

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 $3.87 \pm 0.36^\circ\text{C}$ and $4.97 \pm 0.23^\circ\text{C}$, 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.

83 **2. Material and methods**

84 ***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 $2.1 \pm 0.7 \text{ kg m}^{-2}$ ($1.2\text{--}3.65 \text{ kg m}^{-2}$). 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 ($30 \pm 17 \text{ lux}$)
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 Ripley, 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, $\text{kJ g of wet tissue}^{-1}$) using the
184 relationship established for Greenland halibut juveniles by Ait Youcef (2013):

$$185 \quad Y = 61.7366 - 0.4568X$$

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 \times 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 17 β -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 \times Period of sampling, $F_{2,21} = 5.19$, $P < 0.05$). Temperature had no
232 effect on FCE (Temperature \times 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 ($26.0 \pm 2.6\%$ vs.
234 $23.4 \pm 3.6\%$, 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 17 β -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 17 β -estradiol, females were pooled
256 and their plasma 17 β -estradiol was regressed against their length (Fig. 5). The increase in
257 plasma 17 β -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 17 β -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-ketotestosterone 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.

396 **5. Conclusion**

397

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

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

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416 **Authorship statements**

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

419 Lambert: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology,
420 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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622 **Figure Caption**

623

624 **Figure 1:** Cumulative proportion of survival (Kaplan-Meier analysis) in juvenile Greenland
625 halibut reared at 4.0, 5.5, or 7.5°C for one year. The censored data, unlike the complete data,
626 correspond to individuals who survived until the end of the experiment but who could have
627 died due to temperature effect after the end of the experiment. Number of died females (F),
628 males (M), and fish of undetermined sex (NA) are indicated in parentheses below the
629 temperature. Each point represents one fish.

630 **Figure 2:** Length (A) and mass (B) plotted against degree-days in juvenile Greenland halibut
631 reared at 4.0, 5.5, or 7.5°C for one year. Each point represents the average length or mass in
632 one tank. Different superscript letters (a, b) indicate significant differences between slopes.

633 **Figure 3:** Fulton condition index plotted against degree-days in juvenile Greenland halibut.
634 Individuals reared at 4.0, 5.5, or 7.5°C for one year were pooled in the analyse due to absence
635 of temperature effect. Each point represents the average condition index in one tank. Linear
636 regression: $R^2 = 0.72$; $F_{1,70} = 179.69$, $P < 0.001$.

637 **Figure 4:** Length (A) and mass (B) plotted against degree-days for female (black line and
638 squares) and male (white circle and dotted line) juvenile Greenland halibut reared at 4.0 (1),
639 5.5 (2), or 7.5°C (3) for one year. Each point represents one juvenile. * indicates a significant
640 difference ($P \leq 0.05$) between slopes.

641 **Figure 5:** Linear regression of plasma 17β -estradiol as a function of female juvenile length
642 ($1.1316 + 0.1025x$, $R^2 = 0.37$, $F_{1,117} = 67.71$, $P < 0.001$). Data for each temperature and each
643 sampling date were pooled because there was no temperature effect or sampling date effect to
644 test for the size effect. Statistical analysis was done on log-transformed data.

645

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 \pm 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 \pm SD.

654 **Table 4:** Average food intake per juvenile (g d^{-1}) per temperature and experimental period.
655 The results are expressed as mean \pm SD. The tank is the statistical unit. Different superscript
656 letters (a, b) indicate differences among the means (significant temperature \times 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 \pm 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 \pm 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	<i>df</i>	<i>df(error)</i>	F	<i>P</i> 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	R ²	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	***
	<i>Slope homogeneity</i>		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	***
	<i>Slope homogeneity</i>		2	66	7.74	***

Table 3

Temperature	Fulton condition index			One-way ANOVA			
	4.0°C	5.5°C	7.5°C	<i>df</i>	<i>df(error)</i>	F	<i>P</i> 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			
	♀	♂	<i>df</i>	<i>df(error)</i>	F	<i>P</i> 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	R ²	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	***
		<i>Slope homogeneity</i>		1	76	1.99	> 0.05
		<i>ANCOVA</i>		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	***
		<i>Slope homogeneity</i>		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	**
		<i>Slope homogeneity</i>		1	86	2.38	> 0.05
		<i>ANCOVA</i>		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	***
		<i>Slope homogeneity</i>		1	76	3.36	> 0.05
		<i>ANCOVA</i>		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	***
		<i>Slope homogeneity</i>		1	86	2.71	> 0.05
		<i>ANCOVA</i>		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	**
		<i>Slope homogeneity</i>		1	86	1.73	> 0.05
		<i>ANCOVA</i>		1	87	0.82	> 0.05

Table 7

Sex	Fulton condition index		One-way ANOVA			
	♀	♂	<i>df</i>	<i>df(error)</i>	F	<i>P</i> 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	<i>df</i>	<i>df(error)</i>	F	<i>P</i> 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

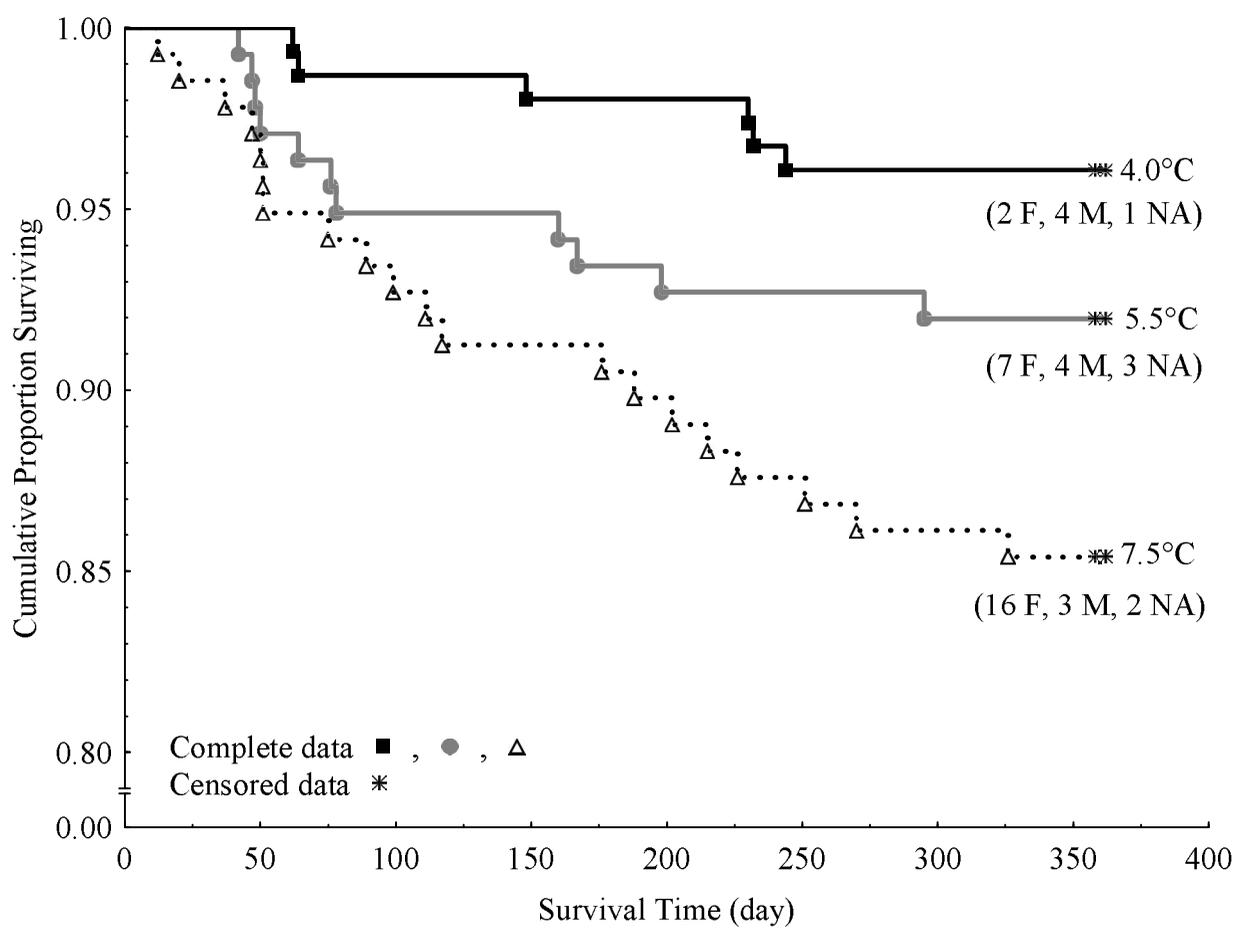


Figure 2

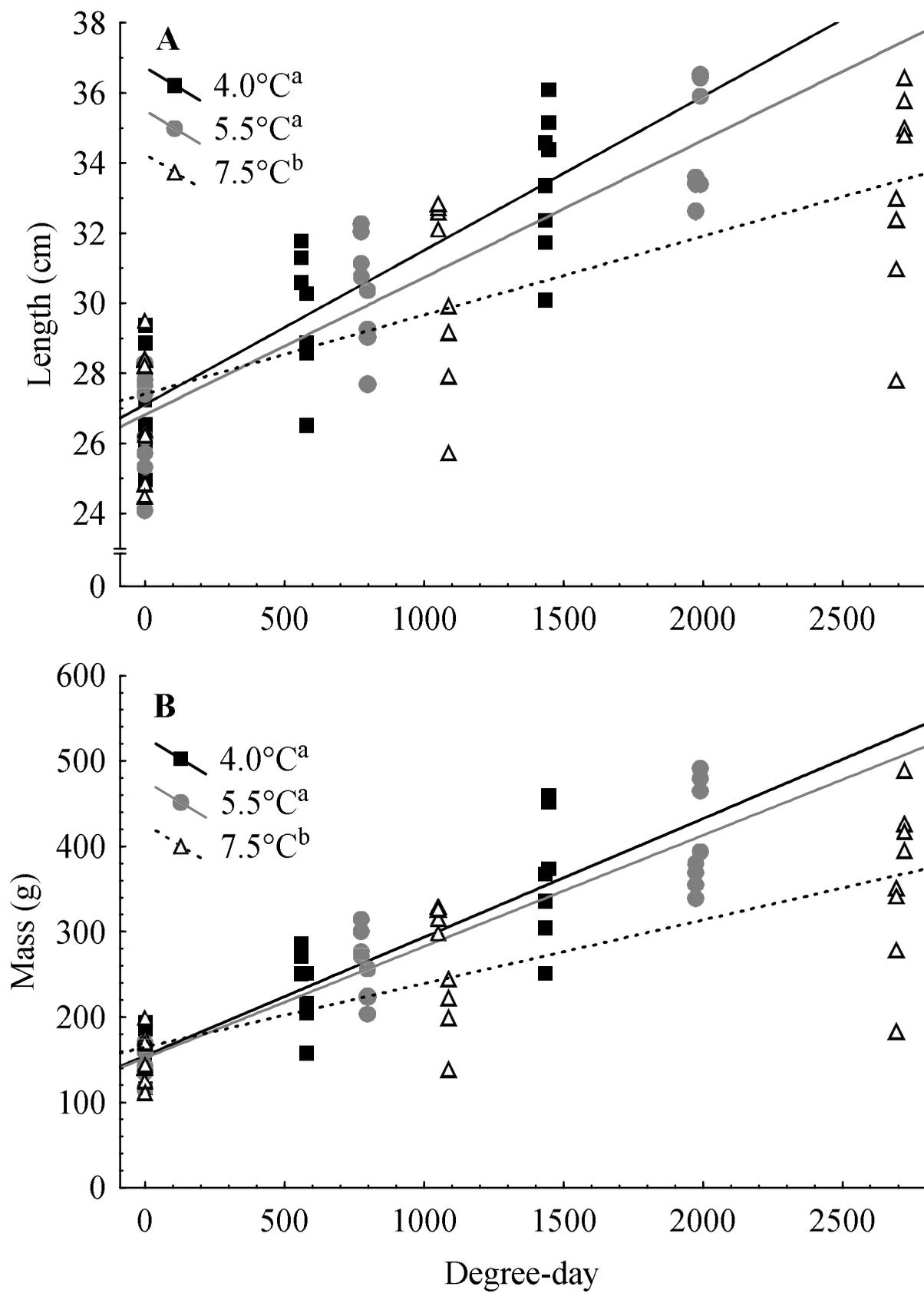


Figure 3

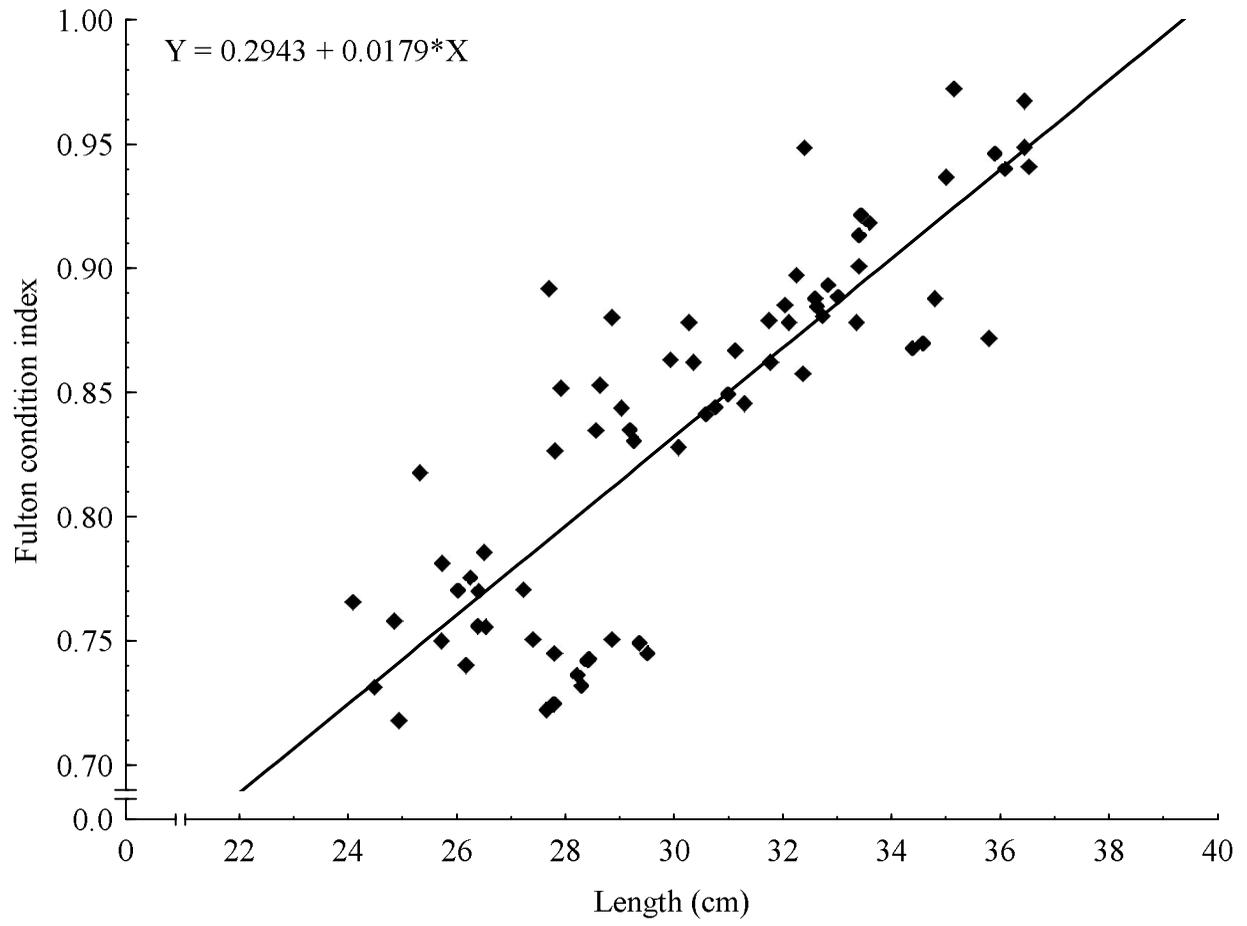
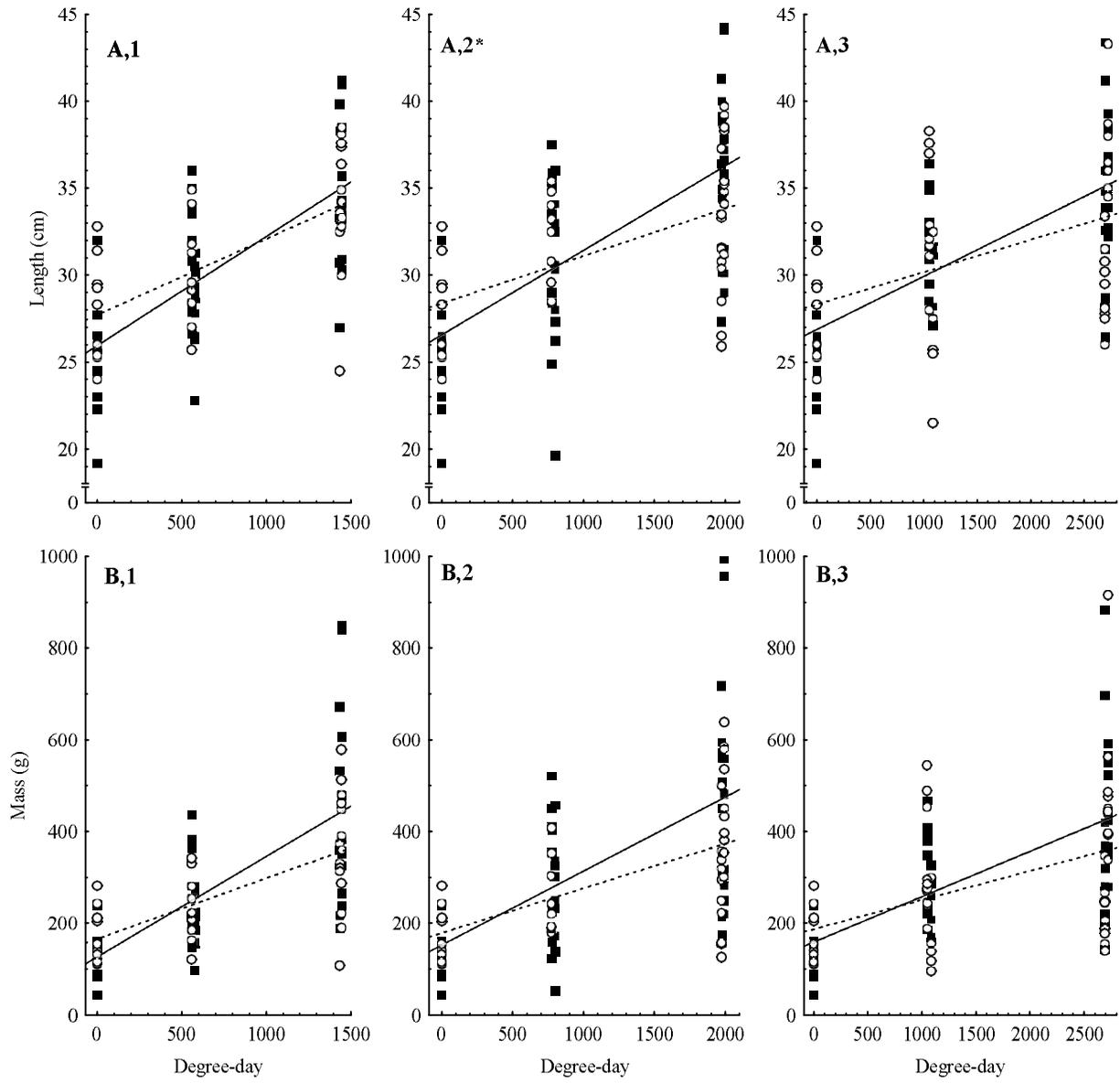
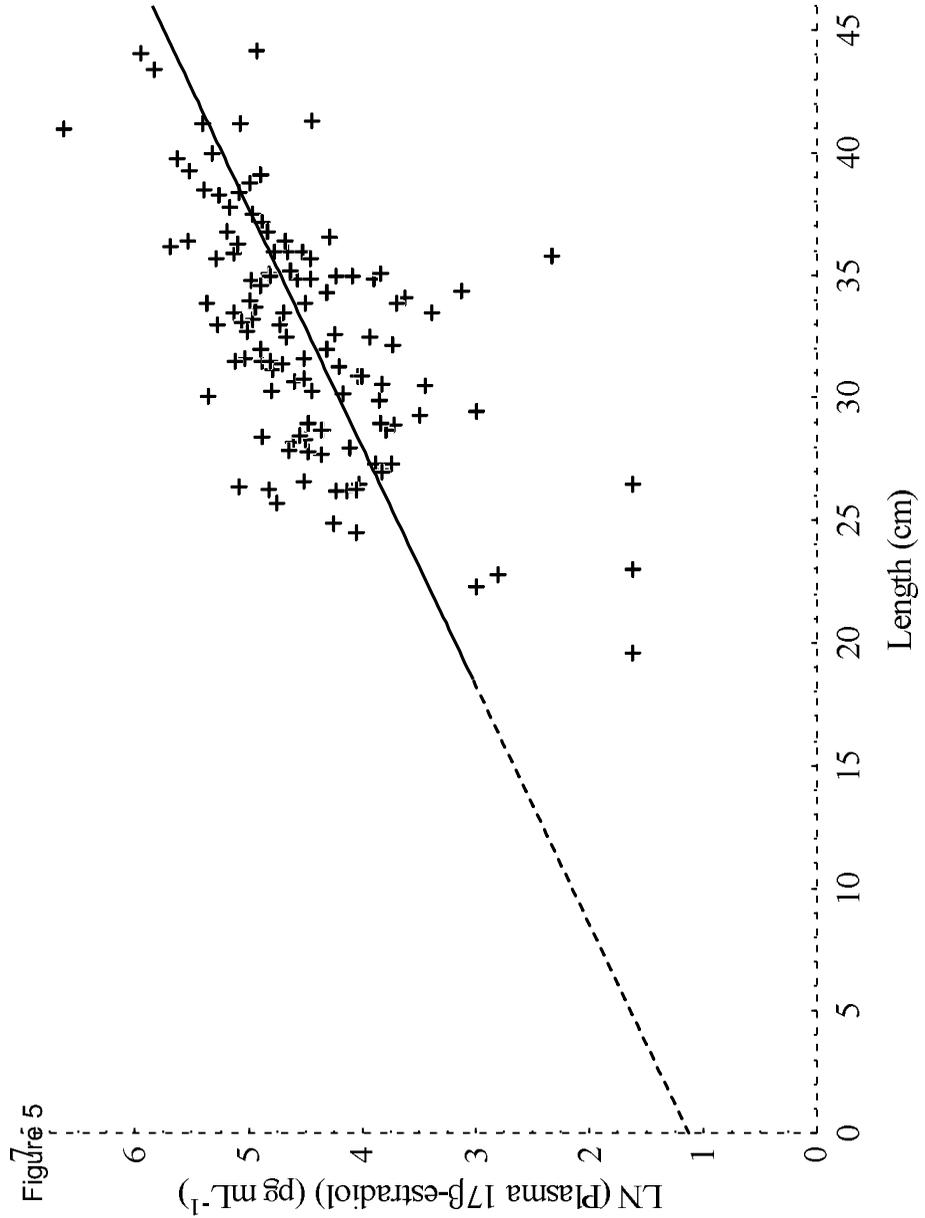


Figure 4





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.

