Juvenile Greenland Halibut (*Reinhardtius hippoglossoides*) growth in the context of rising temperature in the Estuary and Gulf of St. Lawrence

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1 **Abstract**

2 In a context of climate change, understanding the influence of temperature on fish species

3 growth is important for the management of fisheries. The effect of increasing temperature on

4 the growth of juvenile Greenland halibut (*Reinhardtius hippoglossoides*), a cold-water

5 species, circum-continental in the Arctic, was investigated on juveniles that had been captured

6 in the wild and kept in captivity. Mortality rate increased with higher temperature, from 4.5%

7 at 4.0°C to 15.2% at 7.5°C. Relative growth (normalized in degree-days) was lower at 7.5°C

8 than at the two other temperatures tested. Food conversion efficiency, muscle energy content,

9 and the Fulton condition index were not influenced by temperature, but food intake

10 significantly increased over time at 5.5°C. No clear difference in growth trajectories between

11 sexes was highlighted. Overall, the results suggest that optimal temperature conditions were

12 exceeded at 7.5°C and that any further increase in temperature would significantly decrease

13 survival and impair juvenile growth. With the current deep-water warming trends in the

14 Estuary and Gulf of St. Lawrence, recruitment and commercial fishing of Greenland halibut

15 may be impaired in the near future.

16 **Keywords: Greenland Halibut, growth, temperature, juveniles, fisheries, mortality**

17 **1. Introduction**

18 Among all factors influencing fish physiology, temperature is certainly one of the most

19 important. In ectotherms, metabolic processes are closely linked to water temperature (Fry,

20 1971; Fonds *et al.*, 1992; Burel *et al.*, 1996). Thus, fish growth is temperature-dependent (*e.g.,*

21 Brett and Groves, 1979; Jobling, 1993). A rise in temperature is usually accompanied by an

22 increase in basal maintenance costs and an increase in the efficiency of metabolic processes

23 (Xiaojun and Ruyung, 1992; Pörtner *et al.*, 2010; Horodysky *et al.*, 2015). It is generally

24 observed that growth and temperature are positively correlated when temperatures are below

25   an optimum that varies among species, but that any further increase beyond that optimum

26   destabilizes metabolism and leads to a reduction in growth (Fonds *et al.*, 1995; Neuheimer *et*

27   *al.*, 2011).

28   Interrelationships between temperature, metabolism, and growth are complex. When basal

29   metabolic rate increases due to a rise in temperature, fish may compensate by consuming

30   more food (Fonds *et al.*, 1992; Russel *et al.*, 1996), by adjusting their food conversion

31   efficiency (Russel *et al.*, 1996; Imsland *et al.*, 2001; Van Ham *et al.*, 2003), and/or by

32   modifying energy assimilation efficiency (Xiaojun and Ruyung, 1992; Russel *et al.*, 1996).

33   Without proper compensation, the scope for growth should decrease while basal metabolic

34   needs increase (*Cynoglossus semilaevis*, Fang *et al.*, 2010). In laboratory studies on flatfish,

35   temperature and food availability have been identified as major factors influencing growth

36   rate (*Pleuronectes platessa*, *Platichthys flesus*, Fonds *et al.*, 1992; *Paralichthys olivaceus*,

37   Fonds *et al.*, 1995; *Scophthalmus maximus*, Van Ham *et al.*, 2003; *Cynoglossus semilaevis*,

38   Fang *et al.*, 2010).

39   Fish are remarkably sensitive to temperature changes (Steffel *et al.*, 1976; Claireaux *et al.*,

40   1995), and Greenland halibut (*Reinhardtius hippoglossoides*) has shown shifts in its

41   distribution that are associated with changes in bottom water temperatures (Morgan *et al.*,

42   2013; Wheeland and Morgan, 2019). Over the last few decades, bottom water temperatures in

43   the Estuary and Gulf of St. Lawrence (EGSL) have been rising due to increased warm water

44   pulses entering through Cabot Strait (Galbraith *et al.*, 2019). Bottom waters of the St.

45   Lawrence Estuary, the main nursery of Greenland halibut in the EGSL (Ait Youcef *et al.*,

46   2013), have increased by nearly 1°C between 2010 and 2018, from 3.56 to 4.60°C at 200 m

47   and from 4.73 to 5.63°C at 300 m. Since 2015, these changes have been particularly notable,

48   with average temperatures above 4.5°C and 5.4°C at 200 m and 300 m, respectively, while the

49   1981–2010 averages were 3.87 ± 0.36°C and 4.97 ± 0.23°C, respectively (Galbraith *et al.*,

50   2019). In 2015, a reduction of 45% in the growth of two-year-old juveniles was observed

51   (DFO, 2019).

52   Greenland halibut is a species, circum-continental in the Arctic, that inhabits cold (−0.5 to

53   6°C; Bowering and Nedreaas, 2000) and deep waters generally between 130 and 1600 m and

54   sometimes to 2200 m (Scott and Scott, 1988; Boje and Hareide, 1993; Bowering and Power,

55   1995; Bowering and Nedreaas, 2000). In flatfish, juvenile growth is determinant for

56   recruitment (*e.g.,* Van der Veer *et al.*, 1994, 2000, 2015). This is especially true for Greenland

57   halibut, which is characterized by slow growth, late maturity, and a long juvenile period

58   (Morgan *et al.*, 2003; Treble *et al.*, 2008; Albert, 2016). The EGSL population is one of the

59   southernmost in the species' range, with bottom temperatures among the warmest where

60   Greenland halibut is found. The growth rates of juveniles in the EGSL are the highest

61   recorded for Greenland halibut (Ait Youcef *et al.*, 2015). Temperature and food abundance

62   are the main factors explaining this characteristic (Bowering, 1983; Ait Youcef *et al.*, 2015).

63   Based on the absence of a temperature effect on length increment between 1- and 2-year-old

64   juveniles despite differences of 1.5°C among areas and periods studied, Ait Youcef *et al.*

65   (2105) suggested that the optimal temperature range for juvenile growth may have been

66   reached in the EGSL. In such a scenario, and in the current context of warming waters of the

67   St. Lawrence, temperature conditions could become deleterious for juvenile growth and

68   impair recruitment.

69   Greenland halibut is a species with high commercial value and is subjected to a sustained

70   fishery across the North Atlantic (Victorero *et al.*, 2018). Changes in the structure of the

71   population have already been observed, especially since 2015, with decreases in juvenile

72   abundance and growth as well as a sharp decrease in the number of fish above the minimal

73   catch size in 2017 and 2018 (Bourdages *et al.*, 2016; DFO, 2019). Along with the

74   establishment of a minimum catch size of 44 cm since the mid-1990s, changes in growth due

75 to temperature changes could have a considerable impact on the biomass available for capture

76 and therefore on the Greenland halibut fishery.

77 In the present study, we tested the effect of temperature on juvenile growth using juveniles

78 captured in the wild and acclimated to controlled conditions. The temperature conditions

79 tested were 4.0, 5.5, and 7.5°C, which would allow us to compare current field conditions as

80 well as a temperature increase that is anticipated in the near future (Galbraith *et al.*, 2019).

81 Our hypothesis is that the temperature conditions for optimal growth will be exceeded at

82 7.5°C.

2. Material and methods

2.1 Capture, rearing conditions, and experimental design

85 Greenland halibut juveniles were caught in the St. Lawrence Estuary (48° 39′ 11″ N, 68° 28′

86 37″W) at the end of May in 2016 and 2017. Captures were made aboard the CCGS *Leim* with

87 a Comando-type trawl (3″ trawl bucket; 2″ mesh size extension and pocket). The tows lasted

88 30 minutes and had a speed of 2–3 knots. In May 2016, depths varied between 175 and

89 275 m, with most captures at 235 m (n = 210; mass = 135.7 ± 52.8 g; length = 25.7 ± 3.2 cm);

90 in May 2017, depths were between 162 and 333 m and most captures at 324 m (n = 243; mass

91 = 175.5 ± 57.5 g; length = 28.4 ± 3.0 cm). Juveniles between 20 and 32 cm were selected.

92 According to Ait Youcef *et al.* (2015) and Bourdages *et al.* (2016), these lengths should

93 correspond to two-year-old juveniles. Fish were driven the 34 km from Rimouski to the

94 Maurice-Lamontagne Institute (DFO; 48° 38' 25″ N, 68° 9' 21″ W) in insulated aerated tanks.

95 Wild fish were acclimated to captivity conditions for two months at 5.0°C, which corresponds

96 to the temperature at which juveniles were captured. During this period, fish were trained to

97 feed in captivity and post-capture mortalities ended. The total post-capture mortality was 59%

98 and 58% in 2016 and 2017, respectively.

99   Because of unbalanced sex ratios (higher numbers of females, 60%), the experiment was run

100   on two consecutive years with new fish captured each spring. At the end of the acclimation

101   period (July), 10 juveniles were sacrificed at random for histological and physiological

102   measurements. The rest of the individuals (200 in 2016 and 233 in 2017) were randomly

103   placed in 850 L circular tanks (4 tanks per temperature, per year: total of 8 per experimental

104   temperature conditions) where the temperature was gradually adjusted over 2–3 days (-0.5°C

105   d-^{1}) to obtain the three targeted temperatures (4.0°C [4.1 ± 0.3], 5.5°C [5.5 ± 0.3], or 7.5°C

106   [7.5 ± 0.3]). Lengths and masses were similar among the three temperature treatments at the

107   beginning of the experiments (Table 1). According to Ait Youcef *et al.* (2015), 4.0 and 5.5°C

108   represent, respectively, the lowest and the highest part of the range characterizing the average

109   bottom temperatures where Greenland Halibut juveniles are usually captured during

110   Department of Fisheries and Oceans Canada (DFO) surveys in the EGSL. In the current

111   context of deep-water warming in the EGSL, 7.5°C was chosen as the highest temperature.

112   The average number of fish per tank was 18 and varied from 13 to 25 juveniles, for an

113   average density of 2.1 ± 0.7 kg m^{-2} (1.2–3.65 kg m^{-2}). Rearing tanks were supplied with

114   natural seawater (10 L min^{-1}, salinity 27.2 ± 1.6) and oxygenated with bubblers (oxygen

115   saturation > 80%). Because juveniles are found in mesopelagic habitats (Ait Youcef *et al.*,

116   2013) with small measurable quantities of light (disphotic zone), low intensity (30 ± 17 lux)

117   red light was provided that followed natural photoperiod variations at this latitude. Greenland

118   halibut juveniles were fed individually to satiety twice a week with capelin (*Mallotus villosus*)

119   and shrimp (*Pandalus borealis*); once a month, multivitamin-enriched additives (Vita-Zu

120   Small Bird Tablet, no Vitamin A added # 5TLC, Mazuri®) that are essential for immune

121   system development were added to the diet. The growth survey lasted for one year.

122   For the 2016–2017 experiment, final sampling data showed that the sex ratio was unbalanced

123   in favour of females. The decision was then made to use the same protocol on juveniles in the

124   2017–2018 experiment to increase the number of males to be sampled. We used similar 60:40

125   female:male ratios for the two experiments and among the three temperatures tested. Total

126   mortalities during these two experimental years was 10%.

127   Experimental methods complied with the regulations of the Canadian Council on Animal

128   Care and were approved by the Maurice-Lamontagne Institute animal care committee.

129   *2.2 Samplings*

130   Growth rate was monitored three times during the year: July, December, and July of the

131   following year. At each sampling time, all fish were anaesthetized with a solution of

132   metomidate (Mattson and Riple, 1989; metomidate hydrochloride, Aquacalm, 5 mg L^{-1},

133   Western Chemical Inc., Washington, USA), measured to the nearest 0.1 cm, and weighed to

134   the nearest 0.1 g. We sacrificed 10 fish per treatment at each sampling date, but due to the

135   unbalanced sex ratio encountered in 2016–2017 experiment, this number was raised to 20 in

136   2017–2018. Sacrificed fish were anaesthetized in a solution of MS 222 (tricaine methane

137   sulfonate 0.18 g L^{-1}, Sigma-Aldrich, Co., Missouri, USA, for 5 min) between 12:00 and 17:00

138   to avoid possible biases associated with endocrine circadian rhythms. The individuals were

139   then weighed and measured. Blood was sampled from the caudal artery using a 23-gauge

140   needle and a 1 mL TB syringe (Becton Dickinson & Co, New Jersey, USA), both previously

141   heparinized (ammonium heparin salt, Sigma-Aldrich, Co., Missouri, USA) in a heparin

142   solution at 100 U mL^{-1}. Muscle and gonad samples were excised. Blood samples were

143   centrifuged for 3 min at 4.6 G and plasma was frozen in liquid nitrogen and stored at −80°C.

144   Gonads were fixed in Bouin's solution (Sigma Aldrich, Co., Missouri, USA) and muscle

145   samples were immediately frozen in liquid nitrogen and stored at −80°C.

146   *2.3 Morphological calculations*

147   At three times during the year (July – beginning of the experiment; December; July – end of

148   the experiment), all fish were weighed and measured. Data were reported as the average

149 weight and length per tank, and tank is the statistical unit (n = 8 per treatment). To determine

150 growth relative to sex, only data on sacrificed fish are available because the lack of sexual

151 dimorphism prevented us from identifying sex in live juveniles. For these, n is the number of

152 fish because fish that were sacrificed were sampled from different tanks during the same

153 sampling time. Data are expressed in terms of degree-days to take into account the thermal

154 units accumulated under the different temperature conditions (Neuheimer and Taggart, 2007).

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

156 $$K = (W / L^3) \times 100$$

157 with W being the total mass (g) and L the total length (cm) of fish. The use of the Fulton

158 index was justified since we compared fish in the same size range.

159 *2.4 Feeding calculations*

160 Food intake (FI) and food conversion efficiency (FCE) were calculated for each experimental

161 tank. At each feeding, food was weighed (g) and uneaten food was collected, weighed, and

162 subtracted from the given food mass to estimate food ingested. The total amount of food

163 ingested was divided by the number of fish present for each feeding event and tank to

164 normalize data between tanks. The average FI per fish per day was then calculated for each

165 sampling interval. FCE was calculated by dividing the mass increment (mass difference

166 between two sampling dates) by FI per fish and expressed as a percentage.

167 *2.5 Physiological and histological measurements*

168 For physiological measurements, individuals were considered as the statistical unit. At 4.0 and

169 5.5°C in December 2016, no males were among the euthanized fish. Sexual maturity was

170 determined based on histological observations and sex steroid measurements. Gonads were

171 dehydrated in increasing concentrations of ethanol and embedded in metacrylate JB-4 solution

172 according to the protocol suggested by the manufacturer (Sigma-Aldrich, Co., Missouri,

173   USA). The embedded gonadal tissue was sectioned in 3 μm slices and stained with Lee's

174   methylene blue-basic fuchsin solution. The stage of testis and ovarian development was

175   determined for each individual according to Morrison (1990) and to Rideout *et al.* (2012),

176   respectively. For each individual, one histological section on three different slides was used to

177   discriminate the maturity stage. Sex steroid concentrations were determined using an RIA kit

178   for testosterone and 17β-estradiol (^{125}I RIA Kit # 07-189102 and # 07-138102, respectively,

179   MP Biomedicals, LLC, California, USA) and ELISA for 11-ketotestosterone (ELISA Kit #

180   582751, Cayman Chemical, Inc., Michigan, USA). Undetectable values were assigned the

181   half-value of the smallest standard.

182   The water content of muscle (X) was determined after drying pieces of muscle for 48 h at

183   65°C and converted into total muscle energy content (Y, kJ g of wet tissue^{-1}) using the

184   relationship established for Greenland halibut juveniles by Ait Youcef (2013):

185    $$Y = 61.7366 - 0.4568X$$

186   Plasma cortisol was measured with an RIA kit (^{125}I RIA Kit # 07-221106, MP Biomedicals,

187   LLC, California, USA) and used as a primary stress indicator (Mazeaud *et al.*, 1977).

188   ***2.6 Statistical analysis***

189   Normality and homogeneity of variances were verified by Kolmogorov-Smirnov and Levene

190   tests, respectively. No transformation of data was needed to meet these criteria except for

191   steroid data (11-ketotestosterone, 17β-estradiol, and cortisol), which were log transformed

192   prior to statistical analysis. For linear regressions, normalities were tested on residuals and

193   residual variations were tested using ANOVAs.

194   Data were first analyzed including "year" as a fixed effect. Because both years showed similar

195   results, data from experiments A and B were pooled. Length and mass data were regressed

196   against degree-days and regression slopes were compared. Fulton condition index values were

197 compared using one-way ANOVA (temperature) at each sampling date. Fulton condition

198 index was regressed against length per temperature and slope coefficients were compared. FI

199 and FCE were tested using repeated-measurements analysis of variance (ANOVAR;

200 temperature × period).

201 Physiological analyses were made on sacrificed fish. For the following analyses, individuals

202 were considered as the statistical unit, and sex could be identified. The effects of sex on

203 length and mass were regressed against degree-day units. Effects of sex on the Fulton

204 condition index were tested using one-way ANOVA at each sampling date. The effects of

205 temperature, sampling time, and sex on muscle energy storage and plasma cortisol were

206 analyzed using three-way ANOVAs excluding the first sampling date (July) because the

207 sacrificed fish were finishing the acclimation period and had not yet begun treatment. The

208 effects of temperature and sampling time on plasma concentrations of sexual steroid (11-

209 ketotestosterone in males and 17β-estradiol in females) were analyzed using two-way

210 ANOVAs, and the *a posteriori* Tukey test was used when significant factor effects were

211 found for comparison of means ($\alpha = 0.05$). Since cortisol data were heteroscedastic, we used

212 the Fisher LSD post-hoc test. Mortality was represented using the survival function of Kaplan

213 and Meier (1958); the Chi-square test was performed to test the effect of temperature; and the

214 Gehan Wilcoxon *a posteriori* test allowed us to identify the differences. Statistical analyses

215 were performed with Statistica software (Statsoft v.6.1, Oklahoma, USA).

216 ## 3. Results

217 ### *3.1 Survival, growth, and feeding*

218 Survival of Greenland halibut juveniles varied according to the temperature treatment

219 throughout the experiment ($\text{Chi}^2_2 = 10.62$, $P < 0.01$; Fig. 1). Survival was lower at 7.5°C than

220 at 4.0°C ($P < 0.05$), and intermediate at 5.5°C ($P > 0.05$). Among the mortalities, the sex-ratio

221 (F:M) varied according to temperature. While it was 2:4 at 4.0°C and 7:4 at 5.5°C, many more

222   females died at 7.5°C, with a ratio 16:3 (Fig. 1). Greenland halibut juveniles were similarly

223   sized among the three temperatures on the initial sampling date (Table 1). While mass and

224   length trajectories were similar at 4.0 and 5.5°C, they were significantly lower at 7.5°C than

225   at 4.0 and 5.5°C (Table 2; Fig. 2A, B).

226   The Fulton index was not influenced by temperature (Table 3; slope comparison $F_{2,66} = 1.47$,

227    $P > 0.05$, and covariance analysis $F_{2,68} = 1.20$, $P > 0.05$), but a clear size effect was identified,

228   with the Fulton condition index increasing with fish size (linear regression: $R^2 = 0.72$, $F_{1,70} =$

229   179.69, $P < 0.001$; Fig. 3). Food intake (FI) was significantly higher from December to July

230   than during the first rearing period at 5.5°C, but this was not the case for fish at 4.0 or 7.5°C

231   (Table 4; Temperature × Period of sampling, $F_{2,21} = 5.19$, $P < 0.05$). Temperature had no

232   effect on FCE (Temperature × Period, $F_{2,21} = 1.13$, $P > 0.05$; Temperature, $F_{2,21} = 0.32$,

233    $P > 0.05$), but it was significantly lower during the second rearing period (26.0 ± 2.6% vs.

234   23.4 ± 3.6%, respectively; Period of sampling, $F_{1,21} = 4.48$, $P < 0.05$).

235   *3.2 Physiological and histological measurements*

236   The terminal samplings allowed us to identify the sex and then to study the two sexes

237   separately. Mass and length of males and females were similar at the beginning of the

238   experiment (Table 5). While length and mass did not differ over time between females and

239   males at 4.0 and 7.5°C, length increased more rapidly at 5.5°C in females than in males but

240   mass increases were similar (Table 6; Fig. 4). At the beginning of the experiment, females and

241   males showed similar condition indexes (Table 7) whereas females had a significantly higher

242   Fulton condition index than males in December and after one year (Table 7).

243   Males were still sexually immature in December, a period of the year during which gonad

244   ripening occurs in Greenland halibut. Gonad histology revealed that no males showed signs of

245   gonad maturation at 4.0 or 5.5°C and only one male out of 15 sampled at 7.5°C in December

246   was at the very beginning of the maturation process. Plasma testosterone remained

247 undetectable during the whole experiment at the three rearing temperatures. However,

248 temperature did not affect plasma 11-ketotestosterone concentration, which remained stable

249 during the experiment despite the growth of males, with an overall mean of

250 79.16 ± 127.85 pg mL^{-1}.

251 All female gonads were still immature in December. Plasma testosterone also remained

252 undetectable throughout the experimental period and for all temperature treatments. Plasma

253 17β-estradiol concentration was not influenced by temperature, but it was almost twice as

254 high at the end of the experiment in July than in December (149.7 ± 115.4 pg mL^{-1} vs. $85.7 \pm$

255 41.5 pg mL^{-1}). Because there was no temperature effect on 17β-estradiol, females were pooled

256 and their plasma 17β-estradiol was regressed against their length (Fig. 5). The increase in

257 plasma 17β-estradiol throughout the experiment was clearly associated with the growth of

258 female juveniles during the experiment (Fig. 5).

259 Sex had no effect on the content of energy stored in muscle tissue or on plasma cortisol levels.

260 A global increase in energy stored in muscle tissue was notable between the fish at the

261 beginning of the experiment (4.23 ± 0.74 kJ g of wet muscle^{-1}) and those sampled on the other

262 two sampling dates (December and July – end of experiment; 5.09 ± 0.93 kJ g of wet

263 muscle^{-1}; Table 8). However, temperature conditions had no effect on this variable.

264 Temperature had a slight effect on plasma cortisol, and juveniles kept at 5.5°C showed lower

265 plasma cortisol concentrations (80.9 ± 135.7 ng mL^{-1}) than fish reared at 4.0 or 7.5°C ($93.2 \pm$

266 122.6 ng mL^{-1}; Table 8).

267 **4. Discussion**

268 The main objective of this work was to investigate in experimental conditions the potential

269 effect of current and anticipated temperatures in the EGSL on the growth of juvenile

270 Greenland halibut. Bottom temperatures in the EGSL are rising, and this tendency will

271   continue in the coming years considering temperatures measured in incoming waters and the

272   renewal time of water masses in this system (Gilbert, 2004; Galbraith *et al.*, 2019). Our results

273   demonstrate that increasing temperature could seriously impair juvenile growth and survival.

274   Mortality varied according to temperature: it was higher at 7.5°C (15.2%) than at 4.0°C

275   (4.5%) and intermediate at 5.5°C (10.1%). In the wild, Sünksen *et al.* (2010) showed that the

276   instantaneous mortality rate from 1 to 2 years old was positively correlated with temperature

277   in juvenile Greenland halibut. In our study, many more females held at 7.5°C died compared

278   males (16:3), suggesting that females are more sensitive to elevated temperatures.

279   Despite a greater quantity of thermal energy received in degree-days at 7.5°C relative to 5.5°C

280   and 4.0°C, juvenile Greenland halibut growth did not show the increase expected for

281   ectotherms. This result suggests that optimal temperature conditions may have been exceeded

282   at 7.5°C and that any further increase in observed temperatures in the EGSL would have a

283   deleterious impact on metabolic processes and growth. Growth by thermal energy was similar

284   at 4.0 and 5.5°C, and so we expected a difference in size at the end of the one-year

285   experiment since fish reared at 5.5°C received more energy over time. At the end of the

286   experiment, the difference in degree-days between the 4.0 and 5.5°C conditions was 540

287   degree-days. Greenland halibut is considered to be a slow-growing species, and it is possible

288   that the experiment was not long enough to observe differences at the two lower temperatures.

289   It is also possible that these two temperatures were within or close to the optimal temperature

290   range for growth of Greenland halibut juveniles, so differences in growth would be hardly

291   noticeable and may have plateaued. Indeed, in laboratory studies on *S. maximus* juveniles fed

292   the same ration, such plateaux were observed between 17 and 20°C (Burel *et al.*, 1996) and

293   16 and 22°C (Van Ham *et al.*, 2003).

294   Except at 5.5°C, where females had a greater increase in length than males, our results

295   differed from those obtained in a previous study (Ghinter *et al.*, 2019), where females grew

296   faster than males at 4.0°C. It is possible that different durations of the experiments (ours was

297   one year, theirs 18 months) could explain these differences.

298   Food supply is also a major factor affecting growth, since it is generally assumed that food is

299   the "driving force" supplying the energy to invest in growth (Brett and Groves, 1979; Jobling,

300   1993). Food intake per fish is closely related to temperature (*P. platessa* and *Platichthys*

301   *flesus*, Fonds *et al.*, 1992; *S. maximus*, Burel *et al.*, 1996; *Salmo salar*, Handeland *et al.*,

302   2008). However, the only significant food intake difference we observed was observed at

303   5.5°C, with an increase in feeding rate during the second part of the experiment. Interestingly,

304   this increase in feeding rate did not result in higher growth rate. The FCE, which reflects the

305   fraction of ingested food converted into growth in mass, is known to be influenced by

306   temperature and fish size (*Hippoglossus hippoglossus*, Björnsson and Tryggvadóttir, 1996;

307   *Gadus morhua*, Björnsson *et al.*, 2001; Imsland *et al.*, 2005; *S. maximus*, Van Ham *et al.*,

308   2003; *Salmo salar*, Handeland *et al.*, 2008). Here, it appears that only size is affected by FCE,

309   with a global decrease between the two sampling periods. FCE was variable among tanks,

310   especially during the second half of the experiment. A global decrease in FCE during juvenile

311   growth is consistent with previous results obtained on flatfish (*S. maximus*, Van Ham *et al.*,

312   2003).

313   Muscle makes up about $37 \pm 4\%$ of the body mass in juvenile Greenland halibut < 40 cm (Y.

314   Lambert, *pers. obs.*) and 42 to 51% in adults (calculated from Karl *et al.*, 2018), so it plays an

315   important role in energy storage (Ait Youcef, 2013). Here, we only observed an overall

316   increase in muscle energy content between the beginning and the end of the experiment. This

317   rise reflects a positive effect of the experimental settings relative to the natural environment,

318   and especially that of the *ad libidum* diet. From December until the end of the experiment, all

319   juveniles reached a plateau after which neither temperature nor sex had any effect on muscle

320   energy content. The food supply was thus adequate to meet all energy needs, including basic

321   maintenance metabolism and other needs, even at 7.5°C, when basal energy demands should

322   have been higher. No individuals had to draw extra energy from their tissues. It is therefore

323   possible that better food assimilation (conversion of food energy into net energy available for

324   maintenance and growth) at higher temperatures occurred, as has been shown in other fish

325   species (*Dicentrarchus labrax*, Hidalgo *et al.*, 1987; Russel *et al.*, 1996; *Silurus meridionalis*,

326   Xiaojun and Ruyung, 1992).

327   Although long debated (see Bolger and Connolly, 1989; Lloret *et al.*, 2014), especially

328   concerning application criteria, the Fulton condition index is a simple and widely used proxy

329   reflecting the "well-being" of a fish, with the general idea that a heavier weight for a given

330   length corresponds to better condition. The similar Fulton condition indices between all three

331   temperatures support the idea that the food supply met all the juveniles' energy needs. The

332   increase in the Fulton condition index throughout the experiment is related to the increase in

333   juvenile size. With allometric growth (the b-value of the weight–length relationship being

334   different from 3; Lloret *et al.*, 2014), correlation between the condition factor and length is

335   expected (Bolger and Connolly, 1989; Cone, 1989); this has already been demonstrated in

336   juvenile Greenland halibut (Ait Youcef, 2013). Nevertheless, females showed higher Fulton

337   indexes than males throughout the experiment. This is consistent with the results of Ghinter *et*

338   *al.* (2019), where females, whose growth was greater, reached the maximum plateau faster

339   than males at 4.0°C.

340   Plasma cortisol levels, a primary stress indicator (Mazeaud *et al.*, 1977), were significantly

341   higher in juveniles kept at 4.0 and 7.5°C than at 5.5°C, although the difference remained

342   small. In their study of salmonid fish *(Salmo trutta* and *S. gairdneri)*, Pickering and Pottinger

343   (1989) measured responses to acute stress (handling or 1 h confinement) ranging from 40 to

344   200 ng mL^{-1}. Thus, in view of our results, it seems that fish held at 5.5°C were slightly less

345   sensitive to manipulation than were those at the other two temperatures. Sensitivity to stress

346   has been shown to vary according to sex in juvenile Greenland halibut (Ghinter *et al.*, 2019),

347   but we found no effect of sex in our study.

348   Histological and hormonal analyses clearly showed that a rise in temperature did not promote

349   sexual maturation at a smaller size in either males or females. Sexual steroids remained at

350   very low concentrations despite the presence of significant variations. There are no data about

351   sexual steroid concentrations in adult Greenland halibut. In Atlantic halibut (*H. hippoglossus*),

352   a species that also reproduces during winter, plasma 17β-oestradiol concentrations in females

353   increased from less than 3 000 pg mL^{-1} in summer to more than 20 000 pg mL^{-1} during the

354   reproductive period (Methven *et al.*, 1992), while it remained lower than 400 pg mL^{-1} in the

355   fish we surveyed. We did observe a gradual rise that must be due to the progressive and

356   increasing secretion of sex hormones along with the development of follicles, which takes

357   place throughout the growth of juveniles (*Leucoraja ocellate*, Sulikowski *et al.*, 2005).

358   Testosterone concentrations remained undetectable in both sexes, and in males,

359   concentrations of 11-ketotesterone did not exceed 900 pg mL^{-1}, which is far below the

360   concentrations reported for Atlantic halibut during milt release (> 2 000 pg mL^{-1}, with peaks

361   up to 16 000 – 18 0000 pg mL-^{1}; Methven *et al.*, 1992; Norberg *et al.*, 2001). Gonad histology

362   confirmed the absence of advanced differentiated germ cells in the testes and ovaries, and

363   none of the juveniles studied reached the adult stage during the survey. At the end of the

364   experiment—independent of the temperature conditions—only 4.0% of females exceeded 44

365   cm and 28.1% of males were above 35 cm, which are respectively the sizes at which 50% of

366   fish should have reached sexual maturity in the EGSL (DFO, 2018).

367   Very few studies on Greenland halibut have been pursued under laboratory conditions,

368   including experiments involving temperature conditions. However, the effect of temperature

369   on juvenile growth has already been addressed in the wild in some studies analyzing

370   oceanographic survey data. In the offshore waters west of Greenland, the mean lengths of the

371 1- and 2-year-old Greenland halibut were positively correlated with ambient temperature,

372 which varied between 1.0 and 4.0°C (Sünksen *et al.*, 2010). These authors found an average

373 increase in mean length of 1.6 cm °C^{-1} for 1-year-old and 1.5 cm °C^{-1} for 2-year-old

374 juveniles. In the EGSL, in a narrow and upper range of temperatures among sites, surveys,

375 and years (mean annual bottom temperatures 4.95–5.14°C), no correlation between juvenile

376 growth and temperature was noted, possibly because of the very stable temperature conditions

377 that prevail throughout the year (Ait Youcef *et al.*, 2015).

378 It is important to keep in mind that our study was done under stable experimental conditions,

379 without food limitations or any other energy expenditure such as food foraging or predator

380 avoidance. In nature, an increase in temperature also means a decrease in dissolved oxygen. In

381 the St. Lawrence Estuary, these rates are already very low and close to the critical threshold

382 for this species (Dupont-Prinet *et al.*, 2013). Thus, with increasing bottom temperatures,

383 dissolved oxygen levels could become dangerously low for the survival of juveniles. In the

384 wild, Greenland halibut carry out extensive vertical migrations (Vollen and Albert, 2008;

385 Albert *et al.*, 2011) to feed on epibenthic and bathypelagic prey (Bowering and Lilly, 1992;

386 Dawe *et al.*, 1998; Solmundsson, 2007; Dennard *et al.*, 2009). This foraging activity, which is

387 metabolically very demanding, could be altered by the modification of abiotic parameters

388 generated by increased temperature. The consequences of increasing temperatures on growth,

389 survival, and thus recruitment to the population would be much greater than those predicted in

390 this experimental study, where dissolved oxygen was maintained above 80% saturation.

391 Greenland halibut, especially the young stages, have been shown to change their distribution

392 to maintain preferred thermal habitats, migrating deeper or shallower depending on

393 temperature conditions (Morgan *et al.*, 2013; Wheeland and Morgan, 2019). Such migration

394 possibilities would be very limited in the EGSL due to the geography and bathymetry of this

395 region.

5. Conclusion

Although the temperature was higher, no increase in juvenile growth occurred at 7.5°C, as would be expected in ectotherms, so the optimal temperature range for growth might have been exceeded. The absence of significant variations in FI, FCE, and muscle energy reserves between temperatures suggest that food supply or/and assimilation efficiency in experimental conditions were sufficient to sustain growth and maintain condition at every temperature treatment.

These results are in accordance with the hypothesis of Ait Youcef *et al.* (2015), which stated that, within the EGSL, an optimal temperature of around 5.0°C would have been reached. It would also support hypotheses by Bowering (1983) and Ait Youcef *et al.* (2015), which stated that the higher growth rates recorded in the EGSL for this species would be partly due to ambient temperatures found in this region. However, the results of our study suggest that a further increase in bottom temperature could affect the commercial Greenland halibut fishery in the EGSL by decreasing fish growth and increasing natural mortality of juvenile fish.

Authorship statements

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

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

421 Audet: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology,

422 Project administration, Resources, Supervision, Validation, Writing – review and editing.

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Figure Caption

Figure 1: Cumulative proportion of survival (Kaplan-Meier analysis) in juvenile Greenland

halibut reared at 4.0, 5.5, or 7.5°C for one year. The censored data, unlike the complete data,

correspond to individuals who survived until the end of the experiment but who could have

died due to temperature effect after the end of the experiment. Number of died females (F),

males (M), and fish of undetermined sex (NA) are indicated in parentheses below the

temperature. Each point represents one fish.

Figure 2: Length (A) and mass (B) plotted against degree-days in juvenile Greenland halibut

reared at 4.0, 5.5, or 7.5°C for one year. Each point represents the average length or mass in

one tank. Different superscript letters (a, b) indicate significant differences between slopes.

Figure 3: Fulton condition index plotted against degree-days in juvenile Greenland halibut.

Individuals reared at 4.0, 5.5, or 7.5°C for one year were pooled in the analyse due to absence

of temperature effect. Each point represents the average condition index in one tank. Linear

regression: $R^2 = 0.72$; $F_{1,70} = 179.69$, $P < 0.001$.

Figure 4: Length (A) and mass (B) plotted against degree-days for female (black line and

squares) and male (white circle and dotted line) juvenile Greenland halibut reared at 4.0 (1),

5.5 (2), or 7.5°C (3) for one year. Each point represents one juvenile. * indicates a significant

difference ($P \leq 0.05$) between slopes.

Figure 5: Linear regression of plasma 17β-estradiol as a function of female juvenile length

($1.1316 + 0.1025x$, $R^2 = 0.37$, $F_{1,117} = 67.71$, $P < 0.001$). Data for each temperature and each

sampling date were pooled because there was no temperature effect or sampling date effect to

test for the size effect. Statistical analysis was done on log-transformed data.

646   **Table 1**: Initial average length and mass of juveniles (average per tank; N = 8 for each

647   temperature treatment). The results are expressed as mean ± SD.

648   **Table 2**: Summary of linear regression parameters for lengths and masses with degree-days

649   (Figure 2). ** = $P \leq 0.01$; *** = $P \leq 0.001$. The results of the slope comparisons are indicated

650   for each temperature. Different superscript letters (a, b) indicate significant differences.

651   **Table 3**: Average Fulton condition index of juveniles at each temperature and sampling date

652   (average per tank; N = 8 for each temperature treatment and sampling date). The results are

653   expressed as mean ± SD.

654   **Table 4**: Average food intake per juvenile (g d^{-1}) per temperature and experimental period.

655   The results are expressed as mean ± SD. The tank is the statistical unit. Different superscript

656   letters (a, b) indicate differences among the means (significant temperature × period

657   interaction, see the Results section).

658   **Table 5**: Initial average length and mass of female and males juvenile Greenland halibut. The

659   results are expressed as mean ± SD.

660   **Table 6**: Summary of linear regression parameters for lengths and masses with degree-days

661   (Figure 4). * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

662   **Table 7**: Average Fulton condition index of juveniles at each sampling date. The results are

663   expressed as mean ± SD. ** = $P \leq 0.01$.

664   **Table 8**: Summary of ANOVA analyses. The statistical unit is the individual. * = $P \leq 0.05$;

665   *** = $P \leq 0.001$.

Table 1

Temperature	Initial conditions			One-way ANOVA			
	4.0°C	**5.5°C**	**7.5°C**	*df*	*df(error)*	F	*P* value
Length (cm)	27.1 ± 1.5	26.6 ± 1.6	27.1 ± 1.8	2	21	0.32	> 0.05
Mass (g)	156.6 ± 25.0	149.6 ± 19.6	155.5 ± 29.5	2	21	0.18	> 0.05

Table 2

Length	equation	R²	df	df (error)	F	P value
4.0°C[a]	27.1106 + 0.0044x	0.72	1	22	55.65	***
5.5°C[a]	26.8153 + 0.0039x	0.82	1	22	103.04	***
7.5°C[b]	27.4153 + 0.0022x	0.53	1	22	24.70	***
Slope homogeneity			2	66	6.10	**
Mass						
4.0°C[a]	154.5493 + 0.1390x	0.75	1	22	66.62	***
5.5°C[a]	152.0815 + 0.1304x	0.87	1	22	152.95	***
7.5°C[b]	164.7043 + 0.0746x	0.61	1	22	34.22	***
Slope homogeneity			2	66	7.74	***

Table 3

| Temperature | Fulton condition index | | | One-way ANOVA | | | |
	4.0°C	5.5°C	7.5°C	df	df(error)	F	P value
July (begining)	0.75 ± 0.02	0.75 ± 0.03	0.75 ± 0.01	2	21	0.08	> 0.05
December	0.85 ± 0.03	0.87 ± 0.02	0.86 ± 0.04	2	21	0.67	> 0.05
July (end)	0.88 ± 0.05	0.92 ± 0.03	0.89 ± 0.04	2	21	1.86	> 0.05

Table 4

Temperature	Period	
	July–Dec.	Dec.–July
4.0°C	2.07 □0.57[a]	2.85 ± 1.14[a,b]
5.5°C	2.84 ± 0.71[a]	3.83 ± 1.28[b]
7.5°C	2.85 ± 1.07[a,b]	2.55 ± 1.02[a,b]

Table 5

Sex	Initial conditions		One-way ANOVA			
	♀	♂	df	df(error)	F	P value
Length (cm)	25.3 ± 3.4	27.7 ± 3.00	1	18	2.80	> 0.05
Mass (g)	128.7 ± 52.6	170.9 ± 60.6	1	18	2.76	> 0.05

Table 6

Length		Equation	R²	df	df (error)	F	P value
A,1 (4.0°C)	♀	25.9445 + 0.0063x	0.48	1	43	38.92	***
	♂	27.7152 + 0.0044x	0.43	1	33	24.89	***
		Slope homogeneity		1	76	1.99	> 0.05
		ANCOVA		1	77	0.18	> 0.05
A,2 (5.5°C)	♀	26.5639 + 0.0049x	0.41	1	48	33.92	***
	♂	27.3799 + 0.0027x	0.31	1	38	17.15	***
		Slope homogeneity		1	86	3.90	*
A,3 (7.5°C)	♀	26.8730 + 0.0031x	0.45	1	43	35.46	***
	♂	28.3180 + 0.0019x	0.19	1	43	10.14	**
		Slope homogeneity		1	86	2.38	> 0.05
		ANCOVA		1	87	0.24	> 0.05
Mass							
B,1 (4.0°C)	♀	126.3498 + 0.2191x	0.44	1	43	33.25	***
	♂	165.8713 + 0.1320x	0.43	1	33	24.95	***
		Slope homogeneity		1	76	3.36	> 0.05
		ANCOVA		1	77	0.88	> 0.05
B,2 (5.5°C)	♀	152.1798 + 0.1615x	0.36	1	48	27.09	***
	♂	178.6109 + 0.0975x	0.35	1	38	20.10	***
		Slope homogeneity		1	86	2.71	> 0.05
		ANCOVA		1	87	2.19	> 0.05
B,3 (7.5°C)	♀	158.6343 + 0.0990x	0.41	1	43	29.92	***
	♂	186.9149 + 0.0635x	0.19	1	43	10.12	**
		Slope homogeneity		1	86	1.73	> 0.05
		ANCOVA		1	87	0.82	> 0.05

Table 7

Sex	Fulton condition index		One-way ANOVA			
	♀	♂	df	df(error)	F	P value
July (begining)	0.75 ± 0.06	0.78 ± 0.10	1	18	0.59	> 0.05
December	0.89 ± 0.10	0.84 ± 0.08	1	88	6.69	**
July (end)	0.95 ± 0.11	0.88 ± 0.13	1	108	7.70	**

Table 8

|		ANOVAs		
Muscle energy	*df*	*df(error)*	F	*P* value
Sampling date	1	188	0.29	> 0.05
Sex	1	188	0.13	> 0.05
Temperature	2	188	0.73	> 0.05
Sampling date × Sex	1	188	2.26	> 0.05
Sampling date × Temperature	2	188	0.28	> 0.05
Sex × Temperature	2	188	0.62	> 0.05
Sampling date × Sex × Temperature	2	188	0.46	> 0.05
Cortisol				
Sampling date	1	188	3.68	> 0.05
Sex	1	188	2.35	> 0.05
Temperature	2	188	3.33	*
Sampling date × Sex	1	188	0.57	> 0.05
Sampling date × Temperature	2	188	0.13	> 0.05
Sex × Temperature	2	188	0.22	> 0.05
Sampling date × Sex × Temperature	2	188	2.03	> 0.05
11-ketotestosterone (♂)				
Sampling date	1	84	0.31	> 0.05
Temperature	2	84	2.41	> 0.05
Sampling date × Temperature	2	84	1.54	> 0.05
17β-estradiol (♀)				
Sampling date	1	104	11.66	***
Temperature	2	104	0.66	> 0.05
Sampling date × Temperature	2	104	0.67	> 0.05

Figure 1
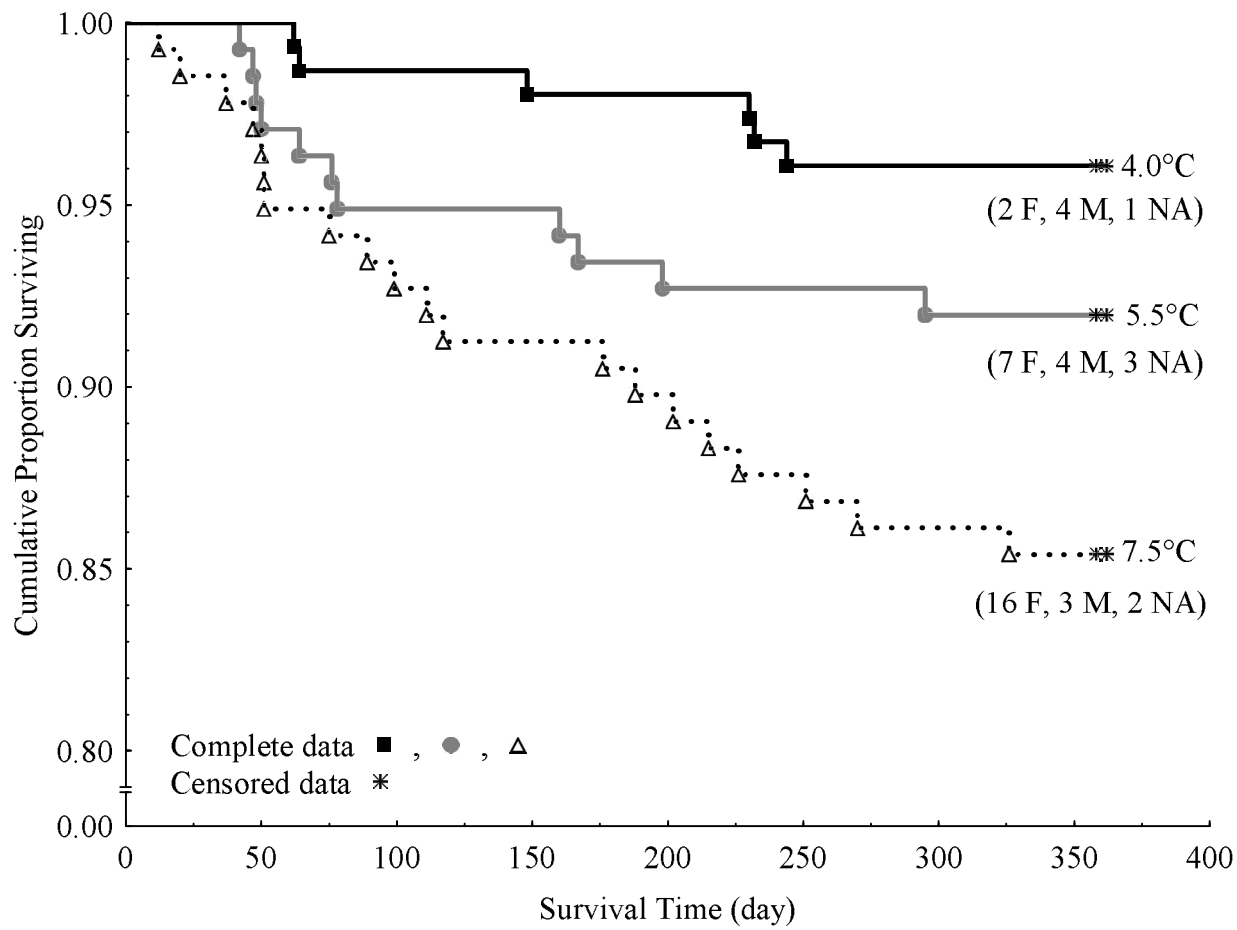
Figure 1
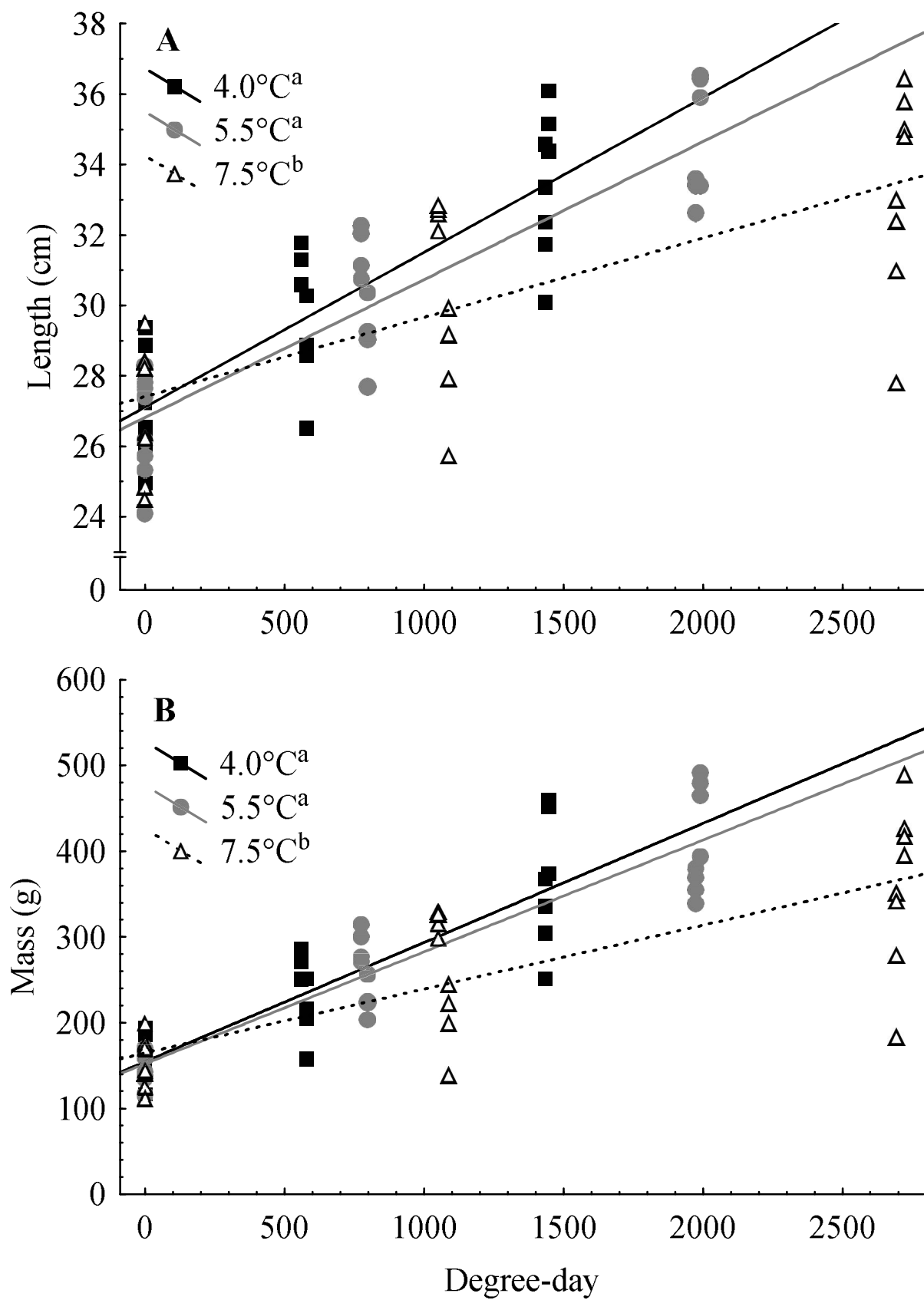
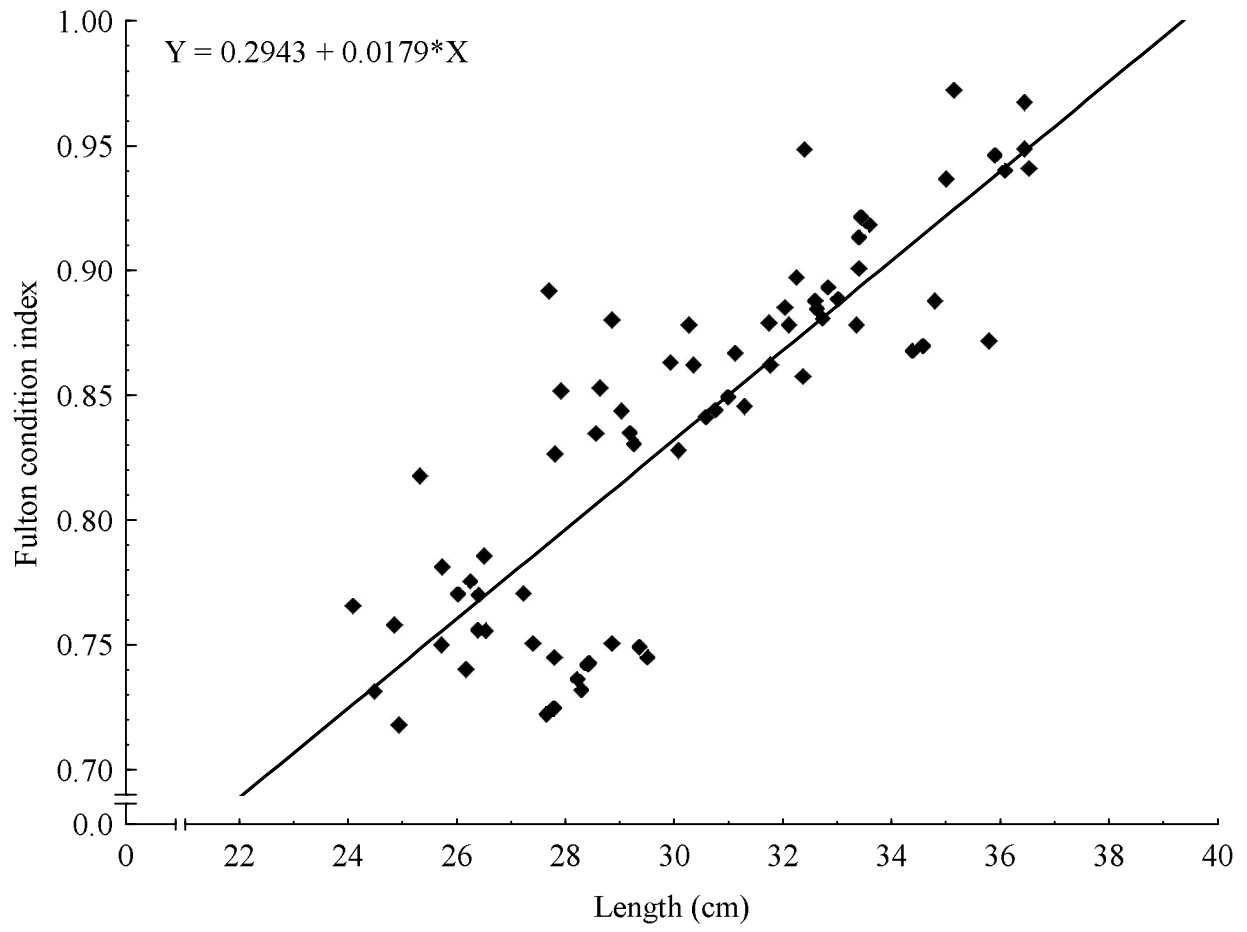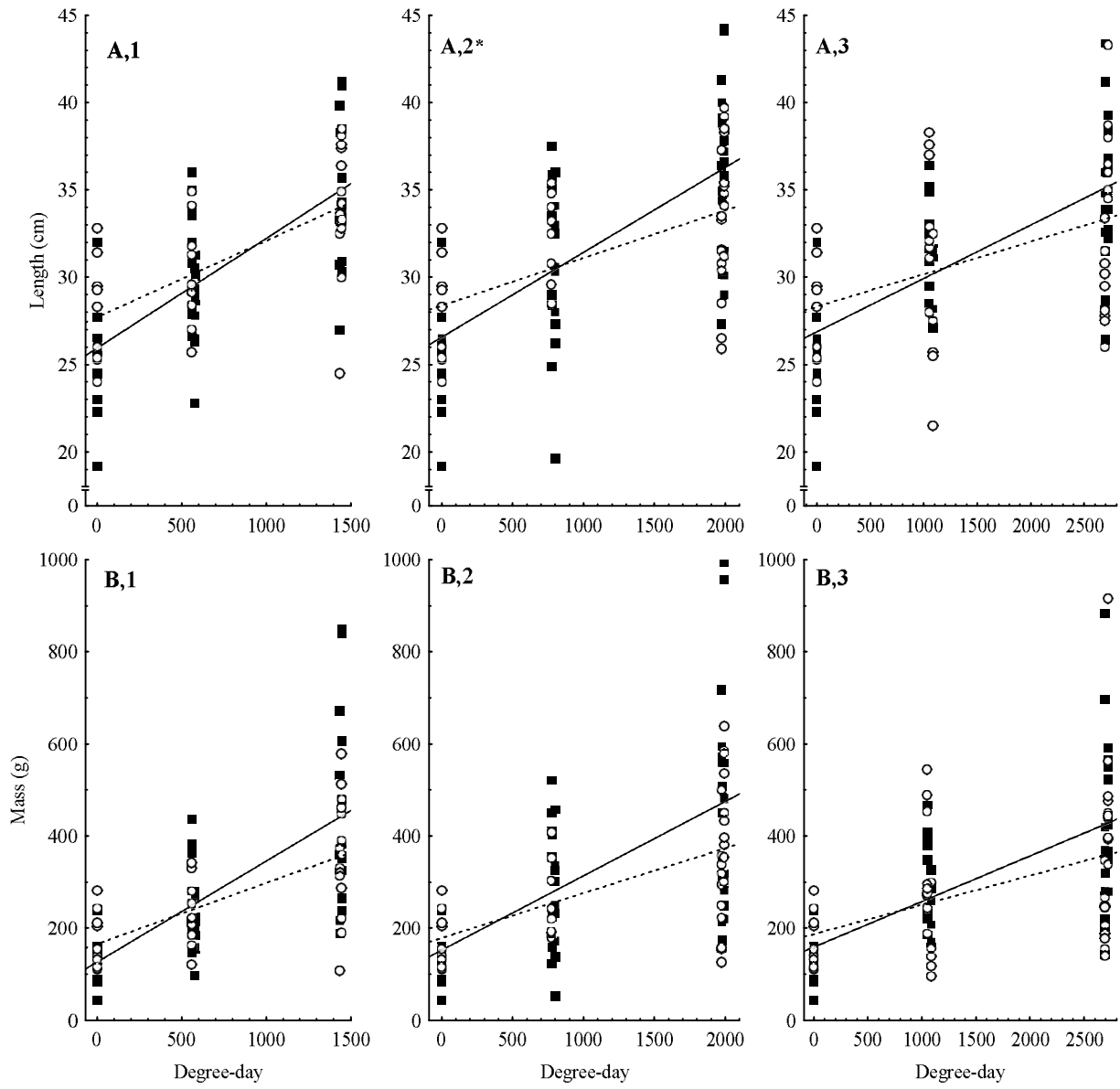Figure 2

Figure 3

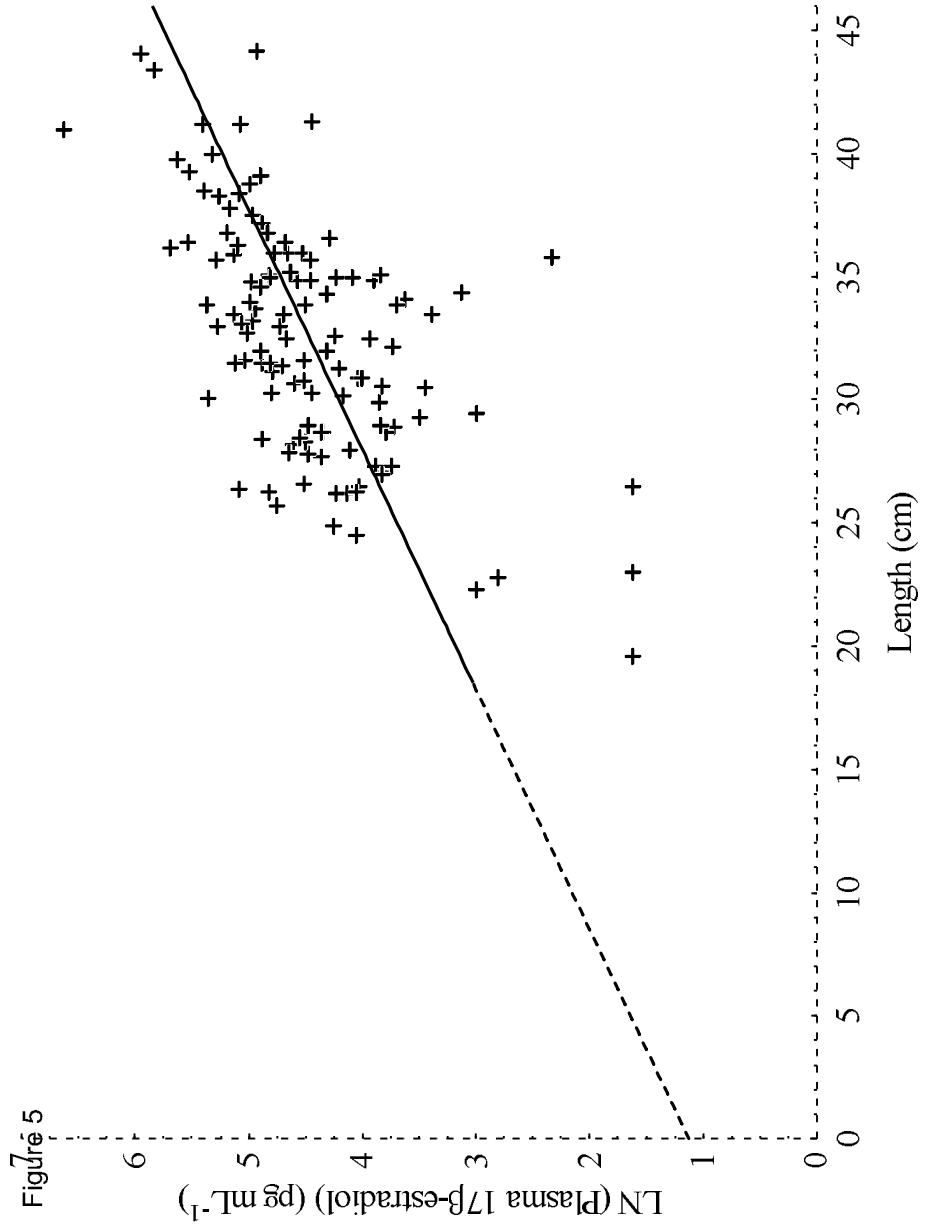$Y = 0.2943 + 0.0179*X$

Figure 4

Figure 5

Authorship statements

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

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

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

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☒ Ç⊔ś Ăĵ Ć⊔▫ınℓ ŕ śľ▪Ăńś Ć⊔ĂĊ⊔śŦ ⊔Ăōś ▪▫ ⊩▪▫Ŏ▪ ľ▫▧ᎫśĠ⊦źⅢĂľ⊦Ă▟⊦Ġ′nśℓŒ ▫ń▦śńᴅ▪Ă▪ńś▓ĂĠᴅ▪ℓ ⊔Ⅱ⎘ Ć⊔ĂĆ▫ĵ▐ ▕ ⊔Ăōś Ă♫Ꭻ́śĂńŕ Ġᴅ⊣źĵ́ś▪ľś Ć⊔ś Ŏ▫ⅿⱵ▪ńᵹⱵⓘnᴅŕ ⊣Ć⊔ℓ ᠗Ăᴊśⁿ

□Ç⊔ś Ăĵ Ć⊔▫ınℓ ŕ śľ▪Ăńś Ć⊔ś źᴀ▗Ŏ⊔⊦źⅢĂľ⊦Ă▟⊦Ġ′nśℓŒ □Ꭻśń▫Ă▪́nśᴅ▓ĂGᴅ▪ℓ ⊔Ⅱ⎘ Ŏ⊔Ⅱ⊦⊔▧ĂŦĽś ľ▫▪ℓ⊦Ⱶśńŕ Ă▕ ᠗▫ĠᴅGᴅĂ▧ľ̾▗źśĠᴅ⊦⊦Ġ′nśℓŒⱵ