

**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  $6^{\circ}\text{C}$ ; 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  $1.5^{\circ}\text{C}$  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 ( $-0.5^{\circ}\text{C}$   
105  $\text{d}^{-1}$ ) to obtain the three targeted temperatures ( $4.0^{\circ}\text{C}$  [ $4.1 \pm 0.3$ ],  $5.5^{\circ}\text{C}$  [ $5.5 \pm 0.3$ ], or  $7.5^{\circ}\text{C}$   
106 [ $7.5 \pm 0.3$ ]). 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  $5.5^{\circ}\text{C}$   
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,  $7.5^{\circ}\text{C}$  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 ( $10 \text{ L min}^{-1}$ , salinity  $27.2 \pm 1.6$ ) 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<sup>-1</sup>,  
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<sup>-1</sup>, 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<sup>-1</sup>. 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  $\mu\text{m}$  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  $17\beta$ -estradiol ( $^{125}\text{I}$  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  $65^{\circ}\text{C}$  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 ( $^{125}\text{I}$  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,  $17\beta$ -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 $\beta$ -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  $79.16 \pm 127.85$  pg mL<sup>-1</sup>.

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 $\beta$ -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 ( $149.7 \pm 115.4$  pg mL<sup>-1</sup> vs.  $85.7 \pm$   
255  $41.5$  pg mL<sup>-1</sup>). Because there was no temperature effect on 17 $\beta$ -estradiol, females were pooled  
256 and their plasma 17 $\beta$ -estradiol was regressed against their length (Fig. 5). The increase in  
257 plasma 17 $\beta$ -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 ( $4.23 \pm 0.74$  kJ g of wet muscle<sup>-1</sup>) and those sampled on the other  
262 two sampling dates (December and July – end of experiment;  $5.09 \pm 0.93$  kJ g of wet  
263 muscle<sup>-1</sup>; 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 ( $80.9 \pm 135.7$  ng mL<sup>-1</sup>) than fish reared at 4.0 or 7.5°C ( $93.2 \pm$   
266  $122.6$  ng mL<sup>-1</sup>; 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<sup>-1</sup>. 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 $\beta$ -oestradiol concentrations in females  
353 increased from less than 3 000 pg mL<sup>-1</sup> in summer to more than 20 000 pg mL<sup>-1</sup> during the  
354 reproductive period (Methven *et al.*, 1992), while it remained lower than 400 pg mL<sup>-1</sup> 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<sup>-1</sup>, which is far below the  
360 concentrations reported for Atlantic halibut during milt release (> 2 000 pg mL<sup>-1</sup>, with peaks  
361 up to 16 000 – 18 0000 pg mL<sup>-1</sup>; 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<sup>-1</sup> for 1-year-old and 1.5 cm °C<sup>-1</sup> 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  $17\beta$ -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 ( $\text{g d}^{-1}$ ) 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

<b>Length</b>	equation	R <sup>2</sup>	df	df (error)	F	P value
4.0°C <sup>a</sup>	27.1106 + 0.0044x	0.72	1	22	55.65	***
5.5°C <sup>a</sup>	26.8153 + 0.0039x	0.82	1	22	103.04	***
7.5°C <sup>b</sup>	27.4153 + 0.0022x	0.53	1	22	24.70	***
	<i>Slope homogeneity</i>		2	66	6.10	**
<b>Mass</b>						
4.0°C <sup>a</sup>	154.5493 + 0.1390x	0.75	1	22	66.62	***
5.5°C <sup>a</sup>	152.0815 + 0.1304x	0.87	1	22	152.95	***
7.5°C <sup>b</sup>	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 <sup>a</sup>	2.85 ± 1.14 <sup>a,b</sup>
5.5°C	2.84 ± 0.71 <sup>a</sup>	3.83 ± 1.28 <sup>b</sup>
7.5°C	2.85 ± 1.07 <sup>a,b</sup>	2.55 ± 1.02 <sup>a,b</sup>

Table 5

<b>Sex</b>	<b>Initial conditions</b>		<b>One-way ANOVA</b>			
	♀	♂	<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

<b>Length</b>		Equation	R <sup>2</sup>	df	df (error)	F	P value
<b>A,1 (4.0°C)</b>	♀	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
<b>A,2 (5.5°C)</b>	♀	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	*
<b>A,3 (7.5°C)</b>	♀	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
<b>Mass</b>							
<b>B,1 (4.0°C)</b>	♀	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>B,2 (5.5°C)</b>	♀	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>B,3 (7.5°C)</b>	♀	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

	<b>ANOVAs</b>			
<b>Muscle energy</b>	<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
<b>Cortisol</b>				
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
<b>11-ketotestosterone (♂)</b>				
Sampling date	1	84	0.31	> 0.05
Temperature	2	84	2.41	> 0.05
Sampling date × Temperature	2	84	1.54	> 0.05
<b>17β-estradiol (♀)</b>				
Sampling date	1	104	11.66	***
Temperature	2	104	0.66	> 0.05
Sampling date × Temperature	2	104	0.67	> 0.05

Figure 1

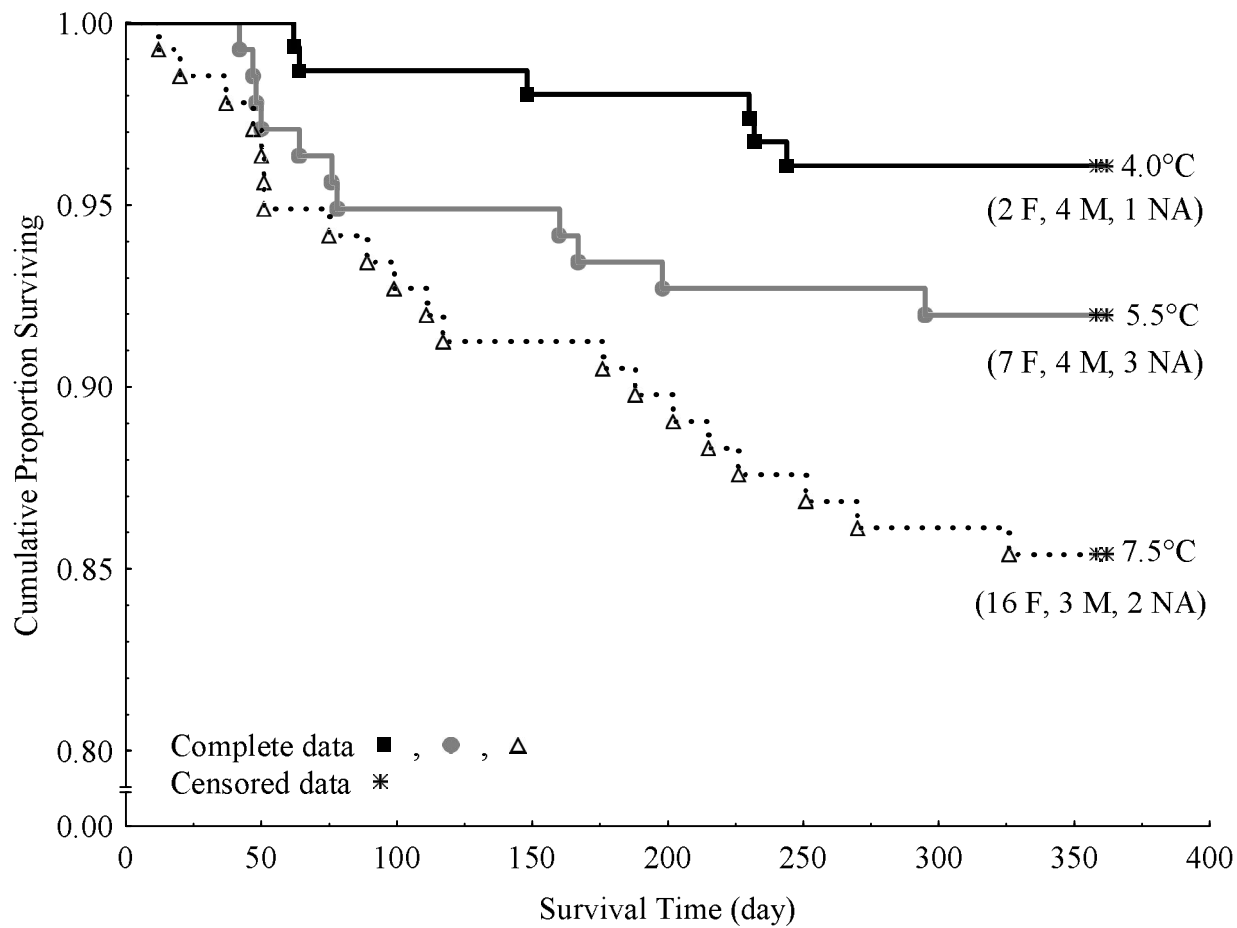


Figure 2

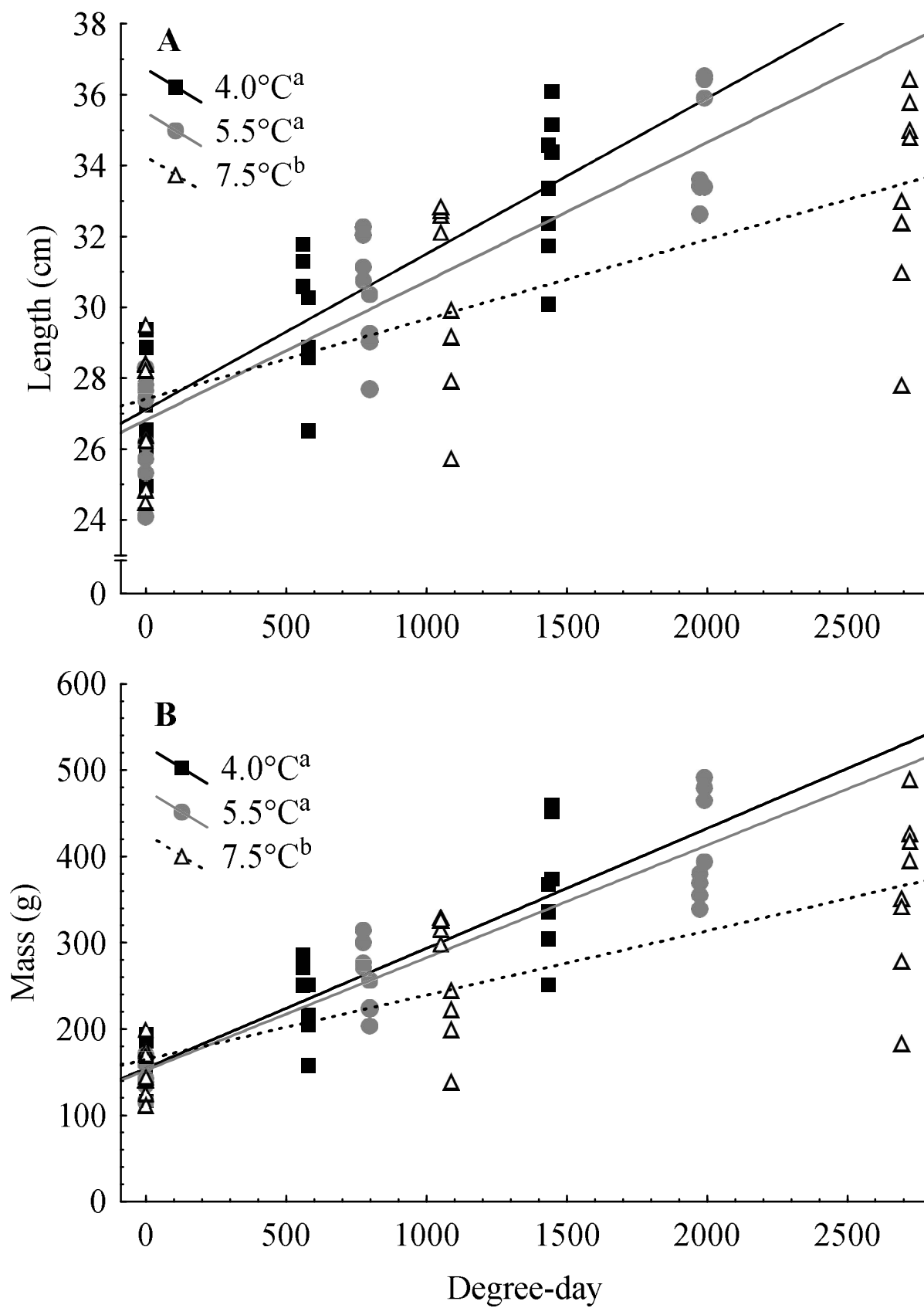


Figure 3

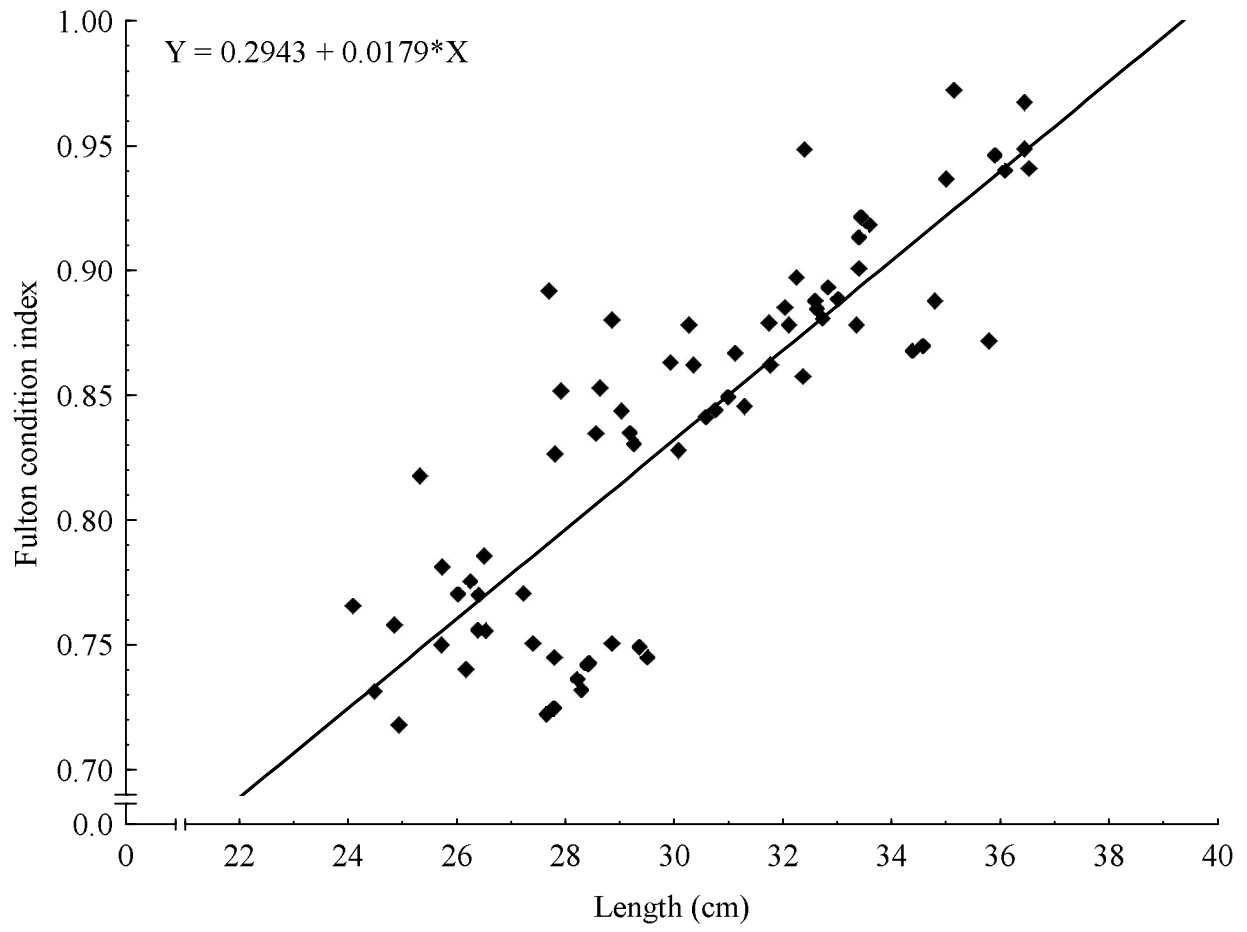
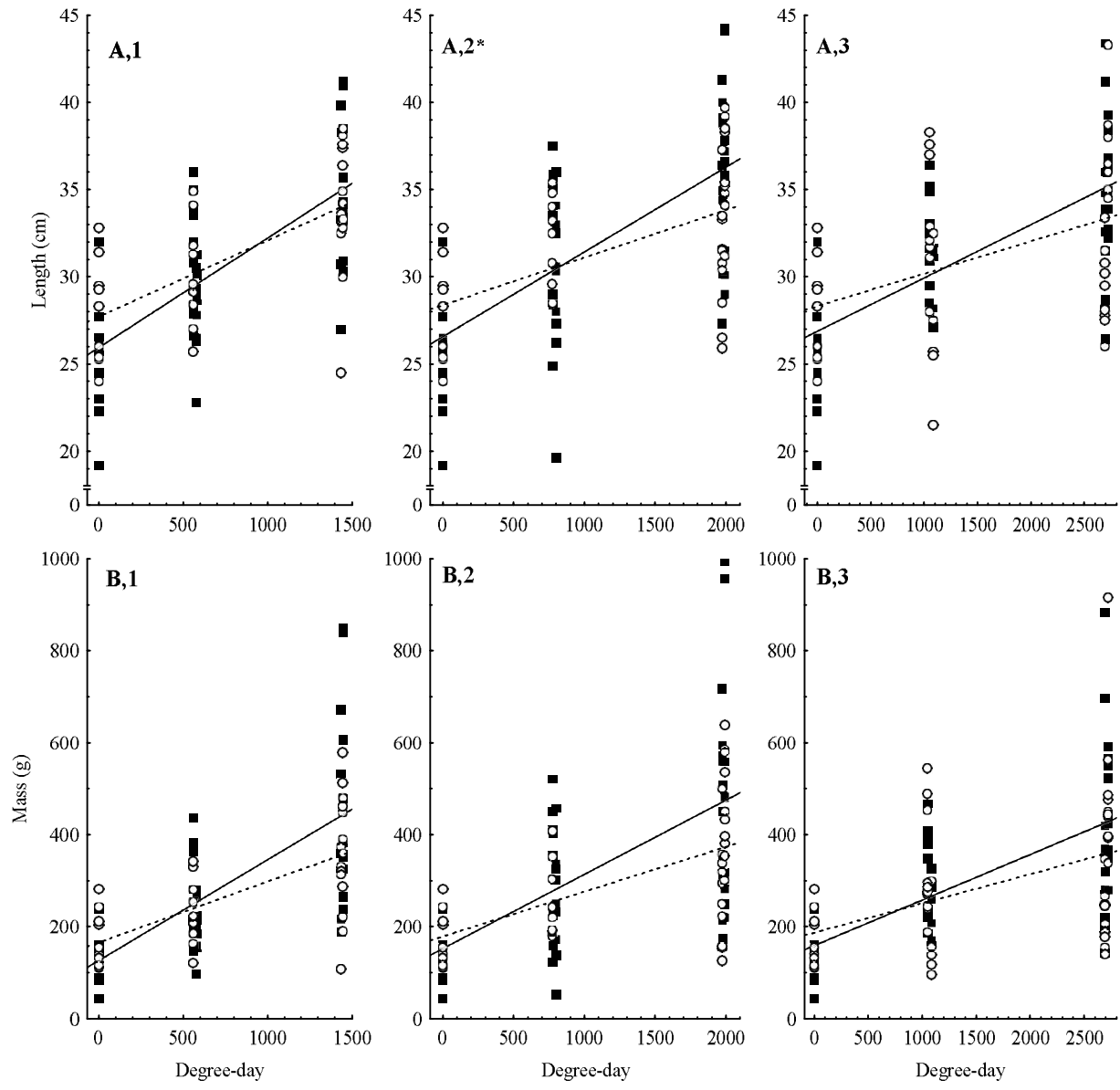
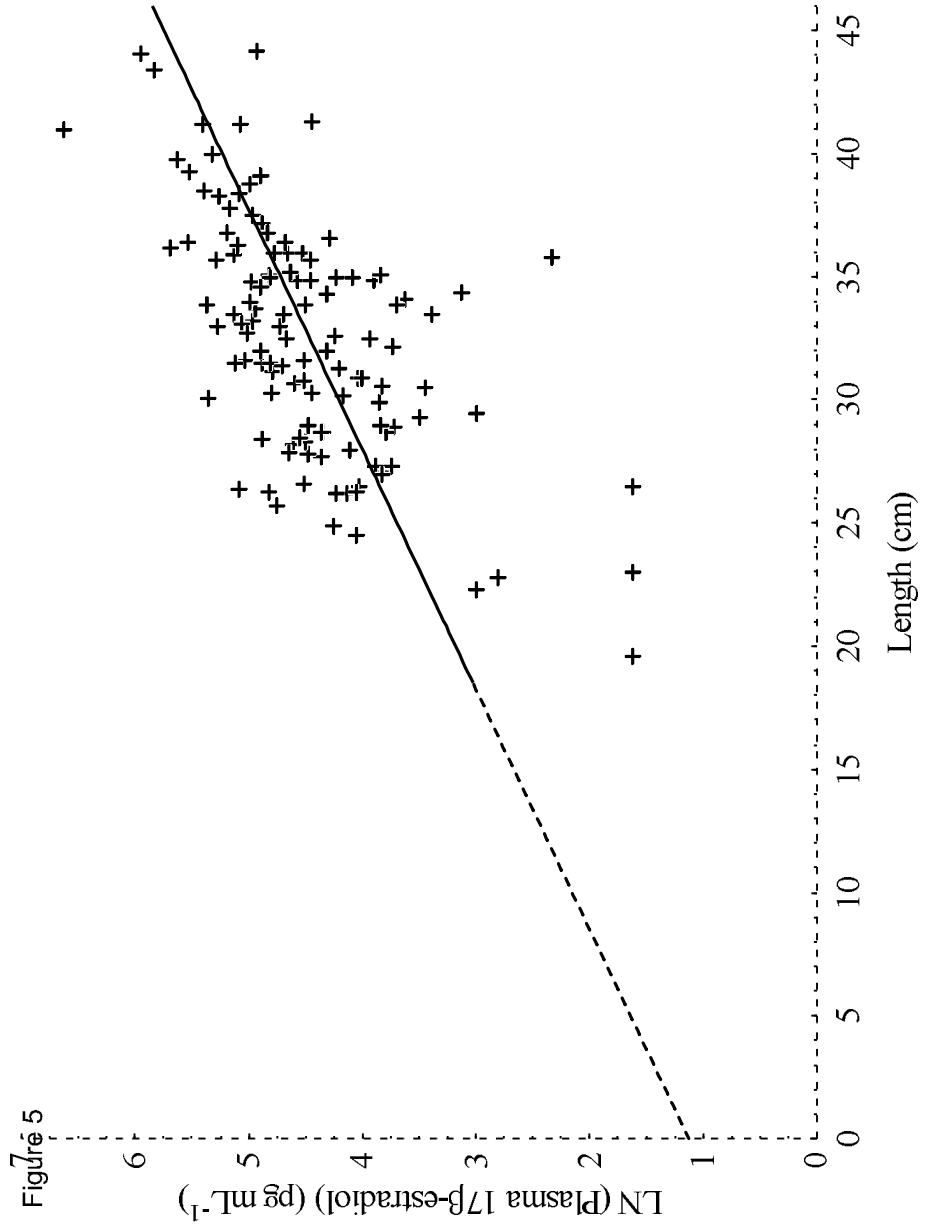




Figure 4





### **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.

