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

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Corresponding Author:	Martin Castonguay, Ph. D. Pêches et Océans Canada, Institut Maurice-Lamontagne Mont-Joli, Québec CANADA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Pêches et Océans Canada, Institut Maurice-Lamontagne
Corresponding Author's Secondary Institution:	
First Author:	Brian Boivin, M.Sc.
First Author Secondary Information:	
Order of Authors:	Brian Boivin, M.Sc.
	Martin Castonguay, Ph. D.
	Céline Audet, Ph.D.
	Scott Pavey, Ph.D.
	Mélanie Dionne, Ph.D.
	Louis Bernatchez, Ph.D.
Order of Authors Secondary Information:	
Abstract:	The influence of salinity on habitat selection and growth in juvenile American eels Anguilla rostrata 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.

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

B. BOIVIN§, M. CASTONGUAY*|, C. AUDET§, S. PAVEY†,

M. DIONNE¥ AND L. BERNATCHEZ†

§Université du Québec à Rimouski, 310 Allée des Ursulines, Rimouski, Québec G5L 3A1 Canada, |Institut Maurice-Lamontagne, Pêches et Océans Canada, 850 Route de la Mer, Mont-Joli, Québec, G5H 3Z4 Canada, †Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, G1V 0A6 Canada, ¥Ministère des Forêts, de la Faune et des Parcs, 880 chemin Sainte-Foy, Québec, Québec, G1S 4X4 Canada

*Author to whom correspondence should be addressed: Tel: +1 418 775 0634; fax: +1 418 775 0740; email: martin.castonguay@dfo-mpo.gc.ca

Salinity preference in *A. rostrata* glass eels

23

24   **ABSTRACT**

25

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.

43

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

45   differences.

46

47

INTRODUCTION

48
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. Eelsresiding 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*

106 *al*., 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 elversfrom 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 watersSampling 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 $16 \cdot 9 \pm 0 \cdot 2°C$. 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 $22 \pm 0·4°C$. Total body length (± 1 mm) and wet mass ($\pm 0·02$ 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 CO_2 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 ± S.D.

220

221   *Behavioural experiments*

222   A mixed logit model, $\mathrm{logit}(P_{ijklm}) = \mu + R_i + B_j + RB_{ij} + T_l + \varepsilon_{m(ijkl)}$ (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 $\square_{m(ijkl)}$ 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 ($P = 0\cdot22$) and slight water temperature variations

233   ($P = 0\cdot47$) 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 ($P > 0\cdot75$) throughout the experiments.

236

237   *Growth experiments*

238   Since total length and body weight data (log transformed) were highly correlated

239   ($y = 0\cdot254x + 1\cdot977$, $R^2 > 0\cdot95$), 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 $5\cdot27\times 10^{-5}$ and $1\cdot97$ 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 ($P < 0\cdot0005$) 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 ($P < 0\cdot05$, Table II). There was no significant difference in locomotor

275   activity among the other rivers ($P > 0\cdot05$). An overall decline in activity was noticed

276   between the first ($16\cdot2 \pm 2\cdot3\%$) and second ($13\cdot1 \pm 1\cdot8\%$) sampling times, although this

277   difference was not significant ($P > 0\cdot05$).

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 ($1\cdot41 \pm 0\cdot05$ *vs* $1\cdot40 \pm 0\cdot05$). The choice effect was not

302   significant ($P > 0\cdot05$), 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 ($P < 0\cdot0001$) for glass eels from the Mersey

309   River ($15\cdot8 \pm 10\cdot7\%$) compared to those from Grande-Rivière-Blanche ($39\cdot1 \pm 16\cdot5\%$)

310   although this was unrelated to salinity ($P > 0\cdot05$). 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: $1\cdot13 \pm 0\cdot16$; > Saint-Jean: $1\cdot01 \pm 0\cdot12$; > Grande-Rivière-Blanche: $0\cdot90 \pm 0\cdot10$,

317    $P < 0\cdot0001$), between all rivers as glass eels continued their migration away from the

318   Sargasso Sea. While the batch effect was not significant ($P > 0\cdot05$), the river $\times$ batch

319   interaction was ($P < 0\cdot0001$, 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 ($0\cdot92 \pm 0\cdot10 - 0\cdot88 \pm 0.10$, $P > 0\cdot05$) but significantly decreased over

322   time for glass eels from the Saint-Jean River ($1\cdot06 \pm 0\cdot12 - 0\cdot97 \pm 0\cdot10$, $P < 0\cdot001$) and

323   significantly increased for those from the Mersey River ($1\cdot08 \pm 0\cdot14 - 1\cdot18 \pm 0\cdot17$, $P <$

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 × 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

340   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 T_3. 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 ($39 \cdot 1 \pm 16 \cdot 5\%$) compared those from Mersey River ($15 \cdot 8 \pm$

517    $10 \cdot 7\%$) 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 $2 \cdot 46$ times heavier ($3 \cdot 44$ *vs* $1 \cdot 40$ 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   ($\leq 5°C$) 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	$13 \cdot 04 \pm 0 \cdot 02^a$	$61 \cdot 9 \pm 5 \cdot 3$
East	9	$10 \cdot 65 \pm 0 \cdot 02^a$	$64 \cdot 0 \pm 7 \cdot 5$
Saint-Jean	18	$14 \cdot 29 \pm 0 \cdot 03^{ab}$	$77 \cdot 9 \pm 5 \cdot 7$
Grande-Rivière-Blanche	27	$22 \cdot 07 \pm 0 \cdot 03^b$	$62 \cdot 6 \pm 5 \cdot 7$

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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.	Body condition		Pigmentation	
		F	*P*	*F*	*P*
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 ± 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
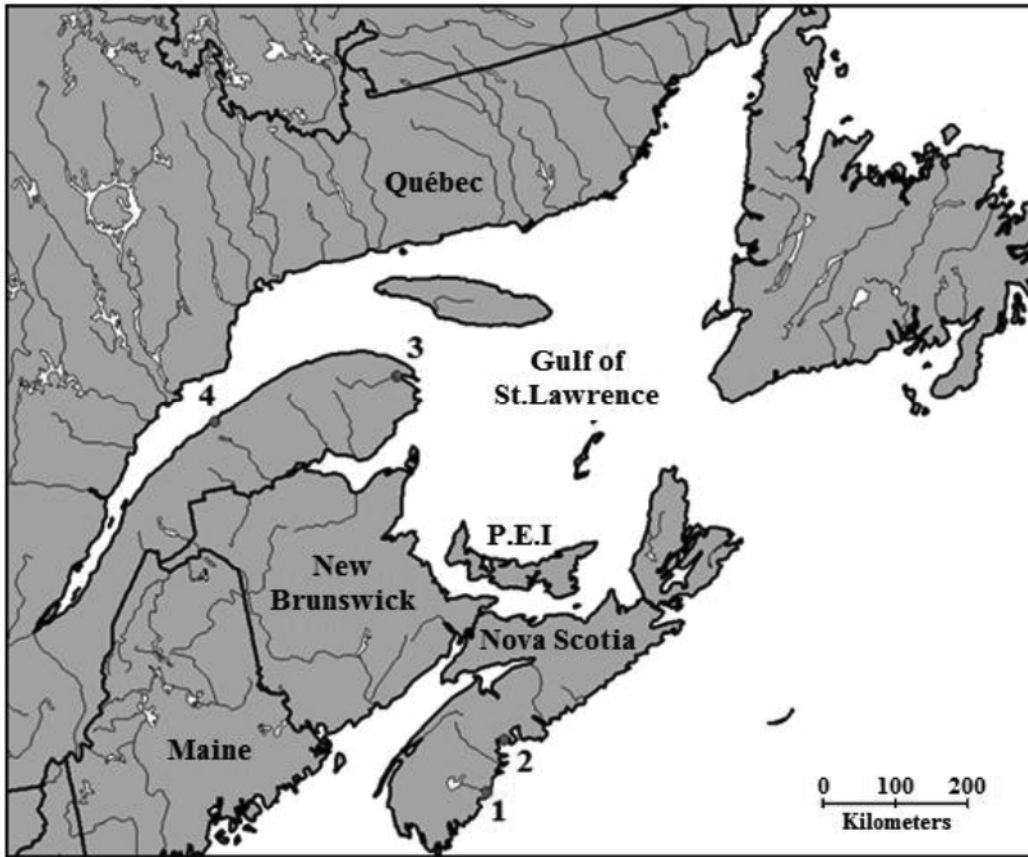
14   given sampling period.

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1   FIGURES

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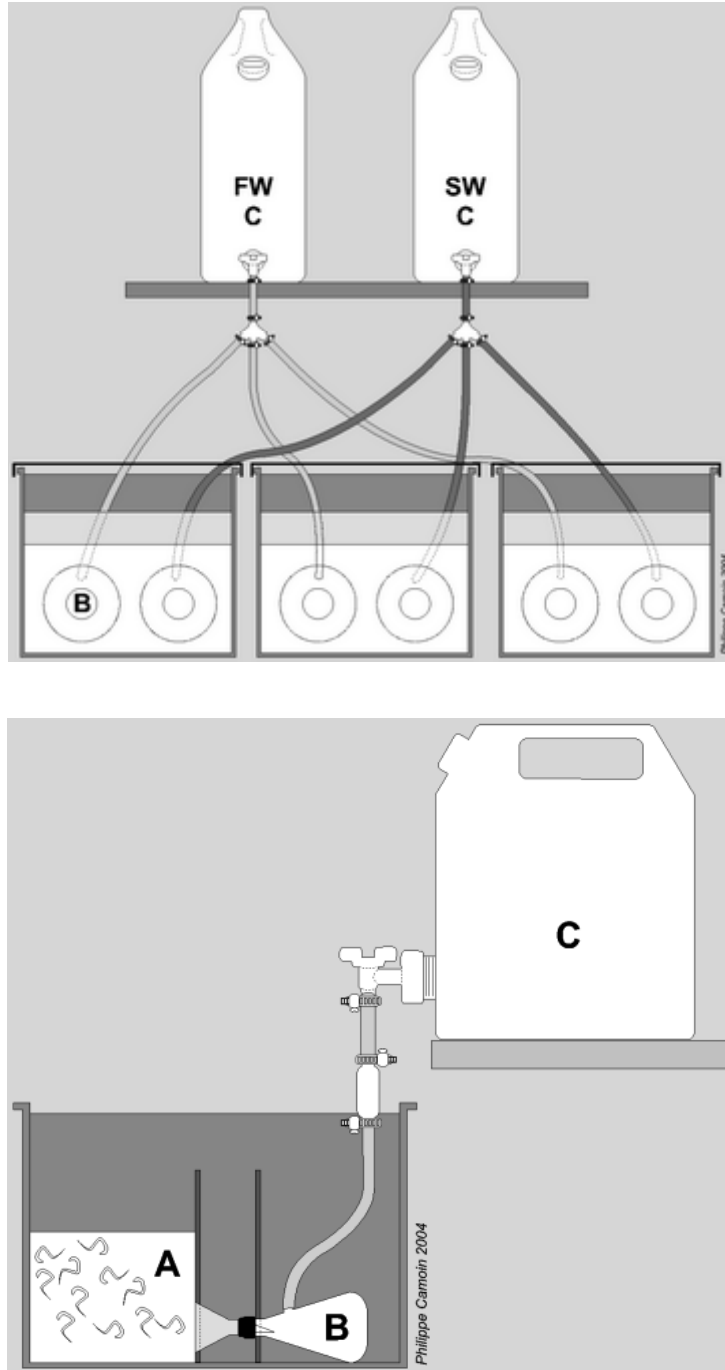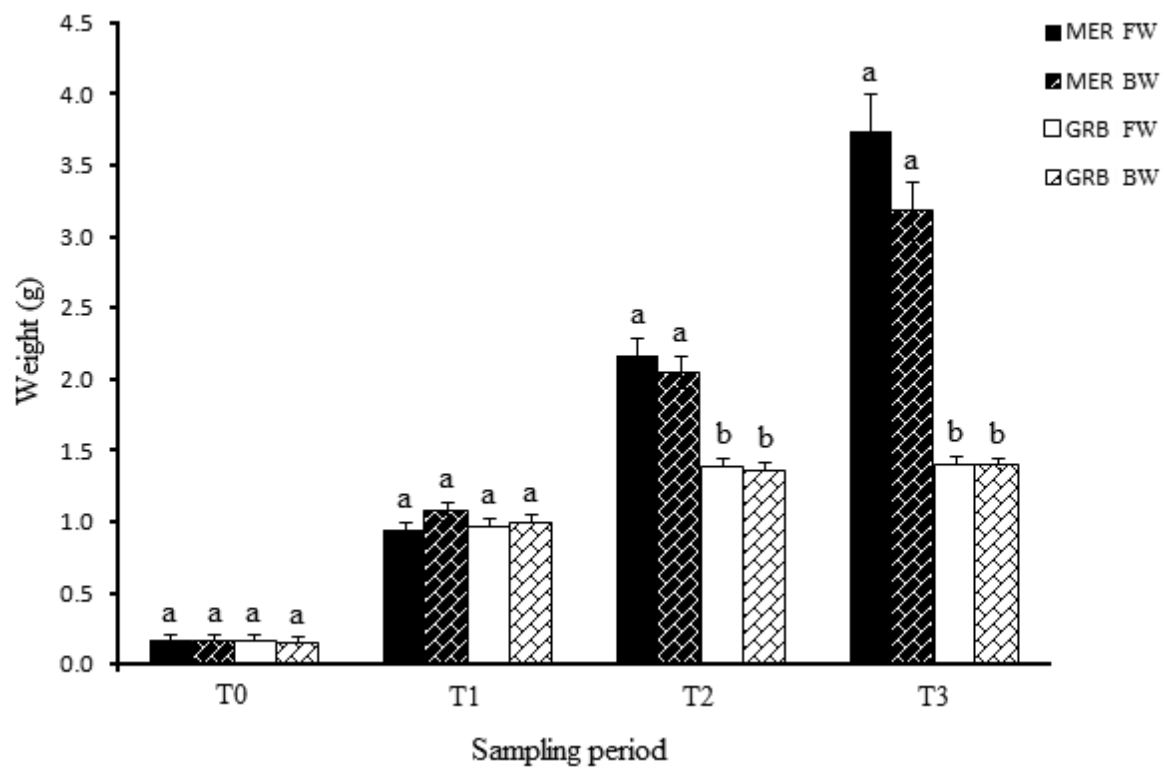3   **Figure 1.**

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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 (± 1 mm) and wet mass (± 0·02 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 ± S.D.) and length (L ± 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 ($P > 0.05$).
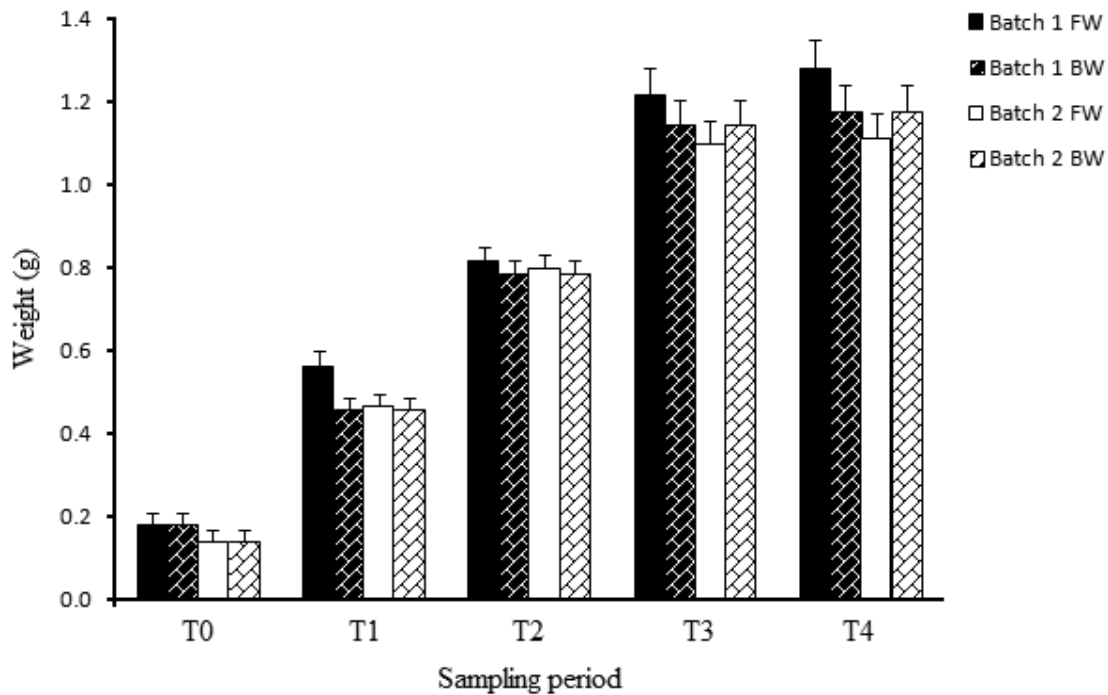

Figure 1. Mean body weight (W $\pm$ SD) of *Anguilla rostrata* juvenile eels from Mersey River (batch 1 and 2) reared in fresh (FW) and brackish (BW) water in 2011. No significant differences were found at any time.

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.	*F*	*P*
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