dynamics in the era of climate change (Heintz et al., 2013; Hurst, 2007; Siddon et al., 2013a). In the Arctic, winter conditions are particularly severe (long, cold, dark, and ice-extensive), and likely require specific physiological adaptations for survival, such as cold tolerance and high lipid storage. Therefore, climate change impacts on species distributions in the Arctic and sub-Arctic may be manifested by a species' ability to capitalize on warmer summer growth conditions (e.g. grow fast while storing fat) while also having the physiology to survive persistently harsh, dark overwintering environments (Copeman et al., 2020, 2017; Geissinger et al., 2021; Geoffroy and Priou, 2020). The vulnerability of polar cod (*Boreogadus saida*) to climate warming is particularly concerning, given their role in channeling energy from plankton to upper trophic levels such as marine mammals, birds, and other fishes in arctic environments (Hop and Gjosaeter, 2013; Whitehouse et al., 2014). Estimates from the Canadian Arctic indicate that polar cod can funnel up to 75% of the carbon between zooplankton and top predators, such as seabirds and whales (Welch et al., 1992). Changes in the distribution and abundance of polar cod will therefore likely lead to broad trophic, subsistence hunting and economic impacts (Huntington et al., 2020; Huserbråten et al., 2019; Marsh and Mueter, 2020). Unfortunately, studying these and other Arctic fish species is logistically challenging outside the summer open-ice period (Geoffroy and Priou, 2020).

Laboratory studies provide a tractable way of examining overwintering processes in high-latitude marine fish that are difficult to sample under the ice (David et al., 2016; Flores et al., 2012). Juvenile gadids have been focal species in laboratory experiments, with results being used to validate several field observations of size-dependent overwintering mortality (McCollum et al., 2003; Shoup and Wahl, 2011; Sogard, 1997). Experimental studies also provide a means of characterizing rates of energetic loss in juveniles during the winter period (Gotceitas, 1999; Sogard and Olla, 2000). Finally, by manipulating overwintering environments (e.g. food, temperature), laboratory experiments can examine impacts on survival and condition, which in turn will allow annual forecasting of fish recruitment under different oceanographic conditions (Geissinger et al., 2021; Gotceitas et al., 1999; Kooka, 2012; Sogard and Olla, 2000). We

performed thermal winter experiments that were simulated in the absence of food. There is little information on the degree of feeding in age-0 polar cod from October through the winter, but low ration feeding and a high proportion of empty stomachs have previously been reported (Geoffroy and Priou, 2020). Therefore, here we have established the lower bounds on winter survival times; further work including the impact of low winter feeding rations will be needed to better constrain polar cod survival and lipid loss models.

The physiological response of polar cod to environmental variability provides an indication as to whether and how these species will persist with continuing climate change. Juvenile gadids must develop, grow and store lipid rapidly during their first year to minimize predation and maximize overwintering survival (Copeman et al., 2008; Geissinger et al., 2021). This is especially important in the Arctic, where the summers are short and the winter-spring season has prolonged temperatures < 0 °C (Bouchard and Fortier, 2008). Food availability is highly seasonal in Arctic systems (Wassmann, 2006), with consumers utilizing elevated summer lipid storage as an efficient strategy to survive extended winter conditions characterized by lower food availability (Copeman et al., 2016; Falk-Petersen et al., 2009; Kattner et al., 2007; Leu et al., 2011). In general, high-latitude fish are presumed to have physiology adapted to growing faster at colder summer temperatures than fish from lower latitudes, but within a narrower range of temperature preference and tolerance (stenothermic, Farrell and Steffensen, 2005; Pörtner and Farrell, 2008). Recent experimental studies have supported these assumptions (Laurel et al., 2016), but it is also clear that the thermal response of polar cod shifts across ontogenetic stages (Koenker et al., 2018b; Laurel et al., 2017, 2018). These results strongly emphasize the need to gather species-specific thermal response information spanning development across multiple critical time periods (Houde, 2008).

There are currently no studies on the overwintering physiology of Arctic gadids or how these and more well-studied sub-Arctic species may respond to changing winter environments resulting from climate change. It is possible that juvenile polar cod utilize alternative developmental and energy storage strategies to maximize overwintering survival compared to sub-Arctic congeners (Copeman et al., 2020). Field studies indicate that saffron cod (*Eleginus gracilis*) overwinter at smaller sizes in the northern extremes of their range (Chukchi and Beaufort seas) than in more southern areas (Gulf of Alaska and southeastern Bering Sea) (Helser

et al., 2017). Furthermore, Copeman et al. (2016) found that age-0 saffron cod from the Chukchi Sea were smaller (< 55 mm, standard length (SL)) and had a much higher lipid concentrations (19 $mg.g^{-1}$ wet weight (WWT)) at the end of their first summer than fish in the Bering Sea (> 75 mm with 12 $mg.g^{-1}$ lipid). These findings suggest that gadids living at high latitudes are selected to store seasonal pulses of food as lipid energy rather than prioritizing accelerated growth. Comparative studies of gadids also indicate that age-0 polar cod store higher concentrations of lipid in their tissues (31 $mg.g^{-1}$ WWT) than other gadid species (i.e. saffron cod, ~16 $mg.g^{-1}$, Copeman et al., 2020, 2017).

The goal of this study was to determine the size-, temperature- and condition-dependence of overwintering survival in juvenile polar cod held under simulated winter conditions in the laboratory. Our specific objectives were to parameterize the rates of juvenile mortality and energy loss (mass, condition, lipid storage) and to determine the minimum energetic state necessary for survival. Further, we aimed to develop lab-based models that can be applied to field observations of contrasting annual pre-winter fish condition to evaluate energetic limitations on overwintering survival. We hypothesize that high energy-density (pre-winter) and cold thermal conditions (winter) are necessary for elevated winter survival of juvenile polar cod in the Arctic.

## 2. **Methods**

### *2.1. Culture of juvenile polar cod*

Broodstock for this experiment were originally collected as juveniles (age-1) in 2014 from the Beaufort Sea (Prudhoe Bay, AK, 70.383°N, -148.552°W) and were then held at the Alaska Fisheries Science Center's (NOAA) laboratory in Newport, Oregon. Broodstock (age-3+) were maintained in a 6-m tank under seasonally adjusted temperatures ranging from 5 °C in the summer to 0.5 °C in the winter. In 2016, fish showed signs of maturity in early March and were checked daily by gently squeezing the abdomen to determine if eggs were freely flowing, clear and hydrated. Egg batches were made by combining the free-flowing eggs of a single female with the milt of three males in a dry, stainless steel bowl nested in crushed ice (following Laurel

et al., 2018). A total of four egg batches were constructed in this manner and then transferred to four corresponding meshed 4-L baskets floating in 2 °C water baths for incubation.

After 4 weeks, egg batches were mixed and transferred to three 400 L upwelling tanks to hatch and be cultured on live food. Larvae were reared at 2 to 3 °C on a 12:12 dark-light cycle and fed enriched rotifers at a density of 5 prey $mL^{-1}$ twice daily for a 4-week period. After 4 weeks, enriched *Artemia* were provided at a density of 1.5 prey $mL^{-1}$ in addition to rotifers. After 10 weeks, live prey consisted entirely of *Artemia* and was supplemented with dry food (Otohime A, Marubeni Nisshin Feed Co., Tokyo) twice daily (Koenker et al., 2018a; Koenker et al., 2018b). At 20 weeks, juvenile fish were transferred to 3-m diameter round tanks with flow-through seawater maintained at 5 °C, and were gradually weaned onto a gel food (diet details as in Copeman et al., 2017 and Copeman et al., 2013). Juvenile fish (40 to 65 mm SL) were available for this experiment at ~7 months after initial egg fertilization in the laboratory. Polar cod were of similar size and lipid density (~55 mm SL; 30 mg lipids. $g^{-1}$ WWT) to those measured in late summer/fall collections in the Chukchi Sea during 2013 (Copeman et al., accepted).

*2.2. Temperature-dependent overwintering experiment*

On October 13, 2016, age-0 juvenile polar cod were transferred from their holding tank and separated into a series of smaller experimental tanks (n = 12; dimensions 66L × 46H × 38W cm) held in an adjacent laboratory. Following transfer, fish were gradually acclimated (temperature change of < 0.5 ℃ per day) to their respective overwintering temperatures (-1, 1, 3 or 5 ℃) and held with flow-through, temperature-controlled seawater over the course of the experiment. The cold end of the temperature range (-1 °C) was chosen based on the lowest thermal habitat that could be maintained in the laboratory. Polar cod have been found in water ranging from -1.5 to 1 °C during the fall/winter in the Beaufort Sea (Benoit et al., 2008, 2014). The warmer range of experimental temperatures represents extreme end-of-century fall/winter warming projections over the northern Bering and Chukchi Shelf (Kristiansen pers. comm., Farallon Institute). Three replicate tanks were used per temperature, with each tank containing 50 fish. Fish were fed during the 12-day acclimation period but were not fed during the simulated overwintering experimental period. All tanks were covered in black tarp to keep fish in dark conditions

throughout the entire experiment, and were checked daily for mortalities using a red-lens flashlight. Mortalities were retained (-80 ℃) for subsequent condition and lipid analysis (see below).

On October 24, 2016, the experiment was initiated by subsampling 10 fish per tank for length (SL, to 0.01 mm) and wet weight (WWT, to 0.01 g). Sampled fish were 42 to 64 mm SL and 0.55 to 2.34 g, and there was no significant difference in mean SL across temperature treatments (ANOVA $F_{3,8} = 1.31$, $p = 0.34$). All sub-sampled fish were frozen (-80 ℃) for later condition analyses based on WWT (n = 10), dry weight (DWT, n = 7) and tissue-specific lipid content (n = 3, sampling numbers as in Table 1). After 28 days of winter conditions (November 22, 2016), 6 fish per replicate tank were measured and frozen for later condition analyses based on WWT (n = 6), DWT (n = 3) and lipid content (n = 3). The final sampling period occurred when each temperature treatment approached 60-50% survival of the population, after adjusting for fish removals due to sampling events. Time to ~50% mortality was temperature dependent and ranged from a low of 94 days at 5 °C to a high of 170 days at -1 °C. At this time, all surviving fish in the tank were euthanized, processed for length and weight (n = 9 to 24 per tank), and frozen for later condition analyses based on WWT (n = 7 to 20), DWT (7 to 10) and lipids (n = 3, Table 1).

*2.3. Condition metrics*

During morphometric and lipid processing, fish were removed from the -80 ℃ freezer in small batches (n < 10) and were kept on ice during sampling to prevent lipid break-down. Fish were rinsed with water, patted dry and immediately measured for SL and WWT (to 0.001 g). Fish intestinal tracts were removed and livers were dissected and weighed on a microbalance (to 0.001 mg). A subset of eviscerated bodies and livers were dried separately at 65 ℃ to a constant weight (~1 week) and reweighed to calculate Fulton's K and hepatosomatic index (HSI) based on DWT (equations below). Conversion factors between tissue-specific WWT and DWT were also calculated and used to express lipids per DWT of tissue analyzed for lipids. Generally, the condition factors below are considered size-corrected within the length range studied in our experiment (see Supplementary Materials A). Liver and body weights were used to calculate the condition factors shown below:

- Fulton's $K_{wet}$ = (Whole body WWT(g)/(SL(cm)$^3$) * 100.
- Fulton's $K_{dry}$ = (Whole body DWT(g)/(SL(cm)$^3$) * 100.
- $HSI_{WET}$ = (Liver WWT(mg)/Whole Body WWT(mg)) * 100.
- $HSI_{DRY}$ = (Liver DWT(mg)/Whole Body DWT(mg)) * 100.
- Concentration of total lipids per WWT = (Iatroscan summed lipid classes (µg))/ (WWT of tissue (mg)).
- Concentration of total lipids per DWT = (Iatroscan summed lipid classes (µg))/(WWT of tissue (mg) * temperature and time-tissue specific conversion factor (DWT:WWT)).

*2.4. Lipid analyses*

Tissue-specific lipid analyses of muscle and liver were conducted for total lipids and lipid classes. Lipid analyses were performed on whole livers and ~300-mg samples of dorsal muscle that excluded the skin. All samples for lipid analyses were stored in chloroform under nitrogen in a -20 °C freezer and were extracted and analyzed within 6 months of sampling. Total lipids were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Lu et al. (2008) and Copeman et al. (2017). Extracts were spotted on duplicate silica-gel-coated Chromarods, and a three-stage development system was used to separate wax esters, triacylglycerols, free fatty acids, sterols and polar lipids. Polar lipids are mostly phospholipids, with minor amounts of other acetone mobile polar lipids. The first rod development was in a chloroform: methanol: water solution (5:4:1 by volume) until the leading edge of the solvent phase reached 1 cm above the spotting origin. The rods were then developed in hexane: diethyl ether: formic acid solution (99:1:0.05) for 48 min, and finally rods were developed in a hexane: diethyl ether: formic acid solution (80:20:0.1) for 38 min. After each solvent development, rods were dried (5 min) and conditioned (5 min) in a constant humidity chamber (~32%) that was saturated with aqueous $CaCl_2$. Following the last development, rods were scanned using Peak Simple software (ver. 3.67, SRI Inc.) and the signal detected in millivolts was quantified with calibration curves using the following commercial standards from Sigma (St. Louis, MO, USA): cholesteryl stearate (wax esters), palmitic acid (free fatty acids), cholesterol (sterols), L-alpha-phosphatidylcholine (polar lipids). A specialized standard was purified by column chromatography to be used for triacylglycerols (*Boreogadus saida* liver oil), using methods from Ohman (1997). Calibrated relationships between lipid class areas and standard lipid amounts (µg) had correlations with an $r^2 > 0.98$ for all classes.

Tissue-specific lipid concentration (mg. $g^{-1}$ WWT) was converted back to whole-body lipid concentrations for comparison with field samples and to examine the relationship between fish length (SL mm) and whole-body total lipids (mg). Field samples were half-body homogenates of polar cod minus the digestive tract and the head (see methods in Copeman et al., accepted). Laboratory tissue-specific values were converted to whole-body concentrations by multiplying the tissue-specific liver and muscle concentrations (mg. $g^{-1}$) by the mass of the liver (g) or the whole-body weight minus liver weight (g), respectively. This gave the total lipid (mg) in the whole fish, which was then divided by the mass of the whole fish (g). Digestive tracks were assumed to have negligible weight, as the experiment was run during food deprivation.

*2.5. Temperature and size effects on survival*

Prior to pooling tanks into temperature populations for survival analyses, we performed Kaplan-Meier survival curves to examine tank effects within each temperature treatment (Fig. 1). A log-rank test with $\chi 2$ statistic was used and found no statistical difference between tanks (Holm-Sidak family error rate of 0.05). We then used Cox regression to determine the significance of covariates size (SL, mm) and temperature on time to polar cod mortality (SPSS version 26). Events were classified as mortality or removals (censures) due to sampling events. The significance of each covariate, as well as the temperature-dependent hazard function for winter mortality, is reported. A two-way ANOVA at the time of 50% population mortality was used to determine the effects of temperature and survival status on the mean SL (mm) of age-0 polar cod (Fig. 2).

*2.6. Calculation of relative weight and lipid loss*

Relative body-weight loss (WWT, g) and lipid loss (total lipid, mg) were determined by subtracting the measured value at the time of sampling from the predicted value at the beginning of the experiment, assuming no change in SL (mm) (see Fig. 3 and 4). The instantaneous rate of mass loss or lipid loss (percent per day) for each individual was therefore calculated as: ([ln (final mass) - ln (initial mass)]/duration) × 100. Temperature-dependence of weight loss and lipid loss was described using a polynomial function (SigmaPlot 14) with tank means as the level of observation.

*2.7. Effects of temperature on size de-trended condition loss*

Temperature-specific effects on condition metrics over the duration (days) of winter were analyzed by fitting polynomial functions (SigmaPlot 14). To describe the continuous temperature-dependent rate-loss, we fit temperature-specific (-1, 1, 3, 5 °C) linear regressions between condition metrics (Fulton's K, HSI, and lipids per weight) and days of winter (Figs. 5a, 6a, 7c), and then fit a linear regression between temperature and these temperature-specific slopes ($K.day^{-1}$, $ln(HIS+1).day^{-1}$, $(mg.g^{-1}).day^{-1}$) (see Figs. 5b, 6b, 7d). HSI was natural-log-transformed (ln) to allow for a linear model fit between condition and days of winter. Condition factors are expressed per WWT and per DWT (for condition metrics per DWT, see supplementary materials). All analyses were run on tank means (n = 3), with numbers of individuals sampled per tank as in Table 1.

Using models developed in this experiment, we plotted the days to starvation over a wide range of temperatures for both laboratory-reared and field-collected fish that had divergent end-of-summer energetic condition (Fig. 8). We used values from fish collected on the central Chukchi Shelf in a previous cold year (2013) compared to a contemporary warm year (2017) (Copeman et al., accepted). Fish possibly had divergent lipid levels due to different summer temperatures and zooplankton availability on the Chukchi Sea during those years (as discussed in Copeman et al., accepted). Starvation levels (12.4 mg. $g^{-1}$ WWT) and the lipid loss model were determined from experimental treatments. Days to starvation were calculated as the days required to decrease in lipid concentration from end of summer levels (2013, 34.7 $mg.g^{-1}$ and 2017, 16.0 $mg.g^{-1}$) to starvation status, 12.4 $mg.g^{-1}$.

## 3. Results

*3.1. Survival*

The time to 40 to 50% population mortality was significantly different across temperature treatments (ANOVA 50% survival: $F_{3,8} = 92.93$, $p < 0.001$). Polar cod had a mean survival time to ~50% mortality of 170 ± 11 days at -1 °C compared to only 94 ± 1 days at 5 °C (Fig. 1).

There was no significant difference in survival functions between tanks (n = 3) within temperature treatments (i.e. 1 °C, log rank Mantel-Cox, $\chi 2$ (2) =1.106, p = 0.58) and therefore all individuals were pooled into temperature populations for survival analyses. We ran a Cox regression with the covariates of SL (mm) and temperature (°C) to explain days to overwinter mortality (significant model fit, $\chi 2$ (2) = 248.22, $p < 0.001$, Table 2a). The regression coefficients predict the hazard of overwinter mortality with a positive coefficient for temperature (b = 0.722, SE = 0.073, $p < 0.001$) indicating that warmer temperatures were associated with higher mortality, while a negative coefficient for SL (b = -0.28, SE = 0.02, $p < 0.001$) indicated that larger size was associated with reduced mortality (Table 2b).

The hazard ratio reflects the multiplicative change in the probability of overwinter mortality per unit increase in the covariate (Table 2b). A hazard ratio of 1 indicates no change in mortality per unit of the covariate, whereas a ratio greater than 1 is associated with positive slopes and a value less than 1 is associated with negative slopes. For each unit change in SL, the model predicted a 24% decrease in overwintering mortality at any given time during our simulated winter conditions. For the categorical temperature variable, the hazard ratio was assessed relative to the reference -1 °C treatment, which had the longest winter survival times (Fig. 1). Relative to fish at -1 °C, polar cod at 1 °C had a hazard ratio of 2.5, those at 3 °C had a ratio of 19, and finally those at the warmest temperature (5 °C) had a hazard ratio of 72.

For visualization purposes, we compared the size (SL, mm) of polar cod at the time the population reached 50% mortality as a function of survival status and temperature (Fig. 2). There was no significant difference in size due to temperature (ANOVA, $F_{3,18} = 2.66$, p = 0.08). Across all temperatures, fish that died were significantly smaller (mean SL of 53.5 ± 0.8) than fish that survived (mean SL of 61.1 ± 0.7 mm, ANOVA, $F_{1,18} = 51.44$, $p < 0.001$, Fig. 2), emphasizing again that larger size provides a winter survival advantage.

*3.2. Relative weight loss and lipid loss models*

The length-weight and length-lipid relationships for age-0 polar cod at the beginning of the experiment (‘pre-winter’) were compared to the relationships among fish that died during the experiment (‘winter mortality’, Fig. 3). The winter mortality length-weight and length-lipid models are the theoretical lower thresholds that juvenile fish must maintain to survive.

Temperature-dependent weight loss (Fig. 4a, linear model, $r^2 = 0.94$) and lipid loss (Fig. 4b, linear model, $r^2 = 0.68$) both showed a significant negative slope, with elevated percentage loss per day at warmer temperatures. The rate of relative lipid loss (slope = -0.13) was approximately twice the rate of relative weight loss (slope = -0.057), indicating that during starvation, polar cod are utilizing tissue lipids while continuing to maintain body weight, likely by the retention of water to their tissues.

*3.3. Morphometric condition loss models*

Temperature-specific Fulton's K based on both WWT (Fig. 5) and DWT (Supplementary materials B) were accounted for by the duration of winter conditions (temperature-specific $r^2$ ranged from 0.83 to 0.98). The relationship between the rate of change in Fulton's $K_{wet}$ and $K_{dry}$ and overwintering temperature (T, °C) were defined using the following linear regressions:

Loss of $K_{wet}.day^{-1} = -2.13 \times 10^{-3} - 1.72 \times 10^{-4}$ (T), $r^2 = 0.90$ (Fig. 5b).

Loss of $K_{dry}.day^{-1} = -2.92 \times 10^{-4} - 3.37 \times 10^{-5}$ (T), $r^2 = 0.97$ (Supplementary materials B).

Condition measurements taken on mortalities allowed us to define the mean ± SD starvation condition for $K_{wet}$ and $K_{dry}$ as 0.44 ± 0.063 and 0.066 ± 0.0058, respectively.

Temperature-specific HSI based on both WWT (Fig. 6) and DWT (Supplementary materials C) were also accounted for by the duration of winter conditions ($r^2$ ranged from 0.79 to 0.98). $HSI_{wet}$ and $HSI_{dry}$ were ln-transformed and the relationship between the rate of condition loss and winter temperature were defined using the following linear regressions:

Loss of $\ln (HSI_{wet}+1).\ day^{-1} = -0.67 \times 10^{-2} - 0.06 \times 10^{-2} * (T)$, $r^2 = 0.91$ (Fig. 6b).

Loss of $\ln (HSI_{dry}).\ day^{-1} = -1.74 \times 10^{-2} - 0.15 \times 10^{-2} * (T)$, $r^2 = 0.89$ (Supplementary materials C).

HSI metrics taken on fish that died allowed us to define the mean mortality stage for $HSI_{wet}$ and $HSI_{dry}$ as 0.67 ± 0.35 and 0.87 ± 0.5, respectively.

*3.4. Lipid concentration loss models*

At time-0, fish ranged in SL from 42 to 66 mm and showed no significant relationship between length and whole-body lipid concentration per WWT ($r^2$ = 0.002, Supplementary materials A). Temperature-specific tissue lipid concentrations were calculated for whole fish over the duration of the experiment (Table 3) from measurements of tissue-specific levels in liver (Table 4a) and muscle (Table 4b). Most of the lipid loss was due to a proportional decrease in the neutral storage lipids (triacylglycerols, TAG), which decreased from 86% in the liver and 56% in the muscle to 8% and 2%, respectively (Table 4). Fish that died were characterized by low total lipid concentrations (12.4 mg. $g^{-1}$ WWT, whole bodies) and high relative proportions of polar lipid (PL, ~70%) and sterols (ST, ~20%), a state indicative of only membrane structures remaining, with little lipid-based energy storage.

Across all temperatures, we measured a rapid decline in muscle tissue lipid concentrations from 29.1 ± 5.5 mg. $g^{-1}$ WWT at time-0 to 20.8 ± 3.8 after day 28 of simulated winter (Fig. 7b). The opposite trend was measured in liver tissue, which increased in lipid density from 290.3 ± 56.7 on day-0 to 334.9 ± 62.3 mg. $g^{-1}$ at day 28 (Fig 7a). Polar cod have small amounts of liver tissue relative to muscle mass and they store high proportions of lipid (TAG) in their muscle, which explains the general decrease in whole-body lipid concentrations from time-0 (41.4 ± 5.3 mg. $g^{-1}$ WWT) until day 28 (33.1 ± 6.0 mg. $g^{-1}$, Fig. 7c). The same trends were measured for total tissue-specific lipids based on DWT (Supplementary materials D). Both muscle and liver tissue decreased rapidly in lipids from day 28 until 50% population mortality (Fig. 7a,b, Tables 4a,b).

Temperature-specific lipid concentrations for whole fish based on both WWT (Fig. 7c) and DWT (Supplementary materials D) were accounted for by the duration of winter exposure ($r^2$ ranged from 0.86 to 0.99). The temperature-dependent rates of whole body lipid concentration (mg. $g^{-1}$ weight) loss were accounted for using the following linear regressions:

Lipid loss (mg. $g^{-1}$ WWT). $day^{-1}$ = $-18.79 \times 10^{-2} - 2.12 \times 10^{-2} * (T)$, $r^2$ = 0.77 (Fig. 7d).

Lipid loss (mg. $g^{-1}$ DWT). $day^{-1}$ = $-90.18 \times 10^{-2} - 8.76 \times 10^{-2} * (T)$, $r^2$ = 0.64 (Supplementary materials D).

Lipid measurements on fish that died allowed us to define the mean WWT-based and DWT-based lipid composition at mortality as 12.4 ± 2.0 mg. $g^{-1}$ and 65.3 ± 10.9 mg. $g^{-1}$, respectively.

The importance of late-summer lipid storage and winter temperatures for survival of age-0 polar cod is shown in Table 5 and Figure 8. Tissue concentrations of lipids in polar cod from the central Chukchi Sea in 2013 (Copeman et al., 2020) and 2017 (Copeman et al., accepted) were used with the rate of loss equation (mg. $g^{-1}$ WWT). $day^{-1} = -18.79 \times 10^{-2} - 2.12 \times 10^{-2} *(T, °C)$, $r^2 = 0.77$ (Fig. 7d) to calculate survival times. Specifically, we calculated the time for lipids to decline from observed field values to experimentally-determined starvation levels (12.4 mg. $g^{-1}$) (Table 5) across a wide range of continuous simulated winter temperatures from -2 to 6 °C (Fig. 8). Starvation resistance at -1 °C was projected to be over 200 days in high-lipid fish from the colder year 2013, compared to less than 30 days in low-lipid fish from the warmer 2017. Further, small changes in temperature at the cold end of the overwintering range made a large difference in survival projection of high-lipid fish in 2013 (i.e. 30-day difference from -1 to 1 °C), but little difference to the low-lipid fish observed in 2017 (i.e. 6-day difference from -1 to 1 °C, Table 5, Fig. 8). Survival of high-condition fish is dependent on winter temperatures, while poor-condition fish had low survival times across the full range of temperatures (24 to 13-day starvation resistance between -1 to 5 °C) (Fig. 8, Table 5).

## 4. Discussion

Polar cod are adapted to highly seasonal environments, requiring rapid accumulation of summer energy reserves and cold winters to survive periods between productivity peaks. The importance of pre-winter size, lipid storage and winter temperature were highly apparent in our study. Age-0 polar cod conserved lipids and survived longest in our coldest winter temperature treatment (-1 °C). We contend that, in the field, polar cod overwintering success will be highly dependent on summer lipid storage, as well as the availability of cold fall-winter Arctic thermal habitat.

### *4.1. Polar cod thermal habitat*

Results from our study indicate that the optimum thermal habitat (-1 °C) for energy conservation agrees with temperatures of maximum age-0 fish abundance from winter field surveys (Benoit et al., 2008; Geoffroy et al., 2016). Chukchi Sea pelagic larval polar cod are distributed within the warm (0 to 7 °C) upper 20 m of the water column until they metamorphose into pelagic juveniles during the summer (~27 to 35 mm, SL) (Deary et al., 2021; Vestfals et al., 2019). In late summer and early fall, juveniles begin to descend to deeper waters and are found within the top 100 m at average surface water temperatures of ~5 °C (De Robertis et al., 2016; Levine, 2021). During this period, extensive diel vertical migrations have been noted, with polar cod feeding primarily on *Calanus* spp. at the surface at night, and returning to depth during the day, thus avoiding visual predators (Bouchard and Fortier, 2020; Geoffroy and Priou, 2020). After attaining a SL of ~50 mm (August through October), the majority of the Beaufort and Barents seas population descends to depths >100 m for the remainder of the winter season (Benoit et al., 2014; Geoffroy et al., 2016; Geoffroy and Priou, 2020). After November, prey levels in surface waters decrease and vertical migration activity slows. From late fall onward, age-0 polar cod are thought to generally remain in cold (1 to -1 °C) epipelagic layers for the remainder of the winter season (Darnis and Fortier, 2014; Mueter et al., unpublished). Age-0 polar cod in our experiment minimized winter energy loss at temperatures < 0 °C with no apparent acute effects of low temperature prior to reaching the point of energetic starvation (lipids per WWT = 12.4 mg. $g^{-1}$, $HSI_{wet}$ = 0.67, $K_{wet}$ = 0.62). It is likely that they utilize antifreeze proteins in the liver and/or gills as a metabolically cost-effective way to utilize these extremely cold habitats (Chen et al., 1997; Fletcher et al., 2001; Geoffroy and Priou, 2020).

Increased fall temperatures following warm summers may doubly impact polar cod by not only causing them to more rapidly metabolize their limited lipid reserves, but also by delaying the availability of sympagic food. Ice-associated diet sources have previously been shown to sustain many keystone polar organisms throughout the winter (Geoffroy and Priou, 2020; Kohlbach et al., 2017a, 2017b). Danielson et al. (2020) noted that recent decade-scale heat retention in the Bering and Chukchi Seas has resulted in additional fall cooling time required for sea-ice formation. Recent fall and winter ocean-to-atmosphere heat fluxes have been anomalously large and associated with elevated air temperatures and increased southerly winds,

resulting in lower fall/winter sea-ice production and extent (Danielson et al., 2020; Woodgate, 2018).

*4.2. Polar cod lipid allocation*

As predicted, high mortality occurred when polar cod energy reserves (e.g. lipids) were exhausted during winter starvation (e.g. Thompson et al., 1991; Ludsin and DeVries, 1997). Application of laboratory rates to realized variability in field-collected polar cod lipid storage (Copeman et al., 2020) indicates that lipid content has an important bearing on winter survival potential. The annual variation in lipid density of field-collected fish from the Central Chukchi Sea (2013: 34.7 ± 18.7 vs. 2017: 16.0 ± 6.4 mg. $g^{-1}$ WWT) was particularly striking, despite fish being similar in size (2013: 43.4 ± 3.9 vs. 2017: 47.2 ± 10.3 SL, mm) (Copeman et al., accepted). Relative weight loss and lipid loss models demonstrated that polar cod rapidly utilize lipids and substitute water into their tissues to maintain body mass. This strategy is typical of cold-water fish species during starvation periods (Dutil and Lambert, 2000; Maddock and Burton, 1994) and makes lipid a more rapid indicator of changing nutritional status than mass based on wet weight.

Our food deprivation models illustrate that winter thermal conditions are either highly important following summers that support juvenile fish in good nutritional condition (e.g. 2013), or are potentially irrelevant when preceding warm summer conditions result in a low nutritional state. Ecosystem warming and loss of sea ice have direct metabolic effects on polar cod, but also cause a mosaic of cascading indirect food web impacts (Sigler et al., 2016S, 2014). In the neighboring Bering Sea, oscillating thermal conditions have been shown to influence food web dynamics that, in turn, have impacted fish energetic condition, stated as the Oscillating Control Hypothesis (Heintz et al., 2013; Hunt et al., 2011; Mueter et al., 2011). This theory links variable sea/ice extent to the timing of the spring bloom and resultant juvenile walleye pollock (*Gadus chalcogramma*) and Pacific cod (*Gadus macrocephalus*) recruitment across alternating warm and cold phases in the southeastern Bering Sea (Farley et al., 2016; Heintz et al., 2013; Hunt et al., 2011, 2002, 2008). During cold phases, March ice-associated phytoplankton blooms are important in fueling the production of large, lipid-rich zooplankton that have been found to be essential to juvenile walleye pollock fall lipid storage and overwintering survival (Duffy‐Anderson et al., 2019; Kimmel et al., 2018). In contrast, during warm phases, this

ice-edge phytoplankton production is reduced, and is replaced with a later pelagic bloom and reduced numbers of large, lipid-rich copepods in the late summer. It is possible that similar ecosystem dynamics may influence the food web and fall condition of age-0 polar cod in the central Chukchi Sea with continued warming. Regardless, the high prioritization for energy storage observed in small-bodied polar cod suggests that there is a high winter starvation mortality risk in the Arctic (Ivan et al., 2015; Renaud et al., 2018).

High levels of lipid storage in polar cod muscle tissue may offset their limited capacity to store energy reserves in their liver at a small size. Proportionally large, lipid-rich livers are not found in gadids until > 60 mm in standard length, which represents the second year of growth for Arctic gadids, compared to the first year in sub-Arctic gadids (Helser et al., 2017; Laurel et al., 2007). Copeman et al. (accepted) demonstrate that age-0 Chukchi Sea polar cod at a given size (~60 mm) store twice the lipid (mg) of similarly-sized sub-Arctic congeners, which is likely an adaption to a short growing season. Size-dependent overwintering success may be even more pronounced in lower latitude regions than in the Arctic, due to higher predation rates (Laurel et al., 2003; Lough and O'Brien, 2012) and more variable size demographics during late fall/winter at lower latitudes (Geissinger et al., in review).

*4.3. Condition loss models*

The use of multiple different condition metrics allows our data to be broadly applied to fisheries research and other laboratory experiments that may have only one metric, such as length-weight. We present three types of size-corrected condition metrics: Fulton's K, a morphometric condition index based on weight at length (Froese, 2006; Nash et al., 2006); HSI, a morphometric index that expresses liver weight relative to body weight, and is often referred to as a metric of lipid storage (Aune et al., 2021; Guy and Brown, 2007); and lastly, tissue-specific and whole-body explicit measurements of lipid. The ease of performing morphometric condition measures can allow for the processing of larger numbers of individuals. However, morphometric condition in larval and juvenile marine organisms has shown a general lack of sensitivity (Copeman et al., 2018, 2008; Suthers, 1998), likely because it is not measuring changes in highly variable lipid storage.

During the first 28 days of winter, polar cod preferentially catabolized triacylglycerols from muscle tissue, and then depleted storage lipids in their muscle and liver until starvation. Polar cod store high concentrations of lipid in their muscle tissues relative to other sub-Arctic congeners (Copeman et al., 2017), making HSI potentially insensitive to changes in the lipid dynamics of polar cod. At time-0, polar cod had 29.9 ± 1.5 mg of lipid in their muscle tissues, compared to 16.2 ± 1.6 mg in their liver, but by the time of 50% population mortality, almost all the remaining lipid was found in muscle storage (8.9 ± 3.9 mg per fish, Fig 7a,b). For future analyses of field campaigns, age-0 polar cod whole-body homogenates should be saved for lipid analyses to compare with laboratory-developed metrics of nutritional status. Dissections of tiny, fragile liver tissues from previously frozen age-0 polar cod are extremely time-consuming, and if not performed by a skilled technician, can lead to inaccurate determination of HSI.

*4.4. Future research*

These condition loss models are based on the caveat that polar cod are not feeding in appreciable amounts during the winter. Understanding the impact of winter feeding scenarios will be an important component of future laboratory and field research. Small amounts of food during winter can dramatically improve survival of age-0 Atlantic cod, with the addition of minimum rations resulting in prolonged survival and growth at low temperatures (-0.8 to 2.7 °C) (Geissinger et al., 2021). Field studies indicate active under-ice zooplankton production in the Arctic winter (Berge et al., 2020a; Geoffroy and Priou, 2020), but we know of no such field studies in the Chukchi Sea. Polar cod have large eyes that enable more successful feeding at lower light intensities than sub-Arctic gadids (Geoffroy and Priou, 2020; Wagner et al., 1998), and this has been proposed as an additional barrier to the establishment of sub-Arctic gadid populations in Arctic regions (Kaartvedt, 2008). In the waters surrounding Svalbard, Norway, adult polar cod have been shown to forage during the polar night, although at a lower stomach fullness than in summer (Geoffroy and Priou, 2020). However, adults were found to switch from zooplankton to larger prey such as fish (Cusa et al., 2019) during the winter. Prey switching was proposed as a solution to reduced light and polar cod's limited ability to visually capture small zooplankton. Due to gape limitation, it is uncertain whether small age-0 polar cod (30-60 mm) can also successfully switch to larger prey items during the winter. Although polar regions are becoming 'brighter' with climate change (T. Kristiansen, personal communication), the projected

foraging gains to visual feeders will be mostly limited to the summer months, as the polar night will continue to be dark regardless of sea-ice loss (Langbehn and Varpe, 2017).

*In situ* studies of overwintering processes may become logistically less challenging in the future due to increased periods of open water in the Arctic and better technology for sampling fish and their prey (David et al., 2015; Kohlbach et al., 2017b). As polar cod tissue samples become available from these efforts, fall/winter lipid storage and trophic dynamics can be used to ground-truth some of the survival trajectories from this study, all of which have implications for recruitment dynamics in Alaska waters (Hurst, 2007a; Farley et al., 2011; Heintz et al., 2013; Siddon et al., 2013). Currently, efforts to understand the bioenergetics of polar cod rely on models with many assumptions about physiological rates, consumption rates and trophic relationships (Hansen et al., 1993). The simple models we report do not account for trophic dynamics; however, they are stage-specific and provide an understanding of tissue lipid compartmentalization that is not typically captured in traditional bioenergetics models (Munch and Conover, 2002).

## 5. Conclusions

Energy density increases with body size for most fish species during the juvenile phase (pre-reproductive; Martin et al., 2017), but Chukchi Sea polar cod enter their first winter at ~50% the length of similarly aged gadids from the Bering Sea (Siddon et al., 2013a, 2013b) and the Gulf of Alaska (Laurel et al., 2017). The specialized ability of polar cod to store high concentrations of lipid at a relatively small size and to overwinter in extreme cold (-1.5 to -1 °C) may set their distributional limits by allowing them to minimize winter energy loss (Parker-Stetter et al., 2011). Based on summer field surveys of the Chukchi Sea (De Robertis et al., 2016), age-0 polar cod are the most abundant gadid in the region, but changing climate conditions in the summer and winter could disrupt these distribution boundaries and result in a more sub-Arctic summer fish assemblage (Baker, 2021; Mueter et al., 2021). It remains uncertain whether sub-Arctic gadids can successfully overwinter at temperatures routinely < 0 °C, such as those common to the Chukchi and Beaufort Sea shelves.

Our experiments show that successful overwintering for polar cod will be highly dependent on seasonal conditions in both the winter and summer. The survival trajectories described in this study demonstrate how high summer lipid storage and cold winters theoretically improve overwintering success, while also showing that winter foraging is necessary for survival when fish are in poor pre-winter condition or when Arctic winter thermal habitats are warmer. However, the transition from age-0 to age-1 will remain a poorly understood component of population dynamics without increased seasonal observational data (Boudreau et al., 2017; Geoffroy et al., 2016; Geoffroy and Priou, 2020; Heintz and Vollenweider, 2010). Winter studies on diet will be especially critical, as will be information on regional predation pressure that could impact polar cod's strategy for energy allocation (e.g. increased growth), as well as their thermal habitat preferences.

**Acknowledgments**

This work was supported by grant 19-10-04 from the Oil Spill Recovery Institute. We would like to thank Scott Haines for assistance with laboratory experiments. Thomas Hurst, Elizabeth Logerwell, Alex De Robertis, Calvin Mordy and two anonymous peers for helpful editorial reviews on earlier versions of this manuscript. Thanks also to Cynthia Sweitzer for providing help with procurement of permits for polar cod collections. Funding for this project was provided by an Alaska Fisheries Science Center and an Essential Fish Habitat grant to Benjamin Laurel. This manuscript is a product of the North Pacific Research Board Arctic Integrated Ecosystem Research Program [https://www.nprb.org/arctic-program; NPRB publication number ArcticIERP-31].

**Ethical approval:** All animal experiments were conducted at National Oceanic and Atmospheric Administration facilities. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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**Louise Copeman**: Conceptualization, formal analysis, writing original draft, supervision, visualization, and investigation. **Carlissa Salant**: investigation. **Michelle Stowell**: investigation. **Michele Ottmar**: investigation. **Mara Spencer**: investigation. **Paul Iseri**: investigation. **Benjamin Laurel**: Conceptualization, formal analysis, investigation, writing original draft, funding acquisition, and project administration.

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**Fig. 2.** Across all temperatures, polar cod (*Boreogadus saida*) that survived past 50% population mortality (S) were significantly longer in standard length (SL, mm) than fish that died prior to 50% population mortality (M). The mean SL of mortalities was 53.5 ± 0.8 mm, while the mean SL of survivors was 61.1 ± 0.7 mm. The asterisks over the survivor bars indicate that they were significantly longer than fish that died within the same temperature treatment ($p<0.05$).

**Fig. 3.** The relationship between length and (a) weight as well as (b) total lipid for age-0 polar cod at the beginning of the experiment ('pre-winter') and at the time of mortality during the experiment ('winter mortality'). The winter mortality length-weight model is the theoretical lower weight and lipid threshold that juvenile fish must maintain for survival during winter.

**Fig. 4.** Rate of temperature-dependent (a) weight loss and (b) lipid loss for age-0 juvenile polar cod during simulated overwintering laboratory experiments. Experiments were run at -1, 1, 3 and 5 °C until ~50% population mortality. Data are tank means (n = 3 per temperature) of individual weight loss over time. Temperature-dependent weight loss and lipid loss were determined using the difference in weight or lipid at the time of mortality as compared to length-based pre-winter models (See Fig. 3).

**Fig. 5.** Fulton's K condition factor based on WWT of age-0 juvenile polar cod (*Boreogadus saida*) shown (a) over the full overwintering experiment and (b) as a temperature-dependent rate of loss function. Data in 5a are the means of three tank values (± 1 SE) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average Fulton's $K_{wet}$ of 0.44. The temperature-dependent rate of loss function (b) relates the slopes of relationships in 5a to temperature. Correlation coefficients for $K_{WWT}$ in panel (a) were $r^2 = 0.97$ at -1°C, $r^2 = 0.91$ at 1 °C, $r^2 = 0.96$ at 3 °C and $r^2 = 0.98$ at 5 °C.

**Fig. 6.** Natural log of the hepatosomatic index (HSI) based on WWT of age-0 juvenile polar cod (*Boreogadus saida*) shown (a) over the full overwintering experiment and (b) as a temperature-dependent rate of loss function. Data in 6a are the means of three tank values (± 1 SE) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average $HSI_{wet}$ of 0.67 and Ln ($HSI_{wet}$+1) of 0.51. The

temperature-dependent rate of loss function (6b) relates the slopes of relationships in 6a to temperature. Correlation coefficients for $HSI_{WWT}$ in panel (a) were $r^2 = 0.98$ at -1°C, $r^2 = 0.94$ at 1 °C, $r^2 = 0.98$ at 3 °C and $r^2 = 0.79$ at 5 °C.

**Fig. 7.** Total lipids per WWT ($\mu g.mg^{-1}$) shown in (a) the liver, (b) muscle tissue and (c) estimated whole bodies of age-0 polar cod (*Boreogadus saida*) over the full overwintering experiment. Data are tank means (n = 3) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average whole-body total lipid per WWT of 12.4 $mg.g^{-1}$. The temperature-dependent rate of lipid loss in whole fish (7d) relates the slopes of relationships in 7c to temperature. Correlation coefficients for total lipids per WWT ($mg.g^{-1}$) in panel (c) were $r^2 = 0.98$ at -1°C, $r^2 = 0.97$ at 1 °C, $r^2 = 0.98$ at 3 °C and $r^2 = 0.94$ at 5 °C.

**Fig. 8.** Days to starvation based on the lab-determined temperature-dependent lipid loss model: Lipid loss ($mg.g^{-1}$ WWT).$day^{-1} = -18.79 \times 10^{-2} - 2.12 \times 10^{-2} * (T)$, $r^2 = 0.77$ (Fig. 7d). Scenarios are shown for experimental fish, as well as polar cod (*Boreogadus saida*) from the central Chukchi Sea in a cold year (2013, Copeman et al., 2020) and a warm year (2017, Copeman et al., accepted) to demonstrate variability in time to starvation for fish of different nutritional status over a wide range of temperatures. The mean lipid concentration values from fish sampled in the Central Chukchi Sea (68.25 - 70.74°N) are given in Table 5.

**Table 1.** Allocation of samples from age-0 polar cod overwintering experiment. Sampled fish and mortalities were processed for condition metrics based on WWT (n = 526), DWT (n = 374) and detailed tissue-specific lipid classes (n = 140).

| Temperature | -1℃ | 1℃ | 3℃ | 5℃ |
|---|---|---|---|---|
| Replicate tanks | 3 | 3 | 3 | 3 |
| Fish per tank | 50 | 50 | 50 | 50 |
| **Time-0: October 24, 2016** | **Number of fish sacrificed per tank** | | | |
| Total fish sampled per tank | 10 | 10 | 10 | 10 |
| Condition: hepatosomatic index WWT | 10 | 10 | 10 | 10 |
| Condition: hepatosomatic index DWT | 7 | 7 | 7 | 7 |
| Tissue specific lipid class analyses | 3 | 3 | 3 | 3 |
| **Day 28: November 22, 2016** | **Number of fish sacrificed per tank** | | | |
| Total fish sampled per tank | 6 | 6 | 6 | 6 |
| Condition: hepatosomatic index WWT | 6 | 6 | 6 | 6 |
| Condition: hepatosomatic index DWT | 3 | 3 | 3 | 3 |
| Tissue specific lipid class analyses | 3 | 3 | 3 | 3 |
| **50 % mortality** | **Number of fish sacrificed per tank** | | | |
| Total fish sampled per tank | 8-9 | 9 | 5-10 | 5-8 |
| Condition: hepatosomatic index WWT | 8-9 | 9 | 5-10 | 5-8 |
| Condition: hepatosomatic index DWT | 13-15 | 9-19 | 2-7 | 2-5 |
| Tissue specific lipid class analyses | 3 | 3 | 3 | 3 |
| **Mortalities** | **Number of mortalities** | | | |
| Total fish sampled per tank | 13-15 | 15 | 10-16 | 10-14 |
| Condition: hepatosomatic index WWT | 13-15 | 15 | 13-20 | 8-16 |
| Condition: hepatosomatic index DWT | 7-9 | 9 | 4-10 | 4-8 |
| Tissue specific lipid class analyses | 3 | 3 | 3 | 3 |

**Table 2.** The fit of (a) Cox regression analyses for the effect of polar cod SL (mm) and categorical covariate temperature (°C) on survival time as well as the (b) slope and hazard ratio for SL and temperature treatments with reference to -1 °C, the treatment with the longest survival times.

(a)

**Omnibus Tests of Model Coefficients**

| -2 Log | Overall (score) | | | Change From Previous Step | | |
|---|---|---|---|---|---|---|
| Likelihood | Chi-square | df | Sig. | Chi-square | df | Sig. |
| 1103.01 | 248.22 | 4 | <0.001 | 275.00 | 4 | <0.001 |

(b)

**Variables in the Equation**

| | Slope B | SE | Chi-square (Wald) | df | Sig. | Hazard ratio Exp(B) | 95.0% CI for Exp(B) Lower | Upper |
|---|---|---|---|---|---|---|---|---|
| Standard Length (mm) | -0.28 | 0.02 | 171.30 | 1 | <0.001 | 0.76 | 0.73 | 0.79 |
| Reference temperature: -1 °C | | | 102.60 | 3 | <0.001 | | | |
| 1 °C relative to -1 °C | 0.93 | 0.27 | 11.57 | 1 | <0.001 | 2.54 | 1.49 | 4.35 |
| 3 °C relative to -1 °C | 2.95 | 0.37 | 64.97 | 1 | <0.001 | 19.16 | 9.35 | 39.29 |
| 5 °C relative to -1 °C | 4.28 | 0.44 | 95.68 | 1 | <0.001 | 72.21 | 30.63 | 170.20 |

**Table 3.** Mean ± standard deviation of the estimated whole-bodied total lipids, total lipid concentrations per WWT and total lipid concentrations per DWT ($\mu g.mg^{-1}$) from polar cod sampled at time-0, day 28, in surviving cod at the time of 50% population mortality, and in fish that died (n = 9 fish per temperature-time).

| Sampling time | Temp | Total lipid per fish (mg) | Total lipid concentration per WWT ($\mu g.mg^{-1}$) | Total lipid concentration per DWT ($\mu g.mg^{-1}$) |
|---|---|---|---|---|
| Time-0 | -1 | 37.5 ± 12.6 | 42.0 ± 5.6 | 210.8 ± 26.5 |
| | 1 | 43.1 ± 10.2 | 40.0 ± 4.2 | 194.3 ± 20.1 |
| | 3 | 53.5 ± 17.0 | 44.6 ± 3.0 | 217.6 ± 20.2 |
| | 5 | 49.3 ± 15.4 | 38.4 ± 5.6 | 189.4 ± 29.0 |
| Day 28 | -1 | 36.3 ± 12.5 | 36.2 ± 6.3 | 186.4 ± 26.5 |
| | 1 | 27.3 ± 7.9 | 32.1 ± 7.7 | 165.8 ± 36.0 |
| | 3 | 39.7 ± 14.4 | 33.5 ± 3.1 | 178.9 ± 14.5 |
| | 5 | 32.6 ± 10.6 | 30.7 ± 4.8 | 175.6 ± 26.8 |
| Survivors at 50% population mortality | -1 | 8.2 ± 1.9 | 10.9 ± 1.3 | 60.8 ± 7.6 |
| | 1 | 7.8 ± 1.2 | 10.4 ± 1.6 | 59.6 ± 8.8 |
| | 3 | 9.5 ± 3.0 | 9.6 ± 1.3 | 55.8 ± 8.1 |
| | 5 | 11.0 ± 6.5 | 12.0 ± 4.6 | 74.1 ± 28.1 |
| Mortalities | -1 | 5.7 ± 0.8 | 11.8 ± 1.1 | 61.1 ± 5.2 |
| | 1 | 5.2 ± 1.8 | 14.1 ± 6.4 | 75.0 ± 34.3 |
| | 3 | 5.6 ± 1.1 | 12.6 ± 2.4 | 71.0 ± 20.1 |
| | 5 | 6.2 ± 1.5 | 11.6 ± 2.2 | 66.2 ± 12.7 |

**Table 4.** Mean and standard deviation of the lipid composition in (a) liver and (b) muscle for polar cod that were sampled at time-0, day 28, in survivors at the time of 50% population mortality, and in fish that died (n = 9 fish per temperature-time). Data are shown for total lipids per whole liver and muscle (mg), total lipid concentrations per WWT (µg.mg$^{-1}$), total lipid concentrations per DWT (µg.mg$^{-1}$), and proportions of total lipid as triacylglycerols (TAG), free fatty acids (FFA), sterols (ST) and polar lipids (PL).

| Sample type | Time | Temp | Total lipid in tissue (mg) | Total tissue lipid (µg.mg$^{-1}$WWT) | Total tissue lipid (µg.mg$^{-1}$ DWT) | % TAG | % FFA | % ST | % PL |
|---|---|---|---|---|---|---|---|---|---|
| (a) Liver | Time-0 | -1 | 12.1 ± 7.7 | 298.5 ± 52.3 | 485.8 ± 85.1 | 87.3 ± 3.0 | 8.1 ± 2.4 | 1.3 ± 0.6 | 3.4 ± 0.6 |
| | | 1 | 18.1 ± 8.1 | 303.5 ± 78.8 | 494.0 ± 128.2 | 85.7 ± 5.5 | 7.8 ± 2.9 | 2.0 ± 0.8 | 4.5 ± 2.4 |
| | | 3 | 18.8 ± 12.0 | 288.8 ± 41.2 | 469.9 ± 67.1 | 88.9 ± 3.9 | 5.6 ± 1.6 | 1.7 ± 1.0 | 3.9 ± 1.6 |
| | | 5 | 16.3 ± 8.9 | 275.0 ± 63.8 | 458.5 ± 106.4 | 85.1 ± 3.4 | 8.8 ± 2.9 | 2.1 ± 0.8 | 4.7 ± 1.2 |
| | Day 28 | -1 | 14.9 ± 6.9 | 334.6 ± 46.6 | 554.2 ± 77.2 | 89.8 ± 4.9 | 5.5 ± 4.3 | 1.1 ± 0.5 | 3.6 ± 1.4 |
| | | 1 | 9.6 ± 4.4 | 320.7 ± 47.3 | 541.9 ± 79.9 | 91.4 ± 2.2 | 4.7 ± 1.9 | 0.8 ± 0.2 | 3.1 ± 1.6 |
| | | 3 | 16.5 ± 7.6 | 338.7 ± 40.5 | 556.2 ± 66.5 | 89.6 ± 4.1 | 5.6 ± 2.2 | 1.1 ± 0.3 | 3.7 ± 2.0 |
| | | 5 | 14.2 ± 6.3 | 345.6 ± 102.8 | 669.5 ± 199.1 | 91.0 ± 2.9 | 4.7 ± 1.6 | 1.1 ± 0.3 | 3.3 ± 1.5 |
| | Survivors at 50% population mortality | -1 | 0.1 ± 0.1 | 13.3 ± 2.0 | 58.4 ± 8.6 | 0.7 ± 1.7 | 25.2 ± 8.2 | 14.3 ± 3.0 | 59.7 ± 7.6 |
| | | 1 | 0.2 ± 0.2 | 14.3 ± 8.6 | 61.8 ± 37.2 | 5.7 ± 13.4 | 19.8 ± 4.0 | 14.5 ± 4.0 | 59.9 ± 12.4 |
| | | 3 | 0.3 ± 0.4 | 16.8 ± 7.7 | 73.1 ± 33.6 | 6.5 ± 12.4 | 20.4 ± 8.9 | 11.8 ± 2.9 | 61.4 ± 14.7 |
| | | 5 | 0.3 ± 0.3 | 24.2 ± 12.6 | 103.8 ± 54.0 | 17.4 ± 17.0 | 24.7 ± 7.0 | 9.7 ± 4.6 | 48.2 ± 17.6 |
| | Mortalities | -1 | 0.1 ± 0.0 | 11.3 ± 2.4 | 56.0 ± 12.2 | - | 13.2 ± 7.8 | 14.7 ± 1.3 | 72.1 ± 8.5 |
| | | 1 | 0.0 ± 0.0 | 9.0 ± 2.1 | 45.2 ± 10.3 | - | 17.2 ± 12.3 | 15.5 ± 2.9 | 67.3 ± 12.0 |
| | | 3 | 0.1 ± 0.0 | 10.2 ± 3.6 | 53.0 ± 19.5 | - | 16.1 ± 9.0 | 16.8 ± 4.4 | 67.1 ± 7.8 |
| | | 5 | 0.1 ± 0.1 | 13.2 ± 7.2 | 73.0 ± 40.1 | 3.9 ± 8.5 | 18.0 ± 15. 8 | 15.0 ± 5.6 | 63.2 ± 13.4 |
| (b) Muscle | Time-0 | -1 | 25.4 ± 6.6 | 30.7 ± 5.4 | 174.0 ± 30.4 | 56.4 ± 4.82 | 8.2 ± 1.4 | 5.5 ± 1.1 | 29.9 ± 3.3 |
| | | 1 | 25.0 ± 3.5 | 25.0 ± 2.6 | 140.4 ± 14.8 | 52.4 ± 3.9 | 9.6 ± 1.4 | 6.3 ± 0.7 | 31.7 ± 2.9 |
| | | 3 | 34.7 ± 9.0 | 31.7 ± 5.6 | 178.3 ± 31.5 | 58.1 ± 4.4 | 7.3 ± 0.7 | 5.7 ± 0.8 | 28.9 ± 3.1 |
| | | 5 | 33.0 ± 9.1 | 27.6 ± 5.4 | 153.6 ± 30.2 | 54.4 ± 6.5 | 8.6 ± 1.3 | 6.2 ± 0.7 | 30.9 ± 5.8 |
| | Day 28 | -1 | 21. 5 ± 6.5 | 22.8 ± 2.7 | 133.0 ± 15.5 | 50.5 ± 6.3 | 5.8 ± 1.2 | 5.9 ± 0.7 | 37.9 ± 5.4 |
| | | 1 | 17.7 ± 5.7 | 21.5 ± 5.7 | 123.2 ± 32.7 | 39.9 ± 15.6 | 6.7 ± 2.0 | 8.85 ± 4.4 | 44.6 ± 9.6 |
| | | 3 | 23.2 ± 8.0 | 20.6 ± 2.3 | 124.1 ± 14.0 | 49.0 ± 3.3 | 6.1 ± 1.1 | 6.8 ± 0.7 | 38.0 ± 3.6 |
| | | 5 | 18.4 ± 4.9 | 18.4 ± 2.8 | 115.9 ± 17.6 | 42.6 ± 7.5 | 6.6 ± 2.4 | 7.5 ± 1.1 | 43.3 ± 5.4 |
| | Survivors at 50% population mortality | -1 | 8.1 ±1.8 | 10.9 ± 1.4 | 60.5 ± 7.5 | - | 7.4 ± 3.6 | 19.0 ± 1.5 | 73.7 ± 4.0 |
| | | 1 | 7.7 ± 1.2 | 10.3 ± 1.5 | 58.9 ± 8.8 | 0.7 ± 2.0 | 7.6 ± 1.0 | 19.5 ± 1.4 | 72.2 ± 2.3 |
| | | 3 | 9.2 ± 2.8 | 9.5 ± 1.4 | 54.5 ± 8.0 | 0.1 ± 0.2 | 6.5 ± 4.6 | 18.7 ± 1.4 | 74.7 ± 5.0 |
| | | 5 | 10.6 ± 6.5 | 11.8 ± 4.7 | 73.1 ± 29.2 | 6.9 ± 18.1 | 10.9 ± 4.5 | 17.0 ± 4.3 | 65.2 ± 12.7 |
| | Mortalities | -1 | 5.6 ±0.9 | 11.8 ± 1.1 | 60.3 ± 5.5 | - | 6.0 ± 3.3 | 20.0 ± 1.9 | 74.0 ± 4.6 |
| | | 1 | 4.8± 1.5 | 13.0 ± 2.1 | 68.5 ±10.8 | - | 3.7 ± 2.1 | 20.2 ± 1.2 | 76.2 ± 2.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | 4.8 ±2.1 | 13.1 ± 2.7 | 69.7 ± 5.5 | - | 5.3 ± 2.3 | 19.0 ± 2.2 | 75.7 ± 3.9 |
| | 5 | 6.1 ±1.5 | 11.5 ± 2.4 | 65.3 ± 13.3 | - | 4.3 ± 2.7 | 19.9 ± 1.3 | 75.9 ± 3.6 |

**Table 5.** The importance of energetic condition and winter temperatures for days of starvation resistance in age-0 polar cod. Lipid concentrations in polar cod from the central Chukchi Sea in 2013 and 2017 are used to demonstrate variability in time to starvation for field-collected fish of different nutritional status. The equation for lipid loss ($mg.g^{-1}$ WWT).$day^{-1}$ = -18.79 x $10^{-2}$ -2.12 x $10^{-2}$ * (T), $r^2$ = 0.77 was used to calculate the days to starvation lipid storage levels under the four different experimental temperatures (-1, 1, 3 and 5 °C). Data are mean ± standard error of lipid composition in field-collected age-0 polar cod in the Central Chukchi Sea (68.25 - 70.74°N, Copeman et al., 2020; Copeman et al., accepted) and the model shown in Figure 8.

| Year | Lipids concentration in polar cod from the Central Chukchi Sea | Change in lipid concentration (µg.$mg^{-1}$, WWT) between end of summer and mortality levels | Duration of starvation resistance at -1 °C (days) | Duration of starvation resistance at 1 °C (days) | Duration of starvation resistance at 3 °C (days) | Duration of starvation resistance at 5 °C (days) |
|---|---|---|---|---|---|---|
| 2013 (n = 30) | 34.7 ± 3.4 | 22.6 | 136 | 108 | 90 | 77 |
| 2017 (n = 35) | 16.0 ± 1.1 | 3.9 | 24 | 18 | 16 | 13 |
| Mortality levels | 12.4 | | | | | |

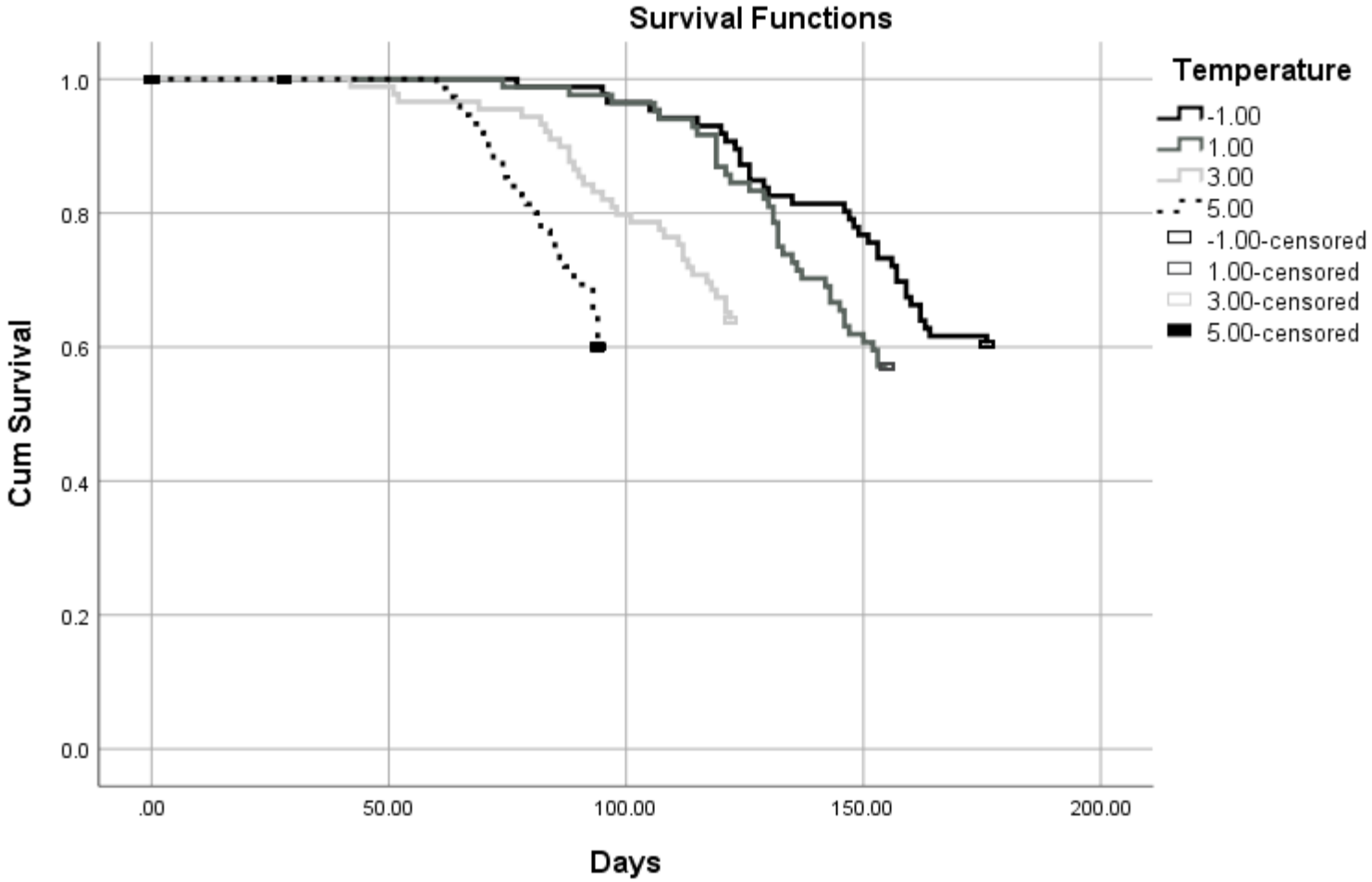


**Fig. 1.** Kaplan-Meier cumulative survival curves for juvenile polar cod (*Boreogadus saida*) in temperature-dependent (-1, 1, 3 and 5 °C) overwintering experiments, shown as a function of days of exposure to experimental conditions. Data are shown using all individual fish pooled by temperature treatment after finding no significant tank effects ($p < 0.05$). Fish removed for sampling were considered censored from the population.

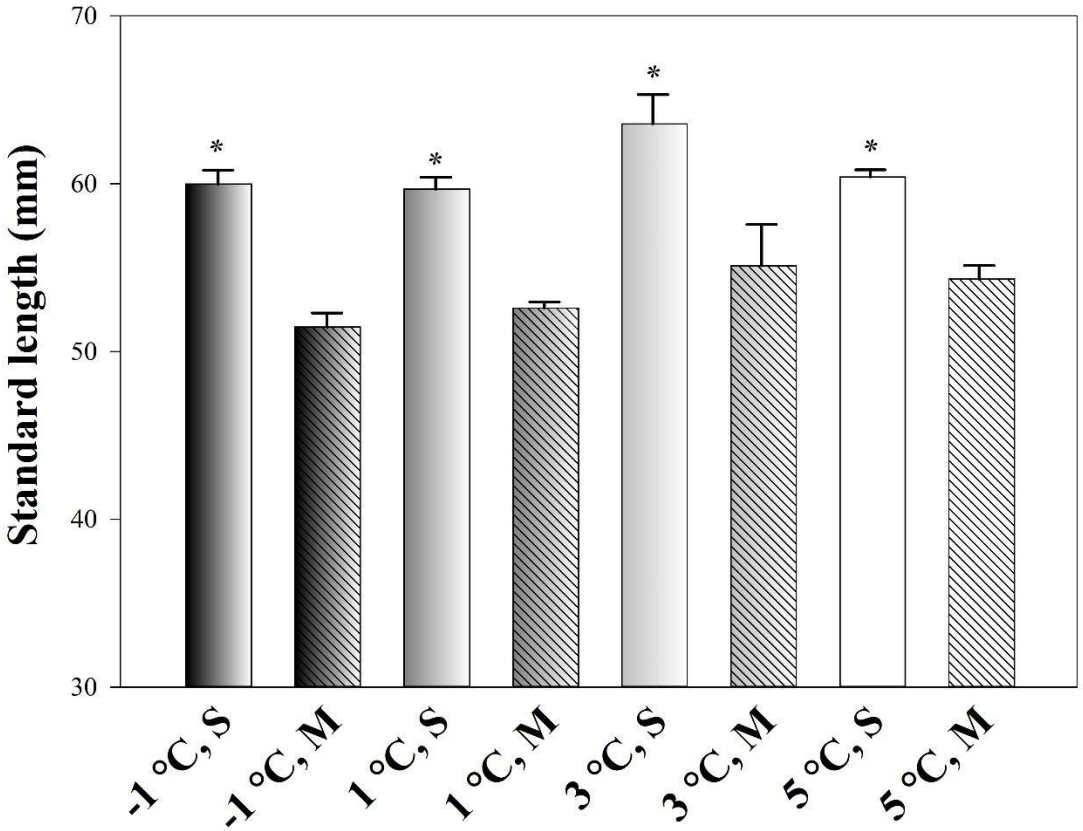


**Fig. 2.** Across all temperatures, polar cod (*Boreogadus saida*) that survived past 50% population mortality (S) were significantly longer in standard length (SL, mm) than fish that died prior to 50% population mortality (M). The mean SL of mortalities was 53.5 ± 0.8 mm, while the mean SL of survivors was 61.1 ± 0.7 mm. The asterisks over the survivor bars indicate that they were significantly longer than fish that died within the same temperature treatment ($p<0.05$).

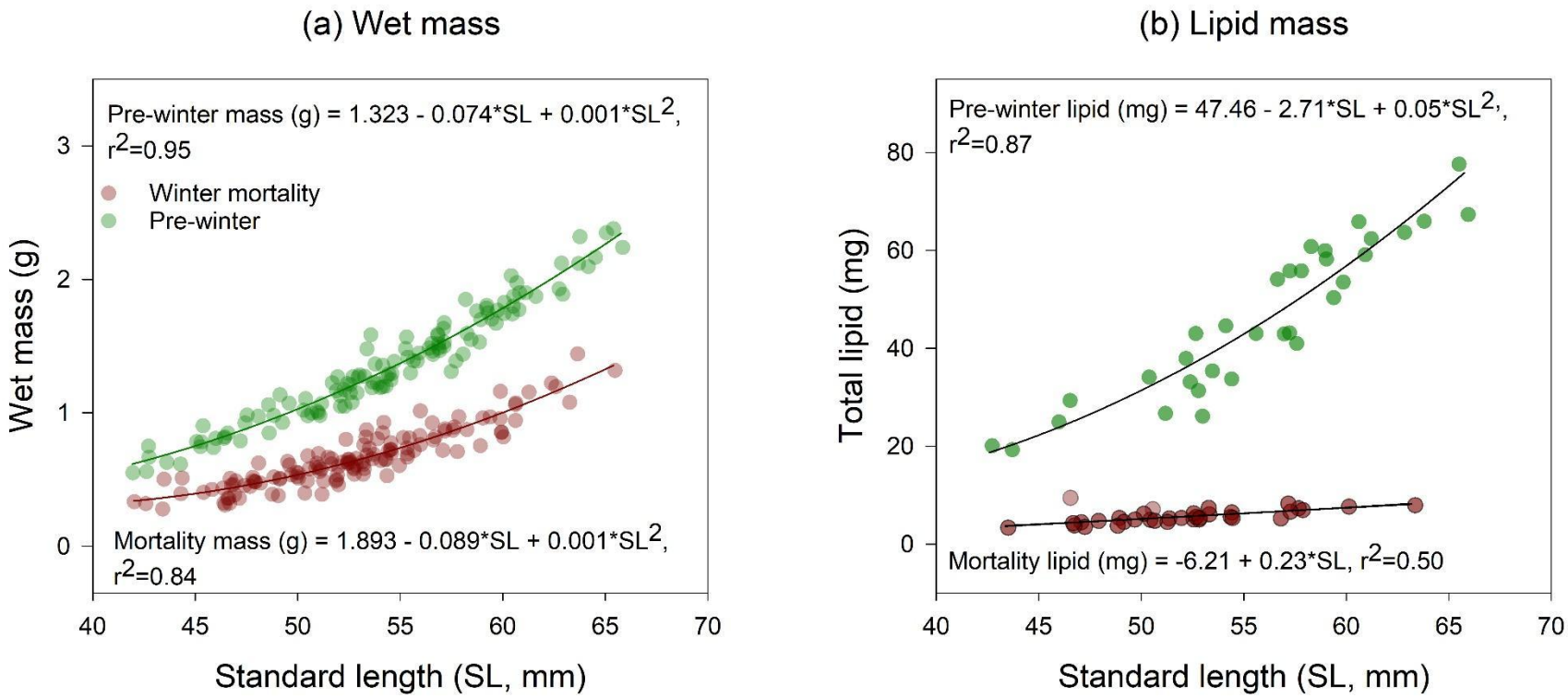


**Fig. 3.** The relationship between length and (a) weight as well as (b) total lipid for age-0 polar cod at the beginning of the experiment ('pre-winter') and at the time of mortality during the experiment ('winter mortality'). The winter mortality length-weight model is the theoretical lower weight and lipid threshold that juvenile fish must maintain for survival during winter.

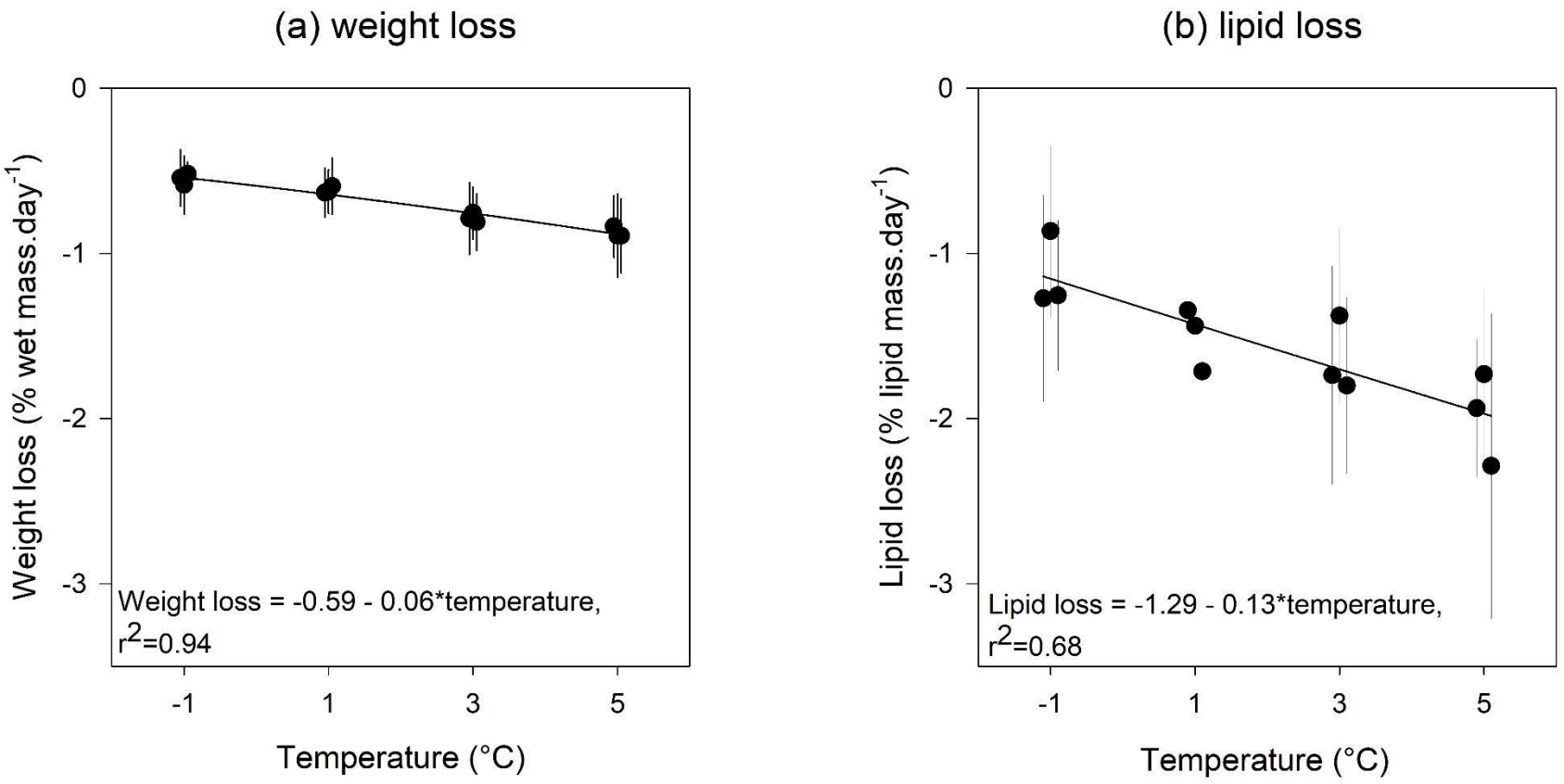


**Fig. 4.** Rate of temperature-dependent (a) weight loss and (b) lipid loss for age-0 juvenile polar cod during simulated overwintering laboratory experiments. Experiments were run at -1, 1, 3 and 5 °C until ~50% population mortality. Data are tank means (n = 3 per temperature) of individual weight loss over time. Temperature-dependent weight loss and lipid loss were determined using the difference in weight or lipid at the time of mortality as compared to length-based pre-winter models (See Fig. 3).

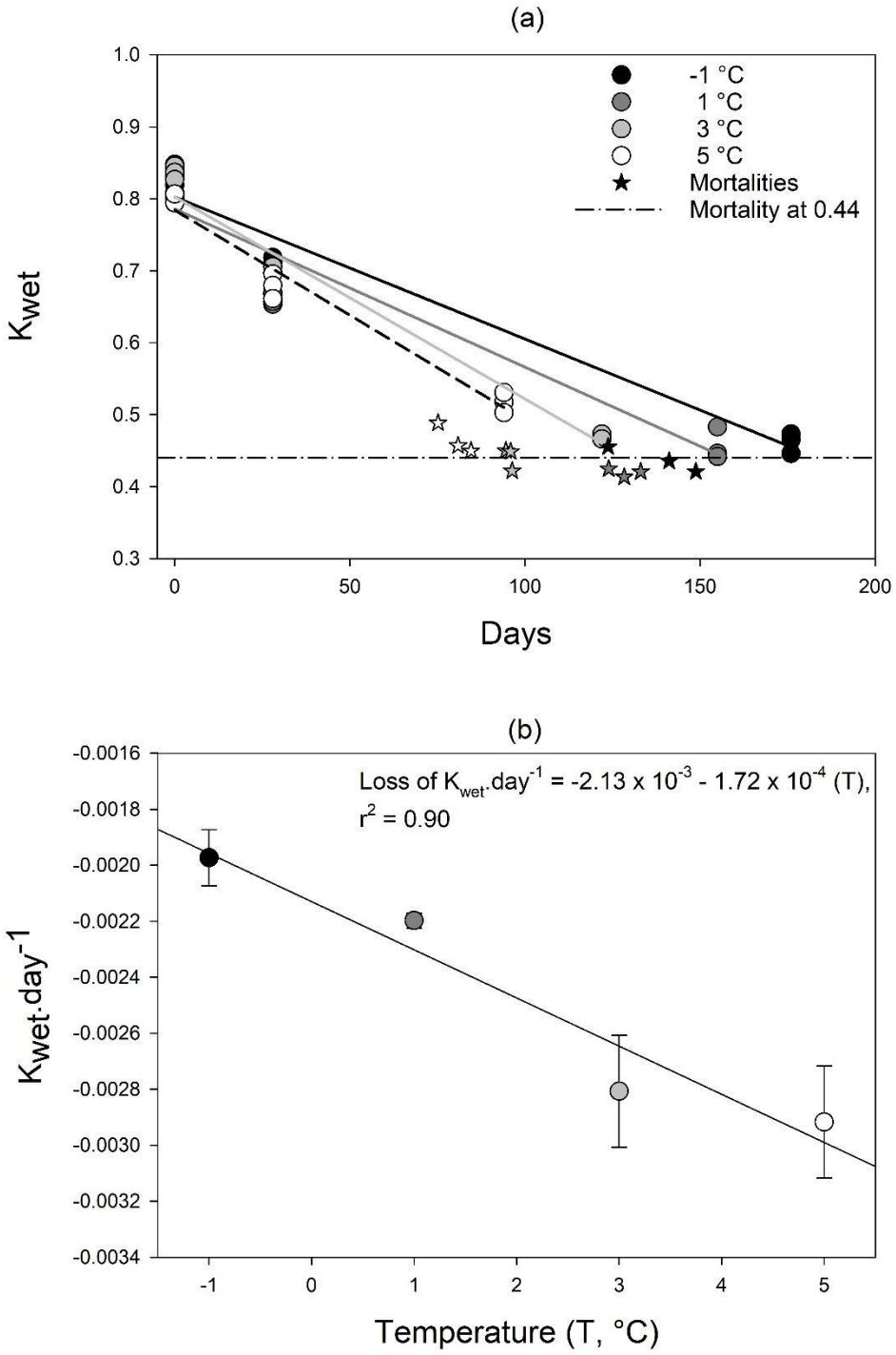


**Fig. 5.** Fulton's K condition factor based on WWT of age-0 juvenile polar cod (*Boreogadus saida*) shown (a) over the full overwintering experiment and (b) as a temperature-dependent rate of loss function. Data in 5a are the means of three tank values (± 1 SE) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average Fulton's $K_{wet}$ of 0.44. The temperature-dependent rate of loss function (b) relates the

slopes of relationships in 5a to temperature. Correlation coefficients for $K_{WWT}$ in panel (a) were $r^2 = 0.97$ at -1°C, $r^2 = 0.91$ at 1 °C, $r^2 = 0.96$ at 3 °C and $r^2 = 0.98$ at 5 °C.

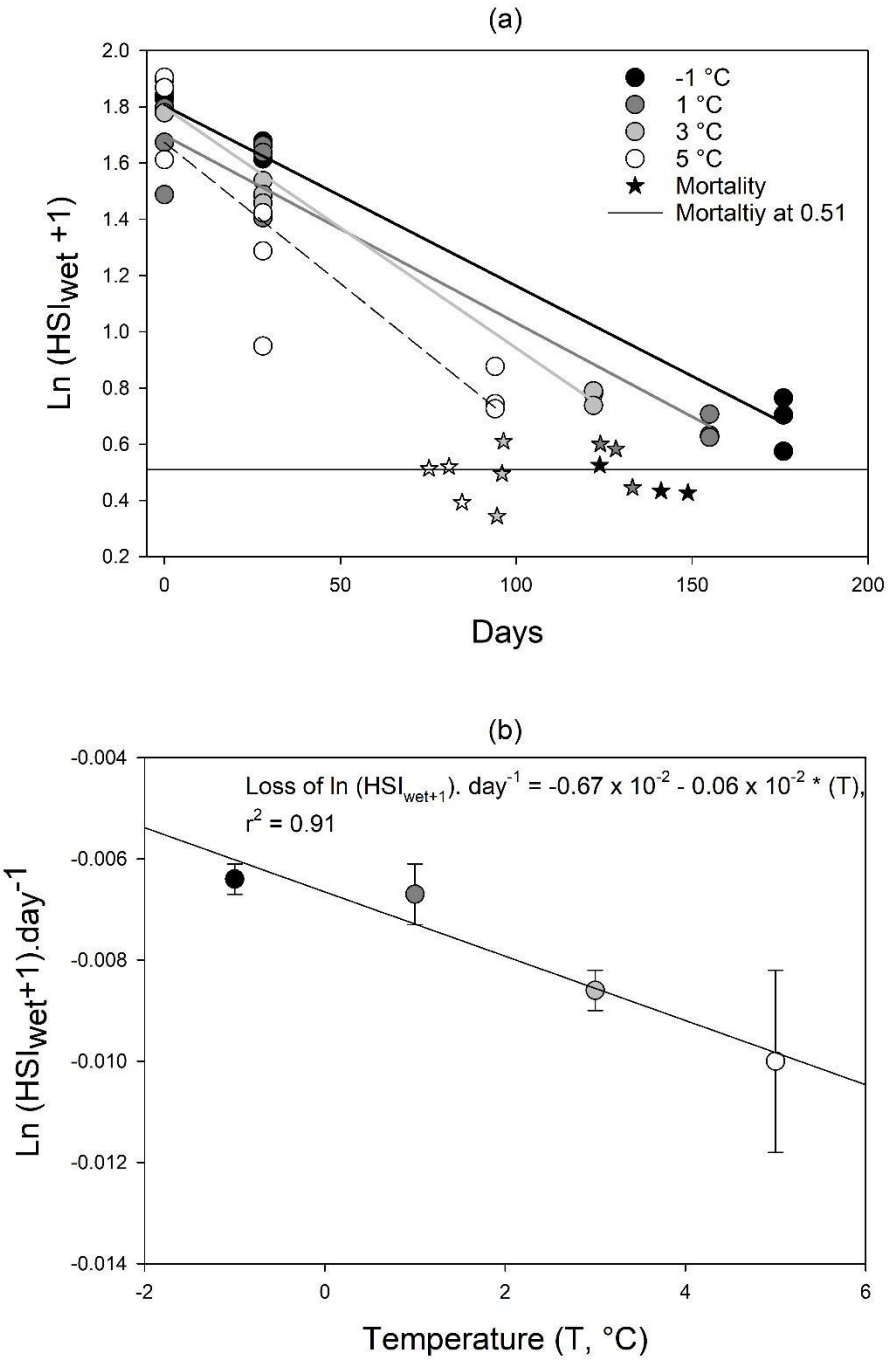


**Fig. 6.** Natural log of the hepatosomatic index (HSI) based on WWT of age-0 juvenile polar cod (*Boreogadus saida*) shown (a) over the full overwintering experiment and (b) as a temperature-dependent rate of loss function. Data in 6a are the means of three tank values (± 1 SE) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average $HSI_{wet}$ of 0.67 and Ln ($HSI_{wet}$+1) of 0.51. The

temperature-dependent rate of loss function (6b) relates the slopes of relationships in 6a to temperature. Correlation coefficients for $HSI_{WWT}$ in panel (a) were $r^2 = 0.98$ at -1°C, $r^2 = 0.94$ at 1 °C, $r^2 = 0.98$ at 3 °C and $r^2 = 0.79$ at 5 °C.

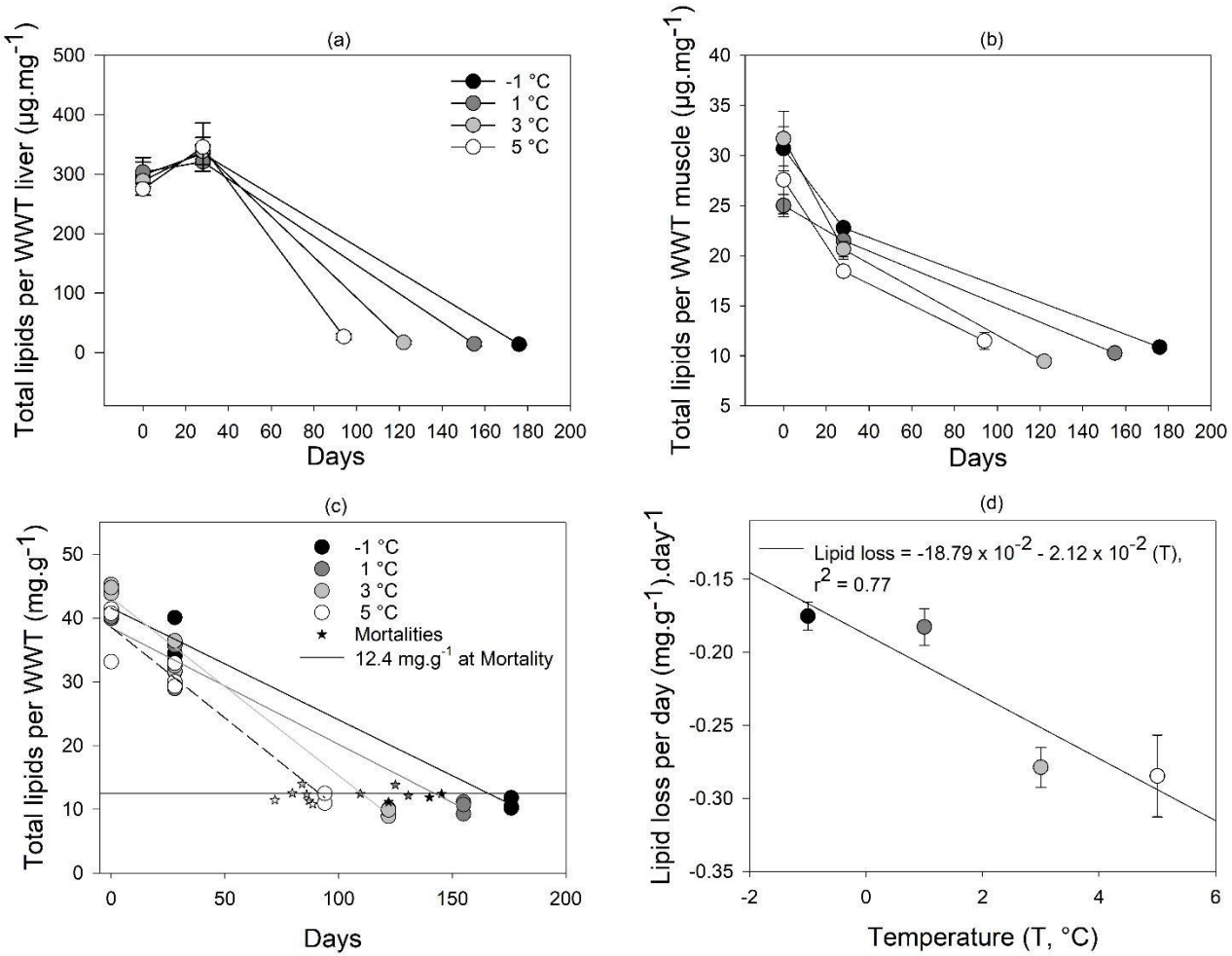


**Fig. 7.** Total lipids per WWT (µg.mg$^{-1}$) shown in (a) the liver, (b) muscle tissue and (c) estimated whole bodies of age-0 polar cod (*Boreogadus saida*) over the full overwintering experiment. Data are tank means (n = 3) for sampled fish (circles) and mortalities (stars) at each temperature treatment (-1, 1, 3, 5 °C). Fish that died had an average whole-body total lipid per WWT of 12.4 mg.g$^{-1}$. The temperature-dependent rate of lipid loss in whole fish (7d) relates the slopes of relationships in 7c to temperature. Correlation

coefficients for total lipids per WWT (mg.g$^{-1}$) in panel (c) were $r^2 = 0.98$ at -1°C, $r^2 = 0.97$ at 1 °C, $r^2 = 0.98$ at 3 °C and $r^2 = 0.94$ at 5 °C.

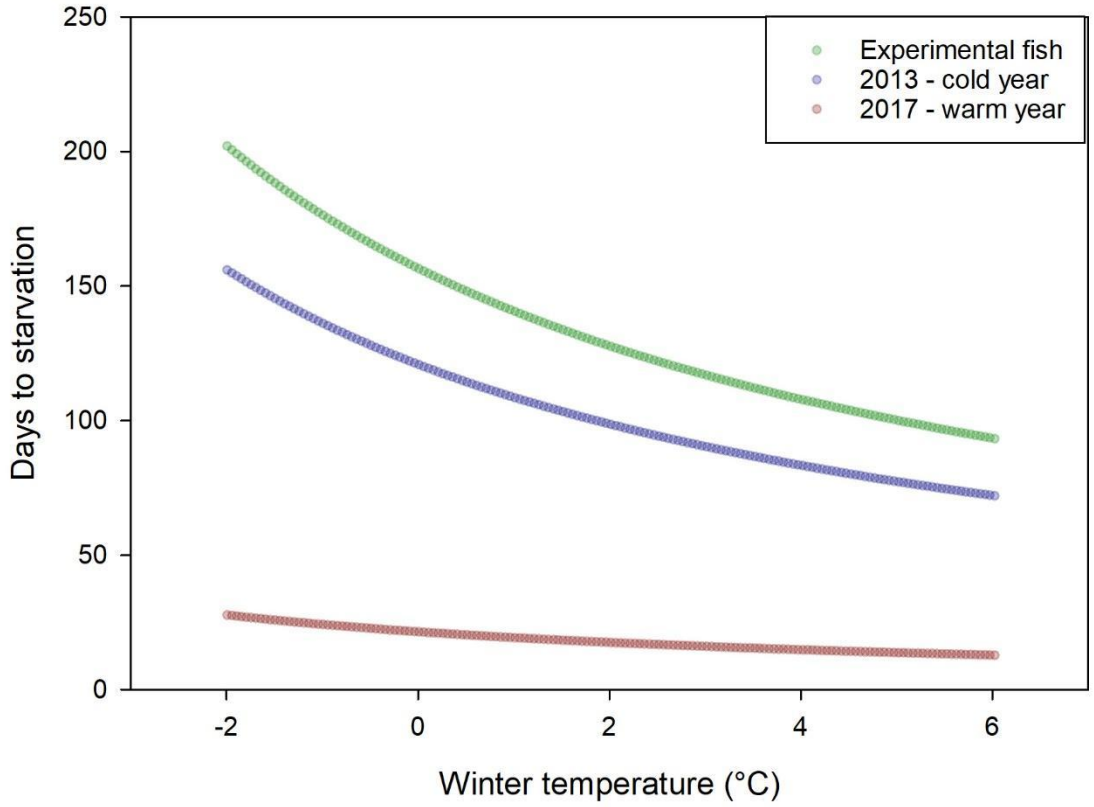


**Fig. 8.** Days to starvation based on the lab-determined temperature-dependent lipid loss model:

Lipid loss ($mg.g^{-1}$ WWT).$day^{-1}$ = -18.79 x $10^{-2}$ -2.12 x $10^{-2}$ * (T), $r^2$ = 0.77 (Fig. 7d).

Scenarios are shown for experimental fish, as well as polar cod (*Boreogadus saida*) from the central Chukchi Sea in a cold year (2013, Copeman et al., 2020) and a warm year (2017, Copeman et al., accepted) to demonstrate variability in time to starvation for fish of different nutritional status over a wide range of temperatures. The mean lipid concentration values from fish sampled in the Central Chukchi Sea (68.25 - 70.74°N) are given in Table 5.

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