Local, seasonal, and yearly condition of juvenile Greenland halibut revealed by the Le Cren condition index

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Abstract

Understanding biological parameters such as growth patterns, condition, and energy reserves is important for a better understanding of the environmental constraints exerted on fish populations. This is especially true for exploited fish stocks in the current context of climate change. Using biological data collected from 2006 to 2009 during bottom trawl research surveys by Fisheries and Oceans Canada in the Estuary and Gulf of St. Lawrence (EGSL) as well as data from 2000 to 2018 in the Northwest Atlantic, our aim was to improve our knowledge on the seasonal condition of Greenland Halibut (*Reinhardtius hippoglossoides*) juveniles and get a better understanding of the divergence in some life-history traits between juveniles captured in these two regions. We validated the use of the Le Cren condition index and evaluated its relationship with energetic status in juvenile (20–32 cm) Greenland Halibut. In the EGSL, juvenile condition was higher in winter and spring compared to summer and fall. Such variations may result from this species' pelagic predation activity and prey availability. Juveniles captured in the EGSL in 2016–2017 were larger but had a lower condition index than those captured in the Northwest Atlantic, but we found no indication of earlier sexual maturation in the EGSL that could explain the sex ratio differences we observed in catches from these two areas.

INTRODUCTION

Overharvesting and environmental changes are major issues in fisheries science, as illustrated by the collapse of Atlantic Cod in the Northwest Atlantic (Harris 1998). To answer these challenges, stock assessments need to be supported—among other things—by biological information on exploited species (Lloret et al. 2014). Developing simple tools to evaluate wild fish condition is a recurrent goal in fisheries management (Ricker 1975; Cone 1989). Stock abundance, biomass, growth, mortality, maturity, and recruitment provide information that is needed to characterize the status of an exploited fish population (reviewed by Young et al. 2006). The Fulton condition index (K; Ricker 1975), Le Cren index (Kn; Le Cren 1951), hepato-somatic index (HSI), and relative mass (Wr; Wege and Anderson 1978) are useful indicators of the physiological status of fish and may also indirectly provide information on environmental quality (Lloret and Planes 2003; Levi et al. 2005; Lloret et al. 2005, 2007; Shulman et al. 2005; Amara et al. 2007, 2009). Indeed, it may be possible to use these indices to better understand the ecological constraints of an environment, such as prey availability (Ogutu-Ohwayo 1999; Yaragina and Marshall 2000; Casini et al. 2006), the presence of parasites (Khan et al. 1997), or alterations in abiotic parameters such as dissolved oxygen, salinity, temperature, or pollution (Adams et al. 1989; Rätz and Lloret 2003; Casini et al. 2006; Edwards et al. 2006; Amara et al. 2007; 2009; Gilliers et al. 2012). Even though fish condition data can provide additional information that may help fisheries biologists with population assessments and resource exploitation decisions, it is seldom used in stock assessment and management (Lloret et al. 2014).

Energy reserves are essential for sustaining growth, migration, reproduction, and other biological processes that ensure survival (Shulman and Love 1999; Sogard and Olla 2000; Lambert et al. 2003). The general condition of a fish is an important descriptor of its health and is usually related to the level of energy reserves; this assumes that a healthy fish in good condition will have more energy reserves than individuals in poor condition (Lloret et al. 2014). In many marine fish species, energy content is mostly stored as lipids, which represent the most efficient form of energy storage, i.e., 38 kJ per g of lipids compared to 24 kJ and 17 kJ per g for proteins and carbohydrates, respectively (Jobling 1993). Depending on the fish species, lipids may be stored in the liver (Atlantic Cod, *Gadus morhua*: Jobling et al. 1991; dos Santos et al. 1993; Lambert and Dutil 1997a; Red Drum, *Sciaenops ocellatus*: Craig et al. 2000; macrourid fishes *Coryphaenoides armatus*, *C. yaquinae,* and *C. acrolepis*: Drazen 2002; Haddock, *Melanogrammus aeglefinus*: Nanton et al. 2001; European Hake, *Merluccius merluccius*: Lloret et al. 2008), in adipose tissue (Atlantic Salmon, *Salmo salar*: Zhou et al. 1996), or in the liver and red muscle (tropical tunas, *Thunnus obesus*, *T. albacares,* and *Kastuwonus pelamis*: Sardenne et al. 2017). In flatfishes, the liver is relatively small and does not serve as a primary energy storage site. In European Plaice, *Pleuronectes platessa*, energy is stored in the carcass (Dawson and Grimm 1980), while in Winter Flounder, *Pseudopleuronectes americanus*, the main storage site is the hypodermal lipid layer under the lateral line (Maddock and Burton 1994). In Greenland Halibut, *Reinhardtius hippoglossoides*, energy is stored mainly as lipids and proteins in white muscle (Ait Youcef 2013; Karl et al. 2018).

When food energy is no longer sufficient to supply basal metabolic needs, energy is drawn from body tissue reserves. When this occurs, lipids and proteins are replaced by water. In certain extreme cases, this can lead to high water content in tissues—a phenomenon called jellied condition—which has been observed in several flatfish species (Broad Flounder, *Paralichthys squamilentus*: Clark 1958; American Plaice, *Hippoglossoides platessoides*: Templeman and Andrews 1956; Haard 1987; Winter Flounder, *Pleuronectes americanus*: Maddock and Burton 1994).

In fishery science, numerous authors have used condition indices, water content, or organ weight : somatic weight ratios to test spatial and temporal variations in the condition, energy balance, and physiological status of exploited populations in different contexts, i.e., global comparisons between different stocks (Rätz and Lloret 2003; Román et al. 2007), more localized population comparisons (Hidalgo et al. 2008; Tomiyama and Kurita 2011), temporal variations including seasonal changes (or changes during the annual cycle; Lloret et al. 2014) (Dawson and Grimm 1980; Lambert and Dutil 1997a, 1997b; Mello and Rose 2005; Tomiyama and Kurita 2011), and interannual and long-term studies (Lambert and Dutil 1997b; Román et al. 2007; Pardoe et al. 2008). Such investigations could be useful to detect possible alterations in the biological parameters of a population and help with decision making for fisheries management and conservation (Murphy et al. 1991; Lambert and Dutil 1997a, 1997b; Rätz and Lloret 2003; Mello and Rose 2005). Despite the economic importance of the Greenland Halibut fisheries (Victorero et al. 2018), such tools have not yet been validated for this species.

Greenland Halibut is a circumpolar species inhabiting cold (−0.5 to 6°C; Bowering and Nedreaas 2000) and deep waters (generally 130–1,600 m and sometimes up to 2,200 m;

Scott and Scott 1988; Boje and Hareide 1993; Bowering and Power 1995; Bowering and Nedreaas 2000). At the southern edge of its distribution in the Atlantic, it is found in the Estuary and Gulf of St. Lawrence (EGSL), especially in the deep Laurentian, Anticosti, and Esquiman channels (from 130 to 500 m). Considered as a separate management unit (Arthur and Albert 1993), the EGSL population has been actively fished for more than 30 years (DFO 2019).

Despite hypoxic conditions in nursery habitats (Gilbert et al. 2007; Ait Youcef et al. 2013) and the negative impacts of hypoxia on Greenland Halibut metabolism (Dupont-Prinet et al. 2013; Pillet et al. 2016), the EGSL population is characterized by a constant juvenile growth rate throughout the year that is the highest recorded for this species (Ait Youcef et al. 2015). Permanent access to abundant food and temperatures close to the optimal growth temperature may explain this high growth rate (Ait Youcef et al. 2015; Ghinter et al. 2021). However, over the last few decades, bottom water temperatures in the EGSL have been rising due to increased warm-water pulses entering through Cabot Strait (Galbraith et al. 2019, 2020). These changes have been striking, with average temperatures of 4.8°C and 5.9°C at 200 and 300 m respectively in 2019, while the 1981–2010 averages were 3.87 ± 0.36°C and 4.97 ± 0.23°C, respectively (Galbraith et al. 2020). In the EGSL, dissolved oxygen, which is already close to the tolerance limit of juveniles, could become critical (Dupont-Prinet et al. 2013; Pillet et al. 2016). In the Northwest Atlantic, the most recent DFO (Fisheries and Oceans Canada) report on oceanographic conditions for the two zones that we are considering indicate that climate indices are evenly spread between positive and negative anomalies, so there is no real trend at this time (Cyr et al. 2021).

The isolated character of the EGSL population relative to the rest of the Northwest Atlantic population is still debated. While many studies based on genetic, meristic, and parasitic criteria support the presence of two distinct populations (Templeman 1970; Fairbairn 1981; Khan et al. 1982; Misra and Bowering 1984; Arthur and Albert 1993; Vis et al. 1997), other studies have revealed strong interrelationships between the different geographic areas (Pomilla et al. 2008; Roy et al. 2014). A recent study showed genetic differences between the EGSL and the Northwest Atlantic population but also the presence of high gene flow that could vary from year to year and contribute to the strong link between the two stocks (Carrier et al. 2020).

The EGSL and Northwest Atlantic stocks are currently managed separately, and the populations diverge in their life history traits and biological parameters. Juvenile growth rate in the EGSL (length increase of 8−12 cm between ages 1and 2) is higher than in the Northwest Atlantic (6−8 cm; see Ait Youcef et al. 2015). In addition, the size at maturity in the EGSL is smaller than that estimated in the Northwest Atlantic, thus the slowdown that occurs when reaching sexual maturity occurs earlier in EGSL individuals. The individuals of these two regions therefore differ according to their size at age, and despite issues with current maturity staging (Nielsen and Boje 1995; Albert et al. 2001; Gundersen 2003; Morgan et al. 2003; Kennedy et al. 2011), the two populations appear to have very different size/age at 50% maturity (L_{50}/A_{50}). In the last decades, the L_{50} of Greenland Halibut in the EGSL was around 36 cm for males and 46 cm for females (DFO 2019). In addition, according to growth models, males and females reach the commercial size of 44 cm at 6 and 7 years old, respectively (DFO 2019). In the Northwest Atlantic, the L_{50} of females was found to vary from 60−88 cm (depending on the region and year), which approximately corresponds to 8- to 12-year-old individuals (Morgan and Bowering 1997). Thus, life history traits diverge between these two management units, causing differences in their biological parameters and catch characteristics. In the last two decades, the average size of fish from EGSL catches was 47.2 cm (DFO 2019). Most catches are composed of adult females, a few immature females, and mature males (about 80% of catches has consisted of females since 1996 [DFO 2019]). In the Northwest Atlantic, catches are mainly composed of individuals between 5- and 9-years-old (Regular et al. 2017), which corresponds to fish from 35–37 cm to 57–59 cm, respectively (Healey 2013). Northwest Atlantic catches are therefore mainly made up of juveniles. Such differences in population structure combined with different fishing pressures are likely to affect the condition of individuals as well as the recruitment potential and productivity of stocks.

In this context, our objectives were 1) to validate the use of the Le Cren index to estimate juvenile condition, 2) to test the hypothesis of an absence of seasonal effects on juvenile condition; and 3) to compare the condition of juveniles captured in the Estuary and Gulf of St. Lawrence and the east coast of Labrador using proxies such as energy reserves to better understand the divergence in the life history traits documented between these two managements units.

MATERIAL and METHODS

Capture and Sampling Design

1) Biological data from the EGSL, 2006–2009.— Biological data on Greenland Halibut juveniles (20–32 cm) were obtained from the annual multidisciplinary bottom trawl research survey conducted in the EGSL (NAFO Division 4RST; summer) by Fisheries and Oceans Canada (DFO) and from annual smaller-scale bottom-trawl surveys conducted in the spring (April–May) and fall (October) in the St. Lawrence estuary (Fig. 1). Within the EGSL, fish belonging to this length range correspond to two-year-old fish (Ait Youcef et al. 2015; Bourdages et al. 2016). For our study, data from 2006 through 2009 were used because complete seasonal sets were available. Detailed information about these surveys is provided in Ait Youcef et al. (2013). Individual fish fork length (± 0.1 cm) and mass (± 0.1 g) were recorded for every fish captured.

In 2009, muscle and liver tissues were sampled on juveniles captured during spring (n = 65, 19.9–31.9 cm) and summer (n = 39, 24.5–30.0 cm). Whole liver was weighed (± 0.1 g, wet mass) and tissue samples were frozen on board at −20ºC for later determination of water and energy contents. All muscle samples were excised on the pigmented side above lateral line.

2) *Biological data from the Northwest Atlantic*, *2000–2018.*— Biological data on Greenland Halibut juveniles (20–32 cm) were obtained from the annual multidisciplinary bottom trawl research survey conducted in the Northwest Atlantic (NAFO Division 2H, 2J; fall) by DFO. Detailed information about the design of these surveys is provided in Doubleday (1981). Individual fish fork length (± 1.0 cm) and mass (± 10.0 g) were recorded for every fish captured.

3) *Biological data from the EGSL and Northwest Atlantic, 2016–2017.*— Greenland Halibut juveniles were caught at five locations in the EGSL and off the coast of Labrador in NAFO divisions 2J and 2H (Table 1, Fig. 1). Sampling locations in the EGSL were relatively deep (> 270 m) compared to depths at the northwest 2J sampling location

(< 200 m), and the range of depths at the 2H sampling site was quite broad (190–374 m). Salinity was similar among the EGSL sampling sites, but dissolved oxygen in the St. Lawrence Estuary (SLE) was almost 50% lower than in Northeast Anticosti (NEA) (Table 1). All captures were made on board the CCGS *Teleost* with a Campelan-type trawl (12.7 mm mesh size) except for the SLE 2017 sampling site, which was made from the CCGS *Leim* with a Comando-type trawl (3-inch trawl bucket, extension and pocket 2-inch mesh size). Tows lasted 15–30 minutes at speeds of 2–3 knots and depths varying between 174 and 374 m. For captures made on the CCGS *Teleost*, depth, temperature, salinity, and oxygen at the sampling sites were directly measured with sensors fixed at the trawl mouth. On the CCGS *Leim*, only depth was directly measured onboard; data from an oceanographic station located close to the trawling sites were used to obtain the other environmental variables (P. Galbraith, Institut Maurice-Lamontagne, Fisheries and Oceans Canada, pers. comm.). Oceanographic data are presented in Table 1. Salinity and oxygen conditions were not available for the CCGS *Teleost* mission in the Northwest Atlantic.

When fish were sorted, a maximum of 10 live juveniles measuring between 20.6 and 33.1 cm were placed in a tank (800 L) filled with pumped seawater. Temperature conditions were kept stable using an insulated tank and heat pump. Prior to tissue sampling, juvenile fish were maintained in the holding tank no longer than 3 h, but most fish were sampled within the first hour following capture.

For tissue sampling, fish were anaesthetized in a solution of MS 222 (tricaine methane sulfonate 0.18 g L^{-1}; Sigma-Aldrich Co., Missouri, USA) for 5 min between 1200 and 1700 to avoid possible biases associated with endocrine circadian rhythms. Individuals were weighed and measured, and blood was sampled from the caudal artery using a 23-gauge needle and a 1-mL TB syringe (Becton Dickinson & Co, New Jersey, USA), both previously treated with a heparin solution (ammonium heparin salt, Sigma-Aldrich Co., Missouri, USA) at a concentration of 100 units mL^{-1} in 0.09% NaCl. Liver, muscle, and gonad samples were excised. For fish collection in the EGSL, liver tissue samples were then frozen in liquid nitrogen and stored at −80°C until analysis. In the Northwest Atlantic, the ship could not carry liquid nitrogen due to logistical constraints; liver tissue was stored in RNA*later* (Sigma-Aldrich Co., Missouri, USA) for further transcription analysis (not presented in this paper), but biochemical analysis could not be performed on these samples. Blood samples were centrifuged for three minutes at 4.6 G and plasma was stored at −20°C. Gonads were fixed in Bouin's solution (Sigma Aldrich Co., Missouri, USA).

Morphological Calculations and Physiological Measurements

The relationship between wet mass and standard length was calculated from the large data sets obtained in the EGSL and Northwest Atlantic in spring, summer, and fall (Table 2). In the absence of an isometric relationship (slope coefficient $b \neq 3$) between length and mass, the Le Cren formulation for condition factor (Le Cren 1951) was selected for the condition index. This index is calculated as

Kn = W / We

where W is the observed mass and We is the estimated mass obtained from the length–mass relationship. To compare the condition of fish from different periods or regions, a single length–mass relationship including all fish from all periods and areas studied was used to estimate We.

When available, the hepato-somatic index (HSI) was calculated as

HSI = (LM/M)*100

where LM is the liver wet mass and M is the fish somatic wet mass.

Liver and muscle water contents were calculated after drying a tissue sample (2–10 g) at 65°C for 48 hours, at which time constant mass was obtained. Energy content was measured with an oxygen bomb calorimeter (Model 1261, Parr Instrument Company, Illinois, USA) standardized with benzoic acid pellets (Parr Instrument Company; 26.44 kJ g^{-1}); results are reported per gram of dry tissue mass.

For fish captured in 2016–2017 in the EGSL and Northwest Atlantic, fish sex was identified and sexual maturity status determined with histology and sex steroid measurements. Gonads were dehydrated in increasing concentrations of ethanol (50, 70, 80, 90, and 95%) and embedded in methacrylate JB-4 solution according to the protocol suggested by the manufacturer (Sigma-Aldrich Co., Missouri, USA). The embedded gonad tissue was sectioned into 3 µm slices that were stained with Lee's methylene blue−basic fuchsin solution (Aparicio and Marsden 1969). Gonad development stage was determined for each individual according to Morrison (1990) for testes and Rideout et al. (2012) and García-López et al. (2007) for ovaries. Sex steroid concentrations were determined using RIA kits for testosterone and 17β-estradiol (^{125}I RIA kits # 07-189102 and # 07-138102, respectively; MP Biomedicals, LLC, California, USA) and ELISA for

11-keto testosterone (ELISA Kit # 582751, Cayman Chemical, Inc., Michigan, USA). The half-value of the smallest standard was assigned to undetectable values.

In 2016 and 2017, analysis of energetic components was only conducted on EGSL individuals. We could not analyze the energetic components of liver tissue from the Northwest Atlantic because of the preservation method used. Hepatic glycogen was measured using the amyloglucosidase digestion method (Carr and Neff 1984). Glucose was measured by colorimetry (Glucose Colorimetric Assay Kit, Cayman Chemical, Inc., Michigan, USA). Total liver lipid concentration was determined using the phospho-vanillin method (Frings et al. 1972). Liver protein concentrations were measured using a protein dye binding method (Protein Assay kit, Biorad, California, USA) according to Bradford (1976). Total liver energy content was calculated using conversion factors of 24, 38, and 17 kJ g^{-1} for proteins, total lipids, and glycogen, respectively (Jobling 1993).

Statistical Analysis

Data normality and homoscedasticity were verified using Kolmogorov-Smirnov and Levene tests, respectively (Quinn and Keough, 2002). For linear regressions, normality was tested on residuals, and residual variations were tested using analysis of variance (ANOVA). Linear length–mass regressions according to season and to population were performed on log-transformed data. No transformation was necessary for the linear regressions of the tissue water and energy content or for the regressions of condition indices and energy content. Year and seasonal effects on condition factor were analyzed using a two-way ANOVA. Because homoscedasticity was not obtained for Kn data, Games & Howell post-hoc tests were applied to each year to identify differences between periods (Sokal and Rohlf, 1995). The inter-seasonal variation of ΔKn was verified using one-way ANOVA. When significant effects were found, a posteriori Tukey mean comparison tests were used ($\alpha = 0.05$). No transformation was needed for fish captured in 2016–2017 except for steroid data, which were log-transformed prior to statistical analysis. Morphometric and physiological data were analyzed using paired two-tailed Student's t-tests. Statistical analyses were performed with Statistica software (Statsoft v.6.1, Oklahoma, USA).

RESULTS

Length–Mass Relationship and Condition Factor

We calculated a single length–mass relationship based on all individuals (2006–2009 in the EGSL; 2000–2018 in the Northwest Atlantic) in this study (linear regression; $R^2 = 0.96$, LN(mass) = 3.2094 * LN(length) - 5.5669, $F_{1,\,10780} = 229343.80$, $P < 0.001$): We = 0.0038 L $^{3.2094}$ (Fig. 2).

In the EGSL, there were significant differences in Kn according to year and season (year × season interaction, $F_{6,\,4146} = 9.3$, $P < 0.001$). Kn decreased from spring to summer for the four years studied (Fig. 3). In 2008 and 2009, Kn remained relatively low and stable from summer to fall, but it decreased in 2006 and increased in 2007 during this same period (Fig. 3). Greenland Halibut juveniles had a higher condition factor in spring, with an average Kn above 1.05. ΔKn varied significantly by season ($F_{2,\,8} = 18.23$, $P \leq 0.001$): ΔKn was negative from spring to summer and positive from fall to spring (Fig. 4). From summer to fall, ΔKn remained close to 0 (Fig. 4).

Comparisons between regions indicated that Greenland Halibut juveniles from the EGSL were significantly larger than those from the Northwest Atlantic (Table 3). However, the Le Cren condition index of juveniles was significantly lower in the EGSL than in the Northwest Atlantic (Table 3).

Tissue Energy Content

In EGSL juveniles captured in 2016–2017, energy compartment analyses showed that liver was mostly composed of lipids (217.43 ± 68.24 mg g^{-1} wet liver) followed by proteins (53.87 ± 10.69 mg g^{-1} wet liver) and glycogen (4.17 ± 4.30 mg g^{-1} wet liver). After conversion into energy values, lipids accounted for most of the energy reserves in the liver (> 80%; 8.26 ± 2.59 g^{-1} wet liver), followed by proteins (10–15%; 1.29 ± 0.26 g^{-1} wet liver) and glycogen, which accounted for the lowest fraction (≤ 1%, 0.07 ± 0.07 g^{-1} wet liver).

Relationships Between Tissue Energy, Water Contents, Kn, and HSI Index

Using juveniles captured in spring and summer 2009, muscle and liver energy contents evaluated with a bomb calorimeter showed significant negative correlation with tissue water content (Fig. 5A and 5B). No relationship between muscle energy content and Kn was found (Fig. 6A), but a significant correlation existed between liver energy content and the hepatosomatic index (Fig. 6B).

Liver water content was converted into total liver energy content (kJ g^{-1} wet tissue) using the relationship between liver energy and water content (see Fig. 5B). A strong correlation was found between estimations of liver energy content (kJ g^{-1} wet tissue) based on liver water content (Y) and total measured protein, lipid, and glycogen contents ($Y = 1.1231*X - 3.0245$, $R^2 = 0.9660$, $P < 0.001$).

Muscle energy content was obtained from the relationship between muscle energy and water content (see Fig. 5A). Using these calculations, we observed no difference in muscle energy content between juveniles from the EGSL and those from the Northwest Atlantic (Table 3), with an overall average of 4.05 ± 0.57 kJ g^{-1} wet tissue.

Sex and Maturity of Fish Captured in the EGSL and Northwest Atlantic

The EGSL and Northwest Atlantic samples (2016–2017) were each represented by approximately equivalent numbers of male and female juveniles overall, with small variations depending on sample site (Table 1). All sampled fish were juveniles, as confirmed by gonad histology, with none of those examined showing signs of sexual maturation. In male juveniles, only immature developmental stages were observed (Fig. 7). Plasma testosterone was not detectable in EGSL fish while low but detectable values were found in Northwest Atlantic individuals (Fig. 8). Plasma 11-ketotestosterone concentrations were lower in male juveniles from the EGSL compared to those from the Northwest Atlantic (Table 3), where they nonetheless remained very low (≤ 160 pg mL^{-1}).

For females, all gonads were immature; we only observed undifferentiated germ cells, i.e., oogonia and primary stage oocytes at the perinucleolus stage. As was the case for males, plasma testosterone was not detectable in EGSL juveniles and was low but detectable in most Northwest Atlantic female juveniles (Fig. 8). No significant variation of 17β-estradiol plasma concentration was noted between the two regions (Table 3). Female plasma 17β-estradiol was very low at all sampling sites (≤ 330 pg mL^{-1}).

DISCUSSION

According to the allometric growth of juvenile Greenland Halibut, the Kn condition index was the best estimator of their general condition. However, the absence of a relationship between Kn and tissue energy content in juveniles from these two areas indicates that muscle water content must be measured along with Kn to fully appreciate the quantity of energy reserves available to juveniles.

Overall, our results indicate that the body condition of Greenland Halibut juveniles varies over the year, with a general decrease in the condition index from spring to fall and a recovery of condition at some point between the fall and spring sampling periods. Kn was different between juveniles from the Northwest Atlantic and those from the EGSL, but tissue energy contents were similar.

Condition Index

Juvenile Greenland Halibut growth in the EGSL is allometric. The slope coefficient of the length–mass relationship was greater than three throughout the year. The Le Cren index (Kn), which is also easy to determine on live fish, avoids the influence of size by comparing the mass of an individual to a standard predicted by the length–mass regression calculated on the population from which the fish was sampled (Le Cren 1951). However, length–mass relationships can vary because of several factors (e.g., populations, geographic sites, year, month, sex), and Kn comparisons can only be made on groups with homogeneous length–mass parameters (Bolger and Connolly 1989). Annual cyclical variations in growth, condition, and energy reserve accumulation have been described for many exploited marine fishes (Atlantic Cod: Eliassen and Vahl 1982a 1982b; Lambert and Dutil 1997a; Schwalme and Chouinard 1999; Mello and Rose 2005; Atlantic Herring: Oskarsson 2005, 2008; Mediterranean Horse Mackerel, *Trachurus mediterraneus*: Tzikas et al. 2007; European Hake: Hidalgo et al. 2008) including flatfishes (European Plaice: Dawson and Grimm 1980; Winter Flounder: McLeese and Moon 1989; Japanese Flounder: *Paralichthys olivaceus*, Tomiyama and Kurita 2011; Greenland Halibut in the North Atlantic: Román et al. 2007). These fluctuations result from ecological and physiological processes, such as feeding, sexual maturation, reproduction, migration, or wintering, which are strongly controlled by abiotic factors, mainly climatic (Lloret et al. 2014). Annual cycle changes are therefore dependent on the life history traits of a species. Here, we only studied fish between 20 and 32 cm—known from prior work (Ait Youcef et al. 2015; Bourdages et al. 2016) to be two-year-old juveniles—settling in nurseries, meaning that no sexual maturation, reproduction, or reproductive migration affected their condition. Indeed, no evidence of gonad maturation was observed: sexual steroid levels were very low, similar to those of juveniles surveyed in captivity from July to December at three different temperatures (Ghinter et al. 2021).

Overwintering is generally a critical period for fish, principally because low temperatures negatively affect fish metabolic processes, such as ingestion, digestion, assimilation, and ultimately fish growth rate (reviewed by Hurst 2007). In many cases, winter is a period of starvation during which fish are forced to use their energy reserves to compensate for the lack of food. Thus, variations in energy reserves as well as in body indices are common during winter (European Plaice: Dawson and Grimm 1980; Winter Flounder: McLeese and Moon 1989; Atlantic Cod: Schwalme and Chouinard 1999; Red Drum: Craig et al. 2000). In the EGSL, Greenland Halibut juveniles settle in deep water, where bottom temperature conditions are relatively constant over the year (Ait Youcef et al. 2015; Galbraith et al. 2019). Ait Youcef et al. (2015) suggested that these characteristics could explain why juvenile growth is constant throughout the year in the EGSL. While Ait Youcef et al. (2015) observed a constant length increment over the year, we found that biotic and abiotic environmental parameters in winter in the EGSL allowed juvenile Greenland Halibut to recover a high condition index prior to the next spring despite inter-annual variability in the condition index during summer and fall. The decrease in Kn after spring suggests a decrease in mass gain relative to the length increment. Such a decrease was observed for Greenland Halibut in the Northwest Atlantic in NAFO divisions 3LNO in a study combining data on adults and juveniles (Román et al. 2007). Greenland Halibut is a voracious top predator that commonly leaves the seabed to feed in the water column on epibenthic and bathypelagic prey (Bowering and Brodie 1991; Bowering and Lilly 1992; Jørgensen 1997; Dawe et al. 1998; Woll and Gundersen 2004; Solmundsson 2007; Vollen and Albert 2008; Dennard et al. 2009; Albert et al. 2011). Thus, its hunting environment is subject to variations during the annual cycle, which could make Greenland Halibut more sensitive to variations of environmental factors than what is observed in other benthic species that feed on benthic sources (Stasko et al. 2016; Giraldo et al. 2018). Accordingly, the pelagic activity of Greenland Halibut has been found to vary over the year; this was hypothesized to be linked with feeding intensity, including prey selection, prey availability, and prey distribution in the water column (Vollen and

Albert 2008; Albert et al. 2011; Boje et al. 2014). Here, only two-year-old juveniles located in the EGSL were studied. Among fish within this size range, prey consumed by juveniles are mainly Northern Shrimp *Pandalus borealis* and fish, mostly Capelin *Mallotus villosus* (Savenkoff 2012; DFO 2019). It is therefore possible that variation in the availability of these prey during the year could contribute to variations in Kn during the year. Indeed, in the Canadian Beaufort Sea, the lower lipid content in Greenland Halibut muscle has been hypothesized to be related to lower abundances of prey (Artic Cod, *Boreogadus saida*), which may explain a drop in feeding rates as well as an increase in energy expenditure in seeking prey (Giraldo et al. 2018). Other top predator fishes living in the deep-sea environment, the macrourids *Coryphaenoides yaquinae* and *C. armatus*, show increased foraging behaviour during periods of prey scarcity (Armstrong et al. 1991; Priede et al. 1994). Despite this, these fishes maintained a constant nutritional condition throughout the year with little or no growth variation (Drazen 2002).

The Kn indices of juveniles from the Northwest Atlantic were higher than for those from the EGSL. Despite the higher Kn, relative muscle energy content (kJ g^{-1} wet tissue) was not different between the two regions. However, the greater Kn indicated that the individuals from the Northwest Atlantic were relatively heavier than those from the EGSL, with potentially a higher muscle mass and therefore higher total energy reserves. Two stations in the EGSL were sampled in summer, when juvenile condition was relatively lower than the condition of spring samples (0.96 ± 0.09 and 0.93 ± 0.06 vs. 1.02 ± 0.04), which confirmed the seasonal comparisons, i.e., a decrease in Kn after spring. The two Northwest Atlantic stations were sampled in fall and showed relatively higher Kn (1.03 ± 0.06 and 1.06 ± 0.05). We do not know whether variations in the condition index occur throughout the year in this region (NAFO Div. 2H, 2J), but this is a factor that cannot be excluded.

The differences in size and mass observed between the two regions were due to a single station of the EGSL—the northeast Anticosti site—where larger individuals were captured. Despite the small number sampled at the northeast Anticosti site, our results agree with the results obtained by Ait Youcef et al. (2015), who reported a larger length increment between ages 1 and 2 at this site but were not able to pinpoint a specific environmental factor that could explain it. If we do not consider the northeast Anticosti site, the size and mass between the two regions (EGSL and Northwest Atlantic) were similar. We cannot discount the possibility of regional differences that could be related to differences in mean ages. Indeed, otoliths were not collected because of known difficulties in age reading using otoliths in Greenland Halibut (Treble et al. 2008), even though new approaches are promising (Albert 2016; Moen et al. 2018). Moreover, no analysis of age classes using length frequencies, such as the one performed for the EGSL (Ait Youcef et al. 2015), has been made in the Northwest Atlantic. Further work to establish the age structure in the Northwest Atlantic remains to be done.

Estimation of Energy Reserves

Water and energy contents were negatively correlated in both muscle and liver. The percentage of water turned out to be a good proxy for energy content; it is relatively easy to measure and therefore to estimate energy reserves in both muscle and liver. Condition indices and HSI usually provide good estimates of fish health status because they are related to tissue energy content as previously shown in other species (Atlantic Cod: Lambert and Dutil 1997a; Winter Flounder: Plante et al. 2005).

In the present study, no correlation was found between Kn and muscle energy content. In a study on fecundity in Greenland Halibut, Kennedy et al. (2009) used the Fulton condition index (K) and HSI and concluded that they may be poor indicators of energy reserves. In the present study, we found a significant correlation between HSI and liver energy content. However, this relationship was weak, and we observed high variability in the liver energy content for the same HSI value. Therefore—strictly in terms of energy content—Kn and HSI do not seem to be reliable indicators of the precise energetic status of juvenile Greenland Halibut. Tissue water content remains one the best indicators to use along with Kn.

By mass, the energy content of the liver is higher than that of muscle tissue, but liver only represents a small percentage of body mass, i.e., from 0.3 to 4.6% (Gundersen et al. 1999, 2001, 2004). In our study, juvenile liver mass was estimated to be 1.3 ± 0.3% of body mass. On the contrary, muscle tissue represents a much larger proportion, from 36.5 ± 3.9% in juveniles (< 40 cm, Y. Lambert, Institut Maurice-Lamontagne, Fisheries and Oceans Canada, pers. comm.) to 47.2 ± 3.9% in adults (Karl et al. 2018). Therefore, muscle represents the main source of energy storage in this species. For example, the expected mass for a 25 cm juvenile would be 120.8 g (We) and estimates of energy content based on an average of 36.5% and 1.3% of body mass for muscle and liver, respectively, would be 229 kJ and 14 kJ. The liver's contribution to total energy reserves in Greenland Halibut is very low, as is the case in other flatfishes such as European Plaice (Dawson and Grimm 1980) and Winter Flounder (Maddock and Burton 1994).

In a study based on a small number of adult Greenland Halibut, Karl et al. (2018) showed that lipid content varies inversely with muscle water content, and that muscle has a relatively high level of lipids compared to other fish species, thus identifying Greenland Halibut as a fatty fish (> 10% fat). During periods of fasting, European plaice (Dawson and Grimm 1980) and Winter Flounder (McLeese and Moon 1989; Maddock and Burton 1994) use their muscle lipids, which results in Winter Flounder in a proportional increase in muscle water content, sometimes up to 94.6%. When muscle lipids are exhausted, muscle proteins are catabolized, with a further increase in water content. In the present study, muscle water and energy contents varied from 69 to 85% and 3.5 to 9.5 kJ g^{-1} of wet mass, respectively. This suggests that juveniles had not exhausted their lipid reserves and that they were not starved prior to sampling.

Histological and hormonal analyses clearly showed that only juveniles were used in our comparisons. Despite differences between the two regional populations, hormonal concentrations remained low throughout the sampling periods. In the present study, sex steroid levels remained 100 times lower for estradiol in females, 10 to 100 times lower for 11-ketotestosterone in males, and testosterone remained at concentrations 10 times lower than concentrations observed in Atlantic Halibut during reproduction (Methven et al. 1992). We found no evidence of earlier sexual maturation in the EGSL nor differences in growth that could explain why more females and smaller fish are now captured in the EGSL.

Conclusion

We validated the use of the Le Cren index, Kn, to evaluate the general condition of juvenile Greenland Halibut. In the EGSL, Kn varied depending on time of year, with results indicating a decline throughout the summer and the potential that recovery might occur during winter or early spring, in advance of the earliest sampling period analyzed herein. Factors that influence these variations throughout the year remain unclear, but prey availability should be an important factor for such a voracious bathypelagic predator. The absence of a significant relationship between Kn and muscle energy content indicates that the evaluation of muscle water content is important in assessing the precise energy status of juvenile Greenland Halibut. When more energy is available in juvenile tissues, more is available to sustain the energetic costs of growth. Using the tools highlighted in this study, we found that juveniles from the EGSL and the Northwest Atlantic appear to have divergent length–mass relationships. This needs to be confirmed by further studies where fish age could be assessed. Adding the use of Kn and muscle energy content to monitoring processes would help document the general status and growth potential in juveniles of this important commercial species.

FUNDING

This project was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC Strategic grant No. STPGP/ 478827-2015 to L. Bernatchez, C. Audet, R. Tremblay, P. Sirois, Y. Lambert, M.J. Morgan, and K. Praebel), and Fisheries and Oceans Canada (DFO).

ACKNOWLEDGMENTS

The authors are grateful to J. Gauthier and H. Bourdages for their help in organizing and assisting in sampling, as well as for their help in analyzing databases. We thank E. Lee and R. Gagné for their invaluable help during sampling, in the organization of oceanographic missions, and in the laboratory. We thank RAQ for its financial and scientific support in this project.

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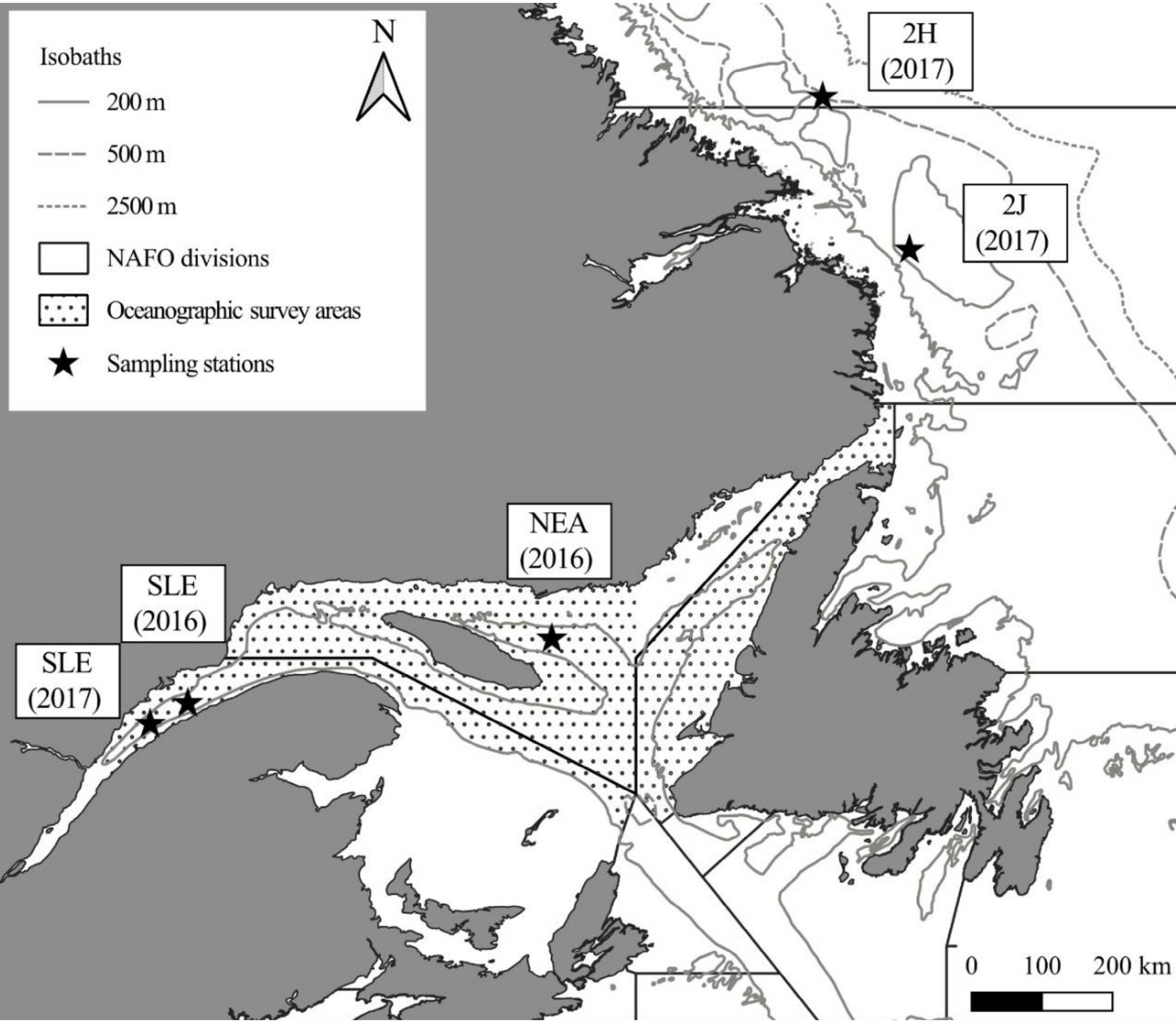
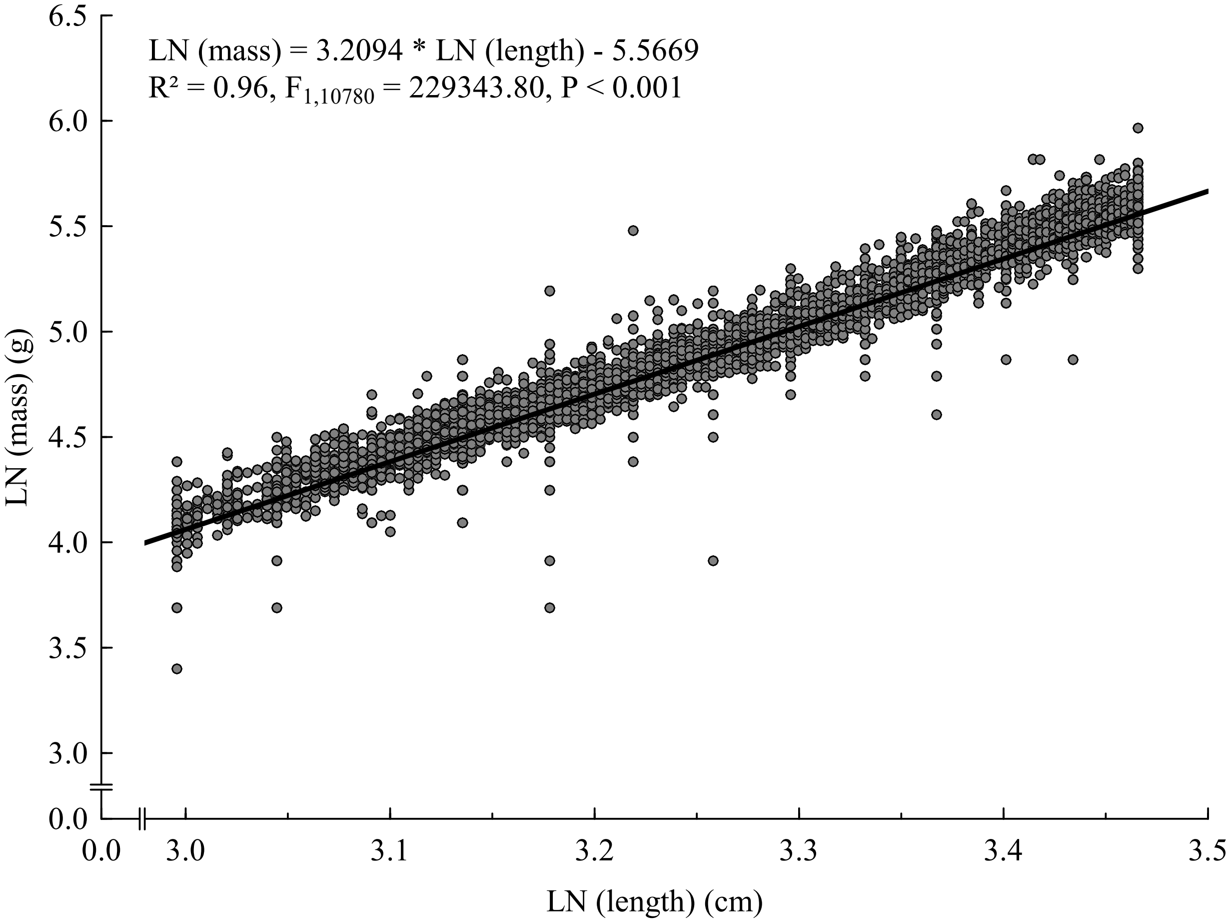
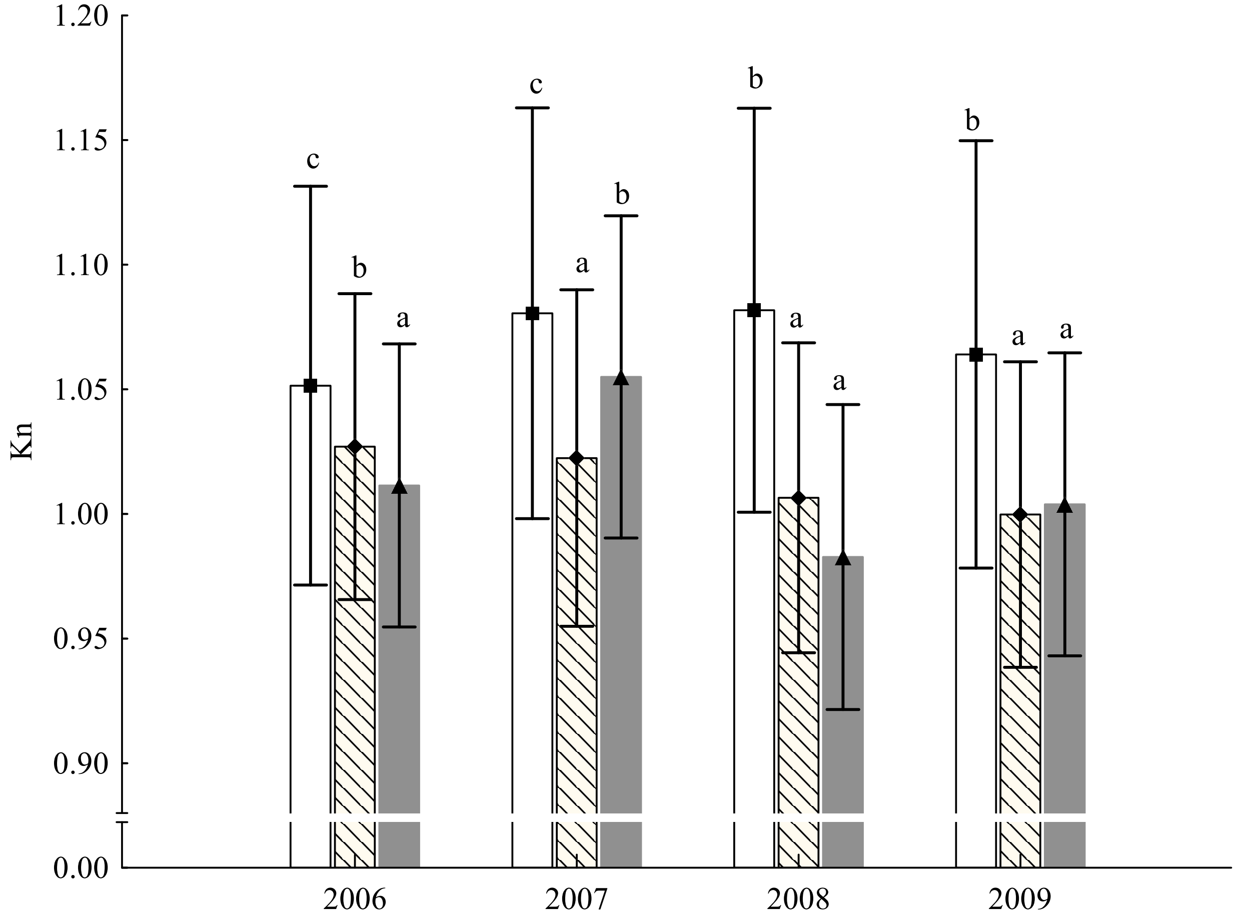
Location	Coordinates	Date	CCGS vessel	Duration (min)	Depth (m)	Temperature (°C)	Salinity	Oxygen (µmol L^{-1})	N_{tot}	N♀	N♂
Estuary and Gulf of St. Lawrence											
SLE	N48° 53' 44" W67° 56' 07"	August 21-23 2016	*Teleost*	20 - 30	271 ± 29	5.5 ± 0.2	34.46 ± 0.08	58.92 ± 8.19	10	6	4
NEA	N49° 38' 15" W61° 29' 24"	August 29 2016	*Teleost*	20 - 30	278 ± 1	6.3 ± 0.1	34.59 ± 0,09	95.09 ± 7.88	9	5	4
SLE	N48° 39' 27" W68° 36' 11"	May 22-25 2017	*Leim*	30	323 ± 6	5.5	34.40	52.25	10	3	7
Northwest Atlantic											
2H	N55° 26' 20" W56° 41' 40"	October 28 2017	*Teleost*	15	269 ± 92	2.2 ± 1.0	-	-	10	5	5
2J	N53° 52' 50" W55° 09' 30"	November 03-05 2017	*Teleost*	15	177 ± 14	-0.1 ± 0.0	-	-	10	4	6

Season	Equation	R^2	*df*	*df (error)*	F	*P* value
Spring	3.3151x - 5.8504	0.97	1	2117	76737.74	< 0.001
Summer	3.2424x - 5.6688	0.96	1	1511	32378.82	< 0.001
Fall	3.1707x - 5.4233	0.97	1	524	16566.32	< 0.001
Slope homogeneity			2	4152	14.70	< 0.001
Region						
EGSL	3.2372x - 5.6237	0.97	1	4156	117011.4	< 0.001
NW Atl	3.1948x - 5.5406	0.96	1	6222	141195.2	< 0.001
Slope homogeneity			1	10778	9.5	0.0021

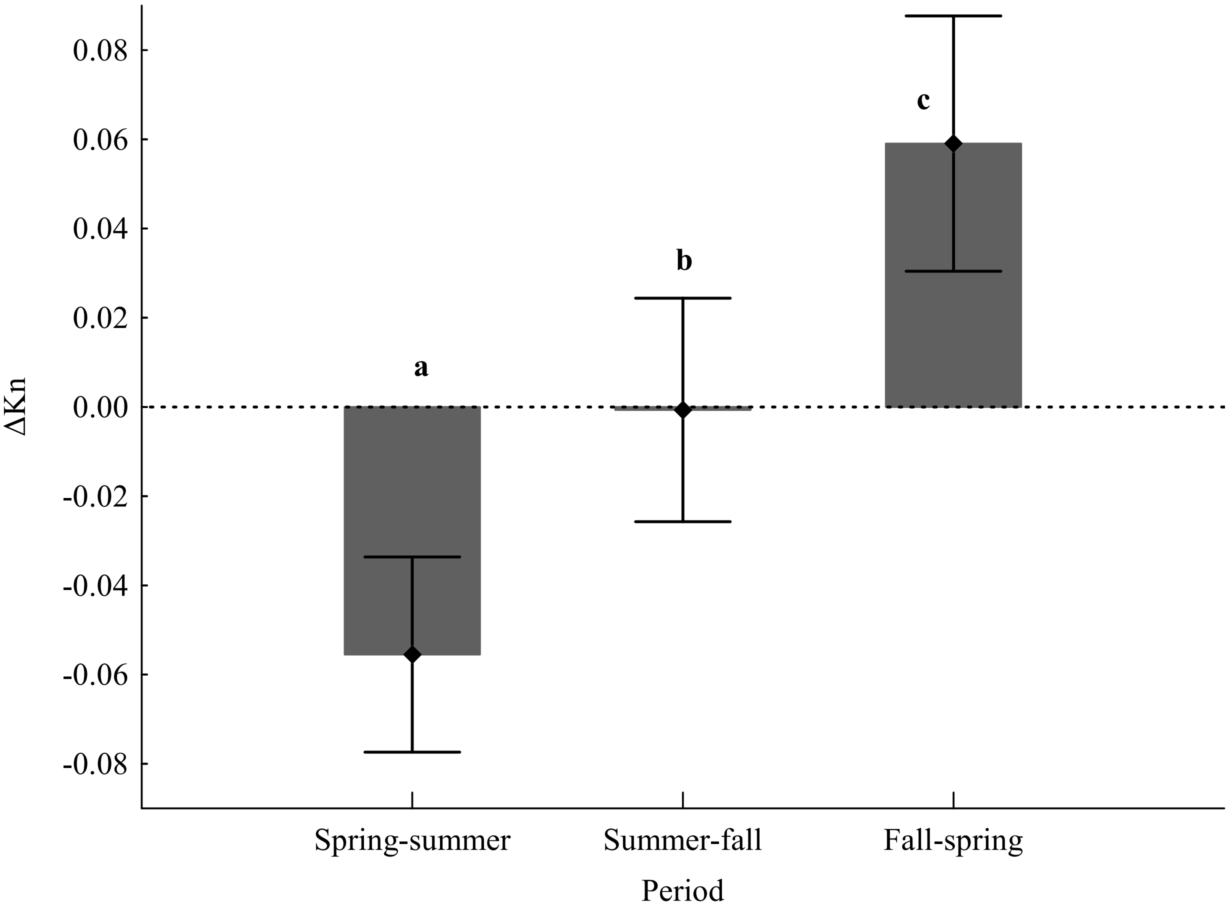
	EGSL	**NW Atl**	**t–test**		
			df	t	*P* value
♀-17β-estradiol (pg mL^{-1})	43.90 ± 85.37	17.16 ± 15.91	21	0.87	0.3943
♂-11-ketotestosterone (pg mL^{-1})	22.52 ± 9.46	68.21 ± 49.77	23	-3.57	0.0016
Length (cm)	28.3 ± 2.7	25.6 ± 3.1	47	3.18	0.0026
Mass (g)	177.3 ± 48.9	143.9 ± 54.8	47	2.24	0.0298
Kn	1.00 ± 0.08	1.08 ± 0.06	47	-4.01	0.0002
Muscle energy (kJ g^{-1} wet)	4.05 ± 0.66	4.05 ± 0.44	47	-0.01	0.9928

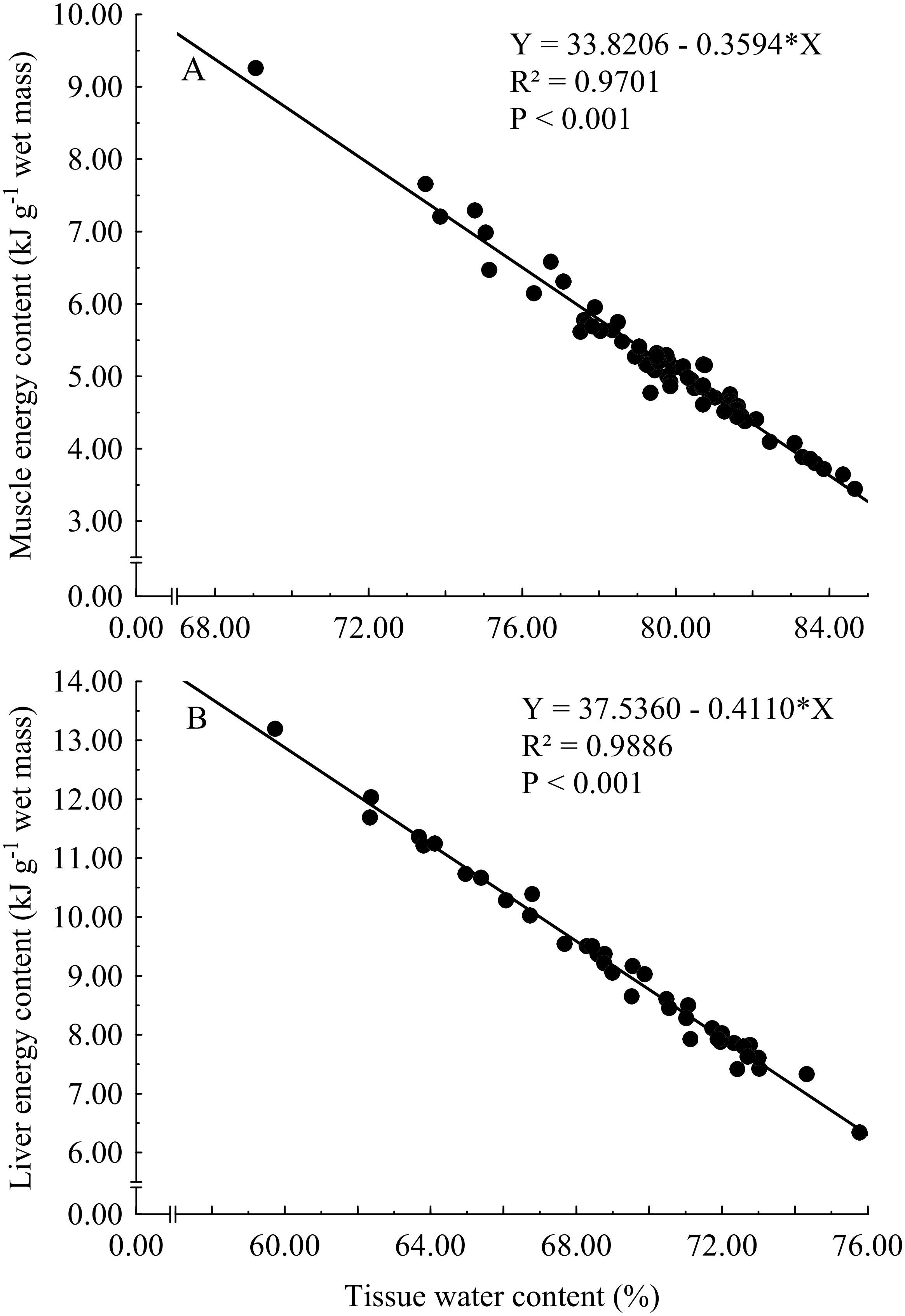

Isobaths
200 m
500 m
2500 m
NAFO divisions
Oceanographic survey areas
Sampling stations
N
2H (2017)
2J (2017)
NEA (2016)
SLE (2016)
SLE (2017)
0
100
200 km

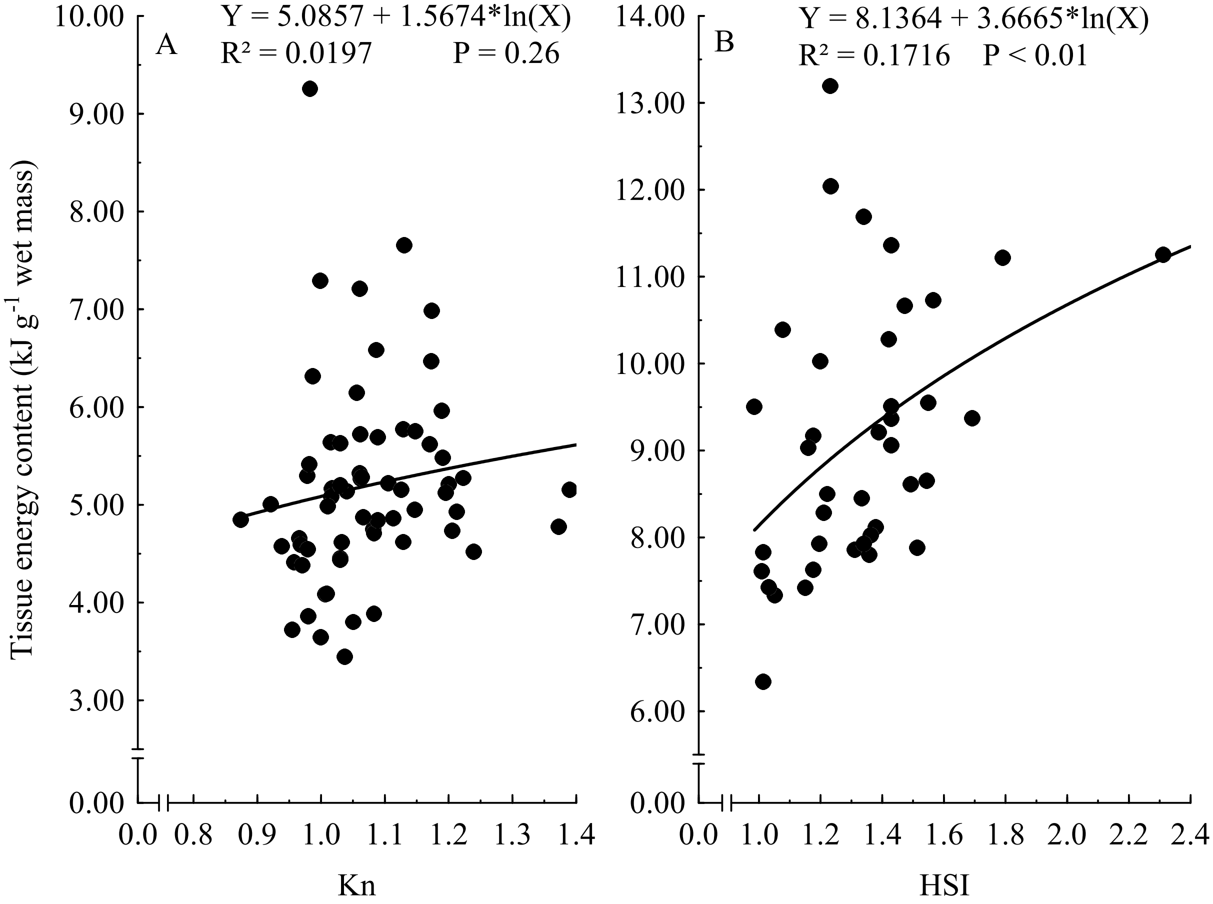

LN (mass) = 3.2094 * LN (length) - 5.5669
R² = 0.96, F1,10780 = 229343.80, P < 0.001
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0.0
LN (mass) (g)
0.0
3.0
3.1
3.2
3.3
3.4
3.5
LN (length) (cm)



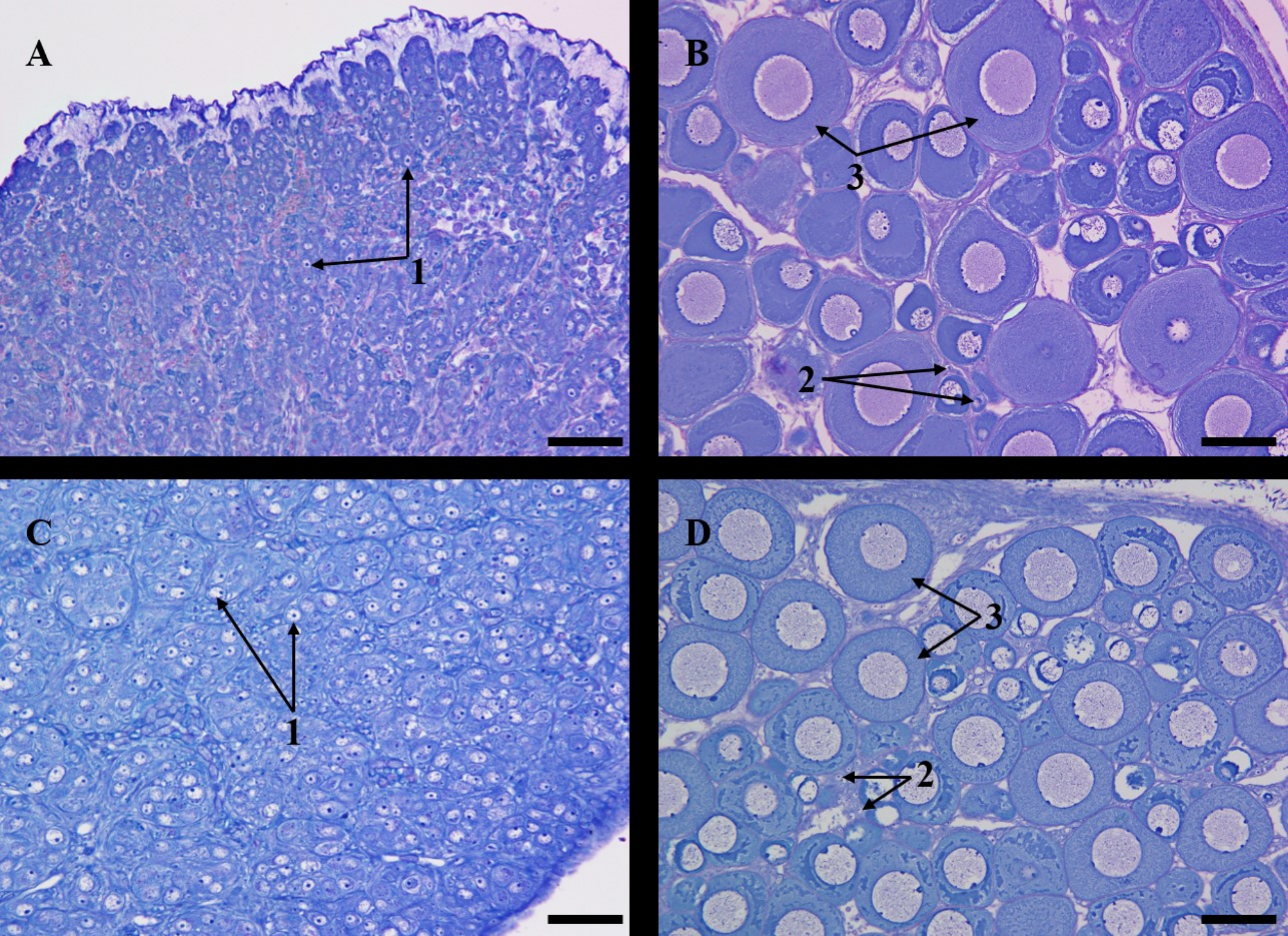
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.00
Kn
c
b
a
c
a
b
b
a
a
b
a
a
2006
2007
2008
2009

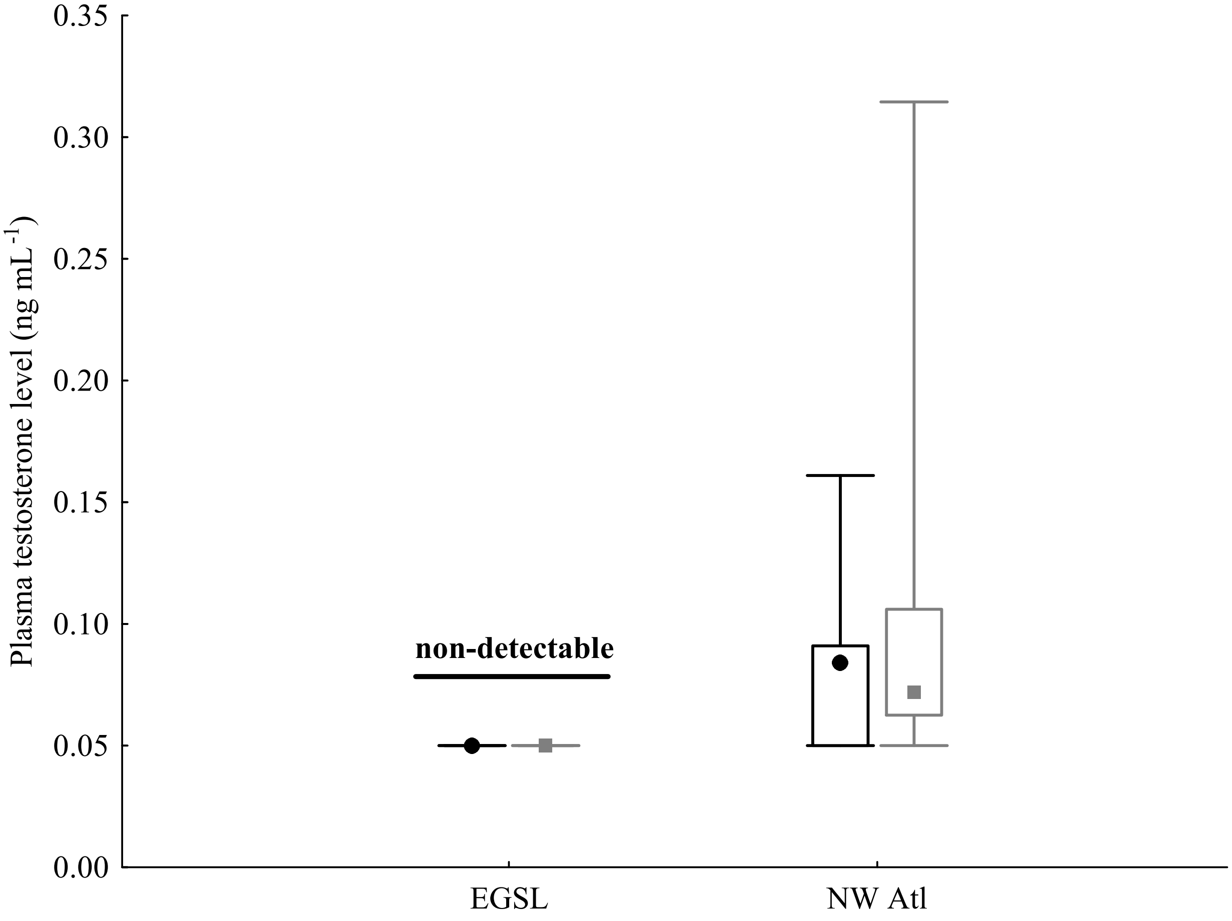

0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
ΔKn
a
b
c
Spring-summer
Summer-fall
Fall-spring
Period


A
Y = 33.8206 - 0.3594*X
R² = 0.9701
P < 0.001
Muscle energy content (kJ g-1 wet mass)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
0.00
0.00
68.00
72.00
76.00
80.00
84.00
B
Y = 37.5360 - 0.4110*X
R² = 0.9886
P < 0.001
Liver energy content (kJ g-1 wet mass)
14.00
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
0.00
0.00
60.00
64.00
68.00
72.00
76.00
Tissue water content (%)



A
Y = 5.0857 + 1.5674*ln(X)
R² = 0.0197 P = 0.26
B
Y = 8.1364 + 3.6665*ln(X)
R² = 0.1716 P < 0.01
Tissue energy content (kJ g-1 wet mass)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
0.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
0.00
0.0
0.8
0.9
1.0
1.1
1.2
1.3
1.4
Kn
0.0
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
HSI


A
1
B
3
2
C
1
D
3
2


Plasma testosterone level (ng mL^{-1})
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
non-detectable
EGSL
NW Atl