Quantifying interannual variability in the condition of Young-of-Year Pacific herring (*Clupea pallasi*) in the Strait of Georgia, BC

by

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B.Sc. (Honours), Queen's University, 2015

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Abstract

The condition of juvenile fish relates to their overall health and is a strong predictor of survival and eventual recruitment. Condition can be quantified and interpreted in a variety of ways covering different time scales and levels of biological organization. Here I (i) quantify interannual variability in the condition in Young-of-Year (YOY) Pacific herring (*Clupea pallasi*) in the Strait of Georgia, BC, from 2013-2016, and (ii) examine the extent to which the condition of an individual fish varies depending on which condition metric is used. Chapter 1 provides a general background on the concept of measuring condition in fish, as well as the basic biology of Pacific herring and their importance in Strait of Georgia ecosystem. In Chapter 2, I report the condition of YOY herring from 2013-2016 using six metrics: (i) Fulton's K, (ii) the residuals from a length:weight regression, (iii) the RNA:DNA ratio, (iv) recent growth estimated via otolith microstructure analysis, (v) lipid content, and (vi) the ratio of two essential acids DHA:EPA. Four of these metrics (Fulton's K, length:weight residuals, and growth from RNA:DNA and otolith increments) indicate a decrease in condition over the four years. In contrast, lipid content suggests an increase across the four years, while DHA:EPA suggests a decrease in 2015 but no change over the other three years. The observed interannual variability in condition can be partly linked to unfavourable changes in temperature and zooplankton community composition in 2015 and 2016, and to the propensity of juvenile fish to prioritize energy storage over somatic growth before a period of prey scarcity, such as their first winter. This dataset is further examined in Chapter 3, wherein I examine variability in condition of individual fish based on the different metrics used. Individual herring are ranked based on their scores from the six

different metrics of condition, and the distribution of these rankings are examined to assess the degree of intercorrelation among the metrics. Based on this model, as well as pairwise Spearman rank correlations between the six metrics, I conclude that there is little intercorrelation between metrics, and that a fish that scores highly in terms of condition in any one metric will not necessarily score highly for the other metrics. These findings underscore the importance of choosing condition metrics carefully, based on the nature of the question being asked.

Table of Contents

List of Tables

List of Figures

Acknowledgments

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1. General introduction

1.1. Introduction to the concept of fish condition

In their comprehensive literature review on fish condition, Ferron and Leggett (1994) describe condition as a property that can include nutritional status, health, size, and growth rate at a morphometric, histological, biochemical or physiological level. A study by Shelbourne (1957) relating health to weight for a given length of larval plaice (*Pleuronectes platessa*) was the first to define condition of fish as a property related to the health of a fish (Ferron & Leggett 1994). Over the intervening years, a wide range of proxies have been used for estimating fish condition, ranging from simple equations comparing actual size to some idealized size (Bolger & Connolly 1989), to complex measurements of metabolic rate (e.g. Moyano et al. 2018).

Studies in both temperate and tropical systems have shown that scoring high on a condition factor can be a good predictor of larval survival and eventual recruitment (Frank & McRuer 1989, Suthers 1998). In particular, Frank & McRuer (1989) argue that abundance data should be corrected for condition before being used to estimate recruitment. Aspects of condition such as growth during the early life history stages of fish can even affect biological processes later in life: for instance, growth rate in juveniles can affect the age at which fish reach reproductive maturity (Hutchings 1993).

Quantifying condition can aid our understanding of how fish respond to changes in their environment, and how future changes in ocean conditions may affect condition and survival. For instance, it has been shown that changes in temperature and zooplankton

availability can affect the growth of larval and juvenile fishes (McGurk 1984, Clemmesen et al. 2003, Peters et al. 2015). The condition of fish, when represented as quantitative estimate of nutritional status, can also be used to assess their quality as prey for their predators (Osterblom et al. 2008, Pethybridge et al. 2014, Røjbek et al. 2014), since prey quality is important to many attributes of higher predator dynamics, such as breeding success and survival of young seabirds and sea lions (Davoren & Montevecchi 2003, Rosen & Trites 2005). Condition indices are also useful in conservation-based studies to determine the level of protection against endangerment or extinction required by a population (Stevenson & Woods 2006).

1.2. Study species: Pacific herring (*Clupea pallasi*)

Pacific herring is an ecologically and culturally significant forage fish species in the Strait of Georgia (SoG hereafter). Pacific herring prey mainly on copepods and krill (Foy & Norcross 1999), and are in turn a key prey item for salmon, lingcod and hake, seabirds, and numerous marine mammal species (Therriault et al. 2009, Schweigert et al. 2010, Tinus 2012, Brodeur et al. 2014). Herring are also an important commercial species in BC, as well as being strongly connected to the cultural traditions of many First Nations groups. An understanding of Pacific herring condition is thus of interest to many different stakeholders. These include the Pacific Salmon Foundation, a non-profit organization dedicated to promoting sustainability for salmon and their ecosystem. Part of the current study was funded by the Salish Sea Marine Survival Project (SSMSP), an effort by the not for profit organizations, “Pacific Salmon Foundation” and “Long Live the Kings” to study factors affecting Pacific Salmon in the Salish Sea (Strait of Georgia, Strait of Juan

de Fuca, and Puget Sound). The SSMSP is organized around a series of hypotheses relating to Pacific salmon health and abundance, one of which suggests a link between the quality of prey available to salmon and their overall growth and survival (PSF 2015). This thesis informs a part of this hypothesis by quantifying variability in the condition in Young-of-Year (YOY) Pacific herring.

The main migratory stock of SoG herring spend the first part of their lives within the SoG itself, before migrating seaward to the west coast of Vancouver Island (WCVI), where they feed in the nutrient-rich waters of La Perouse Bank during their first winter. They generally return to the SoG to spawn after recruiting to the spawning stock at the age of 3 years. There is little evidence of fidelity to spawning locations at smaller scales within the SoG (Hay et al. 2001), and thus the SoG herring stock is generally treated as a single group for the purposes of management and stock assessment (e.g. Cleary & Taylor 2016) The annual migration out to the WCVI feeding areas and back to the SoG for spawning continues for the rest of their lives, typically less than 10 years.

1.2.1. Measuring condition in herring

Pacific herring condition can be affected by many factors, including changes in the physical oceanography of their environment. For example, laboratory studies on condition and growth rate in herring and other clupeids have demonstrated a strong linkage to temperature. Baumann et al. (2007) and Peck et al. (2015) discussed the increased metabolic demand on clupeids exposed to higher temperature, and suggest that this may lower their overall condition. Additionally, warmer temperatures have been shown to bring Pacific herring larvae to the point of irreversible starvation more quickly

(McGurk 1984).

While ocean temperature may play a direct physiological role in variability in YOY Pacific herring condition, it is more likely to have an indirect influence by regulating the timing, abundance, and quality of the main prey of herring - crustacean zooplankton. The presence of larger species of copepods and euphausiids is broadly related to low sea surface temperature (SST), both globally (San Martin et al. 2006) and in the NE Pacific (Chiba et al. 2015). Primary productivity is also positively related to zooplankton abundance, and can determine the carrying capacity of Pacific herring (Perry & Schweigert 2008). In accordance with Cushing's (1990) match-mismatch hypothesis, a correlation in the timing of the spring phytoplankton bloom with Pacific herring spawning has been linked to increased abundance of YOY Pacific herring, although not necessarily higher individual weights (Schweigert et al. 2013). It has also recently been proposed that changes in abundance and condition in YOY herring are driven by density-dependent competition between herring (Boldt et al. 2018).

Numerous studies have focused on adult and larval Pacific herring in the Strait of Georgia (McGurk 1984, 1989, Robinson & Ware 1988, Hay 1990, Tanasichuk 1997, Hay & McKinnell 2002, Rose et al. 2008, Huynh & Kitts 2009, Friedenberg et al. 2012). Studies on the young-of-year stage include reports from surveys to estimate abundance and size (Boldt et al. 2017), as well as studies showing that bottom-up drivers are primarily responsible for recruitment variability (Hay et al. 2003, Schweigert et al. 2013, Boldt et al. 2018). This thesis will add to this growing body of knowledge by quantifying

interannual variability in YOY Pacific herring condition in the SoG, as well as using these data to understand how individual condition metrics relate to each other.

1.3. Quantifying condition: Examples of condition metrics

A plethora of metrics have been used to estimate condition factors in fish. This thesis focuses on six metrics that can be subdivided into three groups: morphometry (Fulton's K and residuals from length-weight regressions), growth (RNA:DNA ratios and otolith increment widths), and nutrition (total lipids and DHA:EPA ratios). The metrics selected for this study were chosen in order to meet the following criteria:

(1) Already confirmed to be an accepted estimate of condition in juvenile fish

(2) Can be measured on fish that have been frozen

(3) Requires only part of the body of the fish or uses the whole body in such a way that does not damage any other part of the fish.

Fulton's K: Morphometric condition factors relate the length of a fish to its weight, such that a fish that is heavier for a given length is considered to be in better condition (Bolger and Connelly 1989). Fulton's condition factor relates the weight of a fish to the cube of its length in order to describe its physical condition. Known as "Fulton's K", it was first proposed by Thomas Fulton in 1904, and designated as such by Ricker (1975), who considered Fulton's K to be the most essential condition metric. However, Ricker (1975) also noted that due to the nature of the formula, Fulton's K makes the assumption that fish are experiencing isometric growth only. McGurk (1985) has described other concerns with Fulton's K, particularly that it does not consider any other body dimensions.

Length-Weight Residuals: Examining the residuals of a linear model between the length and weight of individual fish is another way to assess morphometric condition, and without the need to assume isometric growth (Anderson & Neumann 1996). Length-weight residuals are considered a more comprehensive metric of condition compared to Fulton's K because they are calculated from a dataset in a model, which places the fish in context of its subpopulation (Bolger and Connolly 1989). This is the primary condition metric used to date by Fisheries and Oceans Canada for assessment of Pacific herring (e.g. Boldt et al. 2016). However, as with Fulton's K, length-weight residuals do not account for other potentially important aspects of condition (McGurk 1985).

RNA:DNA Ratios: The RNA:DNA ratio in fish tissues indicates changes occurring at a biochemical level. RNA:DNA ratios have been used as growth rate indicators in fish since 1970, when it was first shown that golden shiners (*Notemigonus crysoleucas*) with various growth rates had analogous differences in the concentration of RNA relative to DNA in their muscle tissue (Bulow 1970). The production of RNA is directly associated with the production of proteins, and as such, the concentration of RNA relative to DNA in animal tissue can be taken to indicate the response of an individual to changes in the environment, e.g., decreased food availability and varying temperature regimes (Buckley 1984, Clemmesen 1994). Higher RNA:DNA ratios are considered to represent better condition with the assumption that higher RNA represents increased protein production (Buckley 1984). An instantaneous estimate of protein growth rate (G_i) can be calculated as a function of RNA:DNA and water temperature using the equation: G_i = 0.93*T+4.75*RD-18.18, where T is temperature (°C) and RD is the RNA:DNA ratio

(Buckley 1984). This growth rate equation can also be rearranged to find the critical RNA:DNA ratio below which a larval fish will not recover from starvation (Robinson & Ware 1988). RNA:DNA ratios are thus useful for providing an estimate of both of the nutritional status of a fish as well as its instantaneous growth rate, based on the underlying assumption that the majority of growth can be approximated by measures of protein production.

Otolith microstructure: Examining otolith microstructure offers another way to estimate growth rate and condition. Otoliths, or ear stones, are small calcium carbonate structures found behind the gill operculum of fish. They are formed by the gradual accretion of calcium carbonate, in proportion to individual growth. Daily rings are generally visible in the otoliths of fish less than one year of age, and reflect both age and somatic growth (Panella 1971). The distance between successive rings, or otolith increments, vary depending on the amount of calcium carbonate deposited, and may be affected by temperature, pH stress, food availability, and/or periods of differing growth rates (Campana & Neilson 1985). In juvenile Atlantic herring (*Clupea harengus*), wider increments are indicative of fast growth, while more tightly packed increments indicate periods of slower growth (Brophy & Danilowicz 2002). Measuring the width of daily rings can therefore provide an estimate of growth patterns over an individual's lifetime, and to identify fast and slow growers within a population.

Lipids and Fatty Acids: Lipid analysis can be used to estimate short-term condition values and provides information on the quality of forage fish as prey for higher trophic

levels. In general, increased lipid content of fish tissues is taken to indicate higher condition (Sargent et al. 1988, Iverson et al. 2002, Lane et al. 2010). Variation in condition during times of stress can be identified by quantifying lipid content. For example, Litz et al. (2010) reported decreased total lipid content in Pacific herring during unfavourable oceanographic conditions in 2005. Thus, a common strategy in fish is to increase internal lipid storage in the late summer and fall in order to prepare for a scarcity of prey in winter (Martin et al. 2017).

Lipids can provide additional information by way of fatty acid profiling. Fatty acids play important roles in tissue structure, and are a key mode of energy storage in pelagic fish (Tocher et al. 1985). Additionally, various fatty acids play different roles in nutrition to predators, as some have more energetic value than others. For example, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are omega-3 polyunsaturated fatty acids that have been shown to be important to development in marine fish (Tocher 2003). Many fatty acids, including DHA and EPA, cannot be synthesized by marine fish and must therefore be obtained through diet. Such fatty acids are known as "essential fatty acids" (EFA hereafter). DHA is a primary EFA for larval growth (Watanabe 1993), and DHA deficiencies have been linked to complications such as vision impairment in Atlantic herring (Bell et al. 1995), and reduced egg quality in sea bream (*Sparus aurata*) (Rodríguez et al. 1998a).

Both the total quantity of EFAs consumed, as well as the ratios between them, can affect fish health and condition (Rodríguez et al. 1998b, Sargent et al. 1999). Since these fatty

acids cannot be synthesized and must be consumed, the concentrations of DHA, EPA and other EFAs in the prey of forage fish can therefore affect the ratio of DHA to EPA in the fish themselves (Mourente et al. 1993). DHA and EPA are usually present in different concentrations in the prey of forage fish. Seasonal cycles of phytoplankton produce different dominant polyunsaturated fatty acids, with dinoflagellates richer in DHA, and diatoms generally associated with EPA (Jeffries 1970). These differences can be reflected in the zooplankton grazing upon the phytoplankton. Certain phytoplankton groups are also more nutritious than others for zooplankton. For example, moulting failure in the copepod *Neocalanus plumchrus* has been linked to a diet of DHA-poor diatoms during a large spring bloom (El-Sabaawi et al. 2009a). High DHA:EPA ratios are thus taken to indicate that a fish has had adequate access to nutritious prey (Copeman et al. 2002, Dalsgaard et al. 2003). While variable "optimal ratios" have been reported (Sargent et al. 1999) it is generally held that individuals with higher DHA:EPA can therefore be considered to be in good condition (e.g. Jin et al. 2017). Fatty acid profiles can also provide information on the condition of forage fish as it relates to their value as prey for higher trophic level predators (Dalsgaard et al. 2003).

1.3.1. Measuring multiple condition proxies on individual fish

As mentioned previously, there are many proxies available to estimate condition, with each providing a unique perspective as well as challenges to overcome. One way to obtain a clearer picture of condition is to apply multiple metrics in a single study. Early work in this area often relied on splitting samples into groups and measuring different metrics on different fish. One of the first reported studies to attempt this examined the impact of starvation on striped bass (*Morone saxatilis*) using fatty acids, RNA:DNA

ratios, and various histological and morphometric measures (Martin et al. 1984). They noted depleted fatty acid levels and decreased growth in starving larvae, but did not comment on the relationship of the metrics to each other (Martin et al. 1984). Since then, a number of studies have attempted to intercalibrate various condition indices by applying them to the same individuals. Examples include RNA:DNA and otoliths (Clemmesen & Doan 1996), triacylglycerol content, otolith microstructure, and morphometry (Suthers et al. 1992), Fulton's K, RNA:DNA, and otolith microstructure (Gilliers et al. 2004), and most recently, two metrics using nucleic acids, gut content, and otolith microstructure on Downs herring (*Clupea harengus*) larvae (Denis et al. 2017).

A common conclusion among these studies is that pairs or trios of condition metrics do not consistently provide the same information about the level of condition in the same individual (Suthers et al. 1992, Clemmesen & Doan 1996, Gilliers et al. 2004, Denis et al. 2017). Recent studies have applied up to four metrics of condition on an individual, but not with the specific aim of examining the correlation of metrics to each other (Kerambrun et al. 2012, Duguid et al. 2018). This study is therefore the first to publish results on more than four metrics of condition in individual fish, and to focus explicitly on the degree of intercorrelation between metrics using a rank scores approach (see Chapter 3).

1.4. Study region

The SoG is a semi-enclosed basin between Vancouver Island and mainland BC, with connections to the Pacific Ocean to the north (Johnstone Strait) and south (Juan de Fuca

Strait). The SoG is influenced by freshwater outflows from the Fraser River, primarily during snowmelt periods in late spring (Masson 2002). Two sills at Boundary Pass and south of Victoria restrict the flow of water to the open ocean and are essential to deep water renewal events promoted by tidal mixing (Masson 2002). These renewal events are ecologically significant for phytoplankton and zooplankton communities by mixing nutrients to the surface and altering the distribution of taxa (Mackas et al. 2013). Nutrients enter the system primarily from the Juan de Fuca Strait due to upwelling at the continental shelf (Harrison et al. 1983). Seasonal primary productivity in the SoG is generally predictable, with the main phytoplankton bloom occurring each spring (Masson & Peña 2009), and sporadic smaller blooms over the summer. Strong vertical stratification confines these phytoplankton blooms to the surface (Masson & Cummins 2007).

Temperatures in the SoG are influenced by the El Niño Southern Oscillation (ENSO). Strongly positive temperature anomalies in 1983, 1992, and 1998, and strongly negative anomalies in 1989 and 1999 have been attributed to El Niño and La Niña events, respectively (Masson and Cummins 2007). In general, sea surface temperature (SST) in the SoG has been rising since 1970 at a rate of 0.03˚C y^{-1} (Masson and Cummins 2007). This trend is similar to observations off the WCVI; however, vertical variation tends to be lower in the SoG (Masson and Cummins 2007). In late 2013 a strong positive SST anomaly of up to 3˚C (compared to 1981-2010), corresponding to an anomalous sea level pressure, developed in the NE Pacific (Bond et al. 2015). By September 2014 the SST anomaly had spread to the southern BC coast (Chandler et al. 2017). By 2016, the SST

anomaly was no longer affecting the surface waters of the BC coast, and was detected 100m below the surface at Ocean Station Papa in the offshore subarctic NE Pacific (Ross 2017). Although the SoG was relatively protected from this event, temperatures were elevated in 2015 and 2016 by up to 2˚C compared to a 16-year average (Chandler et al 2017).

The SoG supports a diverse community of zooplankton. Crustaceans such as copepods, euphausiids, and amphipods are the dominant taxa (Mackas et al. 2013). The non-crustacean category is composed mainly of species that prey on these taxa, such as chaetognaths, medusae, and pelagic polychaetes (Mackas et al. 2013). Zooplankton community composition in the SoG also differs noticeably from the WCVI region and consists mainly of subarctic oceanic taxa (Mackas et al. 2013). As such, many of these species are at the upper limit of their temperature tolerances, and a negative correlation between the abundance of subarctic species and temperature anomalies in the SoG has been observed (Mackas et al. 2013). The warming event in the SoG in 2015 is believed to have led to changes in zooplankton community composition in 2016, including a decrease in the historically dominant large copepod *Neocalanus plumchrus,* and an influx of smaller copepods from the southern California current (Galbraith & Young 2017). There was also a slight negative biomass anomaly of the krill *Euphausia pacifica* (Galbraith & Young 2017).

1.5. Structure of this thesis

This thesis was undertaken to examine condition in YOY Pacific herring in the Strait of

Georgia. This work aims to address two questions:

(1) Is there evidence of interannual variability in YOY herring condition from 2013-2016, and if so, to what may it be attributed?

(2) At the individual level: to what extent will different condition metrics indicate the same level of condition when applied on an individual fish?

This chapter is intended to serve as a general introduction to relevant concepts discussed in this thesis, and to provide the context in which these questions were developed.

Chapter 2 focuses on changes in the condition of YOY Pacific herring in the SoG at the population level. Here I quantify interannual variability in herring condition from 2013-2016. Condition factors changed over the four-year period, however the direction of change varied depending on the condition metric used. The interannual variability in fish condition is discussed in relation to environmental conditions, as well as the developmental changes that occur as fish progress through the juvenile stage.

Chapter 3 delves further into the results from the previous chapter on an individual basis and examines how condition metrics relate to one another when tested on the same fish. The metrics are analyzed using a ranking system to test whether a fish with a high score in one condition metric will also have a high score in other condition metrics. The consequences of the lack of correlation between condition metrics is discussed. Chapter 4 summarizes the main results of the thesis and discusses implications and future work.

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2. Interannual variability in condition of Young-of-Year Pacific herring (*Clupea pallasi*) in the Strait of Georgia, BC from 2013-2016.

Abstract

Pacific herring (*Clupea pallasi*) is an ecologically and culturally significant forage fish in the NE Pacific. Although numerous studies have focused on adult and larval Pacific herring, knowledge gaps remain for many areas of the juvenile, or Young-of-Year (YOY) stage. In particular, the extent to which variability in the growth and condition of YOY herring may affect the food quality that they represent to their predators is largely unknown. Here I quantify variability in YOY Pacific herring condition in the Strait of Georgia from 2013-2016 using six metrics that were all measured in each individual: (i) Fulton's K, (ii) the residuals from a length:weight regression, (iii) the RNA:DNA ratio, (iv) recent growth via otolith microstructure analysis, (v) lipid content, and (vi) the ratio of the essential fatty acids DHA:EPA. Interannual variability was quantified using ANOVAs to compare metrics over the four years of the study period. Fulton's K, length:weight residuals, RNA:DNA growth and otolith growth all decreased in 2016 compared to the other three years, while lipid content increased over the entire time period. These changes in condition may be attributed, in part, to both environmental changes as well as variability in herring development over different years. Since fish with higher lipid content are generally considered more nutritious for predators, this study suggests that YOY herring were in better condition with respect to the prey quality that they represent in 2015 and 2016 compared to 2013 and 2014.

2.1. Introduction

The condition of a fish is understood to refer to its physical state with regards to morphology, nutrition, and growth (Ferron & Leggett 1994), as well as the likelihood of that fish surviving to recruitment (Hutchings 1993). Condition is estimated using various proxies of morphometry, growth, and nutrition, referred to hereafter as "condition factors". Tracking changes in condition factors in fish can provide valuable information about the well-being of an individual fish, and the nutritional value that it may represent to a predator. In addition to such individual-level considerations, condition factors can also be used to establish the quality of prey that a population of fish represents to piscivorous predators, such as larger fish, seabirds, and marine mammals (Davoren & Montevecchi 2003, Rosen & Trites 2005, Osterblom et al. 2008).

2.1.1. Ontogenetic effects on condition

As fish transition from the larval to the juvenile stage during metamorphosis, they undergo significant morphological and physiological changes which can also affect condition. For example, the RNA:DNA ratio, a condition factor that can also be used to estimate instantaneous growth rates, has been shown to decrease naturally as a fish progresses from the late larval through the juvenile stages (Buckley et al. 1999, Fonseca et al. 2006, Peters et al. 2015). As juveniles approach their first winter somatic growth slows, coinciding with an increase of storage of lipids - another metric of condition (Biro et al. 2004, Mogensen & Post 2012). Martin et al. (2017) performed a comprehensive study of changes in the energetics of fish from the juvenile stage through to maturity and reproduction. They noted that energy density (as represented by total lipids) tended to

increase as juveniles grew, with more lipids being stored as fish transitioned from a growing to a non-growing period (Martin et al. 2017).

2.1.2. Environmental effects on condition

In addition to these natural changes during fish ontogeny, a variety of environmental stressors can also cause changes in condition. For instance, increases in temperature can increase metabolic stress on fish, as shown by a meta-analysis of 138 studies on teleost fish (Clarke & Johnston 1999). Food deprivation studies have revealed a decrease in proxies of growth in juvenile fish, as represented by RNA:DNA ratios and otolith increment widths (Rooker & Holt 1996, Baumann et al. 2007, Selleslagh & Amara 2012, Peck et al. 2015). Since variable environmental conditions are naturally encountered in the field, fish need some mechanism to withstand, or "buffer" against this variability. For example, higher initial amounts of stored lipids in the muscles and liver of juvenile roach (*Rutilus rutilus*) has been shown to aid recovery after a period of starvation (Van Dijk et al. 2005). Typically, there is some variability interannually in oceanographic conditions, such as those leading to starvation (e.g. food availability). Understanding the response of fish to these yearly changes in temperature, salinity, and zooplankton community structure that occur in the environment is therefore important for predicting year-class strength, which contributes to the overall health of the population.

Numerous field studies have demonstrated interannual changes in condition factors in various fish populations. For example, Alemany et al. (2006) determined that annual changes in wind strength were responsible for driving variability in the growth rates of Alboran Sea sardine (*Sardina pilchardus*) larvae and juveniles, as determined by otolith

increment widths. Murphy et al. (2018) also used otolith microstructure as a proxy of growth rate to examine interannual changes in condition in Newfoundland capelin (*Mallotus villosus*) in 2002, 2006, and 2013. Larval growth was highest in 2013, a period of increased recruitment and population recovery attributed to increased productivity of small copepods (Murphy et al. 2018). Lipids and fatty acids have also been shown to vary interannually, with changes in fatty acid profiles in various forage fish in the California Current system recorded by Litz et al. (2010). In whitebait smelt (*Allosmerus elongatus*) and Pacific herring (*Clupea pallasi*), lipid content doubled from 2005 to 2006, which coincided with increased coastal upwelling and copepod biomass along the Oregon shelf (Litz et al. 2010). Residuals from length:weight regressions have been used to quantify condition in juvenile YOY Pacific herring from the Strait of Georgia (Boldt et al. 2015), and have shown a general trend towards positive residuals over the past 10 years (i.e. when data are pooled across years), indicating improved condition. However, other condition factors with the potential to provide more detailed information have not yet been applied to YOY herring in the SoG. This study was undertaken to address this issue.

2.1.3. Study species and objectives

Pacific herring (*C. pallasi*) is a well-studied species in BC, as it represents substantial ecological, economic, and cultural value. Understanding variability in the condition of YOY Pacific herring can provide information about the status of this population at a crucial life-history stage. The Strait of Georgia herring stock contributes up to 80% of the current herring landings in BC (Cleary & Taylor 2016). In 2016, estimates of the herring spawning stock biomass in the SoG varied from 110 000 to 199 000 tons depending on the model used, and have been increasing since 2008 (Cleary & Taylor

2016). Some evidence of interannual variation in the growth rates of adult Pacific herring (measured using back-calculations of adult mass) was found throughout the 1980s and 1990s, and was linked to ocean temperature and feeding conditions (Tanasichuk 1997). Additionally, when data are pooled across years, residuals of length and weight have been increasingly positive since the early 2000s, suggesting that the average herring is heavier than expected for its given length (Boldt et al. 2015). This study will expand on these results and take a more in-depth look at interannual variation in condition. The objectives are twofold: (i) to quantify the extent to which the condition of YOY herring varied over a four-year period in the SoG, and (ii) assuming that there is variability in condition, to explore what factors may have contributed to such patterns.

2.2. Methods

2.2.1. Data collection

Fisheries and Oceans Canada (DFO) conducts yearly surveys in the Strait of Georgia to collect information primarily on Pacific salmon. The surveys use a large mid-water trawl towed at low speeds (Trudel et al. 2014), and Pacific herring are often captured as bycatch. The YOY herring in this study were collected in this manner by DFO staff on the CCGS *Ricker* and CCGS *Neocaligus,* and various charter vessels, in September 2013, September-October 2014, and June-October of 2015 and 2016. Despite minor differences in trawl dimensions, a 1cm mesh cod-end net liner was consistently used throughout the four years on the various vessels. Whenever possible, up to 10 YOY Pacific herring were collected per station and frozen at -80℃ for further analysis. As herring were only collected as bycatch, however, the overall distribution of the resultant samples was not spatially defined (see Appendix A1). As such, this study was unable to quantify patterns

of spatial variability in herring growth and condition within the SoG. Six metrics of condition were quantified for each individual herring in the study: (i) Fulton's K, (ii) the residuals from a length:weight regression, (iii) the RNA:DNA ratio, (iv) recent growth via otolith microstructure analysis, (v) lipid content, and (vi) the ratio of DHA:EPA.

2.2.2. Morphometric condition metrics: Fulton's K and length:weight residuals

Individual YOY herring were weighed on a top-loading balance while still frozen, and weights were rounded to the nearest 0.01g. Standard length was measured using a tape measure following Boldt et al. (2015), and rounded to the nearest half millimeter (Figure 2.1). Fulton's K (FK hereafter) was calculated using the formula $100*W/L^3$ (Ricker 1975), where W is weight (g) and L is standard length (cm). Length:weight residuals were calculated by plotting a regression of standard $length_{(log)}$ against $weight_{(log)}$, and then obtaining the residuals as the distance of each data point from the regression line (length:weight residuals or LW hereafter). For this dataset, fish were separated by season (summer vs. fall), and pooled across years (2013-2016).

Figure 2.1. YOY herring caught in fall and summer 2015. Left panel illustrates the measurement of standard length using red lines from nose-tip to end of hypural plate at the beginning of the tail. Right panel shows the location of the white muscle tissue biopsy 5mm behind the gill operculum, denoted with a red X.

2.2.3. Growth-based condition metrics: RNA:DNA ratios

White muscle tissue has the highest correlation of protein synthesis to growth rate, and is therefore generally accepted as being the best choice for RNA:DNA studies (Houlihan et al. 1988). RNA:DNA ratios were calculated using 2mm biopsies of white muscle tissue from frozen individual YOY herring, taken 5mm posterior to the right operculum (Figure 2.1). In order to minimize variability, each biopsy was obtained as close to this location as possible (Caldarone et al. 2001). The biopsy punch was rinsed in distilled water and dried in between each use. Care was taken so that the tissue plug was not contaminated by skin, scales, or blood (Caldarone et al. 2001). Biopsies were placed in individual 1.5mL Eppendorf tubes and transferred immediately to liquid nitrogen. Upon removal from liquid nitrogen, tubes were moved to a freezer at-80˚C for long-term storage. A fluorescence-based RNA:DNA analysis was used, after the protocol of Caldarone et al. (2001). Tissue samples were digested with Sarcosil-Tris-EDTA buffer (STEB) to isolate nucleic acids. 200µl of 0.5% STEB was added to frozen samples, which were placed on a vortex to thaw for 30 minutes. 0.1% STEB was then added and samples were centrifuged for 15 mins at 14,000rpm. The resulting supernatant was pipetted in duplicate into a 96-well Optiplate microplate and stained with ethidium bromide. Fluorescence was read after 5 mins using a PerkinElmer plate reader. Finally, an RNase was used to digest RNA so that DNA was all that remained. Fluorescence was then measured again, and the second value was subtracted from the initial fluorescence reading to obtain the RNA:DNA ratio (RD hereafter). Standard curves of RNA and DNA stock solutions were used to convert raw fluorescence values to concentration values, in order to produce the final RD values (Caldarone et al. 2006). For additional consistency, samples were tested in duplicate; samples in which duplicate values differed by more than 20% were

discarded. The remaining values were then used to estimate instantaneous growth rate (G_i) using the equation: $G_i = 0.93*T+4.75*RD-18.18$, where RD is the RNA:DNA ratio (Buckley 1984) and T is temperature (°C) from a CTD cast at the nearest station to where the herring were collected (Appendix A2). Temperatures were averaged over the top 50m to match the depth of the net tows that collected the herring. The resulting growth rate values are referred to as G_i hereafter.

2.2.4. Growth-based condition metrics: Otolith increment widths

Both sagittal otoliths were removed from each individual while the herring were still frozen and kept sealed in an Eppendorf tube before mounting. Otoliths were mounted on glass microscope slides using Crystalbond™, which also acted as a glaze to increase the clarity of the outer rings. As such, polishing was not necessary. Mounted otoliths were photographed using a Leica compound microscope and camera at 4X and 40X magnification. ImageJ software was used to measure the maximum distance from the tip of the rostrum through the core to the posterior end of the otolith (otolith length hereafter), and the width through the core perpendicular to the maximum distance line (otolith width hereafter) (Figure 2.2). A t-test ($p=0.82$) showed that measurements from the left and right otoliths did not differ significantly. For consistency, however, the left otolith was selected whenever possible, while the right otolith was used if the left was either broken upon removal, or was not mounted properly.

Recent growth rate was estimated by measuring the outermost (i.e. most recent) 10 increments from the posterior end of the otolith (Hovenkamp & Witte 1991, Gilliers et al. 2004, Henry et al. 2012). In this study, one increment is defined as a dark zone followed

by a light zone, and is considered to represent one day of growth (Panella 1971). The total straight-line distance from the beginning of the first ring to the end of the 10th ring was measured perpendicular to the angle of the increments (i.e. 10 daily growth increments, L10 hereafter) using the TreeRings package created for ObjectJ plugin, ImageJ software (N. Vischer, S. Natase, University of Amsterdam) (Figure 2.3).

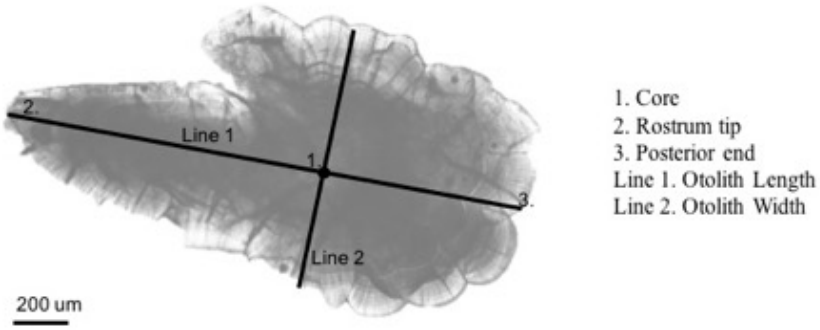

Figure 2.2. A YOY herring otolith photographed using light microscopy at 40X magnification. The various morphological landmarks and the otolith measurements used in the study are also indicated.

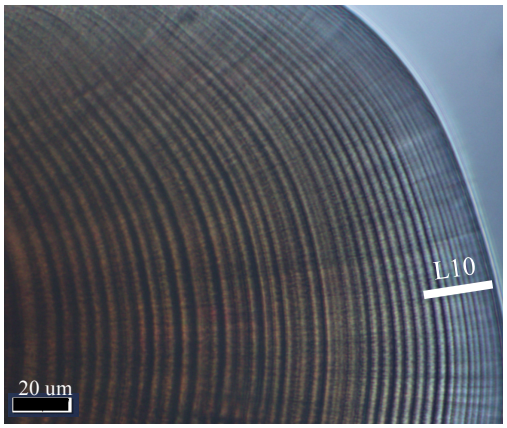

Figure 2.3. A YOY herring otolith photographed at 400X magnification to illustrate the measurement of recent growth rate. White line labeled "L10" denotes the distance between the beginning of the most recent identifiable increment and the end of the 10th increment.

Some studies have used the L10 measurement on its own to estimate recent growth (Hovenkamp & Witte 1991, Gilliers et al. 2004, Henry et al. 2012). However, there has also been some debate about a lag in timing between a change in somatic condition and a resultant change in otolith size, which could lead to an underestimate of the magnitude of change based on otolith increment widths (Fey 2005). In particular, juvenile fish often have irregularly shaped otoliths when compared to the generally round otoliths of many larval fish, on which this technique was developed by Panella (1971). Peck et al. (2015) overcame this issue by employing normalized increment widths using a species-specific constant for sprat (*Sprattus sprattus*). As no such constant has been developed for Pacific herring, however, in this study, otolith growth rates (OGR hereafter) were calculated by taking the residuals from the linear model of otolith widths and L10. This was done to account for variability in increment widths based on the size of the otolith.

2.2.5. Lipid based condition metrics: Total lipids and DHA:EPA

Total lipid content of white muscle tissue (i.e. gram of lipid per gram of tissue) was calculated for each individual, as white muscle is known to be least susceptible to lipid oxidation (Undeland et al. 1998). White muscle tissue was taken from the dorsal side of each herring using a scalpel, taking care to avoid skin and adjacent red muscle tissue. Samples were placed in a -80˚C freezer for at least 24 hours, and then freeze-dried in a Virtis FreezeMobile for 24 hours. Wet and dry mass of the muscle tissue were measured to the nearest tenth of a milligram using a Scaltec microbalance to account for variation in water content. A lipid-free environment was prepared: all glassware was washed in chloroform and methanol, pipettes were heated in a muffle furnace at 450˚C for 8 hours, and any water used was chloroform-extracted by mixing water and chloroform and

allowing chloroform to settle to the bottom to then be removed. Lipid extractions were performed on dry muscle tissue following Parrish (1999). Briefly, muscle tissue was rehydrated with water, and ground in a 2:1 mixture of chloroform and methanol. Samples were then vortexed, sonicated, and centrifuged at 2000rpm for 2-3 minutes. The lower organic layer was removed by pipetting with a short pipette through a longer pipette to minimize disturbance and washed into a new vial using chloroform. This process was repeated three times per sample to ensure that all lipid was collected. Lipid oil was then measured gravimetrically by evaporating all chloroform using nitrogen gas and weighing the oil on a Scaltec microbalance (accurate to 5 decimal places) in order to estimate grams of lipid per gram of dry weight muscle tissue. Values were then log-transformed to meet the assumption of linear models and eliminate skewness. Hereafter, these values are reported as Total Lipid, or TL.

In addition to measuring the total amount of lipid in muscle tissue, fatty acid profiles were compiled on the extracted lipids. After dividing the lipid oil in half (i.e. one half was preserved as a backup sample), and the addition of a known quantity of tricosanoic acid (C23:0) to act as an internal standard, the lipids were derivitized to form fatty acid methyl esters, following Parrish (1999). Samples were suspended in 0.5ml hexane and 1.5ml boron-trifluoride methanol and heated at 85˚C for 90 minutes in a Memert lab oven. Water and 2ml hexane were added, and the upper organic layer was removed to a separate vial by pipette and evaporated. Samples were then suspended in hexane and kept at -80°C until they could be read using a gas chromatograph to determine amounts of fatty acids. Fatty acids were separated into three categories as percentage contributions:

saturated fatty acids (SFA hereafter), mono-unsaturated fatty acids (MUFA hereafter), and poly-unsaturated fatty acids (PUFA hereafter). PUFAs were defined as fatty acids with two or more double bonds. The DHA:EPA ratio was calculated by dividing DHA(ug) by EPA(ug).

2.2.6. Environmental conditions in the Strait of Georgia during the study

The annual State of the Pacific Ocean reports produced by DFO were used to summarize environmental conditions in the Strait of Georgia from 2013-2016 (Chandler et al. 2017). Chandler (2017) collected depth-averaged temperature daily from Nanoose Bay on the east coast of Vancouver Island; the peak temperature from 0-400m is reported for each year. Allen et al. (2016) reported the average timing of the spring bloom in the SoG using ferry observation data. Bloom composition data were reported in Esenkulova & Pearsall (2016), using citizen-science supplemented data. Galbraith and Young (2017) collected and identified zooplankton and reported trends in the community composition in the SoG. Finally, herring spawn dates were determined using dive surveys, data available at http://www.pac.dfo-mpo.gc.ca/science/species-especes/pelagic-pelagique/herring-hareng/herspawn/tables/sogmapf-eng.html (Fisheries and Oceans Canada, Herring Catch Database – Pacific Biological Station, Nanaimo BC).

2.2.7. Data analysis

Herring were divided into six groups based on the time of year during which they were captured: Fall 2013 (September 2013); Fall 2014 (September and October 2014); Fall 2015 (August, September, October 2015); Fall 2016 (October 2016); Summer 2015 (June-July 2015); Summer 2016 (July 2016) (Table 2.1). Note that August samples were

collected in 2015 only (Aug 22-24), and are grouped with Sept/Oct fish due to the consistent temperature, salinity, and chl-a concentrations in the SoG from August-October (Chandler et al. 2017). Interannual variability in YOY herring condition was tested both with and without the August 2015 fish, to ensure that any trends detected were not driven by fish collected closer to summer in one of the four years.

Interannual variability was tested across the four fall seasons for each condition metric separately using a one-way ANOVA with Tukey post-hoc tests, using R studio software (R Core Team 2015). In order to meet the assumption of normally distributed data, total lipid values were logged (TL therefore refers only to logged values). No other transformations were required for the other 5 metrics. Changes in condition were also tested between summer and fall 2015, and between summer and fall 2016.

Table 2.1. Number of YOY herring used in each analysis, separated by year and season

Condition factor	Fall 2013	Fall 2014	Fall 2015	Fall 2016	Total Fall	Summer 2015	Summer 2016	Total Summer
FK	80	38	31	34	183	48	30	78
LW	80	38	32	34	184	48	30	78
OW	66	33	29	31	159	35	28	63
G*i*	68	23	70	6	167	47	14	61
TL	39	36	32	25	132	9	12	21
DHA:EPA	39	36	31	23	129	9	12	21

2.3. Results

2.3.1. Environmental conditions

Environmental trends in the SoG varied substantially from 2013-2016. In general, oceanographic conditions were more typical in 2013 and 2014 (i.e. relative to long-term climatology), with notable changes observed in 2015 and 2016 (Table 2.2). In 2013-2014,

water temperatures, spring bloom dates, phytoplankton community composition, and trends in the zooplankton community did not deviate substantially from the 30-year average (Chandler et al. 2017). In contrast, depth-averaged temperatures from centrally located Nanoose Bay reached a peak of 10.5°C in the summer of 2015, representing a +2˚C deviation from the 15 year average (Chandler 2017). Higher than average temperatures continued during the winter of 2015, preventing mixing of the surface layer to the usual degree, and extended through the summer of 2016 (Chandler 2017).

The spring phytoplankton bloom in 2015 was the earliest of the previous 8 years, commencing on March 17 (Allen et al. 2016). The composition of the bloom was also anomalous in 2015: diatoms of the genus *Skeletonema* dominated strongly, in a bloom that is typically more diverse (Esenkulova & Pearsall 2016). The composition of the zooplankton community in the SoG from 2013-2015 did not show substantial deviations from the 30-year average (Galbraith & Young 2017). In 2016, two dominant crustacean taxa, the copepod *Neocalanus plumchrus* and krill species *Euphausia pacifica* (north region only) experienced negative biomass anomalies compared to the 30-year long term average (Galbraith & Young 2017). Upon examining abundance data from 2013-2016 exclusively (supplied by M. Galbraith, pers comm 2018), *E. pacifica* abundance (number/m^3) was significantly higher in 2015 (ANOVA, $p=0.004$) compared to the other three years, which were not statistically different from each other. Additionally, the abundance of *N. plumchrus* was lower in 2016 compared to 2015 and 2013. 2013 abundances were also higher than 2014 (ANOVA, $p<0.001$) (see Appendix A3).

Table 2.2. Oceanographic conditions in the Strait of Georgia, 2013-2016. Adapted from information contained in Allen et al. 2016, Esenkulova & Pearsall 2016, Chandler 2017, Galbraith & Young 2017, Fisheries and Oceans Canada, Herring Catch Database, Pacific Biological Station.

	2013	**2014**	**2015**	**2016**
Peak depth-averaged temperature (0-400m)	9.5°C	9.5°C	10.5°C	10°C
Spring bloom start date	March 29	March 27	March 17	March 27
Spring bloom composition anomalies	-	-	Limited taxa: *Skeletonema costatum* dominated	-
Zooplankton community anomalies	-	-	-	*E. pacifica* and *N. plumchrus*: Negative biomass anomaly
Herring spawn date	Min: March 2 Avg: March 20	Min: March 3 Avg: March 21	Min: Feb 17 Avg: March 10	Min: Jan 22 Avg: March 13

2.3.2. Fall: Interannual variability in condition

Interannual variability was measured in each condition factor using an ANOVA followed by a Tukey post-hoc test. Results from the Tukey tests are reported in a table at the end of this subsection (Table 2.3).

The average size and weight of the YOY herring used in this study were approximately 9-10 cm and 9-10 g, respectively (Figure 2.4). The greatest variability in both length and weight occurred in 2016, with a range of values from 7-12 cm and 5-15 g (Figure 2.4). Based on ANOVA tests, average lengths and weights did not differ significantly across the four years (Length: p=0.7; Weight: p=0.5).

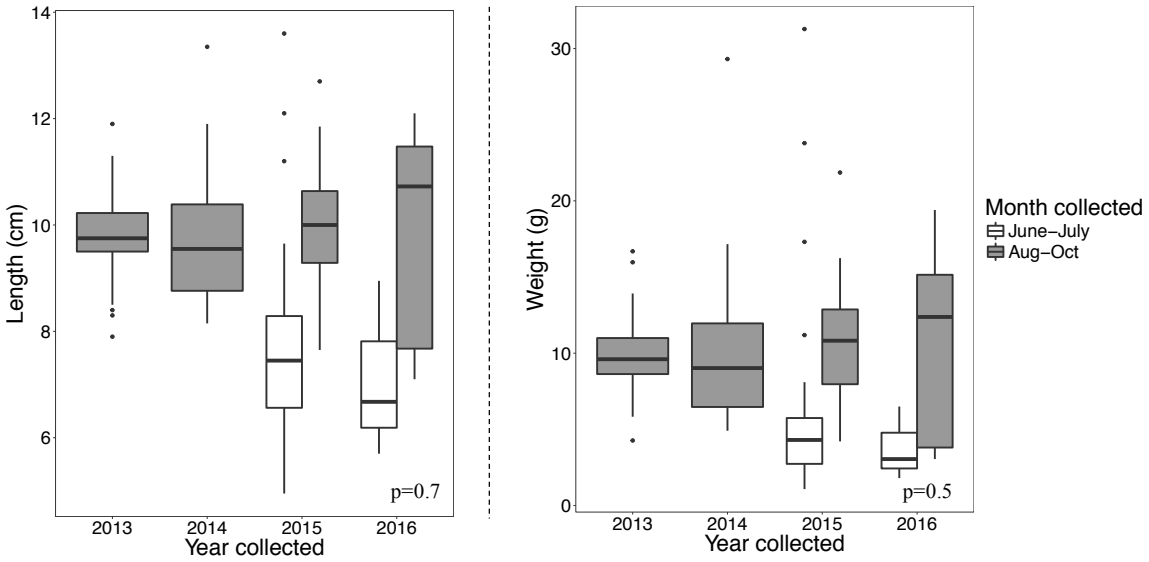

Figure 2.4. Box and whisker plot of length (cm) and weight (g) of YOY herring separated by year and by season. Horizontal lines denote the median (50th percentile), while boxes represent the range between the first and third quartile (i.e. 25th and 75th percentile). Vertical lines extend to the farthest point, no more than 1.5 times the inter-quartile range. Points represent outliers.

The two morphometry-based condition metrics (FK and LW) showed no difference across the first 3 years of the study, and then a significant decrease in 2016, (FK, ANOVA, p=0.01, Tukey post hoc; LW, ANOVA, p=0.007, Tukey post hoc) (Figure 2.5). Removing the August fish from 2015 did not change these trends (Figure 2.5).

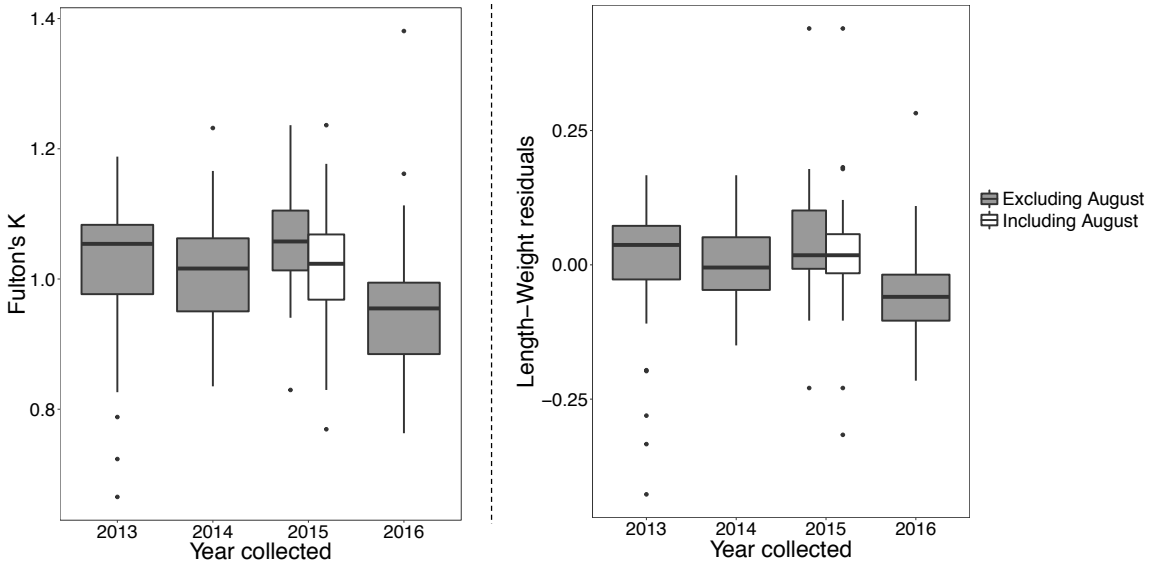

Figure 2.5. Box and whisker plot of interannual variability in morphometry-based condition metrics Fulton's K (FK) and length:weight residuals (LW) from Fall 2013-Fall 2016 with and without August 2015. Both metrics show a decline in 2016. Removing August fish does not change this trend.

The two growth-based condition metrics (OGR and G*i*) generally followed the same trend, albeit with greater variability than seen in FK and LW (Figure 2.6). OGR values were significantly lower in 2016, with no change observed among the previous three years (ANOVA, $p<0.001$, Tukey post hoc) (Figure 2.6). Otolith widths were positively correlated with fish length along two separate intercepts depending on year collected, and were therefore an appropriate proxy for somatic body size. G*i* values decreased in 2015 and 2016 (ANOVA, $p<0.001$, Tukey post hoc), while OGR values also decreased in 2015 when the August values were removed (ANOVA, $p<0.001$, Tukey post hoc) (Figure 2.6).

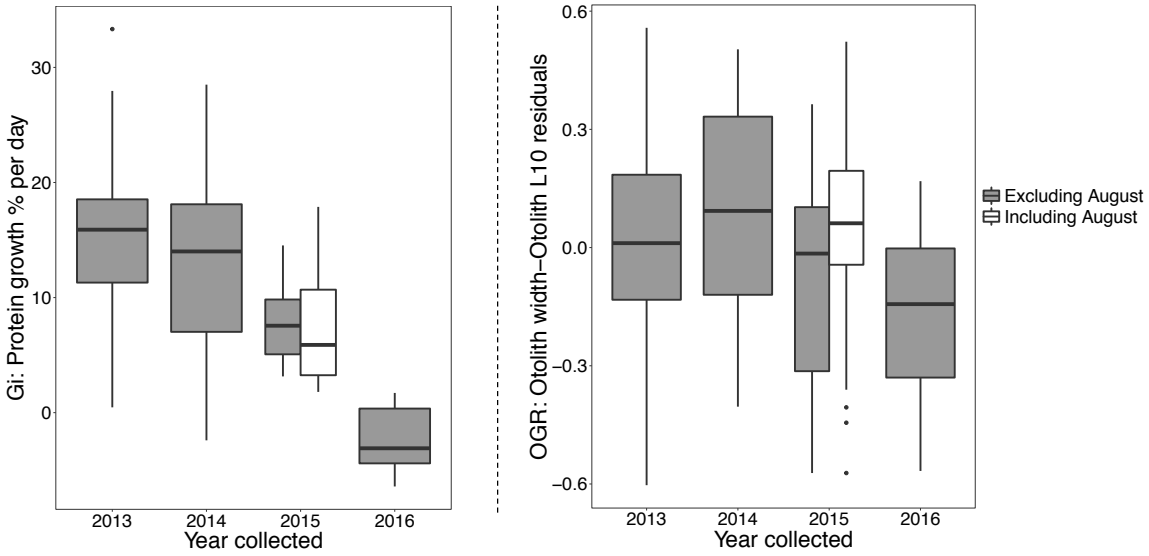

Figure 2.6. Box and whisker plot of interannual variability in growth-based condition metrics from Fall 2013-Fall 2016 with and without the August 2015 fish. There is a similar decreasing trend over the years as in the morphometry-based metrics, though variability is higher, and removing the August fish changed the OGR results such that there is no longer a significant difference between 2014 and 2015 values.

The two lipid-based condition metrics revealed different trends than those seen in the other four metrics (Figure 2.7). Lipid content (gram of lipid per gram of dry muscle tissue) in YOY herring from the SoG were found to increase from approximately 5% in 2013 to 20% in 2016. Once logged to eliminate skewness, TL values increased over the study period (ANOVA, $p<0.001$, Tukey post hoc) (Figure 2.7). Removing the August 2015 fish did not change the general trend, however the 2015 values increased slightly and were no longer significantly lower than the 2016 values (Figure 2.7). DHA:EPA values held steady except for a significant drop in 2015 compared to the other 3 years, with no additional change when August fish were removed from fall 2015 (ANOVA, $p=0.001$, Tukey post hoc) (Figure 2.7).

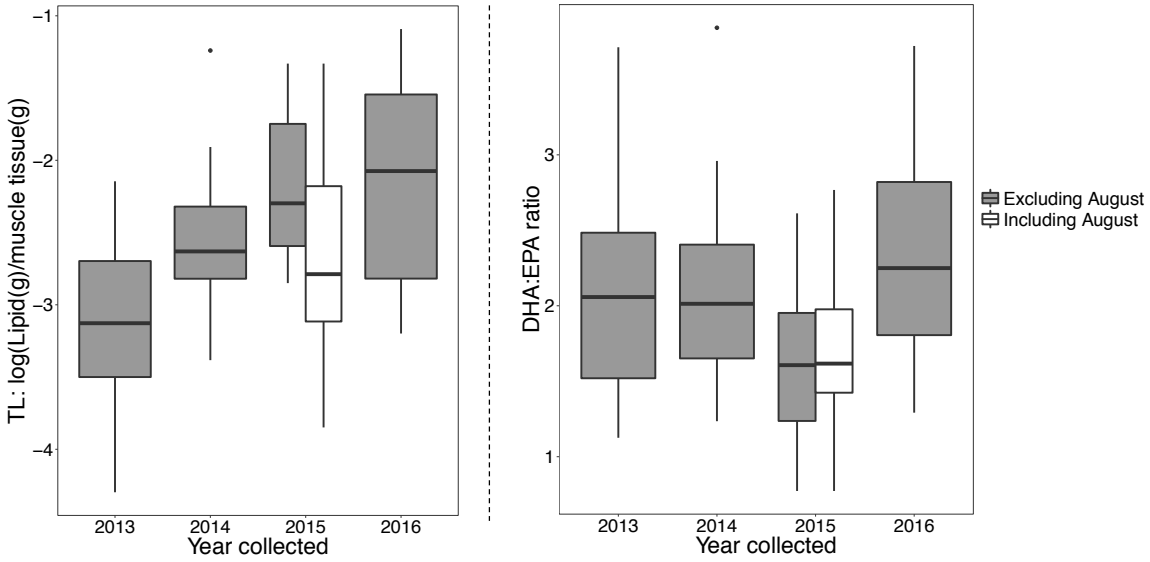

Figure 2.7. Box and whisker plot of interannual variability in lipid-based condition metrics from Fall 2013-Fall 2016, with and without the August 2015 fish. Trends here are different from the previous two groupings of metrics. Removing the August 2015 fish slightly changed the trajectory of the trend in TL, where 2015 values became significantly higher.

The composition of fatty acids showed some interannual variability. The percentage of saturated fatty acids, as well as mono-unsaturated fatty acids remained unchanged throughout the time-period (ANOVA, p=0.27, ANOVA, p=0.18) (Figure 2.8). In contrast, when August values are excluded, polyunsaturated fatty acids comprised a significantly lower percent of total lipid in fall 2015 than in the other three years (ANOVA, p=0.007, Tukey post hoc) (Figure 2.7). When August values are included, there is no significant difference in percent PUFA per year (ANOVA, p=0.49) (Figure 2.8). Fatty acids that are typically taken to be biomarkers of crustacean zooplankton in the SoG were selected following El-Sabaawi et al. (2009): C18MUFA, C18PUFA, C20:1 and C22:1. Percentages of these fatty acids were summed to estimate the overall percent

of fatty acids obtained from krill and copepods, and were not significantly different over the four years of the study (ANOVA, p=0.35) (Figure 2.8).

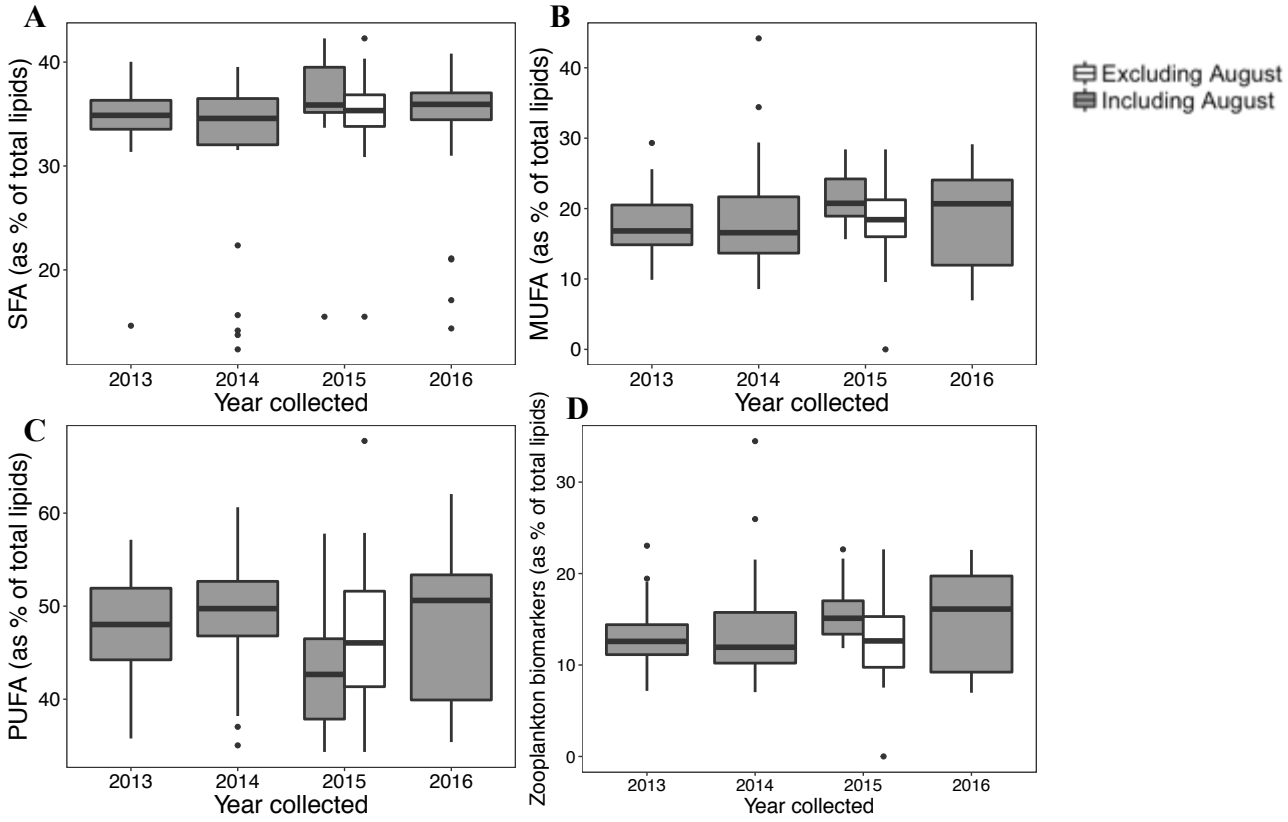

Figure 2.8. Box and whisker plot of interannual variability in fatty acid composition from Fall 2013-Fall 2016. Four groups of fatty acids are shown clockwise from top left: Percent saturated fatty acids, percent mono-unsaturated fatty acids, percent poly-unsaturated fatty acids, and percent fatty acids that can be classified as zooplankton biomarkers. Results are shown with and without August values.

Fatty acids were also analyzed in terms of milligrams of fatty acid per milligram of muscle tissue (dry mass). When separated by degree of saturation, all three types (SFA, MUFA, and PUFA) showed the same increasing trend over the four years, similar to the trend in total lipids. Percentages were used (i.e. instead of the raw values) to better observe interannual differences in fatty acid profiles, as they are not dependant on the initial amount of lipid. Further information can be found in Appendix A4.

Table 2.3. Tukey post-hoc test results for ANOVAs measuring interannual variability in the six condition factors. Left hand column shows pairs of years. Asterisks denote values signifying that pairs are statistically different from each other. (<0.05).

	FK	**LW**	**OGR**	**G*i***	**TL**	**DHA:EPA**
2014-2013	0.932	0.990	0.319	0.202	0.001*	1.000
2015-2013	1.000	0.973	0.788	0.000*	0.004*	0.050*
2016-2013	0.009*	0.010*	0.020*	0.000*	0.000*	0.290
2015-2014	0.973	0.924	0.925	0.000*	0.974	0.065
2016-2014	0.113	0.064	0.001*	0.000*	0.024*	0.276
2016-2015	0.054*	0.016*	0.007*	0.000*	0.010*	0.001*

Excluding August-caught herring

	FK	**LW**	**OGR**	**G*i***	**TL**	**DHA:EPA**
2014-2013	0.932	0.990	0.301	0.202	0.000*	1.000
2015-2013	0.849	0.714	0.539	0.000*	0.000*	0.056*
2016-2013	0.010*	0.009*	0.017*	0.000*	0.000*	0.312
2015-2014	0.668	0.642	0.076*	0.035*	0.060*	0.068
2016-2014	0.113	0.063*	0.000*	0.000*	0.012*	0.298
2016-2015	0.026*	0.012*	0.825	0.002*	0.999	0.002*

2.3.3. Intra-annual variability in herring condition (2015 and 2016)

Variability in condition between the early and late seasons was more common in 2016 than in 2015 (Figure 2.9). In 2016, all condition metrics except for DHA:EPA were significantly different between seasons (Figure 2.9). In contrast, in 2015, FK, LW, and OGR did not show any significant change from early to late in the summer (Figure 2.9). In early 2015, there was strong variability in OGR (Figure 2.9). The same variability can be seen in summer 2016, however the sample size is smaller (2015: n=35; 2016: n=28), and the variability appears mainly as outliers, rather than within the 75th percentile box (Figure 2.9).

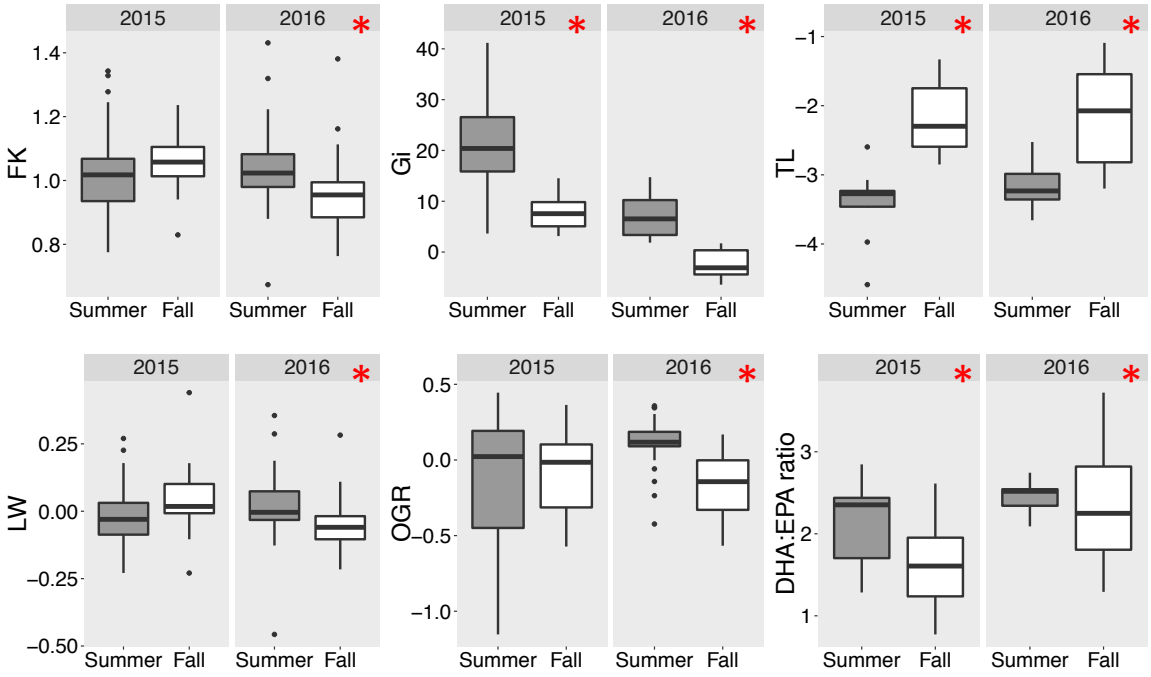

Figure 2.9. Intra-annual differences in condition metrics from 2015 and 2016. Metrics are abbreviated on the y-axes as in Table 2.1. Asterisks denote years where values differ significantly ($p<0.05$) between summer (June-July) and fall (Aug-Oct).

2.3.4. Intra-seasonal variability in herring condition: Fall 2015

In some cases, the amount of variability in condition metrics differed among seasons, which may have been related to the months in which they were sampled. For example, the 2013 herring were consistently the least variable of the entire sample set, and all came from one month of sampling (Figure 2.10). In contrast, herring from 2016 were also only collected from one month, but showed significantly more variability in OGR, TL, and DHA:EPA (Figure 2.10). Herring from 2015 came from 3 different months, and there was occasional strong variability seen in some metrics between August, September, and October, particularly in OGR and TL (Figure 2.10).

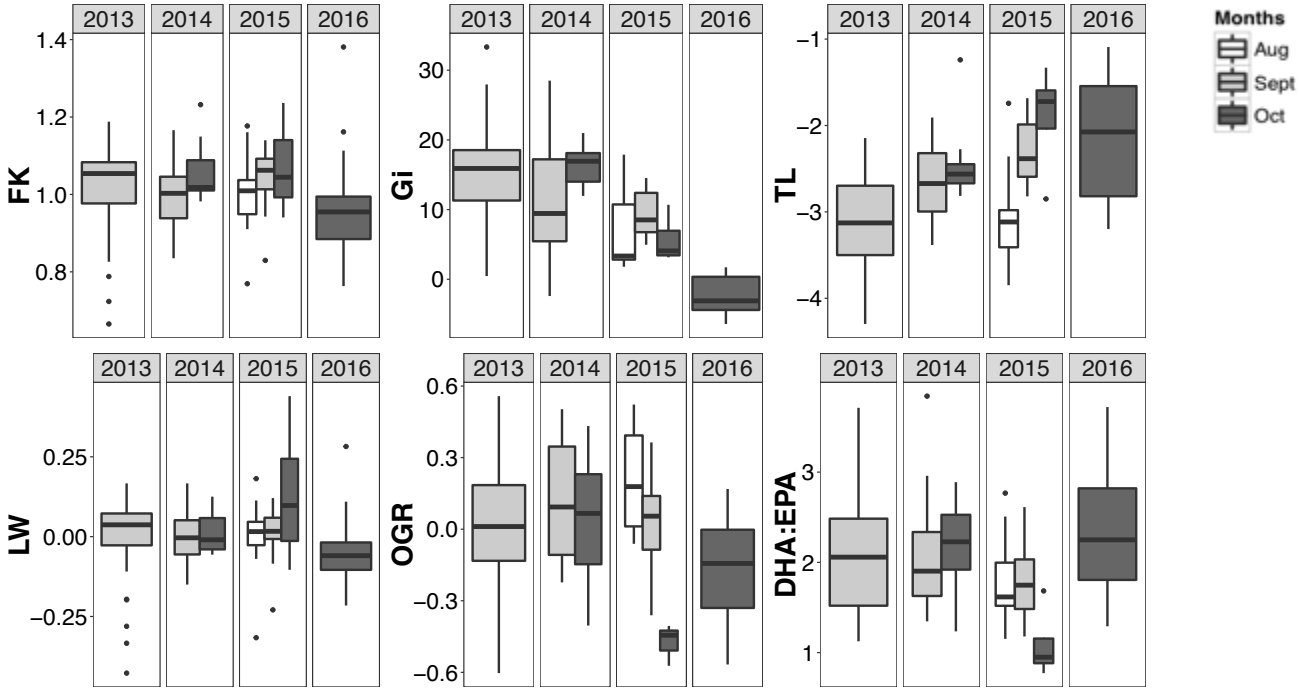

Figure 2.10. Differences in condition metrics between fall months in 2013-2016. Metrics are abbreviated on the y-axes as in Table 2.1.

2.4. Discussion

This research set out to quantify interannual variability in YOY herring condition in the Strait of Georgia. The results showed evidence of change in condition over a four-year period. Fulton's K and LW were lowest in 2016 compared to 2013-2015 (Figure 2.5), suggesting that the condition of YOY herring in the SoG (with respect to their morphometry) decreased in 2016. G*i* and OGR were also lowest in 2016, suggesting that growth rates of YOY herring were lower in 2016 compared to the three previous years (Figure 2.6). Total lipids and DHA:EPA ratio also changed over the four years of the study period, though differently from each other and the other metrics; TL values increased slightly over the four years, whereas DHA:EPA dipped in 2015 (Figure 2.7). The lipid-based results suggest that there was a slight increase in condition over time with respect to the nutritional quality that the YOY herring may have represented to their predators, notwithstanding the decrease in DHA:EPA in 2015. Physiological reasons behind the discrepancies between results from different metrics in individual fish are explored further in Chapter 3 of this thesis; this section will focus on broad scale changes in condition at a group level.

2.4.1. Interannual variability in the condition of forage fish

Interannual variability in condition has been noted in the adult stage of various forage fish in the NE Pacific (Northern anchovy (*Engraulis mordax*), Pacific herring (*Clupea pallasi*), Pacific sardine (*Sardinops sagax*), and whitebait smelt (*Allosmerus elongatus*)) (Litz et al. 2010), and has been described using length:weight residuals of YOY herring in the SoG (Boldt et al. 2015, 2017). The current study also found interannual variability in condition in YOY Pacific herring. Over the four-year period, the six condition factors

showed varying amounts of change. For example, total lipids doubled between years (Figure 2.7). Similarly, Litz et al. (2010) saw the amount of lipid in adult Pacific herring muscle tissue double from 2005-2006, as upwelling increased and feeding conditions improved. However, changes in length:weight residuals were not statistically different in this thesis until 2016 (Figure 2.5). These results differed from the previous study on YOY herring morphometry, which reported length:weight residuals tripling from 2013-2015 (Boldt et al. 2017). It is worth noting that the sampling methods were quite different between these two studies, with spatially targeted sampling along the coasts (Boldt et al. 2017) compared with the random bycatch sampling used for this thesis. This may have contributed to the difference in results. Overall, while the magnitude and direction of variability differed, the presence of interannual variability in condition factors affirms the first objective of this study and allows us to move to the second objective of exploring reasons why such variability in condition may have occurred.

2.4.2. Effects of environmental variability

It is possible to link the variability in herring condition, at least in part, to the environmental variability observed in the SoG across the four years. Four of the six metrics measured in this study suggested that YOY herring condition in the SoG was lower in 2016 than in 2013-2015 (FK, LW, G*i*, and OGR, Figure 2.5, Figure 2.6). Interestingly, there were also several oceanographic conditions that were anomalous in 2016: particularly, lower than average biomass of the copepod *N. plumchrus* and the krill *E. pacifica*, and the longer-than-normal time between both onset and average date of the herring spawn and the start of the spring phytoplankton bloom (Table 2.2). Copepods and krill are important components of herring diets (Haegele 1997, Foy & Norcross 1999),

and the nutrition that fish can obtain has been linked to variability in condition (Foy & Norcross 1999, Norcross et al. 2001) as well as recruitment levels (Flinkman et al. 1998, Beamish et al. 2012, Lusseau et al. 2014). Boldt et al. (2018) found that an increased prey field was generally associated with an initial increase in length:weight residuals in YOY herring, though these values eventually leveled off or decreased slightly. A period of reduced prey availability in 2016 (Table 2.2) may therefore have had a negative effect on the morphometry of herring during that time, as well as their growth rate.

Changes in the zooplankton community in the SoG can be driven by variability in the timing and composition of the phytoplankton bloom in the SoG. As such, Schweigert et al. (2013) applied Cushing's match-mismatch hypothesis to Pacific herring in the Strait of Georgia. Cushing's hypothesis (1990) states that recruitment in fish populations is higher in years when larval fish hatch at a time that matches a high abundance of their preferred prey (and is lower in years when there is a mismatch in timing). It was determined that a relationship exists between herring productivity and the timing of peak zooplankton abundance, expressed as a link between the survival of YOY herring and the timing of the spring bloom in the SoG (Schweigert et al. 2013). In years when the herring spawning season occurs 1-2 weeks before the onset of the spring bloom, there is a trend towards higher abundance of herring in the summer immediately following the bloom (Schweigert et al. 2013). A link can therefore be drawn between spring bloom timing and YOY herring growth and condition, based on the connection of both to the zooplankton community in the SoG (Schweigert et al. 2013, Boldt et al. 2018)

The earliest spawn date for SoG herring in 2016 occurred on January 22, over 2 months before the onset of the spring phytoplankton bloom (Table 2.2). The difference between spring bloom onset and the earliest herring spawning date in 2015 was also large (28 days), but still within the margin of the estimates of Schweigert et al. (2013) for an average year for the SoG herring population. These values have not been weighted for biomass - however upon examining average spawning dates, it can be seen that there was an extra week between the average spawning date and the onset of the spring phytoplankton bloom in 2016 when compared to the other three years (Table 2.2). While their study discussed a connection between spring bloom timing and summer herring abundance (i.e. not herring growth rates, *per se*), spring bloom timing affects prey availability (Schweigert et al. 2013), and prey availability has been linked to growth rates in juvenile fish (Rooker & Holt 1996, Peck et al. 2015). Additionally, Boldt et al. (2018) found lower length:weight residuals in YOY herring when their parents spawned well before the peak spring bloom date. It is therefore possible that the reduction in condition of YOY herring in the SoG in 2016 based on FK, LW, G*i* and OGR was related to this longer than average time gap (i.e. a "mismatch") between herring spawning and the onset of the spring phytoplankton bloom.

The interannual changes observed in DHA:EPA ratios further support the idea that environmental conditions can be reflected in the condition of herring. In general, diatoms are richer in EPA than are dinoflagellates, which are richer in DHA (Dalsgaard et al. 2003). In years when the phytoplankton community is composed primarily of diatoms, fatty acid signatures in zooplankton in the SoG have been shown to be more EPA-rich

(El-Sabaawi et al. 2009b). In 2015, the spring bloom was composed mainly of the diatom *Skeletonema costatum*, and this was the year with the lowest ratio of DHA:EPA in the herring (Figure 2.7). Since YOY herring primarily consume crustacean zooplankton (Foy & Norcross 1999), whose fatty acid signatures can reflect the primary production in the SoG (El-Sabaawi et al. 2009a) it is possible that these two observations are linked. It should be noted, however, that the particular fatty acids that are typically used as zooplankton biomarkers did not significantly change over the course of the study period (Figure 2.8), notwithstanding the decrease in 2016, as expected based on the long-term negative biomass anomalies in *N. plumchrus* reported by Galbraith & Young (2017). Because zooplankton samples were not collected along with the herring used in this study, however, there are limitations to interpreting these trophic markers.

2.4.3. Variability in condition through development of fish

There are many potential linkages between environmental variability and variability in herring condition in this study. However, the increasing trend in total lipids from 2013-2016 does not fit with the general idea that changes in the oceanography of the SoG are the primary drivers of interannual variability in herring condition. Previous studies on juvenile forage fish have shown that lipid stores also decrease as food availability decreases (Frommel & Clemmesen 2009, Litz et al. 2010). Since there was a decrease in *N. plumchrus* (Table 2.2), the preferred prey taxa of YOY herring, it is unexpected that lipids did not decrease over the four years of the study period along with the other four condition metrics.

Age-specificity is an important consideration when choosing condition metrics. Some proxies are better suited to fish of a certain age, and it is therefore preferable not to compare condition factors across life history stages (Buckley et al. 1999). The implications of this can be observed in the results from seasonal comparisons in the current study; for example, there are strong differences between most metrics, including TL, in the interannual comparisons between summer and fall (Figure 2.9). Since SoG herring spawn in March, there is likely an age gap of 2-3 months between the summer- and fall-caught herring used in this study. Juveniles often prioritize the increase of their energy reserves over somatic growth as they age, and as their first winter approaches (Iverson et al. 2002, Biro et al. 2005, Mogensen & Post 2012). The significant decrease in proxies of growth from summer to fall, and corresponding significant increase in total lipids found in SoG YOY herring (Figure 2.9) therefore seems more likely to be related to the difference in age between the two sampling periods, rather than any change in environmental conditions between June and September. For these reasons, interannual changes in condition factors were only compared among fall-caught herring in order to minimize variability in age.

It is still possible that the fall herring used in this study may have been of slightly different ages across the four years. All fall-caught fish were in the same life stage, as determined by the consistent presence of well-formed rostra in their otoliths in contrast to the round shape found in summer-caught (i.e. younger) herring. However, Fall 2016 was the only year in which the entire sample was composed of herring caught in October. In Fall 2013-2015, at least part of the sample was caught in September. The argument could

therefore be made that ontogenetic variability, rather than environmental variability, drove changes in condition between fall-caught herring over four years, as these herring are the oldest of the sampled fish. Further to this point, the intra-annual variability in 2016 and not 2015 could be explained by the larger age gap between summer and fall fish in 2016 compared to 2015 (Figure 2.9).

A study on Pacific herring from Alaska showed that both ontogeny and environmental conditions contributed to variability in lipid content over a three year period (Lane et al. 2010). Similarly, and as discussed above, there were many factors that may have contributed to the interannual variability in this study, and it is thus likely that ontogeny was not the sole driver. For instance, if it were the case that October fish are at a different life-history stage than September fish, October-caught herring from the 2014, 2015, and 2016 should show little interannual variability in condition. Instead, October values were significantly different between years in each of the five metrics other than TL tested in this study, and there was no change from September to October in OGR and TL values from 2014, the only other year with representation from more than one month (Figure 2.10). As such, it likely that processes other than ontogeny alone are influencing variability in condition between years.

In addition to examining calendar dates, which may discount the variable timing of other oceanographic events, this idea can be further explored by examining relationships between various condition factors and standard length of the fish (Figure 2.11). For instance, 57% more variability in TL is explained by fish length in Fall 2016 when

compared to Fall 2015. Both years have similar variability in length (Figure 2.4), but there was only significant variability in age in Fall 2015. Standard length explains a significant amount of variability in OGR in Fall 2015, when there was variability in both age and length, but not in Fall 2016, when there was minimal variability in age (Figure 2.11). If age, and therefore developmental stage, was the sole driver of this variability in condition factor, the results of the linear model between OGR and standard length should have mirrored the results from the test of TL and standard length. The fact that they did not suggests that developmental stage was not the sole driver of the observed variability.

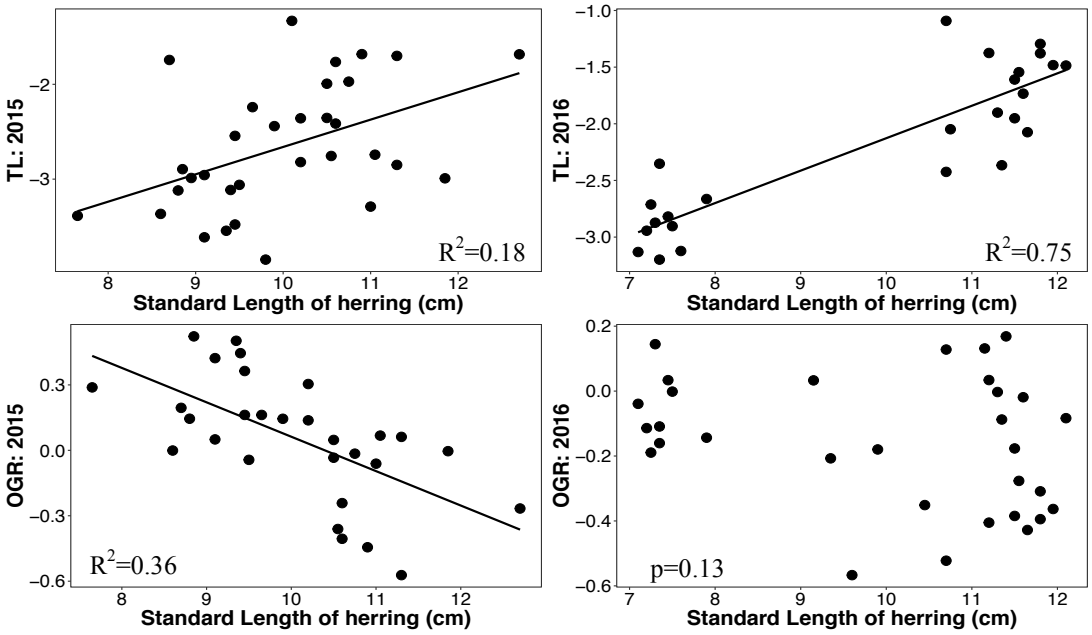

Figure 2.11. Comparing condition factors to the standard length of herring: Clockwise from top left: Total lipids-length in Fall 2015, Total lipids-length in Fall 2016, Otolith growth rate-length in Fall 2015 and Otolith growth rate-length in Fall 2016. TL is significantly correlated to standard length in both 2015 and 2016, but OGR is only significantly correlated ($p<0.05$) to standard length in 2015.

2.4.4. An alternative perspective on the changes in condition factor

Four of the six condition factors (FK, LW, G*i*, OGR) showed a significant decrease in 2016 compared to the other years of the study, while only one (TL) showed an increase (Figure 2.5, Figure 2.6, Figure 2.7). Changes in the environment and changes in the

development of the fish can be used to interpret why this might have occurred, but neither provides a fully satisfactory explanation for the variability in condition in opposite directions. An alternative approach is to examine the processes underlying growth rates and lipid content together, rather than considering them as opposites (i.e. "better" or "worse" condition). Several studies have found growth rate and lipid content of muscle tissue increasing in opposition to each other (Folkvord et al. 1996, Buckley et al. 1999, Peters et al. 2015). For example, a study on starvation in juvenile gobies (*Gobiusculus flavescens*) revealed that RNA:DNA values remained stationary during periods of food deprivation, while lipids decreased (Frommel & Clemmesen 2009). The authors suggest that lipid deposition is indicative of weight-based growth, and that further research should be done to see whether RNA:DNA values are still significantly linked to condition in YOY fish (Frommel & Clemmesen 2009). In their study of RNA:DNA in larval and YOY red drum (*Sciaenops ocellatus*), Rooker & Holt (1996) suggest that the lack of a clear pattern in RNA:DNA values for juveniles compared to larvae is due to the increasing uniformity in growth at a cellular level as fish age. Buckley et al. (1999) suggests that growth in terms of protein production slows naturally as fish reach the juvenile stage, and recommends additional condition metrics be used when studies of RNA:DNA are carried out on juvenile fish.

In the current study, the fall-caught YOY herring may have reached the stage where growth is redirected from protein development (RNA:DNA) to increasing weight (lipids). In 2016, growth metrics were lowest and lipid values were highest (Figure 2.6, Figure 2.7). Rather than interpreting these values as contradictory levels of condition, they may

simply reflect an intensification of the process of redirection from growing in size to increasing lipid stores as reported by Frommel & Clemmesen (2009). By these criteria, herring from 2016 would not necessarily be in "worse" condition than in previous years. Rather, this variability may signify that herring are able to buffer changes in environmental conditions to some degree. Carscadden & Frank (2002) looked at interannual variability in adult Newfoundland capelin (*Mallotus villosus*) and found no significant linkage between FK in capelin and temperature, suggesting that environmental conditions do not necessarily dictate condition in fish. The energy budget models constructed by Martin et al. (2017) describe the tendency for fish to increase their stores of lipids when a period of starvation is imminent, in order to withstand changes in the environment. It may be that in the current study, herring were not in any better or worse condition in 2015 and 2016 compared to the earlier two years, but simply "different" condition, having redirected their energy from growth to lipid storage. Further study would be required to determine whether this is a response to environmental conditions or some other cause. For example, a study rearing herring with specific temperatures and rations, and non-lethally sampling growth rate and lipids at various time points could potentially reveal how relationships between condition metrics change over time.

2.4.5. Implications of the observed variability in condition

The results of this study suggest that YOY herring condition varied over the four years of the study period. Many factors could have contributed to these changes; in addition to the changes in oceanographic conditions and ontogenetic stage discussed here, Boldt et al (2018) link density-dependence to variability in condition of YOY herring. The differing results depending on the condition metric used mean that we cannot conclusively state

whether condition "worsened" or "improved" from year to year. Whereas condition factors are useful for explaining variations in year-class strength, we might predict poor year-class strength of 2016 herring compared to previous years based on the decrease in morphometric condition and growth rates. However, the high total lipids value suggests that these fish may in fact have been better equipped to survive starvation, and the stock assessment forecasts have predicted an increased number of age-2 recruits for 2018, i.e., herring from the 2016 YOY class (Cleary & Taylor 2016).

Comparing growth rate using otoliths in summer- and fall-caught YOY herring revealed a trend towards larger variation in growth rates in the younger, summer-caught fish as compared to the older fish, which were generally fast growing with less variability. This trend may imply that YOY herring in the SoG are subjected to size-selective mortality, where only faster-growing fish are able to survive the June-July period by avoiding predation. Testing this would require polishing otoliths from September- and October-caught herring down so that daily increments from June and July were visible; if size-selective mortality was indeed relevant for this trend, it would be expected that these daily increments would be higher than the average daily increments for the June- and July- caught fish. This would indicate that the fish that survived to fall were the fast growing fish in the summer, and therefore were able to escape predators.

Additionally, condition factors are also useful in estimating prey quality, since fish with higher total lipid content are generally regarded as more being more nutritious (Osterblom et al. 2008) for their predators. In this regard, YOY herring could be said to

have increased in condition from 2013-2016. Ultimately, however, condition in fish is multi-faceted, and can be interpreted in many ways. The next chapter in this thesis undertakes an in-depth analysis of the condition of individual fish, and the extent to which the various condition metrics are correlated within individuals.

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3. Exploring intercorrelation among proxies of condition as measured in Young-of-Year Pacific herring (*Clupea pallasi*)

Abstract

Quantifying the condition of fish can help researchers understand how fish have responded to changes in their environment, and to estimate the quality of prey that they represent to their predators. There are numerous condition metrics used to answer these questions, but typically no more than two or three are applied on an individual fish in a single study. Understanding the intercorrelation of these metrics is important for accurately interpreting condition. This study aimed to quantify condition in YOY Pacific herring (*Clupea pallasi*) using six metrics in each individual: (i) Fulton's K and (ii) the residuals from a length:weight regression, for quantifying morphometry, recent growth via (iii) the RNA:DNA ratio and (iv) otolith microstructure analysis, and (v) lipid content and (vi) the ratio of DHA:EPA to represent condition based on nutritional content. Metrics were compared by ranking the outcomes and examining their distribution, and subsequently using pairwise linear models to test for correlation. The distribution of metrics as plotted in a cumulative distribution function showed that the six metrics were not intercorrelated. Specifically, proxies for morphometry of fish were not correlated with growth indices, and were only related to nutritional proxies where lipid content was high. Additionally, the two growth indices were not correlated with each other. These results confirm that researchers cannot make inferences about a fish's score in a particular metric of condition based on its score for another metric, and suggest exercising caution when selecting proxies in order to choose an appropriate metric for the aspect of condition in question.

3.1 Introduction

3.1.1. Measuring condition of fish

Fisheries scientists assess the "condition" of fish for a variety of purposes. At the population level, measures of fish condition can be used to explore patterns of variability in space and in time, such as in Chapter 2. On an individual basis, the condition of a fish can provide information about its current health, and future likelihood of survival. For example, Cushing's match-mismatch hypothesis centred on the idea that larval fish were more likely to survive to recruitment if they were in better condition, and so the timing of the emergence of larval fish should coincide with high availability of their preferred prey (Cushing 1990). Aspects of condition at the juvenile stage, such as growth rates, have also been linked to recruitment: faster growth in juvenile brook trout (*Salvelinus fontinalis*) has been found to result in earlier reproduction, which is thought to confer increased fitness to those individuals (Hutchings 1993). Knowledge of condition can thus be useful to fisheries scientists; however, for these studies to yield meaningful results, researchers must ensure that condition is accurately interpreted based on the question asked.

Condition is typically reported in terms of various metrics, or condition factors. For example, measurements of length and weight can be used to calculate proxies of morphometry. The earliest example of this is Fulton's K. First proposed by Thomas Fulton in 1904, it states that condition, denoted by K, is equal to the weight of the fish divided by the cube of its length (Ricker 1975). Length-weight regressions are also used to estimate condition in a more descriptive fashion without assuming isometric growth

(Bolger & Connolly 1989). In addition to morphometry, the growth of a fish in early life stages can be used to describe condition. Higher growth rates are considered indicative of better condition under the assumption that fish that grow faster are more likely to survive and reproduce than individuals that grow more slowly (Peterson & Wroblewski 1984, Hutchings 1993). In larvae and juveniles, otoliths grow at a rate closely approximating somatic growth rates, and as such, otolith increment widths can be counted and measured to approximate daily growth (Panella 1971, Campana & Neilson 1985). The ratio of RNA to DNA can also be used to estimate growth rate (Bulow 1970, Buckley et al. 2008). Additionally, lipids and fatty acids can be studied to understand the condition of fish, wherein higher lipid content suggests increased internal energy stores and higher nutritional content (e.g. Iverson et al. 2002, Lane et al. 2010).

3.1.2. Combining metrics of condition

Scoring highly for any one of these condition factors is generally interpreted to mean that a fish is in good condition. The use of multiple condition metrics in a single study can provide a more complete picture by considering different physical or biological aspects of condition occurring across multiple scales. However, if these metrics are not correlated with each other, concluding that a fish is in good condition based on its high score for one metric would not necessarily mean that it is in good condition with regard to other aspects of condition. In order to ensure accurate interpretation of condition, it is therefore important for fisheries scientists to understand how different aspects of condition relate to each other.

Various laboratory studies have been conducted in an attempt to intercalibrate various condition metrics. For instance, the relationship between morphometry and lipid content in Atlantic Salmon (*Salmo salar*) has been found to be so well correlated that it has been suggested that morphometric measurements can be used as a substitute for lipid content (Herbinger & Friars 1991). Metrics are not always correlated this neatly. For example, there is some debate in the literature about the strength of the relationship between Fulton's K and total lipids in other fish species (Davidson & Marshall 2010, Mozsár et al. 2015). RNA:DNA ratios have been compared to otolith microstructure in the same individual fish, as both can be used as proxies of growth. While there is some degree of coupling between these metrics in young larvae (Clemmesen & Doan 1996, Denis et al. 2017), this relationship often breaks down with age (Gilliers et al. 2004, Denis et al. 2017). Other studies have attempted to compare RNA:DNA to lipids in various species of small pelagic fish (Frommel & Clemmesen 2009, Peters et al. 2015). Peters et al. (2015) noted that internal energy reserves (lipids) and growth (RNA:DNA) uncouple towards the end of the larval stage in sprat (*Sprattus sprattus*), while Frommel and Clemmesen (2009) observed a similar decoupling in juvenile gobies (*Gobiusculus flavescens*), though neither study performed the two measurements on the same individuals. This phenomenon of condition metrics correlating in younger fish but uncoupling with age has been attributed to the observed difference in response rate to starvation in early larvae compared to older juveniles, where early larvae are more likely to experience negative effects of starvation more quickly (Selleslagh & Amara 2012, Peck et al. 2015).

In addition to the aforementioned variability in biological responses of fish, some condition metrics may not be strongly correlated due to inherent differences in the sensitivity of the metrics themselves (i.e. the magnitude of change at which a metric will indicate that condition has increased or decreased). Many field studies employ some measure of morphometry along with metrics of growth and nutrition, such as studies on the influence of habitat quality on condition (Gilliers et al. 2004, 2006, De Raedemaecker et al. 2012, Ciotti et al. 2013), or responses to contamination levels (Henry et al. 2012, Kerambrun et al. 2012). Otolith growth and RNA:DNA ratios have been shown to provide different perspectives on fish condition when compared to Fulton's K in these studies (Gilliers et al. 2006, De Raedemaecker et al. 2012, Ciotti et al. 2013). This was thought to have been caused by the decreased sensitivity of Fulton's K compared to otolith growth and RNA:DNA, meaning that otolith growth and RNA:DNA will reflect less extreme changes over a smaller period of time (Gilliers et al. 2006, De Raedemaecker et al. 2012, Ciotti et al. 2013).

Combining multiple metrics on the same set of individuals can thus provide a more complete picture of condition. However, it is also costly and time-consuming, and other than a few recent studies on juvenile sea bass (*Dicentrarchus labrax*) (Kerambrun et al. 2012), juvenile Sockeye salmon (*Onchoryncus nerka*) (Duguid et al. 2018), and larval Down's herring (*Clupea harengus*) (Denis et al. 2017), most studies have opted for no more than two or three metrics at a time on an individual fish. Consequently, the extent to which multiple metrics are correlated in an individual juvenile fish has not been

rigorously tested, and thus the degree to which any one metric can be used to predict the outcome of another in an individual juvenile fish is therefore not yet fully understood.

3.1.3. Objectives of this study

This study examined the degree of correlation among six different metrics of condition within individual young-of-year (YOY) Pacific herring from the Strait of Georgia. The six metrics used were (i) Fulton's K, (ii) the residuals from a length:weight regression, recent growth via (iii) the RNA:DNA ratio and (iv) otolith microstructure analysis, (v) lipid content, and (vi) the ratio of DHA:EPA. We began with the assumption that a high score in any one of these six metrics should also mean high scores across the other five metrics, too. While this is typically not the case in most studies, including the results shown in Chapter 2, there has not yet been a study that has explicitly quantified the degree of intercorrelation among metrics within individuals. Previous work suggests that this hypothesis will break down at some point, whether due to the age of the fish (e.g. Clemmesen & Doan 1996, Peters et al. 2013), the sensitivity of the different metrics (e.g. De Raedemaecker et al. 2012, Ciotti et al. 2013), or other causes. This study aimed to improve understanding of how various aspects of growth and condition are interconnected by determining how this assumption loses validity.

In Chapter 2 this dataset was used to quantify interannual variability in YOY herring condition, with conflicting results depending on the metric of condition used (Chapter 2). Here, the dataset was used to examine the relationships between condition factors applied to the same individual fish by attempting to answer two related questions:

(1) Do individuals that score high (or low) for one condition metric also score high (or low) for the other five metrics?

(2) How strongly are these metrics correlated to each other when studied in pairs?

3.2. Methods

3.2.1. Data collection and laboratory analysis

Laboratory analyses were conducted on YOY herring from Fall 2013-2016 (Table 3.1) to measure Fulton's K (FK hereafter), length:weight residuals (LW hereafter), RNA:DNA ratios as indicators of growth (G*i* hereafter), otolith growth rate from increment widths (OGR hereafter), percent lipid per gram of muscle tissue (TL hereafter) and the ratio of the fatty acids docosahexaenoic and eicosapentaenoic acid (DHA:EPA hereafter). See Chapter 2 for detailed account of sample collection and laboratory methods. Note that in this dataset, length:weight residuals were calculated on lengths and weights separated by season.

Table 3.1. Number of herring in each measurement group for all six metrics

	Fall 2013	Fall 2014	Fall 2015	Fall 2016
FK	80	38	16	34
LW	80	38	17	34
Gi	80	34	17	6
OGR	66	33	14	31
TL	39	36	17	25
DHA:EPA	39	36	16	23

3.2.2 Intercorrelation among condition metrics: A Cumulative Distribution Function (CDF) approach

For each of the six condition metrics, each individual herring was ranked on a scale from lowest to highest according to the relative magnitude of the metric's value. Individuals were excluded if they were missing values for any of the six metrics, leaving 84 herring

to be included in the final ranking analysis. The individual with the highest FK value (for example) would be given the score of 84 for that metric, while the individual with the lowest FK value would be ranked number one. The rank scores for each individual for all six metrics were then summed to give an overall value (termed "Aggregate rank condition," ARC value hereafter) for that individual. A higher ARC value thus indicates better overall condition relative to all other individuals. The 84 herring were then sorted by their ARC value, and new ID numbers from 1-84 (i.e. lowest ARC value to highest ARC value) were assigned to each fish ("Rank ID" hereafter).

The plot of ARC values and their respective Rank IDs takes the form of a cumulative distribution function (CDF). CDF plots describe the probability that a variable X will be equal or less than a variable Y, and are typically read using percentiles (Downey 2014). In normally distributed datasets, the rarer values at each end of the X-axis are seemingly flat, and the most common values appear as a steep vertical section in the middle of the X-axis, giving the graph a sigmoidal shape. In a dataset where there is uniform distribution of percentiles, data is plotted in a linear fashion (Downey 2014).

In this dataset, Rank IDs were assigned based on the ARC value: the CDF therefore describes the probability of a particular Rank ID being assigned to a specific ARC value. If the six condition metrics were highly inter-correlated, individuals should have maintained their rank order across all six metrics (e.g., the top-ranked individual for FK would receive an 84, and should also score 84 for the other five metrics). In a perfectly inter-correlated scenario, we would predict that a plot of ARC value versus Rank ID

would therefore fall along a linear path, since the rankings for each of the six metrics would be in a similar range and there would be an equal probability of finding an ARC value at any point in the range (Figure 3.1, blue line). If the condition values were not related to each other, however, the six rankings making up each ARC would no longer be within a constrained range and the probability of either very low or very high ARC values would decrease. We would therefore see sigmoidal curves representing a normal distribution of ranks across the six metrics (Figure 3.1, grey region). Slopes of the lines representing different scenarios can be quantified to compare different scenarios. All data analysis was performed using R studio (R Core Team 2015) and ggplot2 for R (Wickham 2009).

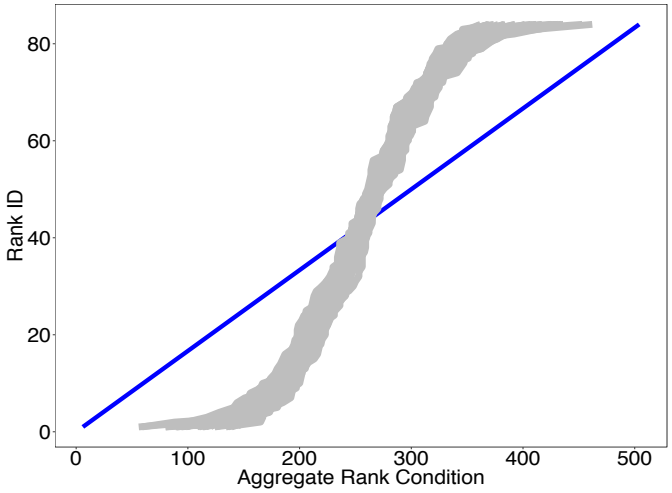

Figure 3.1. Theoretical cumulative distribution function model of herring condition across six metrics. The individual rank in all six metrics is compared to the ARC values across all metrics. If condition in each metric is very strongly related a linear line would be expected (blue). The grey region indicates the range of values from generating random rank orders and summing them in groups of six in order to simulate ARC values where condition metrics are not inter-correlated.

3.2.3. Pairwise correlations between condition metrics: Spearman rank correlations

In order to further explore relationships between condition metrics, pairs of metrics were examined using pairwise correlations. Though both linear regressions and Spearman rank

correlations were applied, Spearman's Rho was deemed the most appropriate test due to its comparison of ranks rather than raw values (Whitlock & Schluter 2009). FK and LW were both metrics of morphometry incorporating length and weight. G*i* and OGR both served as proxies of growth, and TL and DHA:EPA provided measures of lipids in a fish. Spearman rank correlations were calculated on datasets separated by year using rank scores from the same individuals in a pairwise manner (i.e., two metrics at a time). This was done in order to answer 3 questions:

(1) Are faster-growing individuals also more robust in size? This was examined by comparing ranked G*i* and OGR scores with ranked scores from FK and LW. Ranked values from the four years were tested in four possible combinations.

(2) Are individuals that are morphologically the most "robust" also in the best condition in terms of lipids? This was examined by comparing ranked FK and LW scores with ranked TL and DHA:EPA scores.

(3) Are the fastest-growing individuals also in the best condition in terms of lipids? This was examined by comparing ranked TL and DHA:EPA scores with ranked G*i* and OGR scores.

Pairs of condition factors were compared with all fall values pooled together across the four years, as well as individually by year, which totaled 75 possible combinations. A Bonferroni correction was therefore applied by multiplying the p-values *post hoc* by the number of combinations, i.e. 75, to reduce type I errors (Whitlock & Schluter 2009).

Correlations were also re-run after standardizing the data such that each metric had a mean of 0 and a standard deviation of 1, in order to determine whether additional correlations might be evident when the difference in variability between metrics is removed.

3.3. Results

3.3.1. Intercorrelation of metrics using a cumulative distribution function

ARC values (i.e. an individual's summed rankings across all six condition metrics) followed sigmoidal patterns when plotted against Rank IDs in both the overall cumulative distribution model or as separate cumulative distribution models for each of the four years of the study period (Figure 3.2). Standardizing the raw condition metric values to a mean of 0 and a standard deviation of 1 before ranking them had no effect on the trend, and thus these values are not shown.

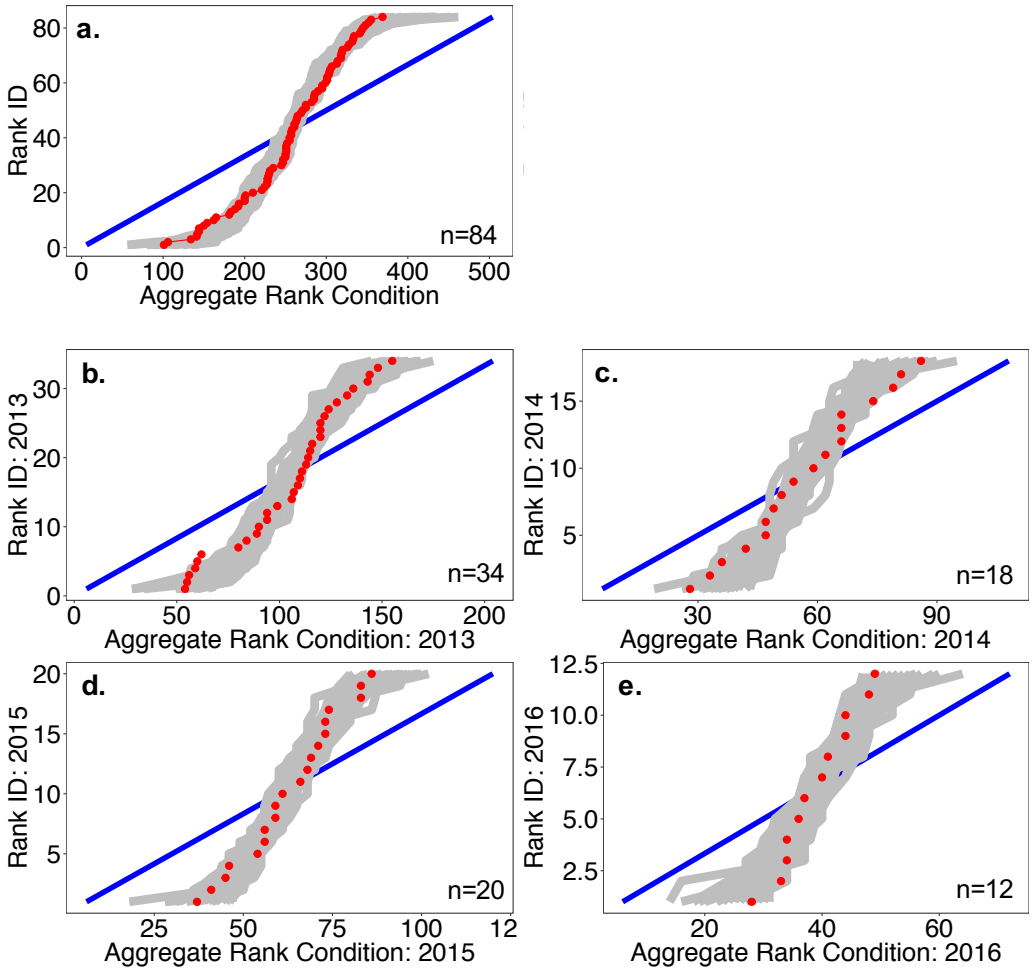

Figure 3.2. CDF models of condition metric rankings. Here, the individual rank in all six metrics is compared to the sum total of rankings across all metrics (FK, LW, G*i*, OGR, TL and DHA:EPA). YOY herring condition is pooled across years (panel a) as well as separated in four different years (panels b-e). Blue lines represent hypothetical values in perfect order. Thick grey lines represent random bootstrapped values, while the actual values are shown as red circles. In all four years, actual condition metric rankings are sigmoidal in shape, and are more similar to bootstrapped random values than the perfect order lines

3.3.2. Pairwise spearman correlations between metrics

The results of the Spearman rank correlations are reported below. Only significant results are listed here ($p<0.05$); see appendix for complete list of p-values, and Spearman rho coefficients (Appendix B1).

FK and LW were used to represent morphology. They were significantly positively correlated in all four years of the study (2013: $p<0.01$, rho = 0.98; 2014: $p<0.01$, rho = 0.87; 2015: $p<0.01$, rho = 0.98; 2016: $p<0.01$, rho = 0.61) (Figure 3.3). This pair of metrics was the most highly correlated pair in the study, though the correlation was slightly weaker in 2016 than in other years (Figure 3.3). TL and DHA:EPA both provided information about lipids in an individual. They were significantly negatively correlated in two of four years (2013: $p<0.01$, rho = -0.66; 2016: $p<0.01$, rho = 0.85) (Figure 3.4). The only other significant correlation from separate years was between FK and TL, which were significantly positively correlated in 2016 only ($p<0.01$, rho = 0.68) (Figure 3.5). Three sets of metrics pooled across years were correlated: FK and LW ($p<0.01$, rho=0.86), TL and DHA:EPA ($p<0.01$,rho=-0.52), and FK and DHA:EPA ($p=0.01$,rho=-0.35) (Figure 3.6).

After standardizing values (such that each metric had a mean of 0 and a standard deviation of 1), the following pairs were significantly correlated when data were pooled across years: G*i* and FK ($p=0.054$, rho=0.18), FK and DHA:EPA ($p=0.002$, rho=-0.27), LW and DHA:EPA ($p=0.02$, rho=-0.20) (Figure 3.7). When separated by years (i.e. rather

than being pooled) standardization had no effect on the results, and as such, values are not reported here.

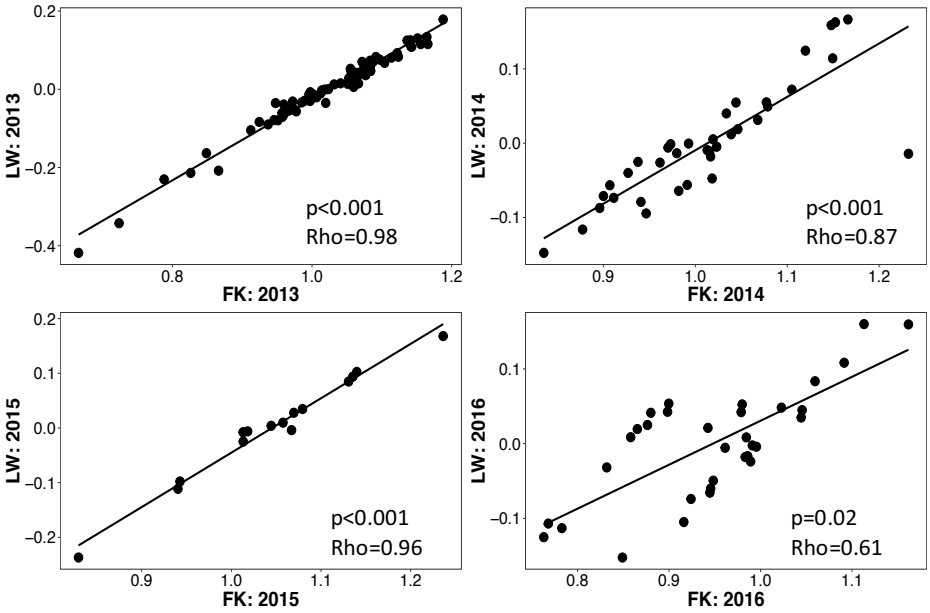

Figure 3.3. Pairwise comparisons between LW and FK, two morphometry-based condition metrics, in four separate years. For figures 3.3-3.7, Spearman rank coefficient is reported, and black lines denote line of best fit and are used only when Spearman correlations are significant for illustrative purpose.

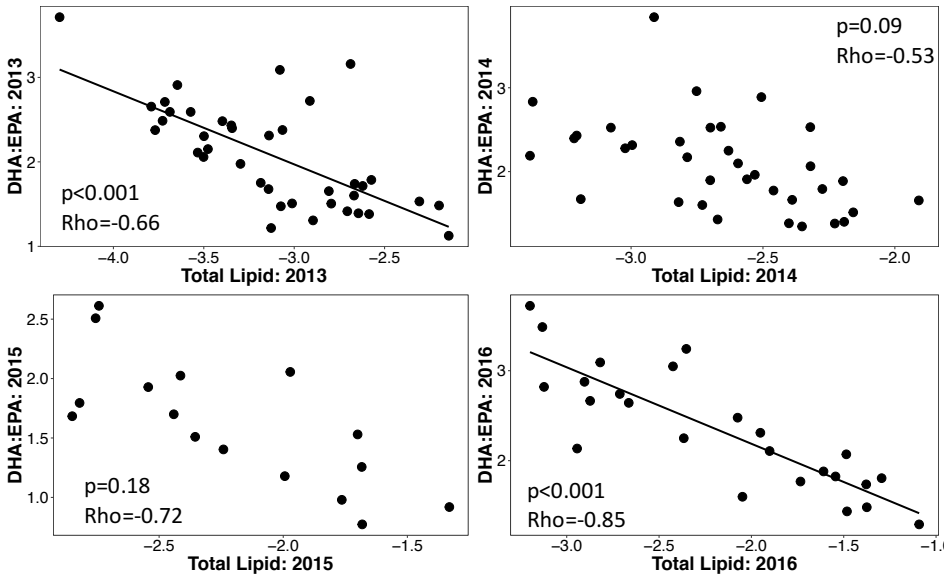

Figure 3.4. Pairwise comparisons between total lipid (mg lipid per gram of dry white muscle tissue, log scale) and DHA:EPA, two lipid-based condition metrics, in four separate years.

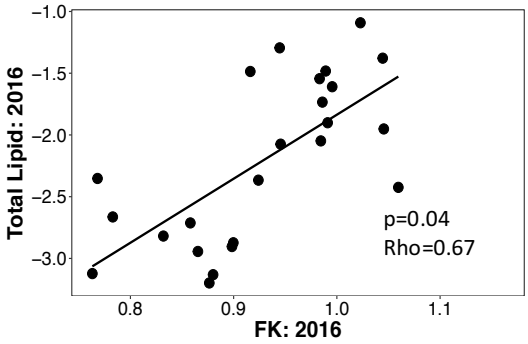

Figure 3.5. Pairwise comparison between Fulton's K and Total Lipids in 2016.

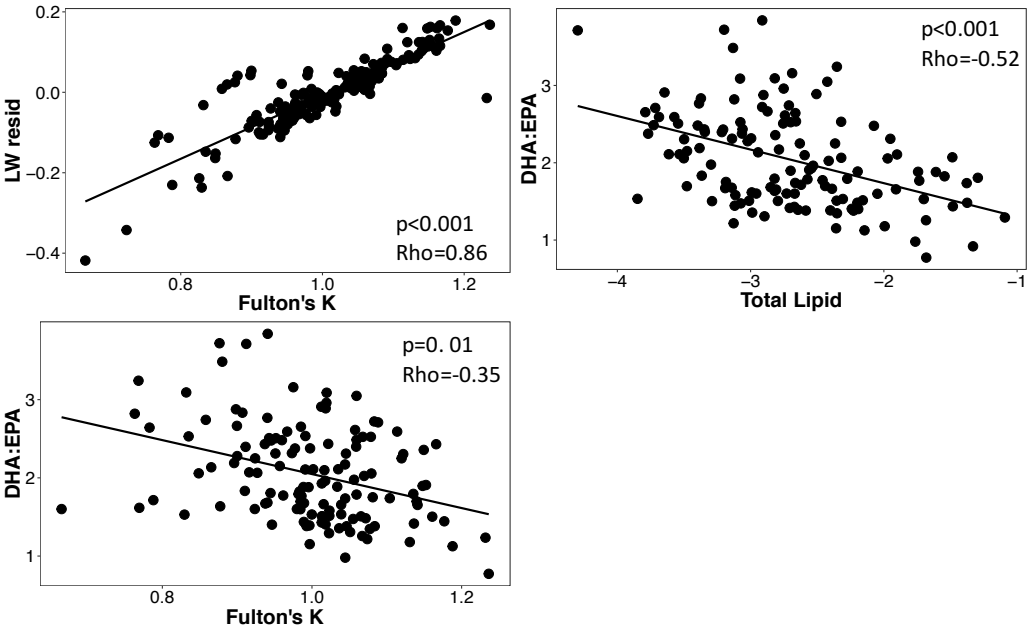

Figure 3.6. Significant pairwise comparisons applied to pooled datasets. LW and FK, TL and DHA:EPA, and DHA:EPA and Fulton's K were significantly correlated.

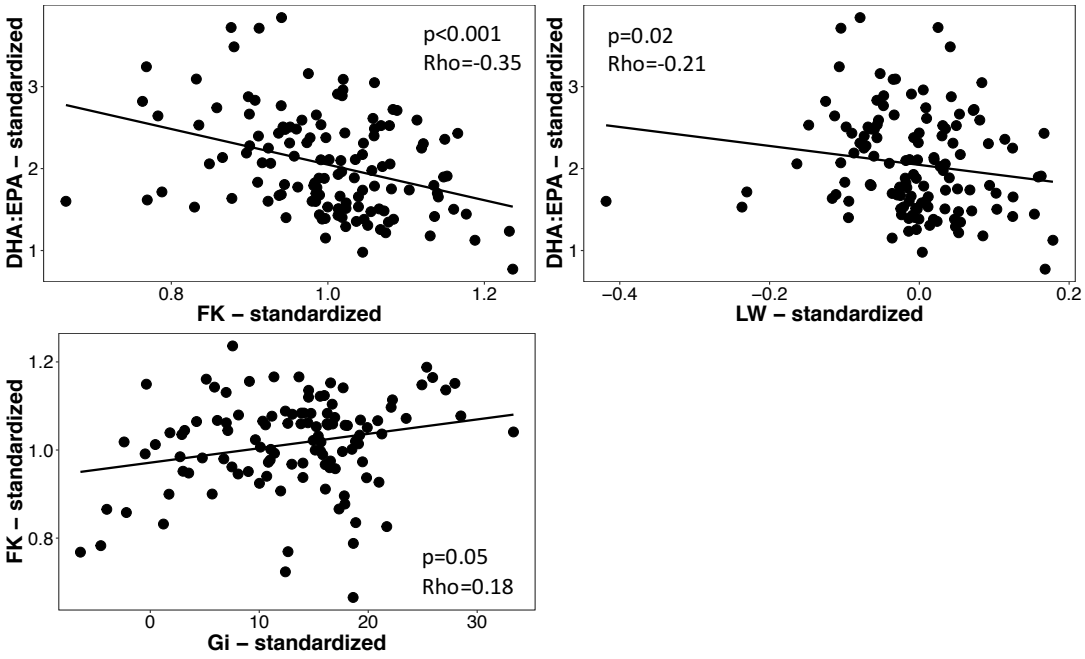

Figure 3.7. Correlated metrics after values are standardized such that the mean of each column is 0 and the standard deviation is 1.

3.4. Discussion

This study compared the degree of inter-correlation among six condition metrics within the same individuals. This method allowed us to determine whether a fish deemed to be in good (or poor) condition using any one metric was also in good (or poor) condition in the other five metrics. The results were analyzed by comparing all six condition metrics together, and then by subsequently splitting them into pairs for a more in-depth examination.

3.4.1. Overall correlation between the six condition metrics

This study began with the basic assumption that any one condition metric should provide the same information about the relative condition of an individual fish (i.e. relative to the condition of other fish in the same population) as any other metric. However, as shown in Figure 3.2, the ARC-Rank ID values followed a trend more similar to patterns produced by the bootstrapped values than those produced by values in a perfect order. These data therefore did not support the hypothesis that a fish should be in essentially the same rank position across all six metrics of condition. Standardizing the values to a mean of 0 and a standard deviation of 1 did not alter this result, suggesting that there was no effect from a difference in variation.

Ferron and Leggett (1994) specified different levels at which condition can be measured in fish, such as morphological, physiological, and biochemical. In the current study, Fulton's K and LW were considered metrics of condition at the morphological level, otoliths and lipids can be classified physiological components at the tissue level of organization, while changes in RNA:DNA take place at a biochemical scale (Ferron &

Leggett 1994). Davidson and Marshall (2010) showed that measuring both Fulton's K and total lipids will not always lead to the same conclusions about the condition of fish, and argued that whereas Fulton's K takes the entire body morphology into account, total lipids measure a more fine-scale process. Frommel and Clemmesen (2009) attempted to intercalibrate RNA:DNA and lipids in juvenile gobies and speculated that the observed lack of correlation was due to the different biological processes measured by each metric: lipids being related to weight-based growth and RNA:DNA being more related to protein-based growth.

Further to Ferron and Leggett's (1994) classification of different condition metrics operating at different organizational levels, Denis et al. (2017) noted that differences in integration times had an effect in their study of four metrics of condition on larval Down's herring. In this study, G*i* measures condition with the shortest integration timing (near-instantaneous), while FK and LW incorporate change over the longest period of time. Differences in integration time make it possible for some measures of condition to be more sensitive than others; for instance, it has been demonstrated that RNA:DNA-based growth responds much more quickly to changes in fish condition than does FK (Gilliers et al. 2004, De Raedemaecker et al. 2012, Ciotti et al. 2013). This was inferred from the increased spatiotemporal variability in RNA:DNA compared to FK in a study on habitat quality of plaice and dab, suggesting that RNA:DNA ratios are more sensitive to changes at a smaller scale (De Raedemaecker et al. 2012). While the current study did not take spatial variability into account, interannual variability in RNA:DNA was indeed

higher than Fulton's K (Chapter 2), which may have been related to this difference in integration time and therefore sensitivity.

The concept of integration time can be applied to help understand the limited relationship between the six conditions metrics used in this study based on the results from the CDF. RNA and DNA turn over on timescales of about 4-5 days, and the G*i* index incorporating temperature-at-capture represents a near-instantaneous growth rate at the time of capture (Buckley 1984). In contrast, the OGR condition metric used in this study is based on the 10 most recent daily growth increments in the herring otoliths. Accounting for the effect of a lag in response to changing environmental conditions (Fey 2005), OGR therefore likely integrated herring condition over an approximately two week period. Timing of lipid turnover can change depending on temperature and type of body tissue (Pethybridge et al. 2014). For example, some studies have found that shifts in fatty acid profiles due to dietary changes occur on the order of six weeks (Kirsch et al. 1998, Litz et al. 2017).

The six condition factors included in the CDF model therefore represent changes in condition at various organizational levels in the fish. In addition to this, temporal differences in turnover leading to variable sensitivity in different metrics may contribute to the lack of support for a linear CDF model (Figure 3.2). Our initial assumption that a fish scoring highly in one metric should also score highly across all metrics is therefore not valid. Dividing the metrics into specific pairs may provide further insight into how this assumption breaks down.

3.4.2. Pairwise correlations between metrics representing similar aspects of condition

The six metrics were grouped into three categories based on the information that each provided about condition: morphology (FK and LW), nutrition (TL and DHA:EPA), and growth (G*i* and OGR). FK and LW were the most tightly correlated pair in the study (Figure 3.3). Both metrics are essentially mathematical formulations of variations in length and weight, so a strong positive correlation was to be expected. The presence of some scatter, particularly in in 2016, may be explained by the slight differences in output between the two metrics, where FK assumes isometric growth while LW allows for allometric growth (Figure 3.3).

TL and DHA:EPA both measure similar physiological aspects of the herring, since lipids are partially composed of fatty acids. They were significantly correlated as well (Figure 3.4), although the correlation was negative, and less strong than the correlation between FK and LW (Figure 3.3). A significant negative correlation between DHA and TL was also observed by Litz et al. (2010) in their study of various forage fish including Pacific herring, Northern anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), and whitebait smelt (*Allosmerus elongatus*), and was thought to be related to variability in the composition of phytoplankton blooms. Diatoms are richer in EPA than DHA, and seasons with fattier fish also had more DHA in the food web (Litz et al. 2010). Further analysis of fatty acids present in the SoG food webs during 2013-2016 would be needed to confirm whether that explanation also applies to this study.

Interestingly, unlike the other two pairs representing similar aspects of condition, G*i* and OGR (both representing growth) were not significantly correlated. Among the three pairs of metrics, however, G*i* and OGR are the least comparable in the way that they are used to measure condition. This is based on differences in both the characteristic timescales over which they integrate growth (instantaneous vs. 10-12 days) as well as the organizational levels on which they measure change (i.e. biochemical vs. physiological). There has been some debate in the literature regarding correlation between RNA:DNA and otolith growth. Clemmesen and Doan (1996) were the first to measure these two condition metrics on the same individual, and found that they correlated well in larval cod (*Gadus morhua*). However a recent study by Peck et al. (2015) applied RNA:DNA and otolith growth metrics on juvenile sprat (*Sprattus sprattus*), and found that changes in otolith growth did not correspond to changes in RNA:DNA. They attributed this to the different timescales over which the two metrics integrate (Peck et al. 2015).

3.4.3. Pairwise correlations between Fulton's K and Total Lipids

When the herring were separated by years, only one pair of metrics was significantly correlated: Fulton's K showed a significant positive correlation to total lipids in 2016 only (Figure 3.5). Of the four years in this study, percent total lipids in muscle tissue was highest in 2016 and lowest in 2013 (Chapter 2, this volume). As discussed earlier, Fulton's K has been found to be strongly related to total lipid content in fish, and was subsequently designated a proxy of energy reserves by Herbinger & Friars (1991). However, Davidson and Marshall (2010) concluded that Fulton's K should not be considered a predictor of total lipids, since it cannot properly separate other contributors to a fish's weight, such as its internal organs. Mozsár et al. (2015) further describe this

variability in the relationship between FK and TL. Their study found correlation between FK and TL only when initial lipid content was quite high, speculating that more extreme changes in length and weight would not be possible with lower lipid content (Mozsár et al. 2015). It is therefore possible that in the YOY herring used in this study, FK is a reliable predictor of TL only when lipids are high in general.

3.4.4. Correlations using a pooled dataset

Correlation among metrics was also tested on values pooled across years. Initial linear regression tests before the Spearman rho tests were calculated showed that pooling the fish often resulted in a statistically significant pair with an R^2 value below 0.15. That is, the two metrics were related statistically but did not explain very much of the variance between each other. The combination of low p-value accompanied by low R^2 was not observed when the pool of samples was separated by year, which suggests that grouping all the herring together regardless of year collected may have introduced a large amount of variance that obscured relevant results. Thus, correlations were tested on datasets separated by year in addition to the pooled dataset.

After calculating Spearman rho coefficients and applying Bonferroni corrections, only 3 of the 15 pooled pairs returned significant results: FK and LW, TL and DHA:EPA, and FK and DHA:EPA (Figure 3.6). Both FK-LW and TL-DHA:EPA were also significantly correlated when separated by year (Figure 3.3, 3.4), so this finding did not contribute new information to this study. As a group, Fulton's K and DHA:EPA were negatively correlated (Figure 3.6), though none of the values from separate years showed any significant correlation. It could be that this is an artifact of the same extra variance that

resulted in pairs with low p-values and low R^2. However, morphometry and lipids have been demonstrated to be related both in previous studies (e.g. Davidson & Marshall 2010, Mozsár et al. 2015), as well as the significant positive relationship between Fulton's K and TL in this study (Figure 3.5). As previously mentioned, total lipids and DHA:EPA ratios have been found to be negatively correlated due to the increased nutritional quality provided by EPA-rich diatoms (Litz et al. 2010). Taken together, these two factors may have contributed to the overall negative relationship found between Fulton's K and DHA:EPA (Figure 3.6).

3.4.5. Pairwise correlations with marginal statistical significance

In addition to the pooled-dataset correlations, the other category of results not reported in the results section were the various combinations of metrics which achieved some marginal statistical significance (i.e. they returned a p-value less than 0.05 before Bonferroni corrections were applied). Bonferroni corrections are used to reduce the rejection of a null hypothesis that is in fact true, however applying the corrections to a set of p-values is a conservative measure that may result in a false conclusion that a pair of values are not significantly correlated (Whitlock & Schluter 2009). To avoid this, it is advisable to examine the results of correlations before they are corrected. In the current study, before applying the corrections, DHA:EPA was negatively correlated to LW in 2013 ($p=0.05$, rho=-0.32) and to FK in 2016 ($p<0.01$, rho= -0.61) (Figure 3.8). These correlations are supported by the negative correlation between TL and DHA:EPA (Figure 3.4), and the correlation between FK and DHA:EPA in 2016 likely contributed to the overall significant correlation between FK and DHA:EPA. However, low rho values suggested that these correlations were weak in terms of variance explained, and the

Bonferroni corrections suggested that they may have been an artifact of the high number of comparisons conducted.

Additionally, before Bonferroni corrections were applied, OGR was significantly correlated to the nutritional proxies in 2016: negatively with TL ($p<0.001$, rho=-0.59), and positively with DHA:EPA ($p=0.01$, rho=0.54). In contrast, G*i* was not correlated with DHA:EPA at all, and the correlation with TL was positive in 2013 ($p=0.03$, rho=0.37) and marginally negative in 2015 ($p=0.07$, rho=-0.69) (Figure 3.8). These results seem contradictory, suggesting that fast growth alternatively allows the inference of high lipids, inferences of low lipids, or no inferences at all. Variable results are also reported in the literature. Peters et al. (2015) noted a decoupling between lipids and growth as their larval sprat matured, while Frommel and Clemmesen (2009) reported a similar uncoupling in juvenile gobies. Whether by statistical artifact or biological decoupling, it is clear that proxies of growth (i.e. G*i* and OGR) do not provide the same information about condition as metrics of nutrition (i.e. TL and DHA:EPA).

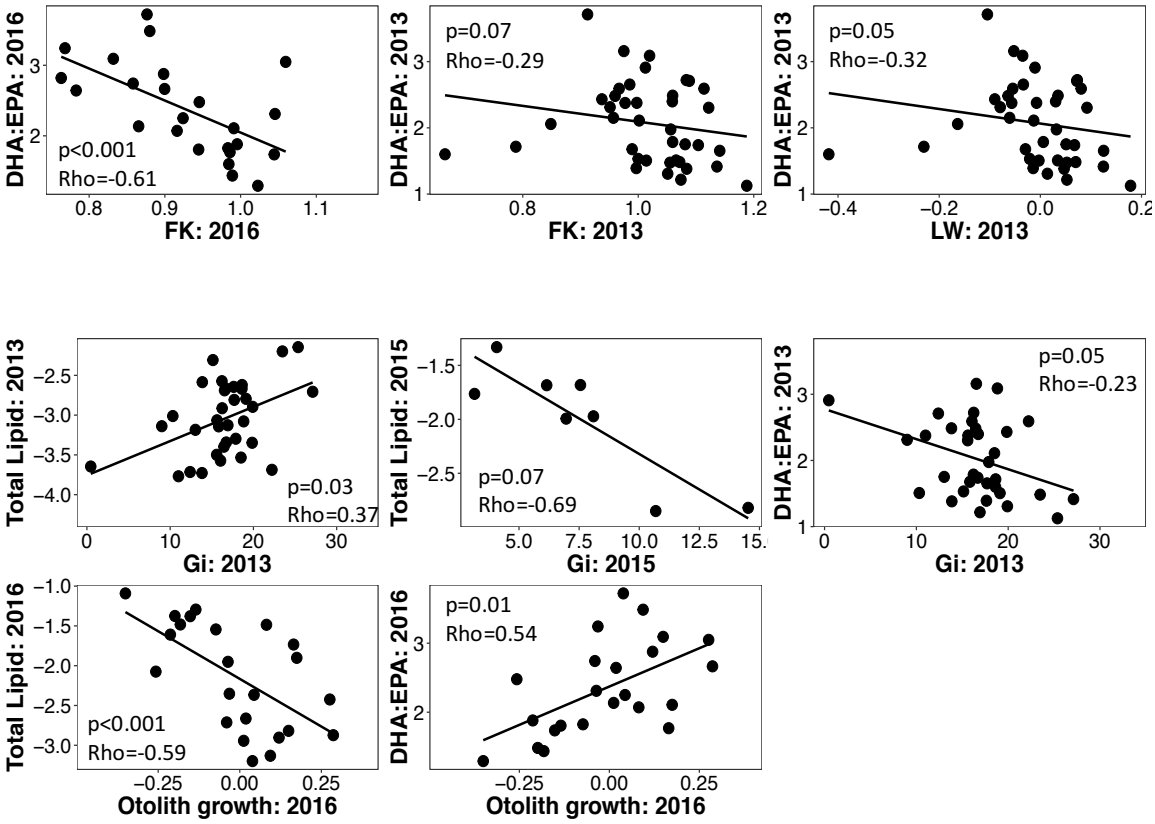

Figure 3.8. Pairs of condition factors that were marginally significantly correlated: i.e. returned a p-value less than 0.05 before Bonferroni corrections were applied, or returned a p-value between 0.07-0.05. Black lines are linear regressions

Finally, no significant correlations (marginal or otherwise) were found between any pair when metrics of condition representing morphology (FK and LW) were compared to the metrics representing growth (G*i* and OGR). This lack of correlation implies, for example, that we cannot say conclusively that a YOY herring with a high G*i* value is in good morphological condition, or that a fish with a high FK value must also have been a fast-growing individual. These two metrics incorporate change on different time scales, as previously discussed by Gilliers et al. (2004, 2006), De Raedemaecker et al. (2012), and Ciotti et al. (2013), who all noted the discrepancy in sensitivity between RNA:DNA and morphometric measures of condition. When values were standardized to a mean of 0,

pooled values of RD and FK became weakly correlated (Figure 3.7), suggesting that a difference in variability between these two metrics may have also contributed to the lowest correlation between them out of all the pairs in this study.

3.4.6. Implications for future use of condition metrics

This study used six metrics of condition to determine the extent to which one metric can be used to make inferences about condition in another metric. In general, the results from the individual Spearman rank correlations can be linked to the degree of similarity between the characteristic time-scales over which each metric integrates. The presence of this fundamental difference between metrics supports the lack of linearity in the CDF model, which showed that the YOY herring did not consistently rank in a similar position across metrics.

While some authors have noted that not all metrics should be considered equal (e.g. Gilliers et al. 2004, De Raedemaecker et al. 2012, Peters et al. 2015), there has not yet been a study comparing them using rankings, or with more than four metrics per individual. The findings of this study confirm that most condition metrics are not strongly intercorrelated. Therefore, a fish confirmed to be in high condition using a single metric cannot be assumed to also be in high condition in other respects. This has general implications for studies of condition in fish. For example, a study assessing the effects of habitat quality on condition would need to ensure that the chosen metric corresponds to the length of time that a fish spends in the given habitat. Similarly, studies on the condition of fish in terms of the nutritional value that they represent to their predators would be advised to examine total lipids, rather than relying on a morphometry-based

measure condition. Finally, these results also demonstrate that rather than studying "condition" as a general concept, it is important to clarify the aspect of condition of interest in a particular study, since one aspect of condition cannot reliably be used to predict another.

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4. Synthesis and suggestions for future research

4.1. Main findings

This thesis was undertaken to quantify variability in condition in YOY Pacific herring. At the interannual scale, four condition metrics (Fulton's K, length:weight residuals, and growth rates estimated from RNA:DNA and otolith increments) showed that condition was lowest in 2016 compared to the other three years, while total lipids were highest in 2015 and 2016. At the individual level, the distribution of metrics as plotted in a cumulative distribution function showed that, in general, the six metrics were not strongly intercorrelated, meaning that a high score in any one metric did not necessarily correspond to high scores in the other metrics. There were, however, some exceptions: proxies of morphometry were significantly correlated with each other, as were proxies that use lipids to represent nutrition. There was some evidence that morphometric measures correlate to nutritional proxies when lipid content was high, but in general, metrics that represent different aspects of condition were not strongly correlated with each other.

4.2. Implications in the context of the Salish Sea Marine Survival Project (SSMSP)

Part of the SSMSP research plan considered whether prey quality, particularly that of YOY herring, could affect the growth and survival of Pacific salmon. The hypothesis in question stated that the availability of higher quality prey would increase the growth and ocean survival rates of Pacific salmon, specifically Coho (*Onchoryncus kisutch*) and Chinook (*Onchoryncus tshawytscha*), whose numbers have been in decline (PSF 2015). One of the objectives of this thesis was therefore to examine nutritional condition of

YOY herring in the form of lipid content and fatty acid profiles to explore one element of this hypothesis. Lipid content (gram of lipid per gram of dry muscle tissue) in YOY herring from the Strait of Georgia was found to have increased from approximately 5% in 2013 to 20% in 2016. Fatty acid profiles did not strongly differ over the four years, with the exception of a decrease in PUFAs in 2015 (Chapter 2). It has been suggested that Coho and Chinook require a diet containing 15%-20% lipids (dry weight) for optimal growth (Higgs et al 1995, Nomura et al. 2004). The results of this study may therefore suggest that YOY Pacific herring represented better quality prey for Pacific salmon in the SoG in 2016 compared to the other years. Further investigation would be required to determine whether the lipid levels in 2013 and 2014 were sufficiently low to affect the health of the salmon. Regardless, the tendency towards interannual variability in lipid content, fatty acid profiles, and other aspects of condition in YOY herring suggests that they should continue to be monitored as potential drivers of growth and survival rates in Pacific salmon.

4.3. Implications for future studies of fish condition: Choosing condition metrics

Having measured six condition metrics on the same set of individuals, it seems reasonable to ask whether any one metric can be considered as "the best." The results of this thesis suggest that there is no singular metric that can provide all of the information necessary for an accurate interpretation of condition in every situation, and that each metric has its own merits and drawbacks to be considered.

Morphometric condition metrics are quick and cost-effective, and are therefore useful for a good first look at condition. They can provide similar information to growth metrics, although with less sensitivity to changes in condition on finer physiological or temporal scales. For example, FK, LW, OGR, and G*i* all followed a similar trend for herring condition from 2013-2016. All three metrics suggest that condition decreased in 2016, and OGR and G*i* suggest a slight decrease in condition in 2015 as well, likely because they detected more subtle changes in condition. In studies of the response of fish condition to changes in habitat quality, FK, otoliths, and RNA:DNA also provided similar estimates of condition, while RNA:DNA-based indicators were found to be the most sensitive to short-term change (De Raedemaecker et al. 2012, Henry et al. 2012). While this relationship can be observed when applied to a subsample of a population, morphometric measures and growth indices were found to be poorly correlated within individuals in this study. These results therefore suggest that inferences about the growth of fish should not be based solely on metrics of morphometry.

Both otolith microstructure and RNA:DNA are longstanding methods for estimating growth in fish, and were effective in this study. However, results from OGR and G*i* measurement were not significantly correlated to each other, which may have been due to differences in the relevant timescales over which the two metrics integrate. Selecting a metric of growth to infer condition should therefore be based on the timescale of interest in any particular study: RNA:DNA can be used to calculated G*i*, instantaneous growth, whereas the method of OGR used in this study, including the most recent ten increments, likely incorporated change over the two weeks before capture (Fey 2005). Researchers

wanting to study growth patterns over a longer time period would therefore be advised to find an alternate condition metric.

There has previously been debate in the literature over the utility of Fulton's K or other morphometric measures for inferring total lipids (Herbinger & Friars 1991, Davidson & Marshall 2010, Mozsár et al. 2015). In this study, Fulton's K was only significantly correlated to lipid content in one of the four years. This happened to be the year that total lipids were at their highest, suggesting that Fulton's K can be used to reliably infer total lipids only when lipid content is high. The variability in this relationship implies that researchers studying lipid content in fish should choose a metric that directly quantifies lipid content, rather than relying on inferences from morphometric measures. Further to this, lipid content by weight in other studies on this subject ranges from 5-10% (Herbinger & Friars 1991) to up to 40% (Davidson & Marshall 2010), but fish are never compared separately by lipid-content class. A laboratory study conducted on fish reared specifically to have varying levels of lipid content could be used to test whether the correlation between Fulton's K and total lipids is dependent on the initial percent lipids in the muscle tissue.

Finally, DHA:EPA was selected as an indicator in this study for its ability to provide information about the diet of the fish, and its relative importance in the developmental processes of young fish (Dalsgaard et al. 2003, Tocher 2003). It was therefore assumed that higher DHA:EPA ratios would indicate fish in good condition. However, comparing DHA:EPA ratios to the other metrics generally resulted in negative correlations (Figure

3.4, Figure 3.8). DHA:EPA has also been used as a proxy of carnivory in copepods by indicating that they have eaten more flagellates than diatoms (El-Sabaawi et al. 2009b), and has been assumed to be an indicator of condition in fish (Copeman et al. 2002, Jin et al. 2017). Thus far, however, DHA:EPA ratios have only been compared to RNA:DNA ratios, with the conclusion being that they do not correlate significantly (Xu et al. 2009). More empirical evidence will therefore need to be gathered before it can be definitively confirmed that a higher DHA:EPA ratio confers increased condition in fish.

Given that there is no singular condition metric representative of all aspects of condition, it is worth considering the continued use of the term "condition" as it applies to studies of fish. To date, "condition" has been used to describe various aspects relating to the physical health of fish; size, growth rate, and nutritional content all were discussed in this thesis. This study demonstrates how these attributes are not guaranteed to be correlated with each other within individuals, which raises the question of whether fisheries scientists should continue to group them together under the catch-all term "condition".

4.4. Caveats and limitations of the current study

In Chapter 2, I concluded that significant interannual variability in condition occurred in YOY Pacific herring from 2013-2016, and suggested that the observed changes may have been due partly to interannual variability in the zooplankton community in the SoG. The evidence in support of this claim was indirect, however. In short, YOY herring condition based on morphometry and growth was better in years when biomass anomalies for the copepods and krill (species which are believed to comprise a significant portion of their

diet - Foy & Norcross 1999), were positive. To better quantify the importance of this link, future studies on the drivers of interannual variability in herring condition should therefore also directly quantify the nutritional content of their zooplankton prey.

As explained in Chapter 1, the YOY herring used in this study were provided as "courtesy samples" collected as bycatch during DFO salmon surveys in the Strait of Georgia. Consequently, this study lacked the ability to impose any sort of spatial sampling structure. The main migratory stock of Pacific herring in the SoG has been shown to represent a single population based on the extent of spawning-site fidelity (Hay et al. 2001, Hay & McKinnell 2002), and is managed as such by Fisheries and Oceans Canada (e.g. Cleary & Taylor 2016). However, spatially targeted sampling of YOY herring via a standardized sampling design would have been preferable in order to better extrapolate these results to the whole SoG population. An example of this targeted sampling would be the recent study by Boldt et al. (2018) which linked herring condition in the SoG to density dependence, and found that length:weight residuals increased in 2016 (i.e. relative to the long-term trend in length:weight residuals), rather than decreasing as in this study. Their sampling takes place in September, and occurs mainly along the coasts, as opposed to the centre of the SoG (Boldt et al. 2017). An increased sample size in the current study, coupled with more targeted sampling, might have helped to reconcile this difference in results.

4.5. Other suggestions for future work

A key question that emerged from the study of interannual variability in YOY herring condition (Chapter 2) was why total lipids indicated a positive trend in condition while four other metrics indicated a negative trend. In previous studies on juvenile fish, trends in growth and lipids have also been found to oppose each other (Frommel & Clemmesen 2009, Peters et al. 2015). As such, I proposed that the decreased growth observed in 2016 as measured by both otoliths and RNA:DNA may have resulted as a consequence of the increased lipids, or vice versa. This reasoning implied that not only do the values of condition metrics change from year to year, the relationship between the metrics can also change. Further work would be needed to confirm this, and to understand the implications for studies of condition if, for example, somatic growth and lipid storage were to be more strongly negatively correlated in times of stress.

Finally, it would be interesting to track the future success of the cohorts of YOY herring that were examined in this study as they reach recruitment age and enter the spawning stock. Boldt et al. (2018) found that LW residuals did not strongly correlate to age-3 recruitment in Pacific herring, however other condition metrics have not yet been tested. DFO surveys indicate that spawning stock biomass of SoG Pacific herring has been increasing since 2016, the year that the 2013 cohort of YOY herring would have reached reproductive maturity. Herring from 2013-2015 were at similar condition levels based on the findings in Chapter 2; it was in 2016 where growth and morphometry were significantly lower from all of the previous years, and lipids were significantly higher than in 2013 and 2014. Estimating abundance of the new recruits to the spawning stock in

2019 will provide some insight into the survival of the 2016 cohort of juveniles, and the extent to which the observed patterns of interannual variability in YOY condition may have played a role.

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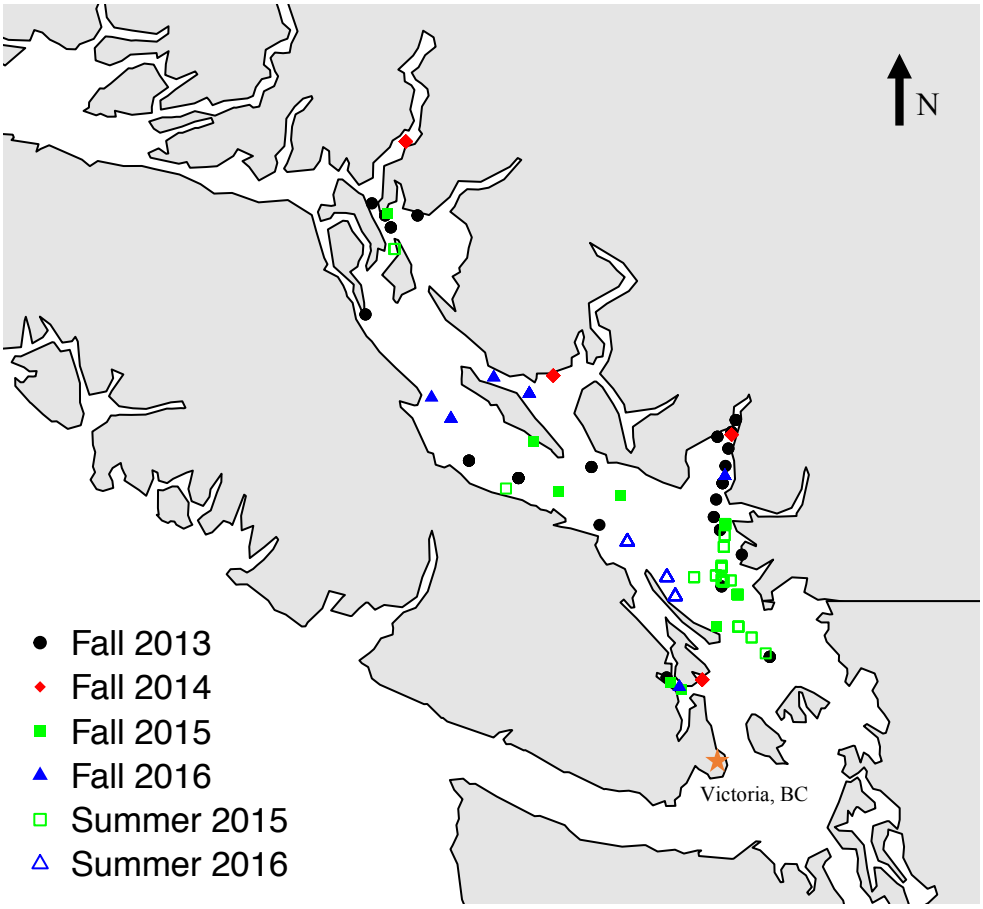
Appendix A



Appendix A1. Map of study area (Strait of Georgia, BC). Points denote location of herring samples collected by DFO Pacific Salmon Survey cruises.

Appendix A2. Table of herring with associated water temperatures (data from CTD cast averaged over the top 50m of the water column at time of capture) and RNA:DNA values used to calculated G*i*

Fish ID No.	Group	T (˚C)	RNA:DNA	Gi
337	Fall 2013	11.66619241	5.462287826	18.61542611
338	Fall 2013	11.66619241	6.112019819	21.70165308
340	Fall 2013	11.66619241	4.153464371	12.3985147
344	Fall 2013	11.66619241	3.436779244	8.99426035
358	Fall 2013	11.59804865	8.57346391	33.33013882
359	Fall 2013	11.59804865	5.203443235	17.32254061
360	Fall 2013	11.59804865	5.520292077	18.82757261
370	Fall 2013	11.66619241	4.824810191	15.58740735
375	Fall 2013	11.66619241	5.307714644	17.8812035
381	Fall 2013	11.97712675	3.809401597	11.05338546
382	Fall 2013	11.97712675	3.609501295	10.10385902
383	Fall 2013	11.97712675	4.814615525	15.82815162
384	Fall 2013	11.97712675	5.005813913	16.73634396
386	Fall 2013	11.97712675	3.654797488	10.31901594
390	Fall 2013	15.19936056	3.769883194	13.86235049
393	Fall 2013	15.19936056	3.077486692	10.57346711
396	Fall 2013	15.19936056	4.266098738	16.21937433
408	Fall 2013	11.66619241	4.750729962	15.23552626
410	Fall 2013	11.66619241	4.90598839	15.97300379
411	Fall 2013	11.66619241	4.511735716	14.10030359
412	Fall 2013	11.66619241	5.268900106	17.69683444
415	Fall 2013	11.66619241	5.057716416	16.69371192
418	Fall 2013	11.97712675	3.832119422	11.16129513
419	Fall 2013	11.97712675	4.902316494	16.24473122
420	Fall 2013	11.97712675	7.190896009	27.11548392
421	Fall 2013	11.97712675	7.368148891	27.95743511
422	Fall 2013	11.97712675	5.290310083	18.08770077
423	Fall 2013	11.97712675	4.091912041	12.39531007
424	Fall 2013	11.97712675	6.135799343	22.10377475
425	Fall 2013	11.97712675	4.586756474	14.74582112
438	Fall 2013	13.67636512	5.046126431	18.50812011
445	Fall 2013	13.67636512	6.490653217	25.36962234
448	Fall 2013	12.42276316	4.774288985	16.05104242
451	Fall 2013	12.42276316	4.05215072	12.62088566

452	Fall 2013	12.42276316	4.69295525	15.66470718
458	Fall 2013	12.50260576	5.571717782	19.91308282
459	Fall 2013	12.50260576	5.773787342	20.87291323
460	Fall 2013	12.50260576	4.624936282	15.4158707
462	Fall 2013	12.50260576	5.558246512	19.84909429
463	Fall 2013	12.50260576	4.943191409	16.92758255
464	Fall 2013	12.50260576	3.07566547	8.056834339
467	Fall 2013	12.42276316	5.318185733	18.63455197
471	Fall 2013	12.42276316	3.504036937	10.01734519
472	Fall 2013	12.42276316	4.969873715	16.98006989
475	Fall 2013	12.42276316	3.67593478	10.83385994
479	Fall 2013	12.42276316	4.860885676	16.4623767
482	Fall 2013	12.42276316	4.685069902	15.62725177
483	Fall 2013	12.42276316	4.138382415	13.03048621
484	Fall 2013	12.42276316	3.425155967	9.642660582
487	Fall 2013	11.97712675	3.871582686	11.34874564
492	Fall 2013	11.97712675	3.797980879	10.99913705
496	Fall 2013	11.97712675	6.938351766	25.91589877
501	Fall 2013	11.97712675	2.951212239	6.976986013
509	Fall 2013	12.50079088	5.440572455	19.28845468
511	Fall 2013	12.50079088	3.294917183	9.096592138
512	Fall 2013	12.50079088	1.477173785	0.462310997
513	Fall 2013	12.50079088	4.290394433	13.82510908
515	Fall 2013	12.50079088	6.323585015	23.48276434
516	Fall 2013	12.50079088	5.09219706	17.63367155
520	Fall 2013	12.50079088	2.273566708	4.245177381
524	Fall 2013	12.50079088	6.05846194	22.22342973
530	Fall 2013	12.42276316	4.129617988	12.98885518
534	Fall 2013	12.42276316	4.875984929	16.53409815
536	Fall 2013	12.42276316	4.829582018	16.31368432
544	Fall 2013	11.84981298	4.693931517	15.13650078
546	Fall 2013	11.84981298	2.747929858	5.892992897
552	Fall 2013	12.42276316	5.418084717	19.10907215
555	Fall 2013	12.42276316	2.137160099	3.524680213
1	Fall 2014	12.17984694	2.863150405	6.747222077
2	Fall 2014	12.17984694	4.499912564	14.52184233
3	Fall 2014	12.17984694	6.690795587	24.92853669
4	Fall 2014	12.17984694	3.841911857	11.39633897
5	Fall 2014	12.17984694	7.443020254	28.50160386

6	Fall 2014	12.17984694	2.94031804	7.113768343
7	Fall 2014	12.17984694	3.019830444	7.491452262
8	Fall 2014	12.17984694	4.926205877	16.54673557
9	Fall 2014	12.17984694	5.488198935	19.21620259
10	Fall 2014	12.17984694	4.315185491	13.64438874
11	Fall 2014	10.24342708	3.017399334	5.679034024
12	Fall 2014	10.24342708	5.922132864	19.47651829
13	Fall 2014	10.24342708	5.570107968	17.80440004
14	Fall 2014	10.24342708	4.770894233	14.00813479
15	Fall 2014	10.24342708	5.204513227	16.06782502
16	Fall 2014	10.24342708	5.581218183	17.85717356
17	Fall 2014	10.24342708	5.791182092	18.85450212
18	Fall 2014	10.24342708	6.240239832	20.98752639
19	Fall 2014	10.24342708	4.340823895	11.96530069
20	Fall 2014	10.24342708	4.771136278	14.00928451
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669	Fall 2014	10.09406875	1.775086197	-0.360856627
671	Fall 2014	10.09406875	2.856210328	4.774482996
672	Fall 2014	10.09406875	1.751629762	-0.472274693
99	Fall 2015	11.20555106	3.224012935	7.555223927
102	Fall 2015	11.20555106	3.883237878	10.68654241
104	Fall 2015	11.20555106	2.490745855	4.072205297
106	Fall 2015	11.20555106	2.296709842	3.150534235
148	Fall 2015	11.68179792	2.835292524	6.151711555
154	Fall 2015	11.68179792	4.59937641	14.53111001
156	Fall 2015	11.68179792	3.00561096	6.960724126
158	Fall 2015	11.68179792	3.244498273	8.095438862
288	Fall 2015	15.56090821	1.412506502	3.001050521
290	Fall 2015	15.56090821	1.390080305	2.894526085
291	Fall 2015	15.56090821	1.16039402	1.803516232
292	Fall 2015	15.56090821	1.356273788	2.73394513
296	Fall 2015	15.56090821	1.861744004	5.134928656
297	Fall 2015	15.56090821	3.82554314	14.46297455
301	Fall 2015	15.56090821	3.927926314	14.94929463
302	Fall 2015	15.56090821	3.441404538	12.63831619
303	Fall 2015	15.56090821	5.254613336	21.25105798
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14-110 2	Fall 2015	11.20555106	2.318362671	3.253385173
14-110 3	Fall 2015	11.20555106	3.224012935	7.555223927

14-110 5	Fall 2015	11.20555106	2.725150885	5.18562919
14-110 6	Fall 2015	11.20555106	3.883237878	10.68654241
14-110 8	Fall 2015	11.20555106	2.490745855	4.072205297
14-110 9	Fall 2015	11.20555106	2.471488449	3.980732619
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14-71 1	Fall 2015	11.68179792	2.583336806	4.954921894
14-71 10	Fall 2015	11.68179792	3.407922065	8.871701874
14-71 2	Fall 2015	11.68179792	3.430511964	8.979003895
14-71 3	Fall 2015	11.68179792	4.296185131	13.09095144
14-71 5	Fall 2015	11.68179792	4.254282662	12.89191471
14-71 7	Fall 2015	11.68179792	4.116160596	12.2358349
14-71 9	Fall 2015	11.68179792	3.259734879	8.167812741
42-17 1	Fall 2015	15.56090821	3.779573004	14.2446164
42-17 4	Fall 2015	15.56090821	4.545138761	17.88105375
42-17 6	Fall 2015	15.56090821	3.434816557	12.60702328
42-217 1	Fall 2015	15.56090821	1.543973651	3.625519478
42-217 11	Fall 2015	15.56090821	1.370413189	2.801107283
42-217 2	Fall 2015	15.56090821	1.587998088	3.834635553
42-217 3	Fall 2015	15.56090821	1.421651095	3.044487337
42-217 4	Fall 2015	15.56090821	1.183877176	1.915061221
639	Fall 2016	10.30674118	0.8530427	-4.542777878
640	Fall 2016	10.30674118	0.968635177	-3.993713612
642	Fall 2016	10.30674118	0.459234316	-6.413367702
646	Fall 2016	10.30674118	1.347006105	-2.196451704
647	Fall 2016	10.30674118	2.170235511	1.713887975
648	Fall 2016	10.30674118	2.063368208	1.206268285
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38	Summer 2015	12.57538298	3.430360769	9.809319824
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44	Summer 2015	12.57538298	4.963704965	17.09270476
47	Summer 2015	15.87774339	6.253901399	26.292333
49	Summer 2015	15.87774339	6.809770162	28.93270962
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61	Summer 2015	15.87774339	6.309623931	26.55701502
73	Summer 2015	15.87774339	8.578552072	37.33442369
76	Summer 2015	15.87774339	8.307705209	36.0479011
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197	Summer 2015	16.93590981	6.698977329	29.39053844
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213	Summer 2015	13.47330758	5.949360216	22.60963708
215	Summer 2015	13.47330758	8.086970005	32.76328357
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219	Summer 2015	13.47330758	6.005388565	22.87577173
222	Summer 2015	13.47330758	5.265854392	19.36298441
224	Summer 2015	13.47330758	6.186973297	23.73829921
225	Summer 2015	13.47330758	5.886910916	22.3130029
237	Summer 2015	13.47330758	4.401914925	15.25927194
241	Summer 2015	13.47330758	5.294927022	19.5010794
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41-08 1	Summer 2015	13.47330758	5.327789391	19.65717566
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41-10 1	Summer 2015	15.65959516	6.544277427	27.46874128
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41-10 2	Summer 2015	15.65959516	4.37249342	17.15276724
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6-100 8	Summer 2015	15.87774339	6.450705664	27.22715326
6-100 9	Summer 2015	15.87774339	4.371149283	17.34926045
6-101 1	Summer 2015	15.87774339	5.019800348	20.43035301
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6-101 8	Summer 2015	15.87774339	7.258692185	31.06508923
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6-110 3	Summer 2015	15.87774339	5.907444555	24.64666299
6-110 5	Summer 2015	15.87774339	4.689225603	18.86012297
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6-98 15	Summer 2015	15.87774339	5.604813666	23.20916627
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6-98 18	Summer 2015	15.87774339	7.821677633	33.73927011
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6-98 3	Summer 2015	15.87774339	1.486156742	3.645545877
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6-98 7	Summer 2015	15.87774339	3.049370458	11.07081103
597	Summer 2016	14.01911663	4.18484721	14.73580271

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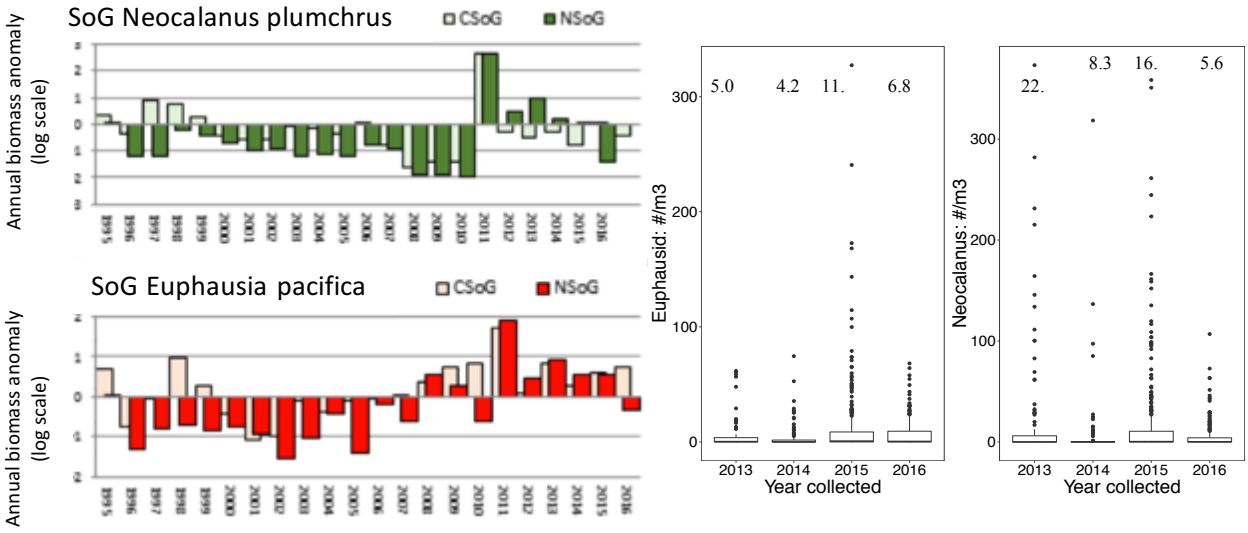
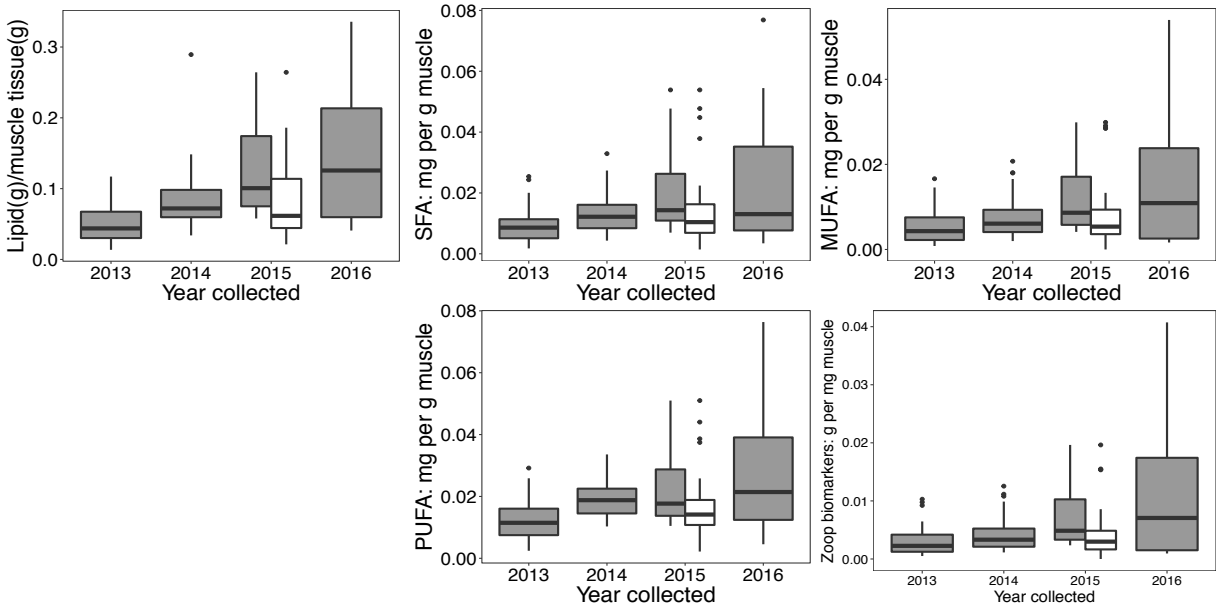
601	Summer 2016	14.01911663	2.940139823	8.823442625
602	Summer 2016	14.01911663	3.063584791	9.409806223
604	Summer 2016	14.01911663	3.512139306	11.54044017
605	Summer 2016	14.01911663	3.270198981	10.39122363
608	Summer 2016	14.01911663	3.772173789	12.77560396
609	Summer 2016	14.01911663	1.85794626	3.683023201
610	Summer 2016	14.01911663	1.741798653	3.131322068
611	Summer 2016	14.01911663	1.472906259	1.854083196
612	Summer 2016	14.01911663	1.642239368	2.658415464
613	Summer 2016	14.01911663	1.97593319	4.243461118
615	Summer 2016	14.01911663	1.780506133	3.315182598
616	Summer 2016	14.01911663	1.820834877	3.506744132



Appendix A3. Zooplankton abundance in the Strait of Georgia. Left panel: Biomass anomalies of *Neocalanus plumchrus* and *Euphausia pacifica*, 1995-2016 (Adapted from Galbraith & Young 2017). Right panel: Box and whisker plots of abundance (number/m^3) of *Neocalanus plumchrus* and *Euphausia pacifica* in various net tows from the Strait of Georgia, 2013-2016 (M. Galbraith, pers. comm. 2018). Mean values are included at the top of each plot.



Appendix A4. Raw quantities of lipids and fatty acids in mass per gram of muscle tissue

Appendix B

Appendix B1. Table of values for condition factor correlations. RD= RNA:DNA growth rate, OGR = otolith increment width growth rate, FK = Fulton's K, LW = Length:Weight residuals, TL = Total lipids, DE=DHA:EPA ratio. Number corresponds with year of collection. Significant values ($p<0.05$) are denoted with *, values with $p<0.01$ are denoted with **

Pairs	p-value	Spearman's Rho	Bonferroni Corrected p-value	Power
RDFK	0.06	0.18	1	0.5
RDFK13	0.28	0.13	1	0.52
RDFK14	0.64	-0.1	1	0.68
RDFK15	0.59	0.25	1	0.67
RDFK16	0.1	0.77	1	0.62
RDLW	0.14	0.14	1	0.5
RDLW13	0.22	0.15	1	0.51
RDLW14	0.56	0.12	1	0.63
RDLW15	0.66	0.21	1	0.71
RDLW16	0.14	0.71	1	0.59
RDOGR	0.8	0.03	1	0.81
RDOGR13	0.54	0.08	1	0.61
RDOGR14	0.92	-0.02	1	0.92
RDOGR15	0.84	0.11	1	0.85
RDOGR16	0.24	0.6	1	0.58
RDTL	0.43	-0.09	1	0.56
RDTL13	0.03*	0.37	1	0.52
RDTL14	0.61	0.11	1	0.66
RDTL15	0.07	-0.69	1	0.58
RDTL16	0.18	-0.66	1	0.57
RDDE	0.05	-0.23	1	0.51
RDDE13	0.06	-0.32	1	0.51
RDDE14	0.18	-0.29	1	0.51
RDDE15	0.1	0.64	1	0.56
RDDE16	0.92	-0.09	1	0.93
FKLW	0.00**	0.86	0.00**	0.51
FKLW13	0.00**	0.98	0.00**	0.52

FKLW14	0.00**	0.87	0.00**	1
FKLW15	0.00**	0.96	0.00**	1
FKLW16	0.00**	0.61	0.02**	0.55
FKOGR	0.33	0.08	1	0.53
FKOGR13	0.44	0.1	1	0.56
FKOGR14	0.1	0.29	1	0.51
FKOGR15	0.66	0.13	1	0.69
FKOGR16	0.21	-0.24	1	0.51
FKTL	0.2	0.12	1	0.51
FKTL13	0.15	0.24	1	0.51
FKTL14	0.19	0.23	1	0.51
FKTL15	0.4	0.24	1	0.56
FKTL16	0.00**	0.67	0.04**	0.57
FKDE	0.00**	-0.35	0.01**	0.52
FKDE13	0.07	-0.29	1	0.51
FKDE14	0.43	-0.13	1	0.56
FKDE15	0.52	-0.18	1	0.61
FKDE16	0.00**	-0.61	0.16	0.55
LWOGR	0.14	0.12	1	0.5
LWOGR13	0.43	0.1	1	0.56
LWOGR14	0.14	0.27	1	0.51
LWOGR15	0.62	0.14	1	0.67
LWOGR16	0.91	-0.02	1	0.92
LWTL	0.52	0.06	1	0.6
LWTL13	0.17	0.22	1	0.51
LWTL14	0.48	0.12	1	0.58
LWTL15	0.52	0.18	1	0.61
LWTL16	0.59	-0.11	1	0.65
LWDE	0.04*	-0.2	1	0.5
LWDE13	0.05*	-0.32	1	0.51
LWDE14	0.54	-0.11	1	0.61
LWDE15	0.64	-0.13	1	0.68
LWDE16	0.86	0.04	1	0.86
OGRTL	0.61	0.05	1	0.65
OGRTL13	0.58	0.09	1	0.64
OGRTL14	0.42	0.15	1	0.56

OGRTL15	0.74	-0.09	1	0.76
OGRTL16	0.00**	-0.59	0.28	0.55
OGRDE	0.07	0.17	1	0.5
OGRDE13	0.93	0.01	1	0.93
OGRDE14	0.41	0.15	1	0.56
OGRDE15	0.58	0.16	1	0.64
OGRDE16	0.01*	0.54	0.66	0.54
TLDE	0.00**	-0.52	0.00**	0.54
TLDE13	0.00**	-0.66	0.00**	0.58
TLDE14	0.00**	-0.53	0.09	0.54
TLDE15	0.00**	-0.72	0.18	0.58
TLDE16	0.00**	-0.85	0.00**	0.82