# Influence of Sex on Growth of Juvenile Greenland Halibut (*Reinhardtius hippoglossoides*) in the Estuary and Gulf of St. Lawrence

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## Abstract

Understanding growth in early life stages is primordial in commercial fisheries management, but studies dealing with growth trajectory divergences occurring during the juvenile stage are scarce. We surveyed growth of individually tagged two-year-old juvenile Greenland halibut captured in the wild, and maintained at 4°C for 17 months. At the end of the experiment, they were still at the juvenile stage (no ovary or testis development through the maturation stage). In the absence of energetic allocation to gonad development, female juveniles were larger than male juveniles, with a greater gain in mass and length. Female juveniles rapidly reached a higher Fulton condition index while maintained in captivity compared to male juveniles. However, no difference in glycogen, lipid, or protein liver composition or muscle energy storage was found between the sexes. Plasma cortisol was higher in male juveniles, suggesting males were more sensitive to captivity than females. Pre-maturation divergences in juvenile growth could participate in or accentuate the size-related sexual dimorphism observed in nature among adults.

**Keywords: Greenland Halibut, growth, sexual dimorphism, juveniles, fisheries**

## 1. Introduction

Size-related sexual dimorphism (SSD) is common among animal species (Shine, 1989; Fairbairn et al., 2007); it has been observed in mammals (Isaac, 2005), birds (Richter, 1983), lizards (Anderson and Vitt, 1990), insects (Fuselier et al., 2007), spiders (Vollrath and Parker, 1992), and fish (Parker, 1992). Factors that control these differences are numerous. In fish, sexual selection (Casalini et al., 2010), parental care (Dupont Cyr et al., 2018), niche separation (Gerritsen et al., 2010), and feeding characteristics (type of food, food intake, food conversion efficiency, search for food, and vulnerability to predators; Blair Holtby and Healy, 1990; Rennie et al., 2008; Horppila et al., 2011) have all been shown to influence SSD. However, the physiological mechanisms that govern these differences remain unclear.

Growth occurs throughout the entire life of fish, but it is not constant over time. Because of variations in energy allocation, fish growth models are characterized as biphasic: the first phase, pre-maturity, is characterized by a relatively high growth rate, whereas in the second phase, post-maturity, somatic growth is slowed due to the trade-off in energy allocation between reproduction and growth (Roff, 1983).

As suggested by Roff (1983), sexual maturation is a turning point in the allocation of energy to growth during which, in most cases, SSD occurs simultaneously with the secretion of sexual steroid hormones, which influence growth (Mandiki et al., 2005; Rennie et al., 2008). Thus, sizes at maturity play an important role in the SSD observed in the nature. Sexual maturation generally occurs when an individual has enough energy reserves to invest in reproduction, survive, and maximize its fitness (Roff, 1982; Stearn, 1992). It is therefore common among bony fishes that males, whose energy investment in reproduction is generally lower than in females (*e.g., Pleuronectes platessa*; Rijnsdorp and Ibelings, 1989), become sexually mature at a smaller size. This is especially true in flatfishes such as plaice (*P. platessa*; Rijnsdorp and Ibelings, 1989), turbot (*Scophthalmus maximus*; Imsland et al., 1997), sole (*Solea solea* and *S. senegalensis*; Dinis et al., 1999; Imsland et al., 2003), Atlantic halibut (*Hippoglossus hippoglossus*; Jakupsstovu, 1988; Hagen et al., 2006), and Greenland halibut (*Reinhardtius hippoglossoides*; DFO, 2018).

Large size differences related to SSD may represent a threat for fisheries, resulting in sex imbalance in catches. Greenland halibut *R. hippoglossoides* (Walbaum, 1792) is a circumpolar species found on continental shelves and slopes of oceans and seas at high latitudes (>45° N; Hedges et al., 2017). As a demersal flatfish inhabiting deep (generally 130 to 1600 m and sometimes up to 2200 m; Scott and Scott, 1988; Bowering and Power, 1995; Bowering and Nedreaas, 2000; Boje and Hareide, 1993) and cold water (-0.5 to 6°C; Bowering and Nedreaas, 2000), Greenland halibut is characterized by slow growth and late maturity (Junquera et al., 1999; Gundersen et al., 2009; Kennedy et al., 2009). Greenland halibut, a species with high commercial value, is intensively harvested throughout the North Atlantic Ocean (Victorero et al., 2018). In the Estuary and Gulf of St. Lawrence (EGSL), 50% of females are mature at 44 cm whereas size at 50% maturity for males is 35 cm (Bernier and Chabot, 2013; DFO, 2018). Since the beginning of the 1990s, these sizes have tended to decrease in both sexes (Morgan et al., 2003; DFO, 2018). Increasing bottom temperature and fishing pressure could explain this trend (Bowering and Brodie, 1991; Barot et al., 2005).

The EGSL stock has been considered as a separate management unit from the Northwest Atlantic since the early 1990s based on studies done by Arthur and Albert (1993). Until 2017, the EGSL stock abundance was considered stable and high even though changes had been observed in biological parameters and stock structure since the beginning of this century

(DFO, 2018). Since the late 1990s, after the introduction of a larger mesh size for gillnets in the fishery and the adoption of a minimum catch size of 44 cm, the percentage of females in commercial catches increased from 57% to 80% (Bernier and Chabot, 2013; DFO, 2018). The early maturation of males in terms of size is largely responsible for this sex ratio imbalance in commercial catches (DFO, 2018).

Many studies have focused on size at maturity and the divergence in growth pattern between sexes following initiation of sexual maturation, but few have investigated the possible differences in growth before the sexual maturation. The aim of the present study was to examine whether growth differences between males and females occur before sexual maturation, and how this may explain differences observed in commercial catches. Juvenile Greenland halibut growth was monitored in experimental tanks for 504 days. Physiological indicators were used to characterize the growth potential of each sex during the experiment.

## 2. Materials and methods

### *2.1 Capture, rearing conditions, and experimental design*

Greenland halibut juveniles were caught in the St. Lawrence Estuary (48° 39′ 11″N, 68° 28′ 37″W) with a Comando-type trawl (three-inch trawl bucket, extension, and pocket with two-inch mesh size) aboard the CCGS *Leim* (Fisheries and Oceans Canada [DFO] survey) at the end of May 2016. The tows lasted 30 minutes at speeds of 2–3 knots; depths varied between 175 and 275 m but most captures occurred at 235 m. Juveniles between 20 and 32 cm were selected. According to Ait Youcef et al. (2015) and Bourdages et al. (2016), these lengths should correspond to two-year-old juveniles. The juveniles were driven from Rimouski to the Maurice-Lamontagne Institute (DFO; 48° 38' 25″N, 68° 9' 21″W), a distance of 34 km, in insulated aerated tanks. Wild fish were acclimated to captivity conditions for two months at

5.0°C, which corresponds to the temperature at which juveniles were captured. During this period, fish were trained to feed in captivity, and post-capture mortalities ceased. The total post-capture mortality was 59%. In July, 10 of the acclimated juveniles were sacrificed at random for physiological measurements and 81 were randomly transferred into five 850 L circular tanks. Weight was variable, with a minimum of 62 g and a maximum of 244 g. The average number of fish per tank was 16 (13–18) for an average density of 1.8 ± 0.3 kg m$^{-2}$. Fish were tagged with an electronic chip (PIT Tag HPT9; Biomark, Inc., Idaho, USA) introduced into the dorsal part of the muscle on the pigmented side, which allowed us to determine the sex of fish *a posteriori* when they were euthanized. The temperature was gradually decreased to 4.0°C (4.1 ± 0.2 °C) within two days (-0.5°C d$^{-1}$), which represents the lowest part of the range characterizing the average bottom temperatures where Greenland halibut juveniles are usually captured during DFO surveys (Ait Youcef et al., 2015). Tagging allowed the *a posteriori* identification of sex. Rearing tanks were supplied with natural seawater (10 L min$^{-1}$, salinity 27.0 ± 1.6) and oxygenated with bubblers (oxygen saturation > 80%). Since juveniles inhabit mesopelagic habitats with small measurable quantities of light (disphotic zone), low red light intensity (30 ± 17 lux) was provided following natural variations of the photoperiod at this latitude. Greenland halibut juveniles were fed individually to satiety twice a week with capelin (*Mallotus villosus*) and shrimp (*Pandalus borealis*). Once a month, multivitamin-enriched additives (Vita-Zu Small Bird Tablet, no vitamin A added, # 5TLC, Mazuri®) that are essential for immune system development were added to the diet. The day after feeding, regurgitations were regularly observed and weighed. Because the feeding technique was close to force-feeding, some individuals may than have eaten more food than they were able to assimilate, resulting in regurgitation. Approximately

7.1 ± 3.1% of the food taken at each feeding was regurgitated. The experiment lasted until December 2017; total mortality was 5% during this period.

Experimental methods complied with the regulations of the Canadian Council on Animal Care and were approved by the Maurice Lamontagne Institute animal care committee.

### *2.2 Samplings*

Growth rate was monitored on four occasions: July 2016, December 2016, July 2017, and December 2017. At each sampling time, all fish were anaesthetized with a solution of metomidate (metomidate hydrochloride, Aquacalm, 5 mg $L^{-1}$; Western Chemical Inc., Washington, USA) (Mattson and Riple, 1989), measured to the nearest 0.1 cm, and weighed to the nearest 0.1 g. In July 2016, December 2016, and July 2017, 10 individuals were randomly sacrificed for tissue samplings. In December 2017, the expected time for the first sexual maturation, 10 males and 10 females were sacrificed. Fish were anaesthetized for 5 min in a solution of MS 222 (tricaine methane sulfonate 0.18 g $L^{-1}$; Sigma-Aldrich, Co., Missouri, USA) between 12:00 and 17:00 to avoid possible biases associated with endocrine circadian rhythms. The individuals were then weighed and measured. 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 of which were previously heparinized (ammonium heparin salt; Sigma-Aldrich, Co., Missouri, USA) in a heparin solution at 100 U $mL^{-1}$. Liver, muscle, and gonad samples were excised. Liver sections were frozen in liquid nitrogen and stored at -80°C for further analysis. Blood samples were centrifuged for 3 minutes at 4.6 G and plasma was frozen in liquid nitrogen and stored at -80°C. Gonads were fixed in Bouin's solution (Sigma Aldrich, Co., Missouri, USA).

### *2.3 Morphological calculations*

The tank was the statistical unit for growth monitoring. Mass (1) and length (2) increments per day were used to describe the growth of juveniles (Hopkins, 1992) using the following formula:

$$WI = (W_T - W_t) / \Delta t \quad (1)$$

$$LI = (L_T - L_t) / \Delta t \quad (2)$$

where $W_T$ ($L_T$) and $W_t$ ($L_t$) are fish masses (lengths) at the end (T) and the beginning (t) of the period considered, and $\Delta t$ is the number of days between measurements.

Fish health condition was estimated using the Fulton condition index (K):

$$K = W / L^3 \quad (3)$$

where W is the total mass (g) and L the total length (cm) of fish.

### *2.4 Physiological measurements*

For physiological measurements, individuals were considered as the statistical unit. In December 2016, no males were present among euthanized fish. Sexual maturity of fish was determined by histology and sex steroid measurements. Gonads were dehydrated in increasing concentrations of ethanol and embedded in metacrylate JB-4 solution according to the protocol suggested by the manufacturer (Sigma-Aldrich, Co., Missouri, USA). The embedded gonadal tissue was sectioned in 3 µm slices and stained with Lee's Methylene blue – basic fuchsin solution. The stage of testis and ovarian development was determined for each individual according to Morrison (1990) and to Rideout et al. (2012), respectively. For each individual, a histological section in three different slides was used to determine the maturity stage. Sex steroid concentrations were measured using RIA kits for testosterone and 17β-estradiol ($^{125}$I RIA Kit # 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). Undetectable values were assigned the half-value of the smallest standard.

Hepatic glycogen was measured using the amyloglucosidase digestion method (Carr and Neff, 1984) followed by the determination of the glucose concentration (Glucose Colorimetric Assay Kit, Cayman Chemical, Inc., Michigan, USA). Total liver lipid concentration was determined using the phospho-vanillin method (Frings et al., 1972). The concentration of liver protein was 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 for protein, total lipids, and glycogen concentrations into energy that were respectively 24, 38, and 17 kJ/g (Jobling, 1993).

The water content of muscle (X) was determined after drying pieces of muscle for 48 h at 65°C and converted into a total muscle energy content (Y, kJ g of muscle$^{-1}$) using a relationship established for Greenland halibut juveniles by Ait Youcef (2013):

$$Y = 61.7366 - 0.4568X \quad (4)$$

Plasma cortisol was measured using an RIA kit ($^{125}$I RIA Kit # 07-221106, MP Biomedicals, LLC, California, USA) and used as a primary stress indicator (Mazeaud et al., 1977).

### *2.5 Statistical analysis*

Normality and homogeneity of variances were verified by Kolmogorov-Smirnov and Levene tests, respectively. No data transformation was needed to meet these criteria except for steroids data, which were log transformed prior to statistical analysis. Length, mass, length increment, mass increment, and Fulton index were analysed using repeated-measurements analysis of variance. Sexual steroids were compared using one-way ANOVAs and maturity stage compared with nonparametric Kruskall-Wallis analyses. Because no males were present in the December 2016 sampling (random sampling in each tank), two-way ANOVAs (sex and sampling time) were first run for liver and muscle energy content variables as well as for cortisol excluding December 2016 data. For all these variables, there were no significant interactions between factors ($P > 0.05$). We then added December 2016 data and checked for sampling time or sex effect using one-way ANOVAs and Student's t tests, respectively. The *a posteriori* Tukey test was used when significant factor effects were found for comparisons of means ($\alpha = 0.05$). Statistical analyses were performed with Statistica software (Statsoft v.6.1, Oklahoma, USA).

## **3. RESULTS**

### *3.1 Fish growth and condition factor*

At the beginning of the experiment, no sex-based differences in length and mass were observed (Fig. 1A, B). The growth pattern was significantly different between male and female juveniles (mass: time × sex, $F_{3,24} = 5.78$, $P < 0.01$; length: $F_{3,24} = 4.63$, $P < 0.05$). Although no difference was found between the two sexes at each sampling time, growth trajectory was steeper for female juveniles, with each measured value being significantly different from the previous ones; this was not the case for males. Mass and length increments were significantly higher in female than in male juveniles ($0.57 \pm 0.22$ g $d^{-1}$ and $0.36 \pm 0.16$ g

$d^{-1}$, respectively; $F_{1,8} = 6.2683$, $P < 0.05$; 0.018 ± 0.005 cm $d^{-1}$ and 0.013 ± 0.006 cm $d^{-1}$, respectively; $F_{1,8} = 5.16$, $P = 0.05$). No significant difference among sampling times or interactions between sampling times and sex were found ($P > 0.05$).

The Fulton index increased sharply during the first fall, after which it remained stable in females but continued to increase from December to July in males (sampling time × sex: $F_{3,24} = 4.64$, $P = 0.01$) (Fig. 1C). At the experiment's end, the condition factor was similar between male and female juveniles (Fig. 1 C).

### *3.2 Maturity stage*

Males were still sexually immature at the end of the experiment (December 2017), with gonads only showing spermatogonia ($P > 0.05$; Fig. 2A). Plasma testosterone remained undetectable during the whole experimental period, and plasma 11-ketotestosterone concentration remained stable and very low, with an average value of 138.14 ± 304.53 pg $mL^{-1}$ ($F_{2,15} = 1.56$, $P > 0.05$).

Female gonads also remained at the immature stage during the experiment, with the presence of oogonia and primary stage oocytes at the perinucleolus stage (see García-López et al., 2007) ($P > 0.05$; Fig. 2B). As observed for males, plasma testosterone remained undetectable, but plasma concentrations of β-estradiol varied over time ($F_{3,27} = 5.87$, $P < 0.01$), with the concentration in July 2017 being higher than in both July and December 2016) (Fig. 3). Globally, β-estradiol concentration remained very low throughout the experiment (<300 pg $mL^{-1}$).

### *3.3 Hepatic and muscle energy storage*

Hepatic glycogen and protein did not vary over time ($F_{3,46} = 2.12$, $P > 0.05$; $F_{3,44} = 1.43$, $P > 0.05$, respectively). Glycogen content was similar between male and female juveniles, with an overall mean of 19.23 ± 9.49 mg $g^{-1}$ (t-test, $t_{48} = 0.17$, $P > 0.05$), while protein content was higher in males than in females (t-test, $t_{46} = 2.96$, $P < 0.01$; 50.62 ± 4.55 mg $g^{-1}$ and 46.19 ± 5.18 mg $g^{-1}$, respectively). Hepatic lipids varied over time (Fig. 4A), with the lowest concentration in July 2016 compared with the three other sampling times (207.84 ± 53.38 mg $g^{-1}$; $F_{3,46} = 9.07$, $P < 0.001$), without significant difference between sexes (t-test, $t_{48} = 0.31$, $P > 0.05$). Total energetic content was also significantly lower in July 2016 compared to the other sampling times ($F_{3,44} = 9.11$, $P < 0.001$) with no sex difference (t-test, $t_{46} = 0.14$, $P > 0.05$; Fig. 4). Lipids accounted for most of the energy reserves in the liver (> 70%; Fig. 5), while glycogen accounted for the lowest fraction (< 7%; Fig. 5). No sex difference was found (t-test, $t_{48} = 0.14$, $P > 0.05$), but the lowest amount of muscle energy reserves was observed in July 2016 (4.06 ± 0.67 kJ $g^{-1}$) and the highest (5.11 ± 0.87 kJ $g^{-1}$) in December 2017 (ANOVA, $F_{3,46} = 4.70$, $P < 0.01$), with intermediate values for the other two sampling periods (4.67 ± 0.91 kJ $g^{-1}$).

### *3.4 Cortisol level*

Plasma cortisol was significantly higher in male than in female juveniles ($t_{47} = 2.50$, $P < 0.05$; Fig. 6A). Plasma cortisol was highest in July 2016 (beginning of the experiment) and lowest in December 2016, with intermediate values in 2017 ($F_{3,45} = 3.88$, $P < 0.05$; Fig. 6B).

## 4. Discussion

One aim of this study was to examine the differences in growth patterns between female and male Greenland halibut prior to the onset of sexual maturity. In general, despite the lack of sexual maturation, the results show higher growth in females relative to males for juveniles caught in the St. Lawrence Estuary.

Rearing Greenland halibut in captivity is challenging, which has limited the number of studies in controlled conditions for this species. Nevertheless, the growth pattern is well known in nature, especially in the EGSL. Here we showed that juvenile growth remained constant over the seasons in both sexes, which is in accordance with results obtained from field data (Ait Youcef et al., 2015). This study highlights differences in growth between female and male juveniles that have never been shown for this species.

Considering the histological and physiological data, it was clear that every fish remained at the juvenile stage throughout the experiment. Some significant seasonal variations in 17β-estradiol concentration occurred in females, but concentrations remained very low (5–276 pg $mL^{-1}$), considering, for example, that the 17β-estradiol plasma concentration in Atlantic halibut (*H. hippoglossus*) may exceed 20 ng $mL^{-1}$ during the spawning season (Methven et al., 1992). Testosterone concentrations remained undetectable in both sexes throughout the experiment. In males, concentrations of 11-ketotesterone remained below 1789 pg $mL^{-1}$, which is far lower than concentrations reported during milt formation in other species. For example, 11-ketotesterone concentration exceeded 2 ng $mL^{-1}$ and peaked at up to 16–18 ng $mL^{-1}$ during the reproduction season in Atlantic halibut (Methven et al., 1992; Norberg et al., 2001). The histological analysis confirmed hormonal results, with no advanced differentiated germ cells in the testes or ovaries. At the end of the experiment, females measured 35.7 ± 1.8

cm and males 32.6 ± 2.7 cm. Only 6.1% of females exceeded 44 cm and 21.7% of males were above 35 cm, which are respectively the size at which 50% of fish should have reached sexual maturity (DFO, 2018).

Contrary to females, males needed more time to reach their maximal Fulton index throughout the experiment. The Fulton index increases with size in juvenile Greenland halibut (DFO, 2018), reflecting their allometric growth, with a gain in mass that exceeds that in length as fish grow. This suggests a poorer condition of males during the first half of the experiment, reflecting their slower growth rate in length and mass. Slower growth in male juveniles has already been observed in flatfish, both under experimental conditions for turbot (*S. maximus*; Imsland et al., 1997) and in nature for southern flounder (*Paralichthys lethostigma;* Fitzhugh et al., 1996).

Relative energy storage was also similar between juveniles of both sexes. Globally, males had a slightly higher liver protein content than females, but the total hepatic energy content remained similar in both sexes. The results showed that liver energy content was correlated with lipid storage in both sexes. In this experiment, juveniles reached a maximum plateau in both liver lipids and energy content after five months. Since they were fed to satiation, we can expect that our results are representative of well-fed juveniles in nature. In juveniles, the liver represents on average only 1.3% of the total body weight (Ait Youcef, 2013) and is considered to have only a limited contribution in energy storage; this has already been observed in other flatfish species (Dawson and Grimm, 1980; Maddock and Burton, 1994). Indeed, in Greenland halibut, most of the energy is stored in muscle tissue (197 kJ in muscle vs 13 kJ in liver in a 25 cm juvenile; Ait Youcef, 2013). The evolution of muscle energy content was quite different than in liver: the rise was slower, constant, and almost linear.

Because of the high proportion of muscle lipids, Greenland halibut is considered as a fatty fish species (Karl et al., 2018). These authors showed that muscle water percentage was negatively correlated with lipid content, which would confirm that lipids—as in other flatfish species (*P. platessa*, Dawson and Grimm, 1980; *Pseudopleuronectes americanus*, Maddock and Burton, 1994)—represent the main energy storage in Greenland halibut juveniles. Even though the relative energy level in the muscle is lower than in liver, muscle tissue represents the largest part of the body (Ait Youcef, 2013). Such a continuous increase in energy storage was expected before the onset of sexual maturation. Thus even though feeding conditions seemed to have promoted constant juvenile growth, it does not seem to have induced early sexual maturation, which has already been observed in aquaculture conditions for *S. maximus* (Imsland et al., 1997).

SSD observed in commercial catches in the EGSL has been associated with differences in the size-at-maturity, with male juveniles becoming adults at smaller sizes than female juveniles (DFO, 2018). In American plaice (*Hippoglossoides platessoides*), somatic growth decreases once sexual maturity is reached (Roff, 1983). Consequently, a larger proportion of Greenland halibut females reach the commercial size of 44 cm (DFO, 2018). In the Northwest Atlantic (Bowering, 1983; Dwyer et al., 2016), the North Atlantic (Bowering and Nedreaas, 2001), and the Arctic (Albert, 2003, 2016), field studies did not highlight growth differences between female and male juveniles, but divergences between growth patterns among adults have been shown. In the EGSL, our results may indicate divergences occurring at the juvenile stage, and juvenile growth then could be partly responsible for SSD observed in adults. According to the work of Roff (1983) on *H. platessoides*, size-at-maturity differs between males and females because of the relative energy investment in gonad development. Furthermore, he specified that the decrease in growth rate of males at the onset of sexual maturation is not only due to their investment into the germinal growth but also to a strategy of minimal foraging to avoid predation risk and so mortality. Imsland et al. (1997) discussed an energy-saving mechanism in *S. maximus* males related to a slow-growing strategy. Contrary to the males, females would need to have a high rate of foraging in order to increase their fitness within the complex equilibrium among risks of predation, fecundity, and survival of the offspring.

Could differences in food intake or food conversion efficiency explain the differences observed between male and female juveniles? In sea bass (*Dicentrarchus labrax*, Saillant et al., 2001) and turbot (*S. maximus*, Imsland et al., 1997), differences in juvenile growth between sexes were thought to be partly associated with food intake. In adult dab (*Limanda limanda*), Lozán (1992) showed that females consumed 73% more food than similar-sized males. In our experiment each fish was fed individually, thus we assumed that they all fed to satiety. Food intake could not be measured per individual, so inferences to sex effect cannot be done. Nevertheless, establishment of a size-dependent hierarchy is common in flatfish juveniles under experimental conditions (*H. hippoglossus*, Jonassen et al., 1999; *S. maximus*, Imsland et al., 1996, 1997, 1998; Fairchild and Howell, 2001), and the resulting behaviours may influence fish juvenile growth in various ways. Although there was no competition for food in the present study, aggressive behaviours were observed. Size heterogeneity seems to increase aggressive behaviours in juvenile fish (Sakakura and Tsukamoto, 1998; Fairchild and Howell, 2001). In our study, there was great variability in size. Since females were the largest, they may also have been more dominant over time. This phenomenon has been observed in cultured Senegalese sole (*S. senegalensis*; Sánchez et al., 2010). Cortisol measurements revealed higher plasma concentrations in males. Considering the hypothesis that females displayed dominance behaviour, the higher plasma cortisol concentrations in males could be due to a higher stress level in subordinate fish (Peters et al., 1988; Sloman et al., 2001; Øverli et al., 2004). Higher cortisol concentrations may also indicate a greater sensitivity to sampling manipulations in males. The significant differences in cortisol levels between the two first sampling dates of the experiment were skewed because no male was sampled in December 2016.

In the EGSL, the sex ratio imbalance in commercial catches has been associated with differences in size at maturity and divergent growth patterns following sexual maturation (DFO, 2018). Similar high SSD was reported for this species in the Arctic (Albert, 2003), in the Northwest Atlantic (Bowering, 1983; Dwyer et al., 2016), and in the North Atlantic (Bowering and Nedreaas, 2001). Our study shows that high SSD could be due to divergences in growth patterns that start from the juvenile stage and which are not influenced by sexual steroids those remaining at very low or undetectable levels. However, this does not mean that precursors or regulators of sexual steroids production may not be involved regulating production or response to growth hormone and growth insulinic factor. Juvenile males experience slower growth than females, and this difference is accentuated at the onset of sexual maturation. The slower growth strategy in males may explain why only a small proportion of males reach the commercial size of 44 cm in contrast to females, whose strategy is to grow rapidly.

## 5. Conclusion

Greenland halibut males grew more slowly than females prior to sexual maturation. Their energy storage was similar to that of females in terms of composition but lower relative to their respective body sizes. Social interaction, food intake, and stress could be partly responsible for the differences observed in this study. These pre-maturation divergences could participate in or accentuate the SSD observed in nature among adults. Growth potentials and sexual dimorphisms are very important features to take into account in terms of fisheries resource management.

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## Authorship statements

Audet: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing.

Lambert: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review and editing.

Ghinter: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft

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## Figure captions

Figure 1: Evolution of (A) mass, (B) length, and (C) Fulton index throughout the experiment at the four sampling dates (July and December 2016 and 2017). Grey: females; black: males. Mean ± SD. Symbols with different letters indicate significant differences.

Figure 2: Histological sections of Greenland halibut gonads. (A) Immature testis containing only spematogonia (1). (B) Immature ovaries containing oogonia (2) and primary stage oocytes at the perinucleolus stage (3). Scale bars = 50 µm (A) and 100 µm (B).

Figure 3: Plasma estradiol concentration in female juveniles. The results are expressed as mean ± SD. Statistical analysis was done on log-transformed data, but non-transformed data are shown in this figure. Symbols with different letters indicate significant differences.

Figure 4: (A) Liver lipid content, and (B) total liver (grey) and muscle (black) energy content throughout the experiment. Data are expressed as mean ± SD. Symbols with different letters indicate significant differences (B: a and for muscle; a' and b' for liver).

Figure 5: Mean total hepatic energy content in Greenland halibut juveniles. Data are expressed as mean ± SD. Grey bars: lipids; hatched bars: proteins; black bars: glycogen.

Figure 6: (A) Plasma cortisol concentration in female and male juveniles and (B) average cortisol level at the different sampling times. Statistical analysis was done on log-transformed data, but non-transformed data are shown in this figure. Data are expressed as mean ± SD. Symbols with different letters indicate significant differences.

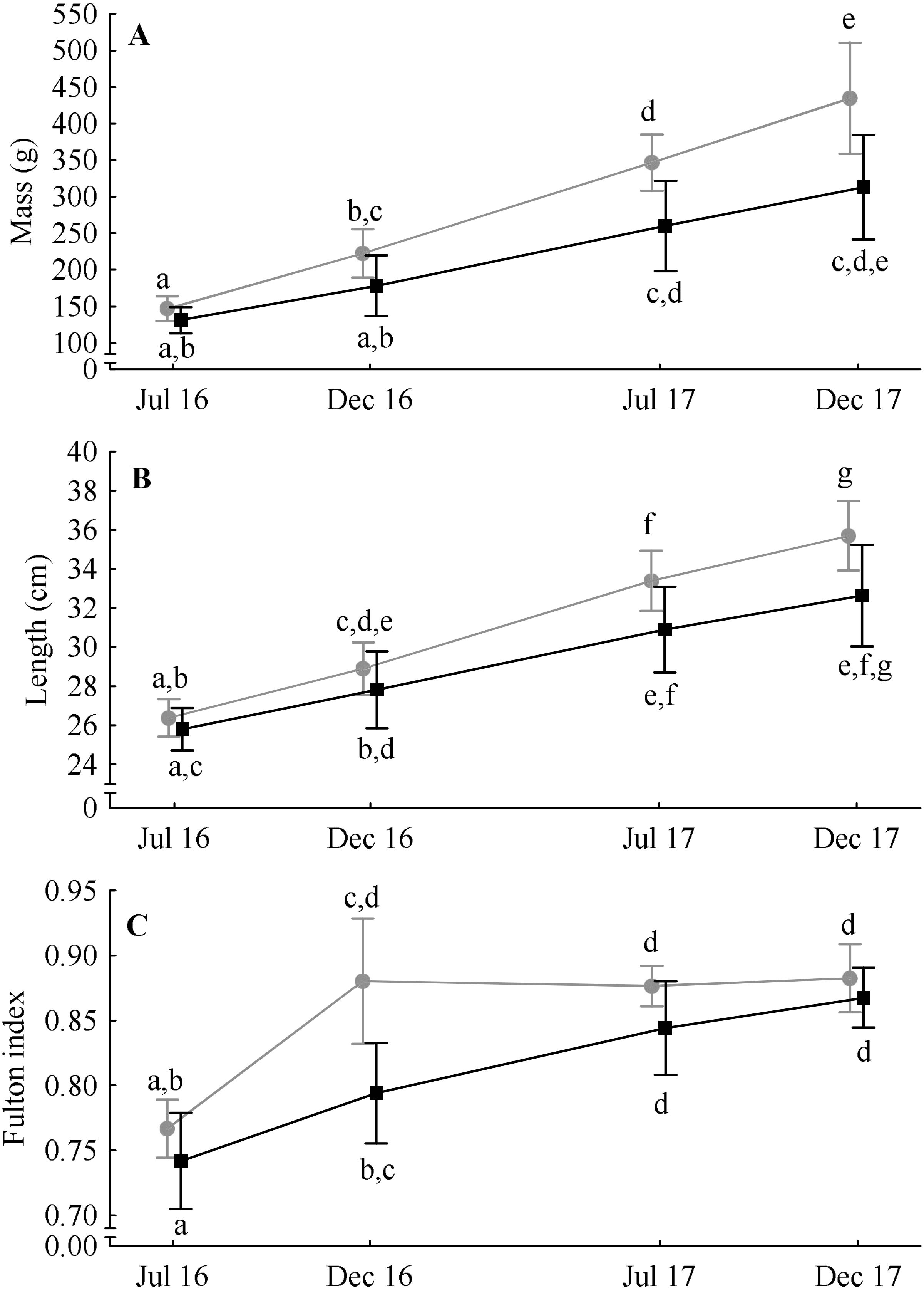
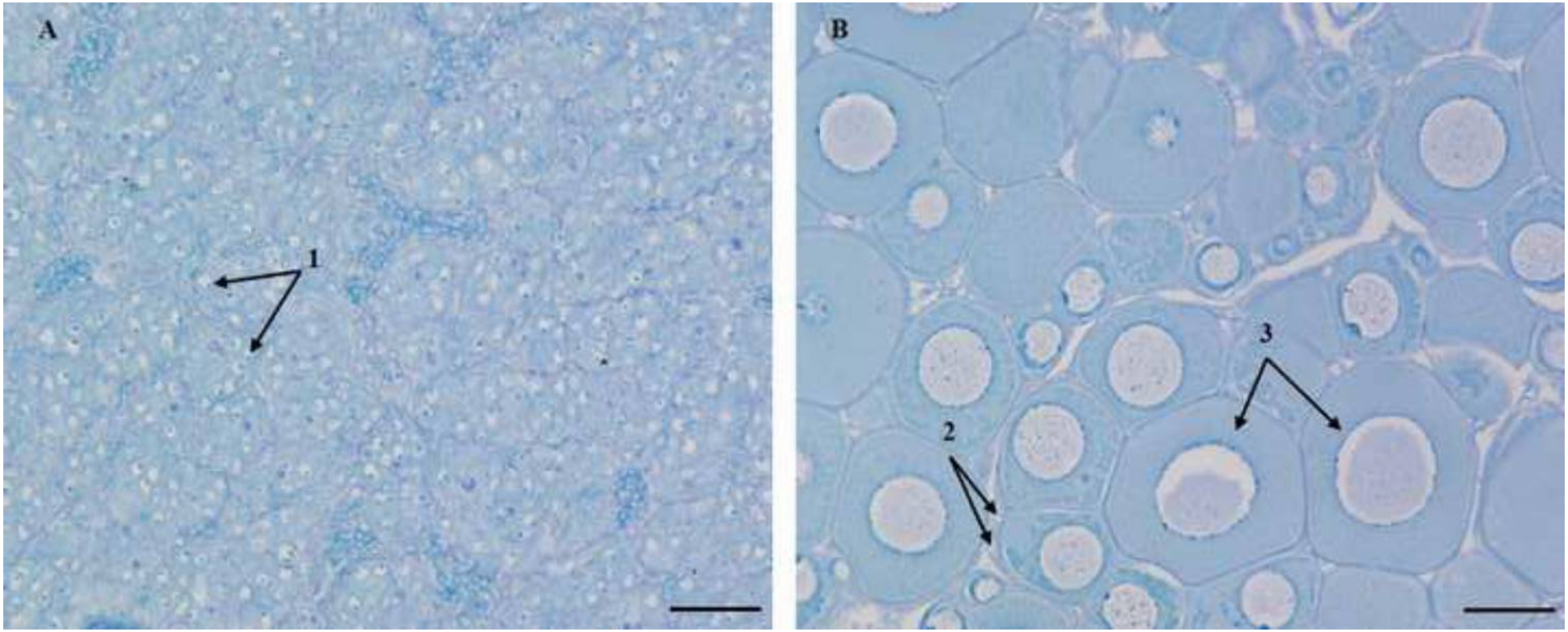

A
Mass (g)
550
500
450
400
350
300
250
200
150
100
0
a
a,b
b,c
a,b
d
c,d
e
c,d,e
Jul 16
Dec 16
Jul 17
Dec 17
B
Length (cm)
40
38
36
34
32
30
28
26
24
0
a,b
a,c
c,d,e
b,d
f
e,f
g
e,f,g
Jul 16
Dec 16
Jul 17
Dec 17
C
Fulton index
0.95
0.90
0.85
0.80
0.75
0.70
0.00
a,b
a
c,d
b,c
d
d
d
d
Jul 16
Dec 16
Jul 17
Dec 17

Figure 2



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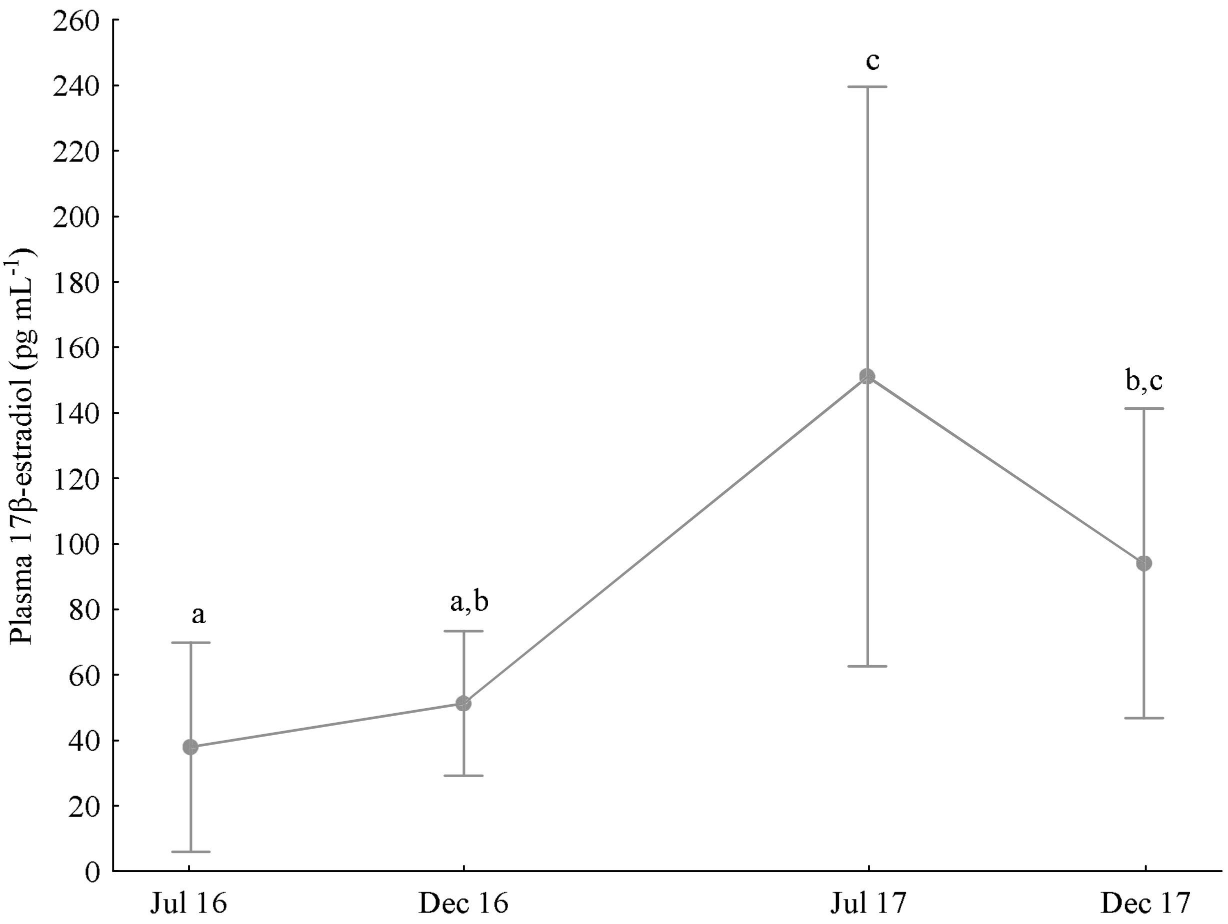
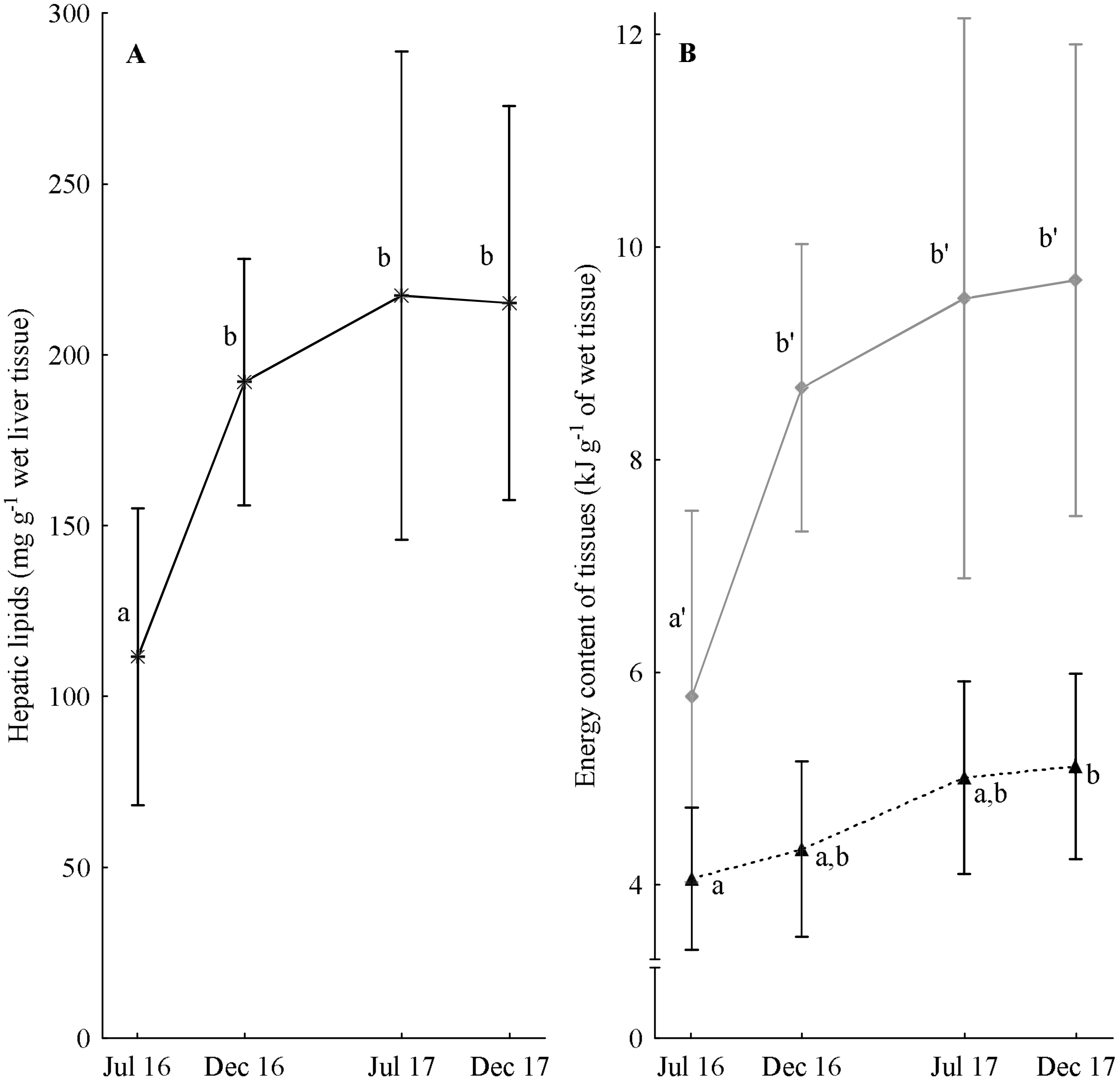
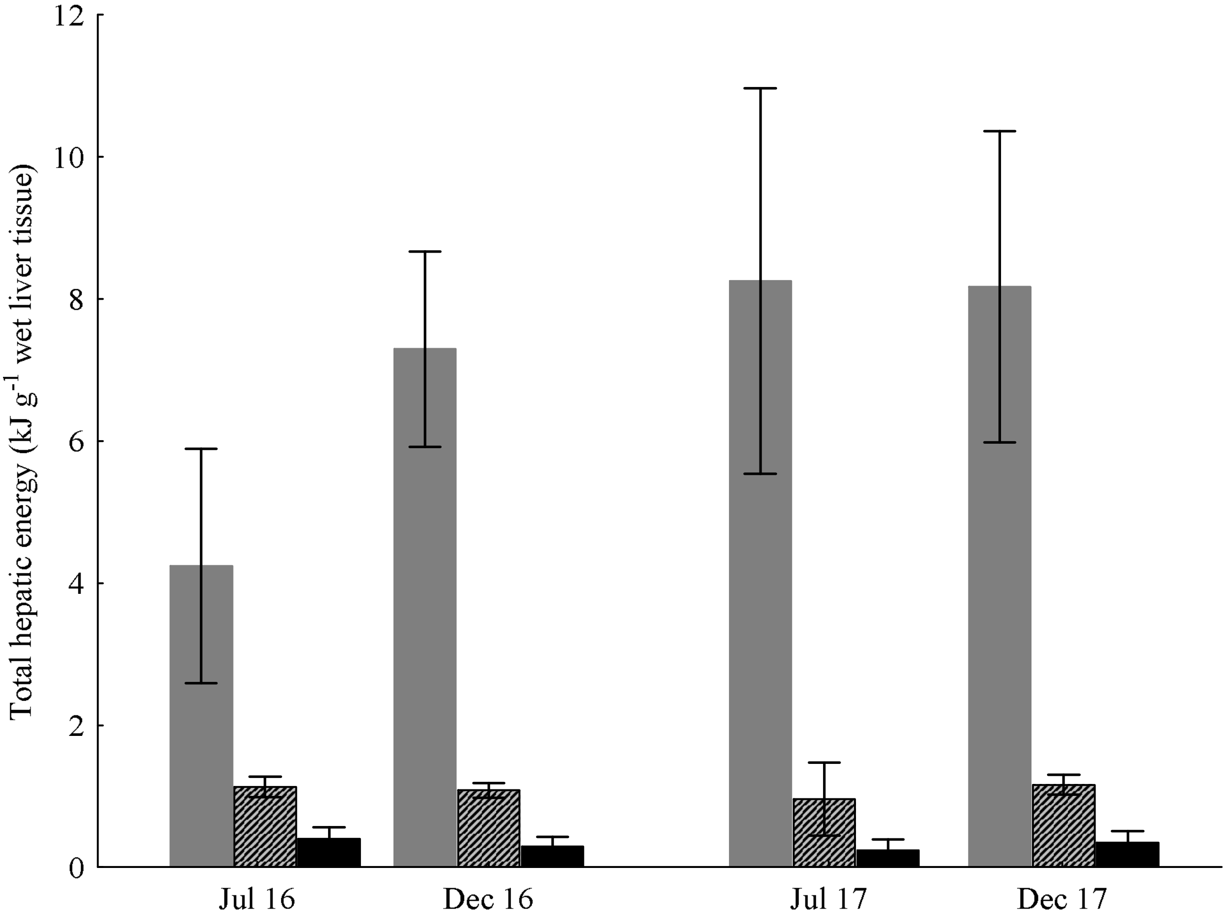
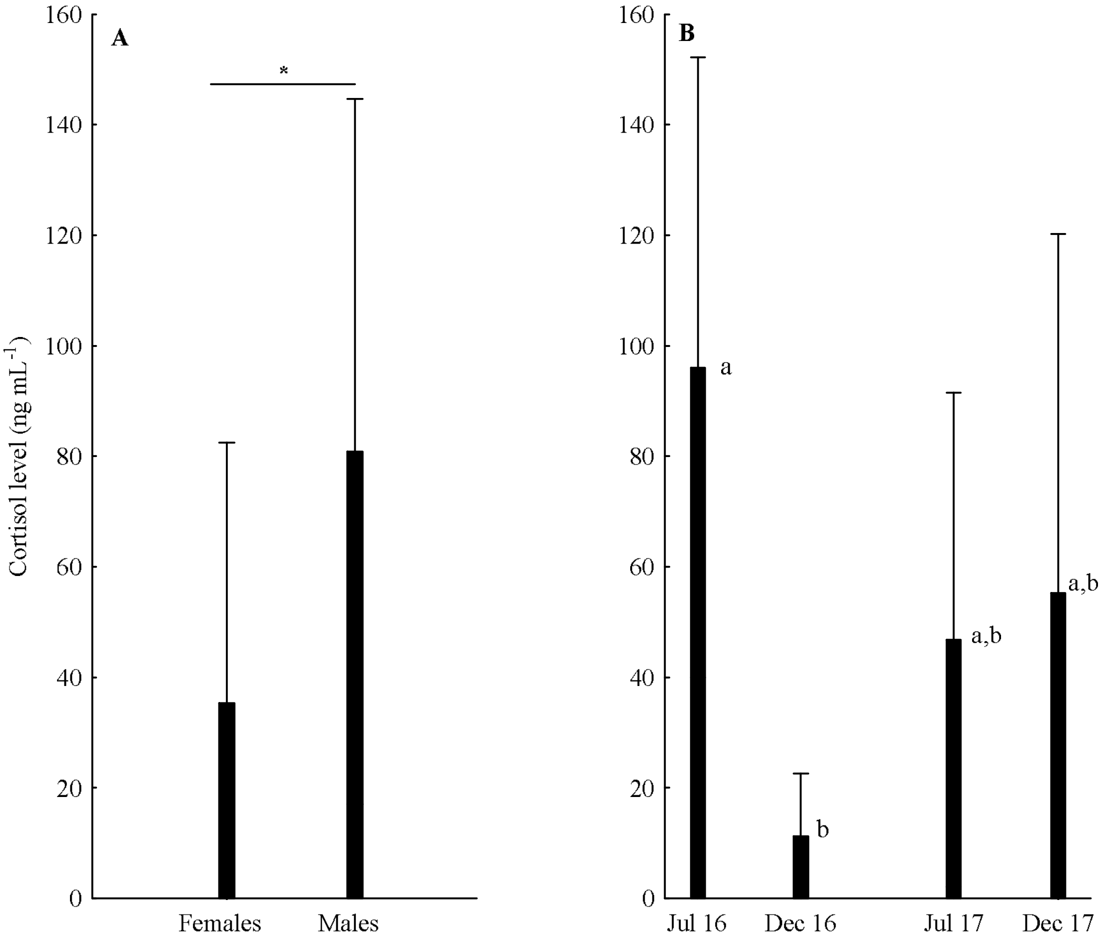

Plasma 17β-estradiol (pg mL$^{-1}$)
260
240
220
200
180
160
140
120
100
80
60
40
20
0
a
a,b
c
b,c
Jul 16
Dec 16
Jul 17
Dec 17

☝✗⛵✿♌✍



A
Hepatic lipids (mg g$^{-1}$ wet liver tissue)
300
250
200
150
100
50
0
a
b
b
b
Jul 16
Dec 16
Jul 17
Dec 17
B
Energy content of tissues (kJ g$^{-1}$ of wet tissue)
12
10
8
6
4
0
a'
b'
b'
b'
a
a,b
a,b
b
Jul 16
Dec 16
Jul 17
Dec 17


Total hepatic energy (kJ g$^{-1}$ wet liver tissue)
0
2
4
6
8
10
12
Jul 16
Dec 16
Jul 17
Dec 17


A
B
Cortisol level (ng mL-1)
0
20
40
60
80
100
120
140
160
*
Females
Males
a
b
a,b
a,b
Jul 16
Dec 16
Jul 17
Dec 17