Estimation of the Relationship between Growth, Consumption, and Energy Allocation in Juvenile Pacific Cod (*Gadus macrocephalus*) as a Function of Temperature and Ration

Ashwin Sreenivasan[1] and Ron Heintz[2]

[1] Corresponding Author: Dr. Ashwin Sreenivasan, Sitka Sound Science Center, asreenivasan@alaska.edu
Current Correspondence Address: Auke Bay Laboratories, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 17109 Pt. Lena Loop Road, Juneau, Alaska-99801

[2] Auke Bay Laboratories, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 17109 Point Lena Loop Road, Juneau, AK 99801, USA

Abstract

Pacific cod (*Gadus macrocephalus*) are generalist predators in the Gulf of Alaska (GOA), and are an important predator on other commercially important species. Efficient management of this species can benefit by knowing how these fish adapt to changing environmental conditions, with a focus on how growth and condition are affected by changes in temperature and diet. We conducted a feeding study to understand the relationship between growth, ration, and temperature, and how these factors interact to affect energy allocation strategies. Since growth and condition of juveniles can determine recruitment into the population, this study focused on growth and consumption of age 1+ Pacific cod held over 4 temperature treatments (4 °C, 8 °C, 12 °C, and 16 °C) and 3 ration levels (unlimited ration, medium ration, and low ration). We also compared cellular nucleic acid (RNA/DNA) ratios, an instantaneous growth index, total-body lipid, and proximate composition between fish. At 4 °C, 8 °C, and 12 °C, fish at medium and low rations had higher growth rates relative to fish at high rations. Higher food consumption appears to negatively affect digestive ability, assimilation efficiency, and nutrient utilization. RNA/DNA was clearly correlated with growth rates at 4 °C and 8 °C, but this relationship did not hold at higher temperatures. A secondary growth study was conducted to test the reliability of the growth/consumption models derived from the main growth study. Temperature influenced energy reserves (lipid) while tissue growth (protein) was influenced by ration level. Average lipid values were higher at 4 °C than at 8 °C or 12 °C, suggesting a predisposition to heightened lipid synthesis at colder temperatures. Longer durations of warmer water temperature in the GOA could consequently affect energy allocation strategies, with dietary changes in the field potentially amplifying this effect in cold and warm years. This energy allocation strategy could be detrimental with warmer temperatures predicted in the GOA.

1.1 Introduction

Efficient management of Pacific cod (*Gadus macrocephalus*) in the Gulf of Alaska (GOA) can be informed by knowledge of how these fish adapt to changing environmental conditions. Pacific cod are abundant in the GOA, and support a commercial fishery valued at ~$389.7M in 2013 (Fissel et al. 2014). Adult Pacific cod play an important ecological role as generalist upper trophic level predators in the GOA (Yang 2004), and are known to forage on commercially important species, including tanner crab (*Chionoecetes bairdi*), shrimp, walleye pollock (*Gadus chalcogrammus*), and juvenile Pacific cod (Jewett 1978, Bakkala 1984, Albers et al. 1985, Urban 2012). Understanding how juvenile Pacific cod adapt to their environments is especially important in the context of the GOA, given the incomplete understanding of how climate, fishing, and food web dynamics interact in this region (Gaichas et al. 2011). In addition, changes in physical forcing factors may have played a large role in increased recruitment of these species (Hollowed and Wooster 1992). For example, a major change in 1977 involved a shift from a cold to a warm regime which had numerous ecological (Hollowed and Wooster 1992) and economic implications (Orensanz et al. 1998). Changing ocean conditions can affect food availability, temperature, and fish growth, in turn possibly affecting survival and recruitment of fish. A shift in ocean circulation patterns in the GOA manifested a number of effects, including an increase in Pacific cod and walleye pollock populations. The resulting increase in recruitment of Pacific cod and walleye pollock led to the development of multimillion dollar fisheries for these species (Jewett 1978, Beamish et al. 2004, Bacheler et al. 2010).

The growth of juvenile Pacific cod under various temperatures and levels of food availability is critical to understanding how cod interact with their environment. Growth is temperature and energy-dependent and maximum growth can only occur if fish encounter an abundant food supply and optimal temperatures. For juvenile fish the relationship between growth, food supply, and temperature is especially important. Maximized growth and energy storage in juvenile walleye pollock can be an

important predictor of recruitment into adult populations (Heintz et al. 2013). Thus, monitoring growth and condition of juvenile Pacific cod sampled during fishery-independent surveys could be useful in understanding how cod populations vary over time.

Despite the importance of these relationships there are relatively few data describing the functional response of juvenile Pacific cod growth to changing temperature and food supply. Hurst et al. (2010) described a functional response between temperature and growth in age-0 Pacific cod fed *ad libitum* rations. Even fewer data exist describing how juvenile Pacific cod allocate the energy they consume between tissue growth and energy storage under different temperatures and ration sizes. Temperature is known to affect lipid storage rates in cold-adapted fish species, including striped bass (*Morone saxatilis*; Egginton and Sidell 1989) and rainbow trout (*Oncorhynchus mykiss*; Egginton et al. 2000), where lipid storage increases with colder temperature. In addition to energy storage, a temperature-dependent lipid storage strategy also has other physiological benefits, primarily as an adaptation to maintenance of metabolic functions in cold temperatures (Desaulniers et al. 1996; Guderley 2004). Increased cellular lipid content enables faster transport of oxygen through the cell relative to cytoplasm, which increases in viscosity due to colder temperatures (Sidell 1998), and lipid could potentially function as an oxygen reservoir (Egginton and Sidell 1989) in addition to an energy store. Thus, laboratory studies designed to understand how fish allocate energy to tissue growth and energy storage are important to establishing benchmarks for indexing the nutritional status of fish caught in surveys, and in understanding metabolic responses of fish with climatic variation.

The nutritional condition of juveniles can be indexed by their lipid content, which is a stored energy reserve (Vollenweider et al. 2011), or energy density (Paul and Paul 1999). Biochemical indices of growth such as nucleic acid ratios (RNA/DNA; Buckley 1979, McLaughlin et al. 1995, Buckley et al. 1999) can be useful tools for monitoring growth in juvenile fish and the nutritional status of fish caught on surveys. While cellular DNA concentrations remain stable, RNA concentrations can increase or

decrease (during nutritional stress) as a function of protein synthesis (Buckley 1984, McLaughlin et al. 1995), indicating growth or lack thereof, as well as nutritional condition (Weber et al. 2003). Understanding how these indices interact with temperature and food supply in juvenile Pacific cod under controlled conditions may provide a valuable step towards relating current environmental conditions to future abundance.

The goal of this study is to describe how juvenile Pacific cod respond to variations in temperature and food availability. The specific objectives include identifying the optimal temperature for growth as a function of ration; developing a model relating temperature, consumption, and growth; calibrating an RNA/DNA growth model that can be used to estimate growth in field-caught specimens; and understanding how temperature influences the energy allocation strategies of juvenile cod. To meet these objectives, two feeding studies were conducted with age-1 Pacific cod held over 4 temperature treatments and 3 ration levels. In addition to measuring growth and consumption rates, white-muscle nucleic acid ratios and whole-body proximate compositions were measured.

1.2 Materials and Methods

Two growth studies were conducted on wild-caught Pacific cod as part of our main study and as a follow-up study designed to test some of the main study assumptions. The main growth study was carried out for the duration of one month, in spring 2013, and the follow-up growth study was carried out during late summer 2013. Juvenile Pacific cod, ranging in size from 135 mm-194 mm total length were obtained by jigging in shallow water at the National Oceanic and Atmospheric Administration's (NOAA) Little Port Walter (LPW) facility (latitude 56.3835, longitude -134.6466) during the month of February 2013. All fish were transported to the Auke Bay Laboratories wet laboratory, where they were initially quarantined for one month in a separate holding tank prior to being placed in the treatment tanks for the main growth study. Fish were held at an ambient temperature during this period. The purpose of the quarantine in the wet laboratory was primarily to ensure that there were no disease

outbreaks that could spread, and also to ensure that only healthy surviving fish were used in the study. Fish were initially fed commercially available 9.0 mm fish food pellets (Bio-Oregon, Longview, WA-98632)[1] during the quarantine period and were then switched to the treatment diet, frozen euphausiids (*Euphausia superba*), two weeks prior to the beginning of the main growth study in order to habituate fish to the treatment food. All fish were maintained on euphausiids thereafter, which is known to be a part of their natural diet.

1.2.1 Main Growth Study: The objective of the main growth study was to examine how instantaneous growth rate (IGR) relates to consumption and temperature. Fish were randomly assigned to 4 temperature treatments: 4 °C, 8 °C, 12 °C, and 16 °C, and held at a 12L:12D photoperiod cycle from April 5 to May 6, 2013. These temperatures were chosen since they encompassed the known range of temperatures Pacific cod can experience in the GOA. Fish were held in 1 of 3 tanks for 12 total tanks. Each 50 liter tank held 5 fish at a flow rate of 4 liters minute^{-1}. Each day during the study the fish in the tanks for a given temperature treatment were offered either a high ration (fed *ad-libitum*), medium ration (11% body weight), or low ration (6% body weight). For the high ration, fish were fed pre-weighed euphausiids in excess. After a period of 3 hours, any unconsumed food was siphoned out from the tanks, blotted dry, and weighed to estimate daily consumption. We assumed that each fish within a tank would have unique consumption rates based on observed behavioral interactions among fish, so that across tanks the entire range of possible consumption rates would be covered.

Growth in mass was measured for all fish that increased in mass during the feeding trial. Lengths and weights of fish were measured at the start of the experiment, after a two-week interval, and finally at the termination of the experiment. Observations at two weeks were used to adjust feeding rates. Fish in each tank were fin-clipped to allow tracking of individual fish growth through the

[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

experimental period. Instantaneous growth rate (IGR) was calculated for each fish in all treatments from the final sampling date (t_2) to the initial date (t_1) using the formula:

$$IGR = 100*(\ln w_{t2} - \ln w_{t1}) / (t_2 - t_1) \qquad \text{equation 1}$$

where w =weight (g). Consumption estimates for fish within a tank were expressed as the percentage of final wet body mass consumed per day using:

$$C_i = \frac{100 \times \left(\frac{\Delta W_i}{\sum \Delta W_i}\right) \times \frac{\sum (F_o - F_r)}{\sum t}}{W_f} \qquad \text{equation 2}$$

Where C_i = estimated consumption of individual fish expressed as percent final body weight per day, ΔW_i = difference in weight of individual fish, $\Sigma\Delta W_i$ = total weight difference of all fish, W_f = the final weight of the fish, F_o = mass of food offered to fish within a treatment tank, F_r = mass of food recovered and Σt = the number of days fish were fed.

1.2.2 Follow-up Growth Study: A follow-up growth study was conducted to test the reliability of the growth/consumption models derived from the main growth study with independent data. The method of estimating consumption in this study introduces dependency in estimating the relationship between growth and consumption, since estimates of consumption depend on the observed growth. In addition an unknown bias is introduced if only a subset of the fish in the tank actually grow. This bias results from the assumption that fish with no growth or negative growth do not consume any food, and attributes all food consumption to fish that had positive growth. However, even fish that had no growth would need to consume a minimum amount of food to maintain basic metabolic functions, which is not accounted for in this estimation.

In this follow-up study, fish were similarly randomly assigned to four temperature treatments: 4 °C, 8 °C, 12 °C, and 16 °C (n=4/tank), and held at a 12L:12D photoperiod cycle from August 20th to

September 13th 2013. Fish were fed a diet of chopped, frozen euphausiids. They were fed arbitrarily selected rations that were designed to fall in between the medium and maximum rations offered in the first experiment. All experimental conditions were identical to the previous study and growth was estimated the same way.

1.2.3 Biochemical Analyses: At the end of the main growth study, surviving fish were sacrificed and analyzed to determine their RNA and DNA content and proximate composition. Sampled fish were euthanized in a solution of tricainemethanesulfonate (MS-222; 0.025 g liter^{-1}), after which wet mass (0.01 g) and total lengths (0.5 mm) of fish were recorded. White muscle plugs (~100 mg) for RNA/DNA were removed from the musculature below the dorsal fin with a scalpel, placed in individual 1500 µL microcentrifuge vials on ice, and then stored at -80 °C until analysis. After muscle plugs were removed, the individual fish were then stored in individual bags at -20 °C until analysis.

All RNA/DNA ratios were measured by a one dye-two enzyme (RNase and DNase) fluorometric protocol modified from Caldarone et al. (2001). Muscle plugs were subdivided, and ~10 mg tissue plugs were placed in individual 1500 µL microcentrifuge vials with 300 µL 2% N-lauroylsarcosil Tris-EDTA buffer, and sonicated using a Branson Sonifier 250 (VWR Scientific, Radnor, PA). Samples were then vortexed for 60 m, diluted with 1200 µL Tris-EDTA buffer, and centrifuged for 15 m at 14000 g using a Sorvall Legend Micro 21 R refrigerated centrifuge (Thermo Scientific, Waltham, MA). Supernatants were then treated with 75 µL ethidium bromide (5 µg ml^{-1}) according to the protocol outlined by Caldarone et al. (2001). A Wallac 1420 microplate spectrophotometer (Perkin Elmer, Waltham, MA) was used to measure total fluorescence, at excitation and emission wavelengths of 355 nm and 600 nm respectively. Samples were sequentially treated with RNase and DNase, and the resulting reduced fluorescence measured to obtain RNA and DNA fluorescence, respectively. Standard curves were constructed using serial dilutions of 18s-28s rRNA (Sigma R-0889) and calf thymus DNA (Sigma D-4764) standards.

Supernatants for RNA/DNA were read on Corning NBS 96-well black flat-bottom microplates (75 µL samples).

Lipid analysis was carried out according to a protocol modified from the Folch's method, outlined by Christie (1982). Approximately ~0.5 g wet sample homogenate was placed in a Dionex Accelerated Solvent Extractor 200, and lipid extracted using 2:1 (v:v) chloroform:methanol. All extracts were then sequentially washed with 0.88% KCl and 1:1 (v:v) methanol:deionized water in a volume that equaled 25% of extract volume. The excess solvent was then evaporated and percent lipid values were gravimetrically determined.

Total protein content was estimated by multiplying total estimated nitrogen content by 6.25, a conversion factor that accounts for the nitrogen content in protein (Craig et al. 1978). Nitrogen was measured using a LECO nitrogen analyzer TruSpec according to the Dumas method (Association of Offficial Analytical Chemists, 2002). Approximately 0.1 g of dried whole-body homogenate was combusted at a temperature of 950 °C, and the expelled nitrogen measured (Sweeney and Rexroad 1987).

Moisture and ash content were measured gravimetrically using a LECO Thermogravimetric Analyzer (TGA) 601. A temperature of 135 °C was used to determine moisture content, and ash content was determined using a temperature of 600 °C.

1.2.4 Statistical analyses: Two different analyses were conducted to evaluate the relationship between temperature and growth. The first analysis entailed regressing the observed average change in mass in each tank against temperature for all the tanks in the experiment. The average change in mass was estimated by dividing the total change in mass for fish in a tank by the number of fish in the tank and the number of days for the trial. A second order polynomial (Growth = $-0.048 + 0.072\ T - 0.004\ T^2$), where T is temperature, was fit to the data using the Gauss-Newton algorithm (Minitab Inc., State

College, Pennsylvania) in order to facilitate comparison of temperature responses between these data and those published previously for age 0+ fish (Hurst et al. 2010) using the equation (Growth = -0.998 + 0.579 T – 0.022 T^2, where T is temperature). This model estimated growth in mass as a function of temperature. The second analysis entailed an Analysis of Covariance (ANCOVA) (Minitab Inc., State College, Pennsylvania) where IGR (equation 1) was the response variable, temperature was the main factor, and consumption (equation 2) was a covariate. Their interaction was also included in the model to evaluate whether or not the relationship between consumption and growth depended on temperature.

In order to determine if the estimated relationship between growth and consumption was biased, the growth and consumption relationships from the previously described ANCOVA were used to predict the growth of fish fed rations equal to those observed in our secondary experiment. Observed consumption was estimated by dividing the total amount of consumed food by the number of fish in each tank and the number of elapsed days. This average daily consumption in the tank was expressed as a percentage of the final average mass of the fish in the tank. The IGR predicted for this consumption from the first model was compared with the highest IGR observed in the follow-up growth study. The underlying assumption was that the lack of independence and bias underlying the growth/consumption models developed from the main experiment did not significantly bias the results if the models could accurately predict growth from the secondary experiment.

1.2.5 Modeling growth with RNA/DNA: A series of 7 linear regression models were compared to identify the best model for predicting the IGR of fish from their RNA/DNA content. In each model the dependent variable was IGR and the independent variables included combinations of wet mass, percent protein (wet mass), and/or temperature along with RNA/DNA. The best model was selected as that with the lowest second order AIC value (AICc) (Wagenmakers and Farrell 2004).

1.2.6 Energy allocation: In order to account for differences in length among treatments**,** an ANCOVA was used to compare the body compositions of all fish (high, medium, and low rations) across all temperature treatments. The response factor was the percent of dry mass allocated to storage as lipid. Temperature, ration level and their interaction were the main factors and the final fish length was a covariate. A similar test was conducted on the percentage dry mass allocated to protein.

The proximate composition of the fish was used to understand how temperature influences body composition. The lipid content of fish from the treatments were compared by a 2-way ANOVA with ration level, temperature and their interaction as main fixed effects.

Linear regressions were used to estimate and compare the relationship between RNA/DNA of fish (high, medium, and low rations) and growth rates at each temperature treatment. Linear regressions relating growth rates to consumption were used to estimate and compare assimilation efficiencies (slope) and standard metabolic rates (intercept) between fish at the medium/low rations and high rations for each temperature. All statistical analyses were made with Minitab v. 14 (Minitab Inc., State College, Pennsylvania).

1.3 Results

1.3.1 Main growth experiment: Comparison of the average growth at different temperatures indicated an optimal growth temperature of 10 °C. Growth calculated as the average grams per day gained by a fish in each of the tanks was fitted (Lack of fit: $p = 0.968$) to the temperatures for each tank using a second order polynomial (Figure 1). Solving the first derivative of the resulting equation (Growth $= -0.048 + 0.072\ T - .004\ T^2$) for zero, yielded a value of 10.02 °C. While data from the 16 °C treatment was not utilized for other analyses (see below), it was utilized for the purposes of estimating optimal growth temperature using the Gauss-Newton algorithm.

Comparison of the individual consumption rates (from equation 2) with IGR (equation 1) in scatter plots demonstrated that IGR of fish from the high ration tanks responded to consumption much differently than fish from the low and middle ration tanks (Figure 2a-c). However, there was a lower correlation between IGR and consumption (medium/low rations) in fish in the 12 °C treatment relative to fish held at 4 °C and 8 °C. In addition, only one fish from each of the medium and low ration tanks survived the 16 °C treatment. Consequently, the analysis of IGR, consumption, and temperature involved two ANCOVAs. The first was limited to fish from the low and medium ration tanks from the 4°, 8°, and 12 °C treatments and the second focused only on fish from the high ration tanks in the 4°, 8°, and 12 °C treatments. There was no evidence of an interaction between temperature and consumption ($p = 0.152$) in the first ANCOVA, indicating that the relationship between IGR and consumption was unaffected by temperature. Removing the interaction term revealed significant effects of temperature ($p = 0.005$) and consumption ($p < 0.001$) on IGR. The interaction between temperature and consumption in the second ANCOVA, examining only the high ration group, was significant ($p < 0.001$). This resulted from a reduced slope in the 4 °C treatment. The existence of this interaction prevented further analysis of temperature and consumption as main effects.

1.3.2 Follow-up growth study: The models relating IGR and consumption for the low and medium ration fish were relatively unbiased in their predictions (Figure 3). The maximum IGR of fish from the follow-up study fell within the confidence intervals for the predicted instantaneous growth in the 4° and 8 °C treatments. The IGR for the 12 °C treatment exceeded the confidence interval predicted from the observed consumption.

1.3.3 Growth Modeling with RNA/DNA: The best model using RNA/DNA to predict growth also included percent protein (wet mass; Table 1). The model using only RNA/DNA and protein had the lowest AIC_c value. The next best model, with $\Delta AIC_c = 0.3944$, included RNA/DNA, protein and temperature. The model with the greatest ΔAIC_c (1.94) included only RNA/DNA. In all, these models

accounted for 59-61% of the total variation in IGR. The correlation between RNA/DNA and growth was inversely related to temperature (Figure 4), decreasing from $R^2 = 0.814$ at 4 °C to $R^2 = 0.700$ and 0.450 at 8 °C and 12 °C, respectively, suggesting that RNA/DNA ratios were less of a reliable predictor of growth at higher temperatures.

1.3.4 Energy Allocation Strategies: As a result of elevated mortality in the tanks held at 16 °C, a proximate analysis could be conducted on only one fish from the high and medium ration tanks. Consequently, the 16 °C treatment was removed from the analysis. Fish held at cooler temperatures allocated an increasing amount of energy to lipid (Table 2; $p = 0.003$). Fish fed at 4 °C allocated an average 14.6% of their dry mass to lipid compared with 11.4% and 10.2% for fish fed at 8° and 12 °C, respectively. Neither ration nor the interaction between ration and temperature affected the lipid content ($p > 0.350$). Conversely, the protein content of fish depended on the ration level ($p = 0.048$), but not the temperature or the interaction between ration and temperature ($p > 0.094$). The protein content increased with ration from 74.4% in the low ration group to 76.1% and 77.2% in the medium and high ration groups, respectively.

1.4 Discussion

The differing effects of temperature and ration level on energy storage and tissue growth has implications for fish condition and energy allocation in the context of changing climate and duration of warming trends in the GOA. While both energy storage and tissue growth are the result of a complex interaction of factors, among the foremost of those factors are temperature and ration. The inverse relationship between energy storage and temperature observed in this study suggests that colder temperatures, leading to higher lipid content, could be beneficial for juvenile cod. While it can be argued that colder temperatures could decrease overall growth rates, higher recruitment has been seen in walleye pollock in years when fish had higher lipid stores (Heintz et al. 2013). These fish evolved in a

scenario where warm water temperature in the summer growing season facilitates a high degree of protein-based somatic growth. As the water temperature starts dropping in the late summer leading into winter, the allocation of energy changes, where fish then start storing greater amounts of lipid relative to protein. Fall decreases in food supply lag behind decreases in temperature (Foy and Paul 1999). Thus energy allocation to growth in soma and storage is balanced against seasonal patterns in temperature and food supply. This overall balanced growth strategy allows for a combination of both somatic growth and lipid (energy) storage in preparation for the winter.

The heightened lipid synthesis with colder temperature observed in fish in this study can be viewed in the context of studies on other cold-adapted fish species, including striped bass (*Morone saxatilis*; Egginton and Sidell 1989) and rainbow trout (*Oncorhynchus mykiss*; Egginton et al. 2000), which showed that a temperature-dependent lipid storage strategy also facilitates specific physiological needs. These can include maintenance of vital metabolic functions in cold temperatures (Desaulniers et al. 1996; Guderley 2004), such as oxygen transport, when cytoplasm tends to increase in viscosity (Sidell 1998). It should be pointed out that heightened lipid incorporation in cell membranes is also normally observed in fish as a response to cold temperatures, in order to maintain membrane fluidity. While the proportion of neutral and polar lipids were not measured in this study, the previously cited studies show that fish can increase intramuscular lipid to aid in metabolic functions, which can contribute substantially to a measured increase in whole-body lipid content.

The elevated lipid storage in fish at the colder treatment, as well as the temperature and ration effect on energy storage and tissue growth, agree with the findings from a recent study (Farley et al. 2015) incorporating growth, energy content, and diet data over multiple years (warm and cold years; 2003-2011) for juvenile Pacific cod in the Bering Sea. The authors found that in warmer years (~6 °C water temperature), Pacific cod tended to be larger but had lower energy density relative to colder

years (~4 °C water temperature), where fish were smaller, but had higher energy density, and consequently higher condition. However, there were also dietary shifts associated with warm and cold years, where fish in warm years had a predominantly piscivorous diet, feeding on low-lipid prey such as walleye pollock, while fish in cold years predominantly fed on large high-lipid prey such as zooplankton. The increased average lipid concentration in fish at the 4 °C treatment in this study, with identical diets across treatments, strengthens the premise that the lipid versus protein storage strategy is temperature-driven, with colder temperatures predisposing cod towards actively increased lipid storage. Any dietary shifts occurring in the wild could further amplify this effect.

Pacific cod fed unlimited rations at all temperature treatments in this study consistently grew at a lower rate than fish fed lower rations. Reduced growth in fish fed unlimited rations across all temperatures suggests a nutritional constraint on growth, for example, reduced digestive efficiency at high consumption levels as observed in Atlantic cod (*Gadus morhua*; Lemieux et al. 1999). This reduced digestive efficiency could cause the interaction between temperature and consumption observed in only the high ration groups across all temperatures. Here, despite high ration levels being consumed, poor nutrient uptake coupled with substantial portions of consumed food being excreted without contributing to growth would skew the relationship between consumption and growth. Reduced digestive efficiency at high rations would necessarily lead to a dome-shaped relationship between growth and consumption, where growth rate increases with consumption, and is maximized at an optimal consumption rate. Beyond this point, growth rate may either not increase or can even start decreasing, as observed in other species, including Atlantic cod (Houlihan et al. 1988), Indian catfish (*Heteropneustes fossilis*; Ahmed 2010), Indian major carp (*Labeo rohita*; Ahmed 2007), rainbow trout (*O. mykiss*; Storebakken and Austreng 1987), and Eurasian perch (*Perca fluviatilis*; Fiogbe and Kestemont 2003). However, while there is an upper limit to growth in Pacific cod, it was not possible to identity this upper growth and optimal consumption limit in this study due to the overestimation bias introduced in

our consumption estimates. Reduced digestive efficiency and nutrient assimilation at higher consumption can be due to numerous factors, including a lack of adequate digestive enzymes such as trypsin, and potentially pepsin (Lemieux et al. 1999). An elevated consumption rate, while reducing digestive efficiency and energy available for growth (Jobling 1986), could also increase energetic consumption due to heightened specific dynamic action (Reddy and Katre 1979), further decreasing energy allocated to growth.

To verify if growth responses to temperature were age-class specific in Pacific cod, we generated growth rates of age 0+ Pacific cod across a temperature range encompassing our treatments, using a juvenile Pacific cod specific growth model ($-0.998 + 0.579\ T - 0.022\ T^2$, where T is temperature) from Hurst et al. (2010). This model estimated growth in mass as a function of temperature. A comparison of estimated age 0+ Pacific cod growth from an alternative study (Hurst et al. 2010) with the observed age 1+ growth from our study in fact showed that growth in age 0+ fish is more sensitive to temperature than age 1+ growth. The comparison of growth between age classes also indicates a slowing of growth as fish age, with age 0+ fish having higher growth rates for a given temperature. This disparity in growth and sensitivity to temperature was observed when growth in mass was compared between age 0+ and age 1+ fish over 4°-12 °C. Estimated growth in age 0+ fish ranged from 0.96% body-weight/day to 2.78% body-weight/day, while in age 1+ fish growth over the same temperature treatments, in the secondary study, ranged from 0.75% body-weight/day to 1.6% body-weight/day. The effects of temperature, depending on age class, would suggest that different age classes should occupy different habitats to maximize their growth efficiency. Given that estimated growth rates for age 0+ fish showed increasing growth with temperature, this could explain the relatively larger numbers of age 0+ Pacific cod observed occupying warm nearshore pelagic waters, such as shallow bays, relative to less prevalent age 1+ fish, which occupy colder and thermally stable deeper waters.

Age 0+ fish have a higher optimum growth temperature than age 1+ fish. The higher estimated optimum temperature for growth in age 0+ fish can be viewed in the context of their occupying a warmer environment, possibly due to predator avoidance and/or food availability, but where they would still have to exhibit rapid growth at this stage in their life history to reduce predation pressure. While the optimal temperature estimated for age 1+ fish in our study (10 °C) is broad-based (since there was a large temperature gap between 8 °C and 12 °C treatments), this serves as a starting point, allowing for further studies with focused narrower temperature ranges. Different growth strategies based on age class have also been observed in walleye pollock, where age 0+ fish maximized growth in the fall, while age 1+ fish maximized energy storage (Heintz and Vollenweider 2010). Age 0+ fish were also found to resume growth earlier than age 1+ fish in the spring.

The strength of the RNA/DNA and growth relationship, which was not consistent across all temperatures, explained the thermal preference for colder waters in age 1+ fish. While highly correlated at the 4 °C and 8 °C treatments, the inconsistency of this relationship at the 12 °C treatment suggested physiological impairment and stress at the warmer temperatures. In nature, while age 1+ Pacific cod could potentially withstand warmer temperatures for periods of time, they could also exhibit behavioral adaptations and avoid prolonged exposure to higher temperatures, based on their physiological responses. This physiological stress ties in with the lower optimum temperatures for age 1+ fish relative to age 0+ fish, and their propensity to stay in deeper, colder and more thermally stable environments.

This temperature-dependent energy storage strategy may have adverse consequences if waters stay warmer for longer periods leading into winter. In this scenario, the duration of warm-water periods would have a greater impact on condition rather than absolute increases in water temperature. While it can be argued that other factors such as habitat also influence growth/energy allocation, among the primary determinants of habitat suitability are food and temperature, in addition to geographical

features. Climate change scenarios predict continued warmer and wetter conditions in the GOA (Spies and Weingartner 2007). Also, the consistent warming trend observed in the GOA (Litzow 2006; Sherman et al. 2009), with temperature on average increasing by 0.37 °C from 1982-2006 (Sherman et al. 2009), suggests that the duration of seasons characterized by warmer water temperatures in the GOA could increase, given that the increased latent heat in the GOA would then take longer to dissipate. While the warmer treatment temperatures in this study were higher than predicted increases in temperature projected for the GOA, this study showed the effect of temperature on allocation strategies in cod. Consequently, while absolute increases in temperature can be important, this study suggests that the duration of warm water periods could in fact have a greater impact on fish condition rather than actual increases in water temperature. Under these conditions, the underlying physiological mechanisms that facilitate this balanced growth strategy could have negative effects. Extended periods of warm water in effect would change the protein-lipid proportions in this growth strategy, where protein-based growth would increase at the expense of lipid-based growth due to the temperature-influence on the allocation strategy. With a longer period of protein synthesis due to warmer temperatures, fish would have shorter periods of time for lipid storage, as waters start to cool later than normal. The cooling period would also be associated with reduced food availability, which could result in reduced energy (lipid) reserves. Consequently, fish would still grow, but may have reduced energy reserves potentially impacting overwinter survival. Reduced energy reserves can force increased foraging behavior, increasing the risk of predation. Protein (muscle tissue) can also be used by fish as an overwinter energy source. However, a breakdown of somatic muscle tissue for energy purposes will negatively affect other factors such as swimming ability, impacting foraging and also making fish vulnerable to predation. Changes in energy-allocation strategies would thus affect growth and body-condition, and ultimately survival and recruitment. Given that increased cellular lipid content can be an

oxygen reservoir and enable quicker oxygen transport through the cell, a lack of lipid reserves could also adversely affect oxygen uptake and delivery during winter, in turn affecting metabolic functions.

The data and overall growth trends in fish from this experiment, in conjunction with the data from Farley et al. (2015), suggest that when other factors including diet remain constant, temperature could influence energy allocation strategies in juvenile Pacific cod, that is, the balance between somatic growth and lipid synthesis and storage. This adaptive energy allocation strategy could be detrimental in the context of ongoing climate change in the GOA. While we have shown that temperature affects energy allocation, it could be the increased duration of warmer water periods in the GOA that may affect Pacific cod rather than the actual increases in water temperature. The lack of a clear RNA/DNA-growth correlation and the visibly stressed behavior observed in fish held at the higher temperatures, additionally suggest that in the long-term, prolonged exposure to higher temperatures was not conducive to growth and condition in juvenile Pacific cod. Similar growth responses to higher temperatures have also been observed in juvenile walleye pollock (Kooka et al. 2007). In the wild, behavioral responses mitigating these temperature effects could be expected in juvenile Pacific cod where fish might be found in shallow warm bays, but might occasionally move into colder waters. These physiological responses (energy allocation strategies) due to temperature should be accounted for in bioenergetic models used to predict ecosystem effects on the growth and condition of cod, so that neither the effects of temperature or ration on growth are underestimated.

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Figure Captions:

Figure 1: Growth in mass of age-1 Pacific cod as a function of temperature. The line of best fit corresponds to the polynomial equation. The x symbol denotes fish at high rations, the closed circles denote fish at medium rations, and the open circles denote fish at low rations.

Figure 2 (a-c): Relationship between instantaneous growth rate (IGR %) and consumption for fish held at 4°, 8°, and 12 °C in the first feeding trial. Lower line in each panel shows relation for fish from high ration tanks while the upper line shows fish from low and medium ration tanks.

Figure 3: Prediction intervals for growth in the secondary growth study. Filled symbols and line show the growth and consumption data and model from the main growth study. The horizontal lines show the 95% prediction intervals for instantaneous growth using the consumption rates observed in the secondary growth study. The open circles show the maximum instantaneous growth observed in each tank from the secondary growth study.

Figure 4: Relation between RNA/DNA and instantaneous growth rate (IGR %) at 4°, 8°, and 12 °C temperature treatments.

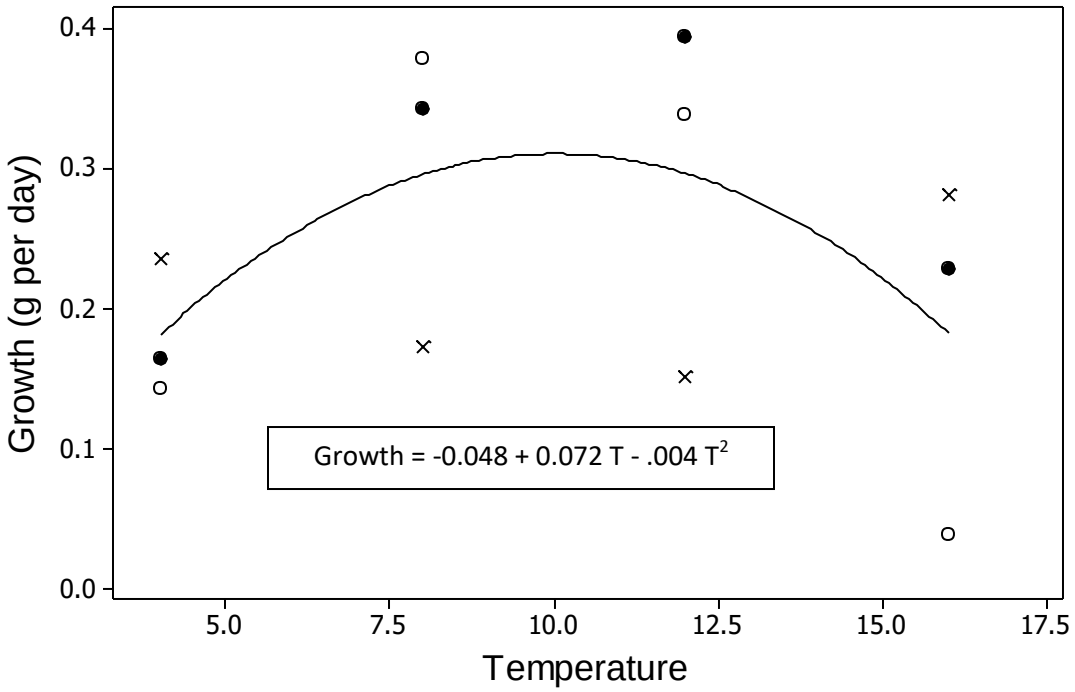
Growth (g per day)
0.4
0.3
0.2
0.1
0.0
5.0
7.5
10.0
12.5
15.0
17.5
Temperature
Growth = -0.048 + 0.072 T - .004 T²

Figure 1.

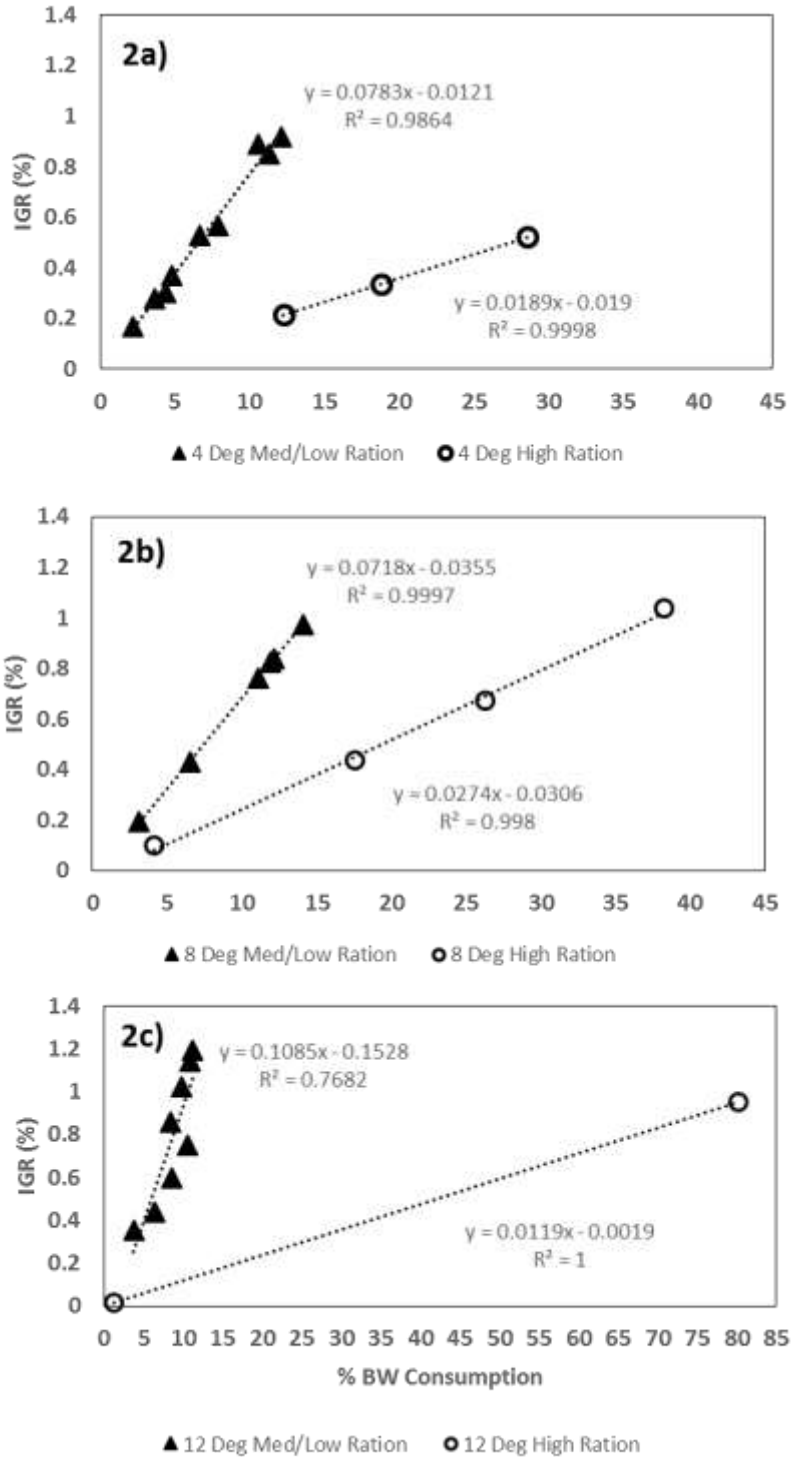
2a)
y = 0.0783x - 0.0121
R² = 0.9864
y = 0.0189x - 0.019
R² = 0.9998
IGR (%)
4 Deg Med/Low Ration
4 Deg High Ration
2b)
y = 0.0718x - 0.0355
R² = 0.9997
y = 0.0274x - 0.0306
R² = 0.998
IGR (%)
8 Deg Med/Low Ration
8 Deg High Ration
2c)
y = 0.1085x - 0.1528
R² = 0.7682
y = 0.0119x - 0.0019
R² = 1
IGR (%)
% BW Consumption
12 Deg Med/Low Ration
12 Deg High Ration

Figure 2 (a-c).

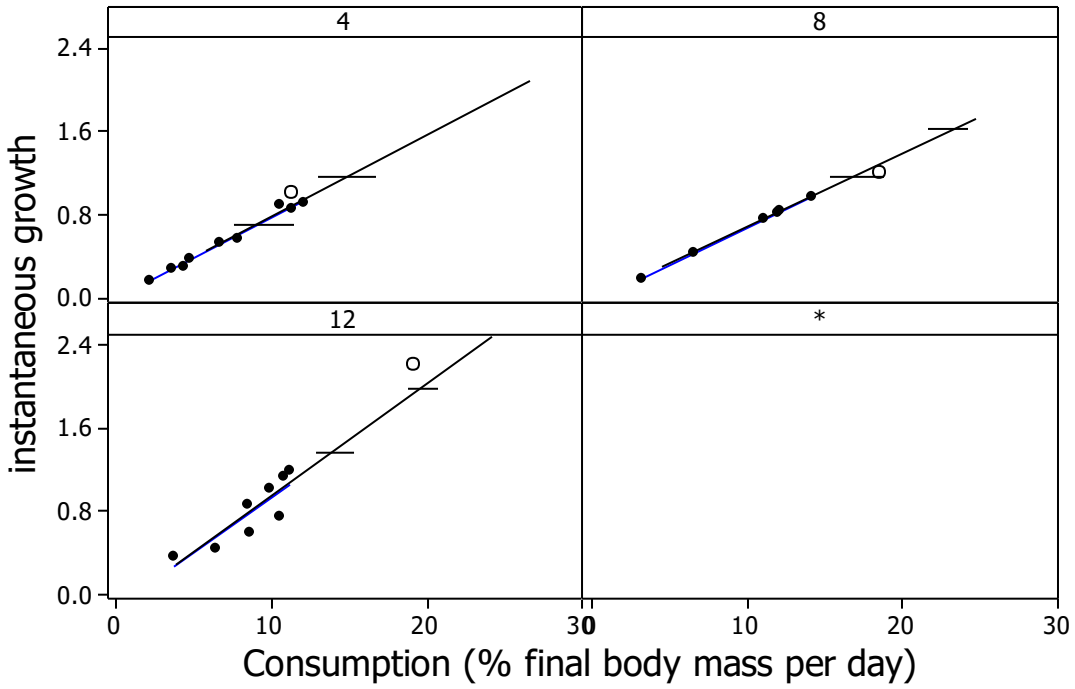
4
8
12
*
instantaneous growth
2.4
1.6
0.8
0.0
0
10
20
30
Consumption (% final body mass per day)

Figure 3.

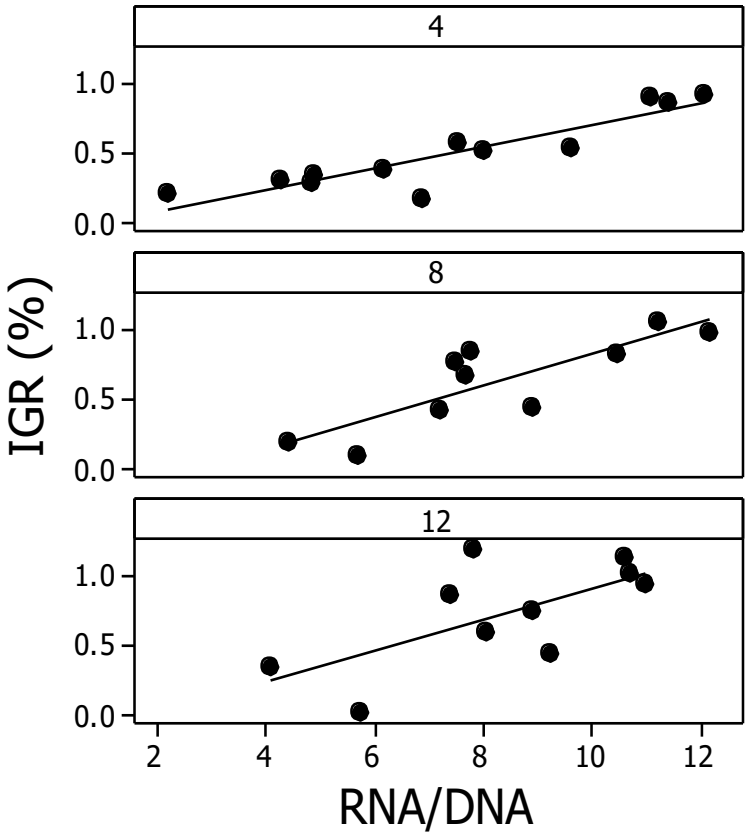

Figure 4.

#	Model Parameters				Intercept	Adjusted r^2	K	AICc	Δ AICc
	RNA/DNA	Protein	Body Mass	T					
1	0.0971 (0.0139)	0.0704 (0.0388)			-1.19	0.616	2	-98.3155	0
2	0.0946 (0.0141)	0.0525 (0.0426)		0.0122 (0.0120)	-1.0049	0.617	3	-97.9211	0.3944
3	0.0932 (0.0142)			0.0183 (0.0110)	-0.2764	0.610	3	-97.7984	0.5171
4	0.0930 (0.0142)		0.0026 (0.0029)	0.0149 (0.0117)	-0.3617	0.608	4	-97.1835	1.132
5	0.0965 (0.0141)	0.0580 (0.0476)	0.0015 (0.0033)		-1.0719	0.606	4	-97.0331	1.2824
6	0.0955 (0.0142)		0.0038 (0.0027)		-0.3178	0.599	3	-96.9485	1.367
7	0.0969 (0.0144)				-0.1638	0.587	2	-96.3778	1.9377

Table 1: Multiple linear regression coefficients (+/-SE) describing the relationship between instantaneous growth rates (IGR), RNA/DNA, protein, body mass, and temperature for Pacific cod. Model selection by Akaike Information Criterion (AICc) value comparison. In all models, $p < 0.001$. RNA/DNA = nucleic acid ratio, T = treatment temperature, K = number of model parameters (including intercept), Δ AICc = difference in AICc with respect to the best fit model. Of the best model parameters (#1), RNA/DNA was significant ($p < 0.001$), but protein was not significant ($p = 0.080$). Equation is of the form $I.G.R.=a_1+b_1+C$, where RNA/DNA-a_1, protein-b_1, and C is a constant.

	4°C	8°C	12°C
Lipid (%)	2.657	2.211	2.024
Protein (%)	14.082	14.682	14.978
Moisture (%)	81.3425	80.5865	80.6075
Ash (%)	2.965	2.931	2.918

Table 2: Comparison of proximate composition (average total-body lipid, protein, moisture, and ash) for all fish (high, medium, and low rations) at 4°C, 8°C, and 12°C temperature treatments