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How does salinity influence habitat selection and growth in juvenile American eels *Anguilla rostrata*? --Manuscript Draft--

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Abstract:	<p>The influence of salinity on habitat selection and growth in juvenile American eels <i>Anguilla rostrata</i> captured in four rivers across eastern Canada was assessed in controlled experiments in 2011 and 2012. Glass eels were first categorised according to their salinity preferences toward fresh (FW), salt (SW) or brackish water (BW) and the growth rate of each group of elvers was subsequently monitored in controlled FW and BW environments for 7 months. Most glass eels (78-89%) did not make a choice, i.e., they remained in BW. Salinity preferences were not influenced by body condition although a possible role of pigmentation could not be ruled out. Glass eels that did make a choice displayed a similar preference for FW (60-75%) regardless of their geographic origin but glass eels from the St. Lawrence Estuary displayed a significantly higher locomotor activity than those from other regions. Neither the salinity preferences showed by glass eels in the first experiment nor the rearing salinities appeared to have much influence on growth during the experiments. However, elvers from Nova Scotia reached a significantly higher mass than those from the St. Lawrence Estuary thus supporting the hypothesis of genetically (or epigenetically) based differences for growth between eels from different origins. Our results provide important ecological knowledge for the sustained exploitation and conservation of this threatened species.</p>

1 **How does salinity influence habitat selection and growth in**
2 **juvenile American eels *Anguilla rostrata*?**

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Salinity preference in *A. rostrata* glass eels

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ABSTRACT

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26 The influence of salinity on habitat selection and growth in juvenile American eels
27 *Anguilla rostrata* captured in four rivers across eastern Canada was assessed in controlled
28 experiments in 2011 and 2012. Glass eels were first categorised according to their salinity
29 preferences toward fresh (FW), salt (SW) or brackish water (BW) and the growth rate of
30 each group of elvers was subsequently monitored in controlled FW and BW
31 environments for 7 months. Most glass eels (78–89%) did not make a choice, i.e., they
32 remained in BW. Salinity preferences were not influenced by body condition although a
33 possible role of pigmentation could not be ruled out. Glass eels that did make a choice
34 displayed a similar preference for FW (60–75%) regardless of their geographic origin but
35 glass eels from the St. Lawrence Estuary displayed a significantly higher locomotor
36 activity than those from other regions. Neither the salinity preferences showed by glass
37 eels in the first experiment nor the rearing salinities appeared to have much influence on
38 growth during the experiments. However, elvers from Nova Scotia reached a
39 significantly higher mass than those from the St. Lawrence Estuary thus supporting the
40 hypothesis of genetically (or epigenetically) based differences for growth between eels
41 from different origins. Our results provide important ecological knowledge for the
42 sustained exploitation and conservation of this threatened species.

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44 Key words: Glass eels; salinity preferences; locomotor activity; pigmentation; geographic
45 differences.

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INTRODUCTION

49

50 Once ubiquitous, the American eel *Anguilla rostrata* (Lesueur, 1817) has suffered a 98%
51 decline in abundance in the upper St. Lawrence River and Lake Ontario since the 1970s
52 while remaining stable or diminishing only slightly elsewhere in eastern Canada
53 (DFO, 2010; COSEWIC, 2012). Such regional disparities are difficult to reconcile with
54 panmixia (Castonguay *et al.*, 1994) as all individuals are presumed to come from a single
55 spawning event which should conceivably translate into homogeneous recruitment trends
56 across regions. A better understanding of the environmental cues leading to habitat
57 selection by glass eels (unpigmented juvenile eels) would be invaluable from both
58 conservation and management perspectives.

59

60 The complex life history of *A. rostrata* begins far offshore in the Sargasso Sea, with a
61 semelparous and panmictic reproduction (Schmidt, 1923; Côté *et al.*, 2013). The willow-
62 leaf-shaped translucent leptocephalus larvae are then advected back to continental waters
63 by oceanic currents (Kleckner & McCleave, 1985) and metamorphose into unpigmented
64 glass eels shortly after reaching the continental shelf. This oceanic migration varies
65 greatly in terms of distance and duration; some glass eels settle all along North America's
66 east coast while some continue beyond to the Gulf of St. Lawrence and Greenland
67 (Tesch, 2003). Glass eels that enter the Gulf of St. Lawrence must rely solely on their
68 diminishing energetic reserves to complete their journey, swimming across several
69 hundred kilometers in often harsh conditions to reach the St. Lawrence Estuary as they
70 are no longer carried by the Gulf Stream (Dutil *et al.*, 2009). This last segment of their

71 journey is estimated to take between 1 and 2 months depending on the final destination
72 (Dutil *et al.*, 2009). After reaching their final settling geographic region, glass eels then
73 use divergent migratory tactics to colonise various coastal habitats (rivers, lakes,
74 estuaries, and marshes) characterised by fresh (FW), brackish (BW), or salt (SW) water
75 (Jessop *et al.*, 2002; Daverat *et al.*, 2006) where they will become fully pigmented and
76 henceforth be classified as elvers for their first year in continental waters.

77

78 In the European eel, *Anguilla anguilla* (L.), the colonisation of coastal habitats is
79 modulated by multiple environmental cues such as temperature (Tongiorgi *et al.*, 1986;
80 Tosi *et al.*, 1988; Edeline *et al.*, 2006), odours (Tosi & Sola, 1993; Sola, 1995; Sola &
81 Tongiorgi, 1998), photoperiod (Bardonnet *et al.*, 2003), and salinity (Tosi *et al.*, 1988,
82 1990; Edeline *et al.*, 2005). Extensive experiments by Tosi *et al.* (1990) demonstrated the
83 dominant influence of salinity over other environmental factors. While glass eels display
84 remarkable tolerance to salinity variations (Wilson *et al.*, 2004; Crean *et al.*, 2005), early
85 experiments (Deelder, 1958) showed that not only did newly arrived eels display no
86 tendency to migrate into FW, but they actively avoided it. An acclimation period
87 therefore seems necessary before FW migration, a phenomenon commonly observed in
88 many fish species (Lucas *et al.*, 2008).

89

90 Various studies with *A. anguilla* have demonstrated glass eels' preference for FW,
91 although a significant number (30–50%) either chose SW or exhibited no preference
92 (Tosi *et al.*, 1988, 1990; Edeline *et al.*, 2005). This inter-individual variability could in
93 part be explained by physiological and genetic factors. Edeline & Elie (2004) and Edeline

94 *et al.* (2006) showed that FW-seeking glass eels had a higher energetic status, higher
95 levels of thyroid hormones and lower cortisol levels than those exhibiting a SW
96 preference. These results suggest that individuals with higher condition factor would
97 therefore maximise their fitness by colonising lower eel density FW habitats, while
98 individuals with lower condition would maximise their fitness by avoiding the energetic
99 cost associated with further inland migration (Edeline, 2007).

100

101 The utilization of these diverse habitats has been shown to result in differential
102 growth. Eels residing in estuarine and coastal habitats grow at a faster rate than those
103 using river and lake habitats (Morrison *et al.*, 2003; Cairns *et al.*, 2004; Jessop *et al.*,
104 2004, 2008; Lamson *et al.*, 2009). Higher productivity of estuarine habitats at higher
105 latitudes (Gross *et al.*, 1988; Kaifu *et al.*, 2013) and lower osmoregulation costs (Tzeng *et al.*,
106 2003) are often cited as partial explanations. However, in controlled experiments,
107 Edeline *et al.* (2005) showed that glass eels with a preference for SW had a higher growth
108 rate than those favouring FW, irrespective of food availability. Furthermore, in controlled
109 experiments, Côté *et al.* (2009) showed differences in growth rate in eels from different
110 geographic origins in eastern Canada. Growth rate is an important life history trait,
111 directly influencing predation, age and size at migration, and female fecundity (Edeline &
112 Elie, 2004; Tremblay, 2004; Davey & Jellyman, 2005; Hutchings, 2006). Various authors
113 (Edeline *et al.*, 2005; Côté *et al.*, 2009) have hypothesised that differential growth could
114 in part be explained by underlying genetic differences, possibly resulting from spatially
115 varying selection (Gagnaire *et al.*, 2012). While much has been learned about the

116 environmental cues affecting *A. anguilla* distribution across various habitats and their
117 influence on growth, such information remains conspicuously absent in *A. rostrata*.

118

119 In this study, glass eels were sampled in four rivers across eastern Canada in order to
120 (i) assess juvenile eel salinity preferences and their locomotor activity for two sampling
121 periods over two years; and (ii) study the influence of salinity, geographic origin, and
122 previous salinity preferences on growth in controlled FW and BW for seven months.

123

124 MATERIALS AND METHODS

125

126 SAMPLE COLLECTION

127 Glass eels were sampled in eastern Canada in 2011 and 2012 at the mouths of four
128 rivers: Mersey River (Nova Scotia; 44°02'45"N, 64°42'30"W), East River (Nova Scotia;
129 44°35'10"N, 64°10'06"W), Saint-Jean River (Québec; 48°46'44"N, 64°23'06"W), and
130 Grande-Rivière-Blanche (Québec; 48°47'18"N, 67°41'50"W) (Table I; Fig. 1). The term
131 glass eel refers to “all developmental stages between the end of metamorphosis and full
132 pigmentation” (Tesch, 2003) while the term elver refers to fully pigmented fish during
133 the first year in continental waters. Sampling occurred twice in both years (once at the first
134 glass eel arrival and a second time 2–4 weeks later) at each location between late March
135 and early July depending on geographic location and local conditions. Glass eels were
136 captured in BW with dip nets except in the Saint-Jean River, where fixed plankton nets
137 were used. Glass eels were sampled at new or full moons during high tides in partnership
138 with professional fishermen (Atlantic Elver Fishery) and with the two government

139 agencies involved in the study (Ministère du Développement durable, de
140 l'Environnement, de la Faune et des Parcs du Québec and the Department of Fisheries
141 and Oceans Canada). Glass eels were then transferred to the Maurice-Lamontagne
142 Institute and placed in a thermostatic chamber at $16.9 \pm 0.2^{\circ}\text{C}$. This temperature was
143 chosen in order to maximise locomotor activity while still being close to natural habitat
144 conditions during the peak migration period. Glass eels were randomly distributed in two
145 40 L tanks filled with BW (salinity 18) equipped with aerators and without shelters. Since
146 glass eels do not feed during their transition to coastal habitats (Dutil *et al.*, 2009),
147 individuals were kept unfed throughout the behavioural experiments. Glass eels were
148 never kept for more than two weeks and were allowed a 48 h acclimatisation period
149 before beginning the experiments. Pigmentation was established according to the 1 to 7
150 scale developed by Haro & Krueger (1988) which has been previously employed in
151 numerous studies (e.g. Sullivan *et al.* 2009) as well as by government agencies like the
152 Ministère du Développement Durable, de l'Environnement de la Faune et des Parcs,
153 Québec.

154

155 BEHAVIOURAL EXPERIMENTS

156 The experimental setup used to assess locomotor activity (% of active eels, i.e. eels
157 that made a choice between FW and SW) and salinity preferences (% of eels that chose
158 FW) and (Fig. 2) was nearly identical to the one used by Edeline *et al.* (2005, 2006) and
159 modified from previous studies (Tongiorgi *et al.*, 1986; Tosi *et al.*, 1990). The concurrent
160 use of three glass tanks ($31.5 \times 27 \times 61$ cm) provided triplicate measurements for each
161 experiment. Each tank was equally divided by a partition into which two funnels

162 (ø 10 cm) were inserted 4.5 cm from the bottom of the tank. Both were connected
163 through a rubber stopper to a 500 mL filtering flask that acted as a trap (B in Fig. 2).
164 Water was gravity-delivered into the neck of the flasks at a rate of 180 mL/min from 15 L
165 tanks (C in Fig. 2), thereby offering a binary choice between flows of FW (salinity 0) and
166 SW (salinity 33). An overflow drain allowed any excess water to be evacuated
167 throughout the experiments. In order to evaluate the experimental bias of the apparatus,
168 control tests with two BW (salinity 18) flows were performed at the arrival of each batch.
169 For all experiments, including control tests, an average of 71 ± 34 individuals, selected to
170 ensure a sufficient number of replicates, were placed at the beginning of each experiment
171 in the BW-filled waiting chamber (A in Fig. 2, salinity 18) for an acclimation period of
172 30 min after which the water flows were activated for a 30 min experimental period. Both
173 the acclimation and experimental periods were conducted in darkness in order to
174 minimize the stress associated with the manipulations and because glass eels are mainly
175 active at night in natural habitats. The number of glass eels in each flask was recorded at
176 the end of each experiment, and individuals were classified according to their
177 preferences: fresh water choosers (FWC), salt water choosers (SWC), and non-choosers
178 (NCH). Locomotor activity was assessed as the percentage of glass eels having made a
179 choice for either FW or SW. Charcoal-filtered dechlorinated tap water was used as FW
180 while BW and SW were prepared by adding either FW or synthetic salts (Instant Ocean)
181 to sand-filtered St. Lawrence Estuary water (salinity 20-25). Non-choosers were reused
182 twice in order to obtain sufficient glass eels, thereby increasing the statistical power of
183 subsequent growth experiments.

184

185 GROWTH EXPERIMENTS

186 Once subdivided according to their salinity preferences (FWC, SWC, and NCH), glass
187 eels were immediately transported to the Laboratoire de Recherche en Sciences
188 Aquatiques (LARSA) at Laval University (Québec City) for growth experiments. All
189 individuals were treated with a formaldehyde solution (0.011% V/V) for 30 min upon
190 arrival to eliminate any potential parasites (Imada & Muroga, 1979; Chan & Wu, 1984).
191 Growth experiments occurred in 2011 and 2012 but due to sampling difficulties resulting
192 in smaller sample sizes only results from 2012 are presented and interpreted in this
193 article. Nevertheless, results from 2011 are included online in the Supporting
194 Information.

195

196 All experimental contingents (FWC, SWC, and NCH) from Grande-Rivière-Blanche
197 and Mersey River were subdivided into triplicate groups of 40–50 individuals per 45 L
198 tank into two independent series of FW (salinity 3 ± 1) and BW (salinity 20 ± 1) tanks
199 with continuous recirculated filtered water. BW was chosen over SW as a growth
200 medium because it is thought to be more representative of natural environments used by
201 eels outside FW habitats (Daverat *et al.*, 2006). Mean initial density was 39 g m^{-2} ,
202 photoperiod was set at 12L:12D (35% light intensity, 60 W light bulbs), and the water
203 temperature was $22 \pm 0.4^\circ\text{C}$. Total body length ($\pm 1 \text{ mm}$) and wet mass ($\pm 0.02 \text{ mg}$) of all
204 glass eels were measured on days 0, 85, 154, and 210 over a 7 month period. Individuals
205 were kept unfed 24 hours beforehand and then anaesthetised with a mixture of eugenol
206 dissolved in ethanol at a 1:10 ratio.

207

208 Tanks were randomly distributed on three-tiered shelves to minimise a possible effect
209 of tank location on growth (Speare *et al.*, 1995). Oxygen content and temperature were
210 monitored continuously, pH daily, and nitrite, ammoniac, and CO₂ contents biweekly;
211 filters were backwashed weekly. Preventive malachite green treatments were conducted
212 on all tanks simultaneously when increased mortalities were noticed (see results).
213 Individuals were fed *ad libitum* once a day, 6 days a week, alternating with frozen
214 bloodworms and brine shrimp cubes. Shelters were placed in each tank to minimise stress
215 and agonistic behaviour. Uneaten food and faeces were removed daily.

216

217 DATA ANALYSIS

218 All analyses were performed with the SAS 9.2 statistical package (SAS Institute Inc.).
219 Statistical significance was accepted at $P < 0.05$. Results are expressed as mean \pm S.D.

220

221 *Behavioural experiments*

222 A mixed logit model, $\text{logit}(P_{ijklm}) = \mu + R_i + B_j + RB_{ij} + T_l + \varepsilon_{m(ijkl)}$ (GLIMMIX
223 procedure, SAS) was used to analyse differences in salinity preferences and locomotor
224 activity where μ is the probability of either being active or preferring FW, i is the river
225 (the origin), j is the batch (i.e. first or second sampling period) while R_i and B_j represent
226 their respective effect. RB_{ij} represents the interaction between the river and batch, l is
227 the tank with its effect T_l , k is the year, m the experiment, and $\varepsilon_{m(ijkl)}$ the random error.
228 This model included the entire dataset in a single analysis (Table II), the unit of
229 replication (sampling unit) was the tank, and statistical analysis was performed using
230 only glass eels that made a choice at the first opportunity. The river and the batch were

231 included as fixed effects while the tanks and the experiments were defined as random
232 factors. The influence of density ($P = 0.22$) and slight water temperature variations
233 ($P = 0.47$) were not statistically significant and therefore not included in the model.
234 Finally, control tests for experimental bias conducted with two flows of BW were also
235 not significant ($P > 0.75$) throughout the experiments.

236

237 *Growth experiments*

238 Since total length and body weight data (log transformed) were highly correlated
239 ($y = 0.254x + 1.977$, $R^2 > 0.95$), statistical analyses were performed on body weight only.
240 The $x^{-3/4}$ transformation was applied prior to statistical analyses in order to achieve
241 normality. Differences in body weight were investigated with a repeated analysis of
242 variance as a function of time ($n = 4$), rearing salinity ($n = 2$), river ($n = 3$), and salinity
243 preferences ($n = 3$) using the MIXED procedure in SAS. Comparisons for each sampling
244 event were made with the Tukey-Kramer multiple comparisons test. Differences in
245 mortality rates between rivers were assessed with the Kruskal-Wallis test. The sampling
246 unit for both repeated ANOVAs and mortality analyses was the tank.

247

248 *Relative body condition and pigmentation*

249 Relative body condition (K_n) was assessed using Le Cren's (1951) equation $K_n = \frac{W}{aL^n}$
250 where W is the wet mass (g) and L the total length (mm). The parameters a and n were
251 estimated to be 5.27×10^{-5} and 1.97 respectively by using the equation $\log W =$
252 $\log a + n \log L$ where n is the slope of the least-square regression and a is a coefficient
253 (10 raised to the power of the intercept).

254
255 Pigmentation and relative body condition were analysed separately as a function of the
256 river, the batch and previous salinity preference using multi-factor analysis of variance
257 (MANOVAs). Since ANOVA is robust to slight departures from normality (Maxwell &
258 Delaney, 2004), and because kurtosis and skewness coefficients were very close to
259 normality, these statistical tests were performed using untransformed data with the
260 individual as the sampling unit. Pairwise comparisons were made with Tukey-Kramer
261 multiple comparisons test. These analyses were realized on a subset of 646 glass eels for
262 which pigmentation data was available (N: Mersey: 241; Saint-Jean: 177; Grande-
263 Rivière-Blanche: 228).

264

265 RESULTS

266

267 BEHAVIOUR EXPERIMENTS

268

269 *Locomotor activity*

270 The percentage of active glass eels (i.e., eels that made a choice between FW and SW)
271 varied from 13 to 22% according to river (Table II). The influence of the river on
272 locomotor activity was significant ($P < 0.0005$) with glass eels from Grande-Rivière-
273 Blanche (St. Lawrence Estuary) displaying a higher activity level than glass eels from the
274 three other rivers ($P < 0.05$, Table II). There was no significant difference in locomotor
275 activity among the other rivers ($P > 0.05$). An overall decline in activity was noticed
276 between the first ($16.2 \pm 2.3\%$) and second ($13.1 \pm 1.8\%$) sampling times, although this
277 difference was not significant ($P > 0.05$).

278

279 *Salinity preferences*

280 The overall influence of the river on salinity preferences was not significant ($P > 0.05$,
281 Table II). Active glass eels from most rivers displayed a preference for FW ($P < 0.05$),
282 with the exception of the East River, although the latter might be attributed to the small
283 sample size. Glass eels from both batches preferred FW, although this preference
284 declined between the first ($71.2 \pm 5.0\%$) and second ($62.6 \pm 5.4\%$) sampling times ($P <$
285 0.05), suggesting that the propensity to migrate to FW decreases with time.

286

287 GROWTH EXPERIMENTS

288 At T_0 , glass eels from Grande-Rivière-Blanche were significantly longer than those
289 from the Mersey River (65.0 ± 3.3 vs. 60.0 ± 3.5 , $P < 0.0001$). Significant time and river
290 effects ($P < 0.0001$), as well as their interaction, were observed on weight (Table III).
291 While there was no difference in mean body weight between origins at T_0 and T_1
292 ($P > 0.05$), by T_2 glass eels from the Atlantic coast grew up to be significantly heavier
293 than those from the St. Lawrence Estuary. The significant river \times time interaction
294 indicates that weight differences gradually increased between origins during the
295 experiments. Indeed, by the end of the experiments, glass eels from the Mersey River
296 were 2.46 time heavier than those from Grande-Rivière-Blanche (3.44 vs 1.40 g). Salinity
297 did not significantly influence growth for either river throughout the experiments
298 ($P > 0.05$). We did observe a significant salinity \times time interaction however (Table III).
299 At T_4 , glass eels from the Mersey River reared in FW tended to be larger than those in
300 BW (3.73 ± 0.27 vs 3.18 ± 0.21 ; Fig. 3) but there were no differences between FW and

301 BW for Grande-Rivière-Blanche (1.41 ± 0.05 vs 1.40 ± 0.05). The choice effect was not
302 significant ($P > 0.05$), meaning that the salinity preference displayed by glass eels in the
303 first experiments did not translate into growth differences. However, the significant
304 choice \times time interaction does suggest a variable influence throughout the experiments.
305 We also observed a significant river \times choice \times salinity \times time interaction.

306

307 *Mortality*

308 The mortality rate was significantly lower ($P < 0.0001$) for glass eels from the Mersey
309 River ($15.8 \pm 10.7\%$) compared to those from Grande-Rivière-Blanche ($39.1 \pm 16.5\%$)
310 although this was unrelated to salinity ($P > 0.05$). Escapement and cannibalism, although
311 unquantifiable, contributed to mortality and no specific cause of death was found for
312 glass eels that died of natural causes despite examination by an expert fish pathologist.

313

314 RELATIVE BODY CONDITION AND PIGMENTATION

315 Le Cren's relative body condition factor (K_n) significantly decreased
316 (Mersey: 1.13 ± 0.16 ; > Saint-Jean: 1.01 ± 0.12 ; > Grande-Rivière-Blanche: 0.90 ± 0.10 ,
317 $P < 0.0001$), between all rivers as glass eels continued their migration away from the
318 Sargasso Sea. While the batch effect was not significant ($P > 0.05$), the river \times batch
319 interaction was ($P < 0.0001$, Table IV). More specifically, the relative body condition did
320 not change significantly throughout the sampling season for glass eels from Grande-
321 Rivière-Blanche ($0.92 \pm 0.10 - 0.88 \pm 0.10$, $P > 0.05$) but significantly decreased over
322 time for glass eels from the Saint-Jean River ($1.06 \pm 0.12 - 0.97 \pm 0.10$, $P < 0.001$) and
323 significantly increased for those from the Mersey River ($1.08 \pm 0.14 - 1.18 \pm 0.17$, $P <$

324 0.0001). Body condition did not vary significantly according to salinity preferences
325 (FWC: 1.02 ± 0.16 ; SWC: 1.02 ± 0.18 ; NCH: 1.00 ± 0.15 , $P > 0.05$) and there were no
326 significant river \times choice interaction either ($P > 0.05$).

327

328 Unlike body condition, no clear pigmentation patterns were found as glass eels
329 continued their migration although there were significant pigmentation differences
330 among all rivers (Mersey River: 2.58 ± 1.19 ; Saint-Jean River: 1.49 ± 1.06 ; Grande-
331 Rivière-Blanche: 3.23 ± 0.72 , $P < 0.0001$). A significant pigmentation increase of 26%
332 (2.06 to 2.79) was also noticed between batches of a given river during the course of the
333 season ($P < 0.0001$) with different rate of increase between rivers ($P < 0.0001$) likely
334 reflecting time differences between samplings. Furthermore, there were no differences in
335 pigmentation between glass eels having exhibited different salinity preferences nor
336 between active and inactive glass eels ($P > 0.05$).

337

338

DISCUSSION

339

SALINITY PREFERENCES

341 One of the main objectives of this study was to assess the salinity preferences of
342 *A. rostrata* juveniles at four sampling sites in eastern Canada. Most glass eels were
343 classified as inactive (making no choice) (78–89%) while most active glass eels
344 significantly preferred FW (62–78%) over SW (22–38%). The salinity preferences of
345 *A. rostrata* observed in this study are similar to those of *A. anguilla*, where the proportion
346 of active FW-seeking eels varies between 50 and 70% (Tosi *et al.*, 1988, 1989, 1990;

347 Edeline *et al.*, 2005). A FW preference is consistent with migration toward riverine
348 habitats and suggests that the most active juvenile eels may seek out low salinity
349 environments in the wild. Decreasing salinity gradients could therefore represent an
350 important environmental cue, guiding active glass eels toward FW habitats. The use of
351 salinity as an orienting sensory cue has been proposed in other fish species, such as
352 *A. anguilla* (Tosi *et al.*, 1988) and the green sturgeon, *Acipenser medirostris* (Poletto *et*
353 *al.*, 2013). Glass eels at both sampling times preferred FW, although this preference
354 declined between the first and second sampling times (71.2 and 62.6% respectively),
355 suggesting that the propensity to colonise FW habitats decreases with time during the
356 migratory season.

357

358 Active glass eels displayed similar FW preferences regardless of their geographic
359 origin, but glass eels from the St. Lawrence Estuary, which had the lowest body
360 condition, had a level of locomotor activity almost twice as high as glass eels from Nova
361 Scotia (Table II), who also had the highest body condition. This difference in locomotor
362 activity is surprising considering that the inverse result (i.e., increasing locomotor activity
363 with body condition) has been demonstrated in *A. anguilla* glass eels (Bureau du
364 Colombier *et al.*, 2007) and could possibly result from endocrine or genetic differences
365 between glass eels of different species. The significant differences in pigmentation
366 observed between rivers (Grande-Rivière-Blanche > Mersey > Saint-Jean) might also
367 have played a role and while its association with salinity preferences has been shown in
368 previous studies (Crean *et al.* 2005), its effect on locomotor activity is unclear and often
369 indirect (Bureau du Colombier *et al.*, 2007). A higher locomotor activity has also been

370 linked to a higher migratory propensity in FW by European glass eels (Edeline *et al.*,
371 2005; Bureau du Colombier *et al.*, 2009). Edeline *et al.* (2005) also found that glass eels
372 that preferred SW to FW during the first behavioural experiments also significantly
373 preferred FW to SW during the second trials. Since in our study, locomotor activity
374 represents the total percentage of glass eels that made a choice for either FW or SW, a
375 higher locomotor activity (i.e. a positive rheotactic response) could then reflect an overall
376 higher migratory propensity more accurately than salinity preference.

377

378 Not making a choice between FW and SW by most glass eels (78–89%) might
379 indicate a low level of locomotor activity and/or a preference for BW. Low activity has
380 been linked to early settlement in coastal and estuarine habitats in *A. anguilla* (Edeline *et*
381 *al.*, 2005; Bureau du Colombier *et al.*, 2007) while a preference for BW likely
382 corresponds with the colonisation of such habitats. Furthermore, Daverat *et al.* (2006)
383 demonstrated using otolith microchemistry that *A. rostrata* in higher latitudes have a
384 greater probability of remaining in BW. They suggested that in the specific case of the
385 Saint-Jean River (QC) up to 85% of juveniles may remain in estuaries and coastal
386 habitats. This proportion is similar to the percentage of glass eels remaining in BW in our
387 behavioural experiments. As such, individuals that exhibited no preference for either FW
388 or SW might represent glass eels predisposed to estuarine or coastal habitat colonisation
389 in the wild but other factors like stress could also explain this low activity. While a strong
390 majority of glass eels were classified as exhibiting no salinity preference, this
391 experimental design did allow a few glass eels to swim between salinities, which could
392 consequently have contributed to a slightly lower than expected locomotor activity.

393

394 This study provides valuable insight into the behaviour exhibited by glass eels.
395 However, the experimental design employed could conceivably have influenced the
396 results. For example, charcoal-filtered dechlorinated tap water was used as FW while BW
397 and SW were prepared by adding either FW or synthetic salts (Instant Ocean) to sand-
398 filtered St. Lawrence Estuary water (salinity 20-25). As such, an influence of water
399 source (tap and river water) on water odours and hence salinity preferences cannot be
400 excluded, although any potential bias was consistent and could not have influenced
401 comparisons between rivers. Furthermore, since the activity levels in our experimental
402 setup were comparable to what has been reported in nature (Daveras *et al.*, 2006), such
403 bias is unlikely.

404

405 BODY CONDITION

406 Relative body condition progressively declined among geographic origins (Mersey >
407 Saint-Jean > Grande-Rivière-Blanche) as glass eels continued their marine migration
408 away from the Sargasso Sea (Fig. 1). Our results are corroborated by Laflamme *et al.*
409 (2012), who found that mean condition factor was the highest in the central distribution
410 range (35–40°N) and gradually decreased as glass eels were sampled north and south
411 along the coast. The decrease in body condition observed in this study likely reflects a
412 longer migration period as well as difficult environmental conditions (Dutil *et al.*, 2009;
413 Laflamme *et al.*, 2012).

414

415 Finally, we observed no differences in condition factor between glass eels having
416 different salinity preferences. Energetic status has been shown to directly influence the
417 upstream migratory behaviour of *A. anguilla* glass eels (Edeline *et al.*, 2006; Bureau du
418 Colombier *et al.*, 2007) and has also been suggested to influence habitat selection in
419 American glass eels (Sullivan *et al.*, 2009). Due to their small size and fasting behaviour
420 during transition to continental habitats, glass eel energy stores are a limiting factor for
421 the successful colonisation of FW habitats. Lower body condition has been linked to
422 reduced locomotor activity, a shift to SW preference, and early settlement in estuaries
423 and coastal habitats (Edeline *et al.*, 2006), while glass eels exhibiting higher relative body
424 condition pursue their migration upstream. This absence of differences in body condition
425 between glass eels of different salinity preferences in our study is therefore surprising and
426 suggests that relative body condition might not accurately predict habitat selection in
427 American glass eels. It is also likely that the dispersion of *A. rostrata* juveniles is at least
428 in part driven by underlying endocrine and genetic factors.

429

430 PIGMENTATION

431 Our results showed decreasing average FW preferences (71.2 – 62.6%) while
432 pigmentation increased on average by 26% during the sampling season. This inverse
433 relationship could partially explain why the less pigmented glass eels from the Saint-Jean
434 River also exhibited the strongest preference for FW although the difference with other
435 rivers was not significant. Our results are in agreement with Edeline *et al.* (2005) who
436 found that non-pigmented glass eels preferred FW, but are contrary to Crean *et al.*
437 (2005), who showed an increasing preference for FW with increasing pigmentation in

438 *A. anguilla*. The use of different methodologies could partially explain these conflicting
439 results. Crean *et al.* (2005) compared average times spent in different salinities while this
440 study and that of Edeline *et al.* (2005) measured the number of glass eels present in a
441 given compartment at the end of the experiments. Another possibility is that the
442 relationship between salinity preferences and pigmentation could be a mere correlation
443 rather than a causal relationship. Pigmentation is a complex trait under the influence of
444 several environmental factors, developing faster with higher water temperatures and more
445 slowly with higher salinities (Briand *et al.*, 2005; Dou *et al.*, 2003). Glass eels from
446 Saint-Jean River were less pigmented than those from Mersey River despite having been
447 captured ~4 weeks later, which could perhaps be explained by cold water temperatures
448 (ca. 5°C) associated with migration through the Gulf of St. Lawrence in May (Dutil *et al.*
449 2009), or by an increased mortality of the more advanced stages during migration. Other
450 variables, such as glass eel arrival in different temporal waves or differences in time
451 spent in the river's estuary before FW migration, might also have influenced
452 pigmentation. Such factors might explain the higher pigmentation observed in glass eels
453 from Grande-Rivière-Blanche.

454

455 INFLUENCE OF SALINITY ON GROWTH

456 Previous studies in controlled conditions involving *A. anguilla* and *A. rostrata* have
457 shown higher growth rates in BW and SW compared to those reared in FW (Edeline *et*
458 *al.*, 2005; Côté *et al.*, 2009). We found no such differences in our study between eels
459 reared in FW (salinity 3) and BW (salinity 20) (Fig. 3) despite the significant salinity ×
460 time interaction which likely reflects the weight differences observed between FW and

461 BW elvers from the Mersey River at T₃. Such a difference however could be due to a
462 difference in mortality between rivers (see below). Nevertheless our results on the effect
463 of salinity on growth should be interpreted with caution. Salinity exerts a manifold
464 influence in numerous species (Boeuf & Payan, 2001). Energetic costs associated with
465 osmoregulation are frequently considered to be lower in isotonic conditions (Boeuf &
466 Payan, 2001), although such costs likely represent only a small (< 10%) fraction of the
467 overall energy budget (Moyle & Cech, 2004; Evans, 2008). Furthermore, Bureau du
468 Colombier *et al.* (2011) found no difference in the energetic cost of osmoregulation for
469 glass eels kept in FW or SW. Salinity could nonetheless influence growth by its influence
470 on food conversion, growth hormone production, and feeding activity, which includes
471 cannibalism in many species (Boeuf & Payan, 2001). When rearing *A. anguilla* glass eels
472 under different salinities and diets, Rodriguez *et al.* (2005) found significant differences
473 in growth related to salinity only when a lower quality diet was employed, suggesting
474 that growth differences could be related to the interaction of diet and experimental
475 salinities in controlled experiments. Nutritional requirements for glass eels and elvers are
476 largely unknown, and many diets might be unsuitable (Rodriguez *et al.*, 2005). Better diet
477 suitability might be sufficient to counterbalance the negative effects of less-than-optimal
478 salinities and thus explain the absence of significant growth differences in our
479 experiments. The higher growth rates in BW and SW observed in natural habitats for a
480 given region (Jessop *et al.*, 2008; Cairns *et al.*, 2009) may reflect the higher productivity
481 of estuaries and coastal habitats and hence food availability compared to FW habitats in
482 temperate latitudes (Gross, 1988; Kaifu *et al.*, 2013). Differential growth associated with
483 salinity reported in previous studies (Edeline *et al.*, 2005) could conceivably be the result

484 of higher rearing salinities (salinity 34), although Côté *et al.* (2009) found that eels
485 exhibited a faster growth rate in BW (salinity 22) than in FW.

486

487 *Fresh and salt-water ecotypes*

488 No difference in growth rate between eels having chosen different salinities during
489 salinity preference experiments was observed. Edeline *et al.* (2005), however, found that
490 *A. anguilla* glass eels that chose SW had higher growth rates than FW or non-choosers
491 regardless of rearing salinities and postulated that genetic factors might be involved. One
492 possible reason for this discrepancy might lie in the methodology used. Edeline *et al.*
493 (2005) sorted glass eels twice in two consecutive behavioural tests before growth
494 experiments and as such likely selected glass eels with the strongest salinity preferences.
495 In our experiments, glass eels used in the behavioural experiments were only sorted once,
496 and we might therefore not have been as selective as Edeline *et al.* (2005). The absence
497 of differences in growth rate between eels that chose different salinities in this study
498 suggests that FW and SW ecotypes, if present in *A. rostrata* as inferred by Castonguay *et*
499 *al.* (1990), likely do not translate into growth differences. Instead, genetically-based
500 regional differences in growth appear more likely (Côté *et al.* 2009, this study).

501

502 *Inter-individual differences in growth rate and mortality*

503 A strong variability in size (0.18–33.1 g) among juvenile eels was observed by the
504 end of our experiments, which might be indicative of hierarchical size effect and/or inter-
505 individual genetically-based growth differences. Growth heterogeneity in glass eels
506 raised in controlled conditions is a common feature in eel aquaculture (Angelidis *et al.*,

507 2005; Rodriguez *et al.*, 2005). Hierarchical size effect, where aggressive larger fish
508 monopolize food, could partially explain some of the growth variation observed despite
509 *ad libitum* feeding. However, differences in growth rate are observed in aquaculture even
510 when eels are graded according to body size, suggesting the influence of underlying
511 genetic factors or a sex-determined effect. Indeed, Côté *et al.* (2014a) recently found
512 evidence of marked growth rate differences between males and females, independent of
513 geographic origin, whereby females showed a bimodal growth distribution (slow-growing
514 and fast-growing) whereas male growth distribution was unimodal and intermediate
515 between female modes. Significantly higher mortality rates were observed in glass eels
516 from Grande-Rivière-Blanche ($39.1 \pm 16.5\%$) compared those from Mersey River ($15.8 \pm$
517 10.7%) in 2012. Because of the marked differences in size, high mortalities could
518 possibly represent a source of bias in our experiments.

519

520 REGIONAL DIFFERENCES IN GROWTH

521 Despite being reared in identical conditions, glass eels from the Atlantic coast (Mersey
522 River, NS) were 2.46 times heavier (3.44 vs 1.40 g) than those from the St. Lawrence
523 Estuary (Grande-Rivière-Blanche, QC) by the end of the experiment. Differences in
524 juvenile eel growth rate between these two regions were previously reported by Côté *et*
525 *al.* (2009), and new results showed that this growth differential was maintained after
526 three years of common rearing (Côté *et al.*, 2014a). Recent population genetic analyses
527 (Côté *et al.*, 2013) found no significant spatial or temporal genetic differentiation among
528 eels collected between 30°N and 48°N for 20 microsatellite loci, thus confirming the
529 panmixia hypothesis in *A. rostrata*. However, the absence of genetic divergence in

530 neutral markers does not necessarily imply the absence of genetic differences in adaptive
531 traits driven by natural selection (Côté *et al.*, 2009).

532

533 Recent studies have shown that selective environmental conditions result in
534 differences in coding genes between glass eels of different geographic origins that also
535 translate in different levels of gene transcription (Gagnaire *et al.*, 2012; Côté *et al.* 2014b,
536 see also Laflamme *et al.*, 2012). Eels enter the Gulf of St. Lawrence as glass eels (Dutil *et*
537 *al.*, 2009) and must rely solely on their energetic reserves to complete their journey
538 across several hundred kilometers. Water temperatures in the Gulf in May are cold
539 ($\leq 5^{\circ}\text{C}$) and have been shown to drastically reduce swimming activity in glass eels
540 (Linton *et al.*, 2007). Glass eels able to complete their migration despite limited energetic
541 reserves and harsh conditions would also be genetically predisposed to lower growth
542 rates. Glass eels predisposed to high growth rates on the other hand would be eliminated,
543 possibly because high growth rate correlates with high metabolism (Burton *et al.*, 2011)
544 and hence insufficient energetic reserves. Locally adaptive alleles could also help explain
545 the eel's ability to colonise such a wide variety of heterogeneous habitats (Gagnaire *et al.*,
546 2012). Furthermore, harsh environmental conditions and long distances from the
547 spawning ground could also explain the much lower glass eel abundance in the St.
548 Lawrence Estuary compared to the Atlantic coast of Canada (Dutil *et al.*, 2009) as well as
549 the observed decreasing energy reserves in our study. Indeed, Gagnaire *et al.* (2012)
550 clearly showed that sea-surface temperatures encountered by glass eels when they
551 approach coastal areas from Florida to the St. Lawrence Estuary areas impose selective
552 pressures that are responsible for shaping allele frequency differences at functional

553 coding genes. Regardless of the exact segregation mechanism, the differences in growth
554 rate observed between eels of different origins in this study and in Côté *et al.* (2009) as
555 well as the results of Gagnaire *et al.* (2012) strongly support the hypothesis of genetic or
556 epigenetic differences among eels from different geographic origins associated with
557 spatially varying selection within an otherwise panmictic context.

558

559 IMPLICATIONS FOR EEL ECOLOGY

560 No significant difference in salinity preference was observed among geographic
561 origins. Despite the small number of sampled rivers, this may suggest that there are no
562 geographic differences in distribution among the various continental habitats used by
563 *A. rostrata* in Canada. Habitat selection, through its influence on population structure,
564 has important implications for eel ecology. The colonisation of estuarine habitats
565 where eel densities are higher is associated with an increased proportion of males
566 which complete their life cycle as soon as the required minimum size for successful
567 migration is reached. In contrast, FW habitat colonisation is associated with lower eel
568 densities and a dominance of larger females maturing at older ages (Krueger &
569 Oliveira, 1999; Goodwin & Angermeier, 2003). Residency in BW habitats increases
570 growth (Morrison *et al.*, 2003; Cairns *et al.*, 2004; Jessop *et al.*, 2008; Lamson *et al.*,
571 2009) which in turn decreases predation risk and age at migration (Edeline & Elie,
572 2004; Tremblay, 2004; Davey & Jellyman, 2005). Edeline (2007) proposed that
573 facultative catadromy could be understood in terms of fitness trade-offs. Residency in
574 the more productive estuarine and BW habitats would provide increased resources at
575 the cost of increased inter- and intraspecific competition (including cannibalism) while

576 the reduced growth rate associated with migration to FW habitats would be
577 compensated by decreased competition. Eels would therefore find different but fitness-
578 equivalent solutions by using opposite migratory behaviours. Lower eel densities
579 driven by the current sharp stock decline could result in a population shift toward
580 estuaries in response to lower intraspecific competition (Edeline, 2007). Moreover, the
581 human-driven selective pressures of recent decades, notably hydroelectric dams and
582 the commercial fishery for St. Lawrence River eels, may have increased selection
583 against upstream migrants and may have displaced the stable state of the conditional
584 strategy which resulted in a decline in the proportion of eels invading FW (McCleave
585 & Edeline, 2009). The eel decline would therefore appear larger in FW than for the
586 population (species) as a whole.

587

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1 TABLES

2

3 **Table I.** Total number (N) of *Anguilla rostrata* glass eels caught for a given sampling
 4 period according to the batch and the river in 2011 and 2012.

Year	Sampling period	River	Batch	N
2011	05/04 - 05/09	Mersey	1	1221
2011	06/09 - 06/12	Mersey	2	568
2012	03/26 - 03/28	Mersey	1	2126
2012	04/20 - 04/21	Mersey	2	1083
2011	—	East	1	0
2011	06/12 - 06/15	East	2	954
2012	—	East	1	0
2012	—	East	2	0
2011	—	Saint-Jean	1	0
2011	—	Saint-Jean	2	0
2012	05/16 - 05/21	Saint-Jean	1	258
2012	05/28 - 06/03	Saint-Jean	2	378
2011	06/29 - 07/03	Grande-Rivière-Blanche	1	124
2011	—	Grande-Rivière-Blanche	2	0
2012	06/02 - 06/06	Grande-Rivière-Blanche	1	321
2012	06/18 - 06/21	Grande-Rivière-Blanche	2	1336

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10 **Table II.** Locomotor activity and salinity preferences of *Anguilla rostrata* glass eels for
 11 2011 and 2012 combined. Act_{TOT} (\pm S.D.): Percentage of glass eel making a salinity
 12 choice (i.e., active glass eels); FW (\pm S.D.): Percentage of active glass eels choosing
 13 freshwater; N_{TOT}: total number of replicate tanks. Different superscript letters represent
 14 significant differences among rivers.

River	N_{TOT}	Act_{TOT} (%)	FW (%)
Mersey	54	13.04 \pm 0.02 ^a	61.9 \pm 5.3
East	9	10.65 \pm 0.02 ^a	64.0 \pm 7.5
Saint-Jean	18	14.29 \pm 0.03 ^{ab}	77.9 \pm 5.7
Grande-Rivière-Blanche	27	22.07 \pm 0.03 ^b	62.6 \pm 5.7

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30 **Table III.** Main effects and interactions explaining mean wet mass differences between
 31 *Anguilla rostrata* glass eels from Grande-Rivière-Blanche and Mersey rivers in 2012.
 32 Statistically significant interactions are in bold.

Effect	d.d.f.	n.d.f.	F	P
River	34	1	42.39	< 0.0001
Choice	33	2	1.88	> 0.05
River × Choice	31	2	1.10	> 0.05
Salinity	34	1	0.54	> 0.05
River × Salinity	32	1	0.58	> 0.05
Choice × Salinity	31	2	0.03	> 0.05
River × Choice × Salinity	29	2	0.43	> 0.05
Time	32	3	739.91	< 0.0001
River × Time	30	3	27.49	< 0.0001
Choice × Time	29	6	2.88	< 0.05
River × Choice × Time	27	6	1.10	> 0.05
Salinity × Time	30	3	4.96	< 0.005
River × Salinity × Time	28	3	1.41	> 0.05
Choice × Salinity × Time	27	6	0.27	> 0.05
River × Choice × Salinity × Time	25	6	2.45	< 0.05

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 34 d.d.f. = denominator degrees of freedom; n.d.f. = numerator degrees of freedom
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42 **Table IV.** Main effects and interactions influencing body condition and pigmentation of
 43 *Anguilla rostrata* glass eels from Grande-Rivière-Blanche, Mersey River and Saint-Jean
 44 Rivers in 2012. Statistically significant interactions are in bold.

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Effect	d.f.	<i>Body condition</i>		<i>Pigmentation</i>	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
River	2	195.06	< 0.0001	151.00	< 0.0001
Batch	1	0.72	> 0.05	90.88	< 0.0001
River × Batch	2	31.41	< 0.0001	40.60	< 0.0001
Choice	2	2.97	> 0.05	1.74	> 0.05
River × Choice	4	1.56	> 0.05	1.70	> 0.05
Batch × Choice	2	0.28	> 0.05	4.78	< 0.05
River × Batch × Choice	4	0.39	> 0.05	0.47	> 0.05

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1 FIGURE CAPTIONS

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3 **Figure 1.** Map of eastern Canada showing rivers sampled in 2011 and 2012: 1) Mersey
4 River, 2) East River, 3) Saint-Jean River, 4) Grande-Rivière-Blanche.

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6 **Figure 2.** Experimental setup used to evaluate both salinity preferences and locomotor
7 activity of *Anguilla rostrata* glass eels. A: Waiting chamber (BW, salinity 18), B: Traps
8 (500 mL filtering flasks), C: Fresh (FW, salinity 0) and salt water (SW, salinity 33) tanks.
9 Reproduced with permission from Edeline *et al.* (2005).

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11 **Figure 3.** Mean body weight ($W \pm SD$) of *Anguilla rostrata* juvenile eels from Mersey
12 River and Grande-Rivière-Blanche reared in fresh (FW) and brackish (BW) water over a
13 7 months period in 2012. Different lowercase letters indicate significant differences for a
14 given sampling period.

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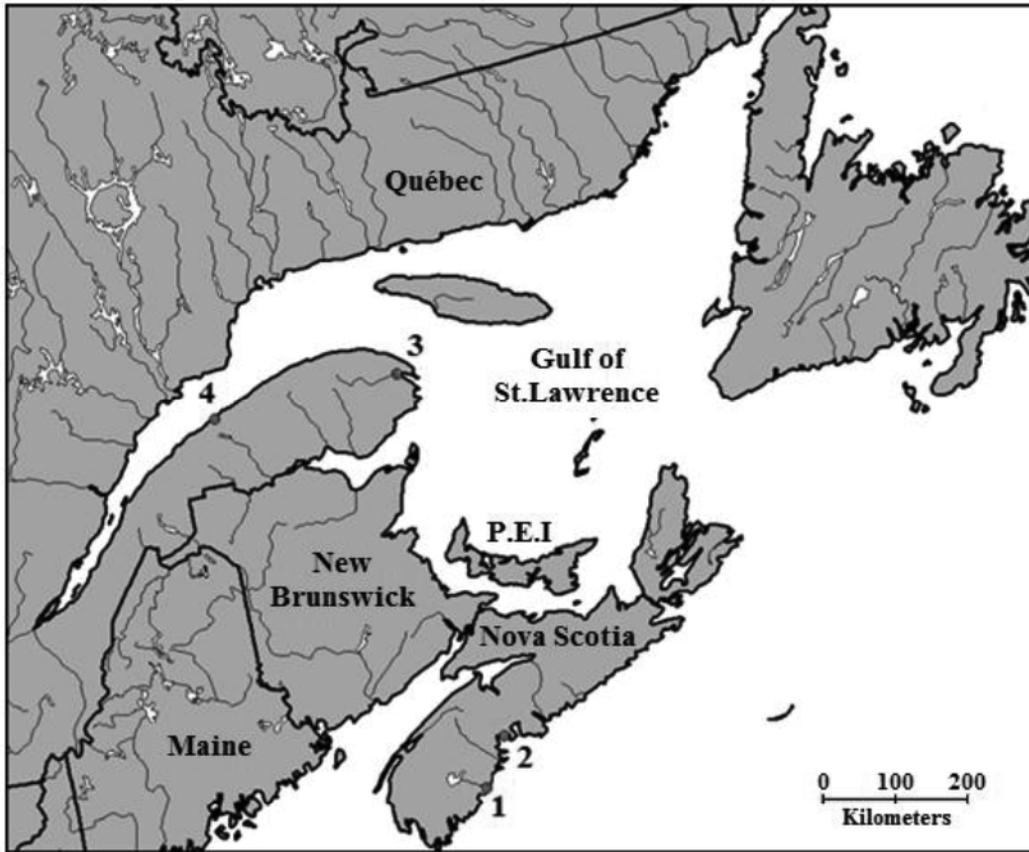
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1 FIGURES



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3 **Figure 1.**

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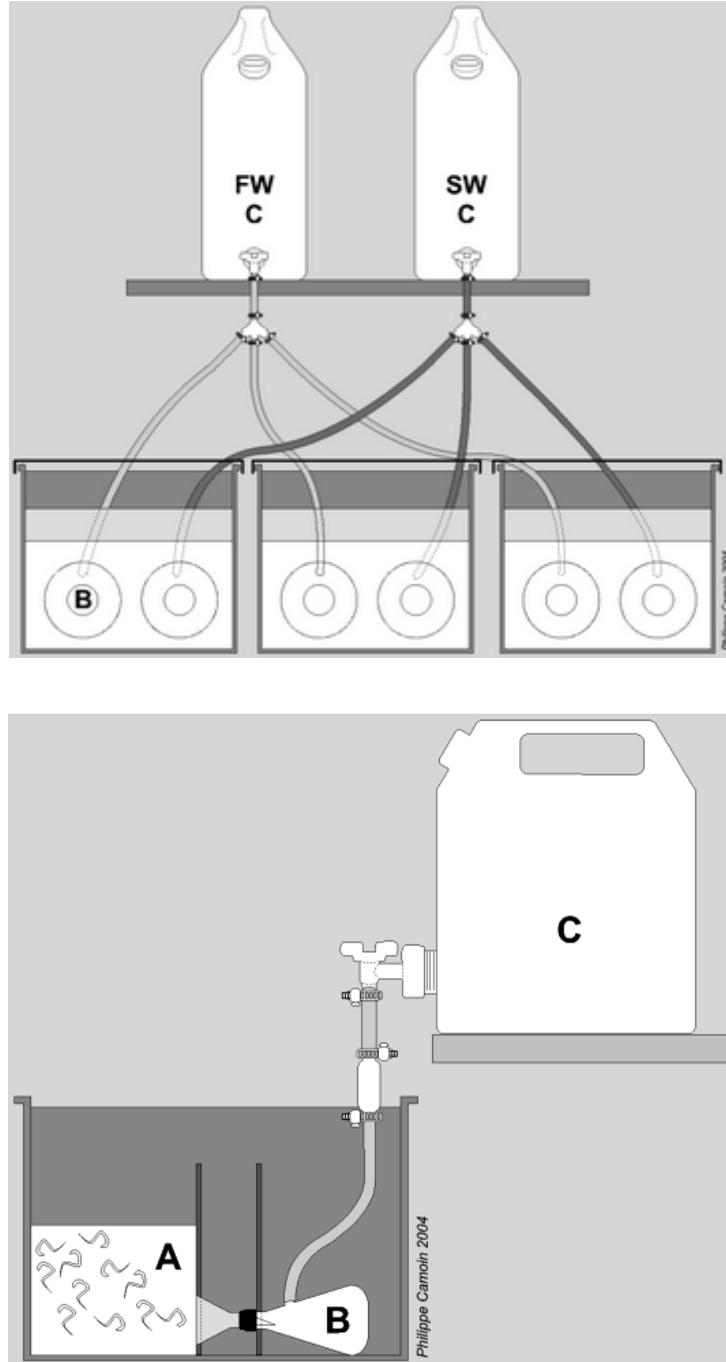
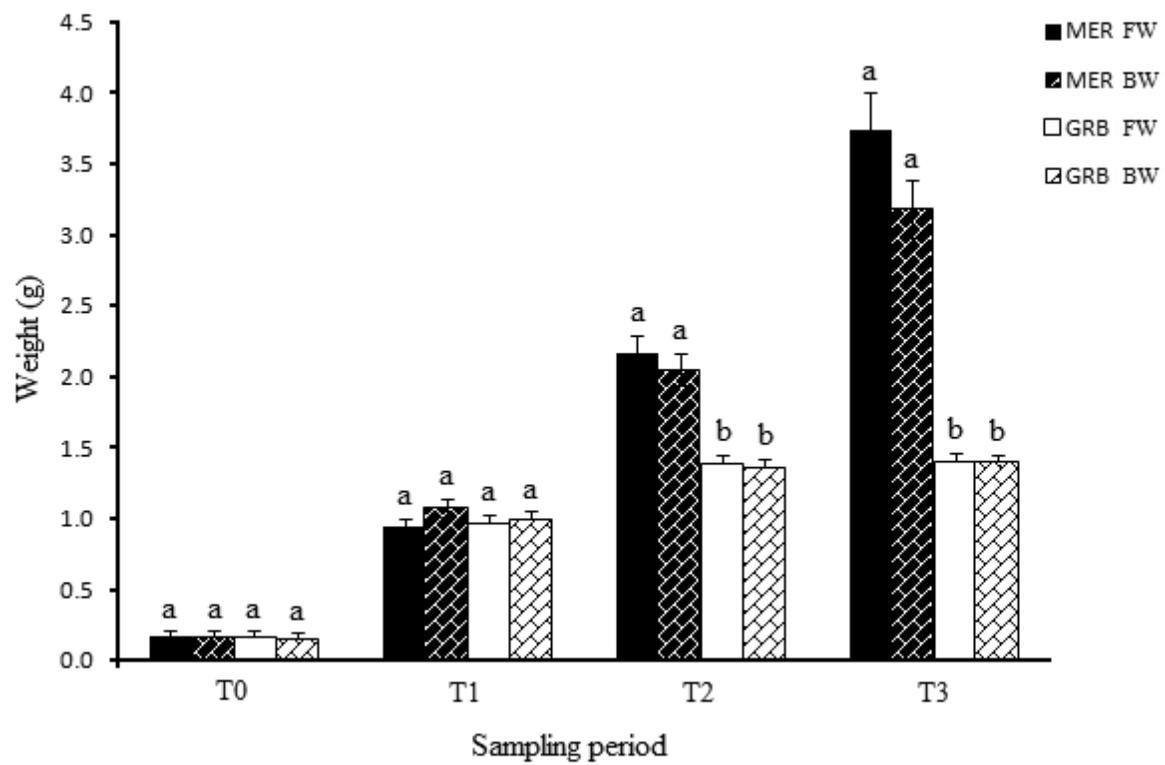


Figure 2.

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39 **Figure 3.**

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SUPPORTING INFORMATION - GROWTH EXPERIMENTS REALIZED IN 2011

MATERIAL AND METHODS

Non-chooser eels from the Mersey River (from both temporal batches) and the East River were subdivided into triplicate groups of 70 individuals per 45 L tanks in two independent series of FW (salinity 3 ± 1) and BW (salinity 20 ± 1) tanks with continuous recirculated filtered water. FW and SW choosers were represented by one tank in either salinity due to the limited number of samples available. Mean initial density was 52 g m^{-2} . Photoperiod was set at 14L:10D (35% light intensity, 60 W light bulbs), and water temperature was set at 17 ± 0.4 °C. Total body length ($\pm 1 \text{ mm}$) and wet mass ($\pm 0.02 \text{ g}$) of all glass eels were measured every 60 days over an 8 month period. Individuals were kept unfed 24 hours beforehand and then anaesthetised with a mixture of eugenol dissolved in ethanol at a 1:10 ratio.

RESULTS

Table I. Mean weight ($W \pm \text{S.D.}$) and length ($L \pm \text{S.D.}$) of *Anguilla rostrata* glass eels captured in 2011 and 2012 at T_0 . N: number of glass eels. Different superscript letters represent significant differences.

Year	River	N	W (g)	L (mm)
2011	East	125	0.13 ± 0.03^a	59.2 ± 3.3^a
	Mersey ⁽¹⁾	129	0.18 ± 0.03^b	61.0 ± 3.1^b
	Mersey ⁽²⁾	81	0.15 ± 0.04^c	59.1 ± 3.7^a
	Grande-Rivière-Blanche	74	0.16 ± 0.03^c	62.6 ± 3.4^c
2012	Saint-Jean	87	0.19 ± 0.03^a	65.2 ± 3.0^a
	Grande-Rivière-Blanche	129	0.17 ± 0.03^b	65.0 ± 3.3^a
	Mersey	128	0.18 ± 0.04^{ab}	60.0 ± 3.5^b

Results for Mersey batches 1 and 2 are shown.

Growth differences were investigated among three groups of non-chooser (NCH) glass eels: Mersey (batches 1 and 2) and East River (batch 2). An overall effect of the group was observed ($P < 0.05$, Table II). The first batch of glass eels captured in 2011 from the Mersey River were significantly heavier than those collected later (Table I), although no differences were found in pairwise comparisons (Fig. 1) once glass eels were separated in tanks. No significant effect of salinity on growth was observed ($P > 0.05$). Interactions between main effects were also not significant with the exception of a group \times time interaction, indicating that the extent of the group effect varied during the experiments. In 2011, average mortality was $28.6 \pm 9.5\%$ with no differences between groups ($P > 0.05$) or rearing salinity ($P > 0.05$).

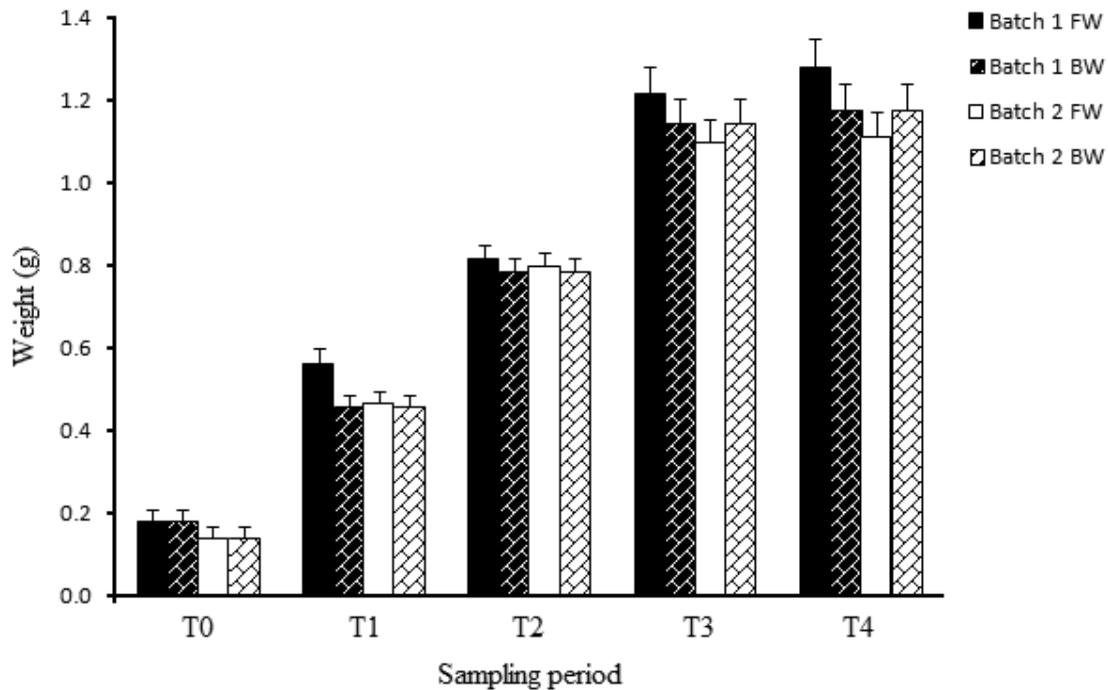


Figure 1. Mean body weight ($W \pm SD$) of *Anguilla rostrata* juvenile eels from Mersey River (batch 1 and 2) reared in fresh (FW) and brackish (BW) water in 2011. No significant differences were found at any time.

Table II. Main effects and interactions explaining mean wet mass differences between non-chooser glass eels from Mersey (batches 1 and 2) and East (batch 2) rivers in 2011.

Statistically significant interactions are in bold.

Effects	d.f.	<i>F</i>	<i>P</i>
Group	2	8.62	< 0.005
Salinity	1	1.28	> 0.05
Group × Salinity	2	0.60	> 0.05
Time	3	345.25	< 0.0001
Group × Time	6	2.62	< 0.05
Salinity × Time	3	0.21	> 0.05
Group × Salinity × Time	6	0.42	> 0.05