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

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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 categorized according to their salinity preferences towards 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. Elvers from Nova Scotia, however, 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 *A. rostrata* from different origins. These results provide important ecological knowledge for the sustained exploitation and conservation of this threatened species.

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Key words: geographic differences; glass eels; locomotor activity; pigmentation; St. Lawrence salinity preferences.

INTRODUCTION

Once ubiquitous, the American eel *Anguilla rostrata* (Lesueur 1817) has suffered a 98% decline in abundance in the upper St Lawrence River and Lake Ontario since the 1970s while remaining stable or diminishing only slightly elsewhere in eastern Canada (DFO, 2010; COSEWIC, 2012). Such regional disparities are difficult to reconcile with panmixia (Castonguay *et al.*, 1994) as all individuals are presumed to come from a single spawning event, which should conceivably translate into homogeneous recruitment

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trends across regions. A better understanding of the environmental cues leading to habitat selection by glass eels (unpigmented juvenile eels) would be invaluable from both conservation and management perspectives.

The complex life history of *A. rostrata* begins far offshore in the Sargasso Sea, with a semelparous and panmictic reproduction (Schmidt, 1923; Côté *et al.*, 2013). The willow-leaf-shaped translucent leptocephalus larvae are then advected back to continental waters by oceanic currents (Kleckner & McCleave, 1985) and metamorphose into unpigmented glass eels shortly after reaching the continental shelf. This oceanic migration varies greatly in terms of distance and duration; some glass eels settle all along North America's east coast while some continue beyond to the Gulf of St Lawrence, Canada, and Greenland (Tesch, 2003). Glass eels that enter the Gulf of St Lawrence must rely solely on their diminishing energetic reserves to complete their journey, swimming across several hundred kilometres in often harsh conditions to reach the St Lawrence Estuary as they are no longer carried by the Gulf Stream (Dutil *et al.*, 2009). This last segment of their journey is estimated to take between 1 and 2 months depending on the final destination (Dutil *et al.*, 2009). After reaching their final settling geographic region, glass eels then use divergent migratory tactics to colonize various coastal habitats (rivers, lakes, estuaries and marshes) characterized by fresh (FW), brackish (BW) or salt (SW) water (Jessop *et al.*, 2002; Daverat *et al.*, 2006; Thibault *et al.*, 2007) where they will become fully pigmented and henceforth be classified as elvers for their first year in continental waters.

In the European eel *Anguilla anguilla* (L. 1758), the colonization of coastal habitats is modulated by multiple environmental cues such as temperature (Tongiorgi *et al.*, 1986; Tosi *et al.*, 1988; Edeline *et al.*, 2006), odours (Tosi & Sola, 1993; Sola, 1995; Sola & Tongiorgi, 1998), photoperiod (Bardonnet *et al.*, 2003) and salinity (Tosi *et al.*, 1988, 1990; Edeline *et al.*, 2005). Extensive experiments by Tosi *et al.* (1990) demonstrated the dominant influence of salinity over other environmental factors. While glass eels display remarkable tolerance to salinity variations (Wilson *et al.*, 2004; Crean *et al.*, 2005), early experiments (Deelder, 1958) showed that not only did newly arrived eels display no tendency to migrate into FW, but they also actively avoided it. An acclimation period therefore appears necessary before FW migration, a phenomenon commonly observed in many fish species (Lucas *et al.*, 2008).

Various studies with *A. anguilla* have demonstrated glass eels' preference for FW, although a significant number (30–50%) either chose SW or exhibited no preference (Tosi *et al.*, 1988, 1990; Edeline *et al.*, 2005). This interindividual variability could in part be explained by physiological and genetic factors. Edeline & Elie (2004) and Edeline *et al.* (2006) showed that FW-seeking glass eels had a higher energetic status, higher levels of thyroid hormones and lower cortisol levels than those exhibiting an SW preference. These results suggest that individuals with higher condition factor would therefore maximize their fitness by colonizing lower eel density FW habitats, while individuals with lower condition would maximize their fitness by avoiding the energetic cost associated with further inland migration (Edeline, 2007).

The utilization of these diverse habitats has been shown to result in differential growth. Anguillids residing in estuarine and coastal habitats grow at a faster rate than those using river and lake habitats (Morrison *et al.*, 2003; Cairns *et al.*, 2004; Jessop *et al.*, 2004, 2008; Lamson *et al.*, 2009). Higher productivity of estuarine habitats at higher latitudes (Gross *et al.*, 1988; Kaifu *et al.*, 2013) and lower osmoregulation costs (Tzeng *et al.*, 2003) are often cited as partial explanations. In controlled experiments,

however, Edeline *et al.* (2005) showed that glass eels with a preference for SW had a higher growth rate than those favouring FW, irrespective of food availability. Furthermore, in controlled experiments, Côté *et al.* (2009) showed differences in growth rate in *A. rostrata* elvers from different geographic origins in eastern Canada. Growth rate is an important life-history trait, directly influencing predation, age and size at migration and female fecundity (Edeline & Elie, 2004; Davey & Jellyman, 2005; Hutchings, 2006; Tremblay, 2009). Various authors (Edeline *et al.*, 2005; Côté *et al.*, 2009) have hypothesized that differential growth could in part be explained by underlying genetic differences, possibly resulting from spatially varying selection (Gagnaire *et al.*, 2012). While much has been learned about the environmental cues affecting *A. anguilla* distribution across various habitats and their influence on growth, such information remains conspicuously absent in *A. rostrata*.

In this study, glass eels were sampled in four rivers across eastern Canada in order to (1) assess juvenile *A. rostrata* salinity preferences and their locomotor activity for two sampling periods over 2 years and (2) study the influence of salinity, geographic origin and previous salinity preferences on growth in controlled FW and BW for seven months.

MATERIALS AND METHODS

SAMPLE COLLECTION

Anguilla rostrata glass eels were sampled in eastern Canada in 2011 and 2012 at the mouths of four rivers: Mersey River (Nova Scotia, 44° 02′ 45″ N; 64° 42′ 30″ W), East River (Nova Scotia, 44° 35′ 10″ N; 64° 10′ 06″ W), Saint-Jean River (Québec, 48° 46′ 44″ N; 64° 23′ 06″ W) and Grande-Rivière-Blanche (Québec, 48° 47′ 18″ N; 67° 41′ 50″ W) (Table I and Fig. 1). The term glass eel refers to 'all developmental stages between the end of metamorphosis and full pigmentation' (Tesch, 2003) while the term elver refers to fully pigmented fish during the first year in continental waters. Sampling occurred twice in both years (once at the first glass-eel arrival and a second time 2–4 weeks later) at each location between late March and early July depending on geographic location and local conditions. Glass eels were captured in BW with dip-nets except in the Saint-Jean River, where fixed plankton nets were used. Glass eels were sampled at new or full moons during high tides in partnership with professional fishermen (Atlantic Elver Fishery) and with the two government agencies involved in the study (Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs du Québec and the Department of Fisheries and Oceans Canada). Glass eels were then transferred to the Maurice-Lamontagne Institute and placed in a thermostatic chamber at 16·9° C, range ±0·2° C. This temperature was chosen in order to maximize locomotor activity while still being close to natural habitat conditions during the peak migration period. Glass eels were randomly distributed in two 40 l tanks filled with BW (salinity 18) equipped with aerators and without shelters. As glass eels do not feed during their transition to coastal habitats (Dutil *et al.*, 2009), individuals were kept unfed throughout the behavioural experiments. Glass eels were never kept for more than 2 weeks and were allowed a 48 h acclimatization period before beginning the experiments. Pigmentation was established according to the 1–7 scale developed by Haro & Krueger (1988) that has been previously employed in numerous studies (*e.g.* Sullivan *et al.*, 2009) as well as by government agencies such the Ministère du Développement Durable, de l'Environnement de la Faune et des Parcs, Québec.

BEHAVIOURAL EXPERIMENTS

The experimental setup used to assess locomotor activity (% of active *A. rostrata*, *i.e.* eels that made a choice between FW and SW) and salinity preferences (% of *A. rostrata* that chose FW)

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

Year	Sampling period	River	Batch	*n*
2011	4 May to 9 May	Mersey	1	1221
2011	9 June to 12 June	Mersey	2	568
2012	26 March to 28 March	Mersey	1	2126
2012	20 April to 21 April	Mersey	2	1083
2011	–	East	1	0
2011	12 June to 15 June	East	2	954
2012	–	East	1	0
2012	–	East	2	0
2011	–	Saint-Jean	1	0
2011	–	Saint-Jean	2	0
2012	16 May to 21 May	Saint-Jean	1	258
2012	28 May to 3 June	Saint-Jean	2	378
2011	29 June to 3 July	Grande-Rivière-Blanche	1	124
2011	–	Grande-Rivière-Blanche	2	0
2012	2 June to 6 June	Grande-Rivière-Blanche	1	321
2012	18 June to 21 June	Grande-Rivière-Blanche	2	1336

(Fig. 2) was nearly identical to the one used by Edeline *et al.* (2005, 2006) and modified from previous studies (Tongiorgi *et al.*, 1986; Tosi *et al.*, 1990). The concurrent use of three glass tanks ($31 \cdot 5 \, cm \times 27 \, cm \times 61 \, cm$) provided triplicate measurements for each experiment. Each tank was equally divided by a partition into which two funnels (diameter of 10 cm) were inserted $4 \cdot 5$ cm from the bottom of the tank. Both were connected through a rubber stopper to a 500 ml filtering flask that acted as a trap (B in Fig. 2). Water was gravity-delivered into the neck of the flasks at a rate of $180 \, ml \, min^{-1}$ from 15 l tanks (C in Fig. 2), thereby offering a binary choice between flows of FW (salinity 0) and SW (salinity 33). An overflow drain allowed any excess water to be evacuated throughout the experiments. In order to evaluate the experimental bias of the apparatus, control tests with two BW (salinity 18) flows were performed at the arrival of each batch. For all experiments, including control tests, a mean $\pm$ S.D. of 71 ± 34 individuals, selected to ensure a sufficient number of replicates, were placed at the beginning of each experiment in the BW-filled waiting chamber (A in Fig. 2; salinity 18) for an acclimation period of 30 min after which the water flows were activated for a 30 min experimental period. Both the acclimation and experimental periods were conducted in darkness in order to minimize the stress associated with the manipulations and because glass eels are mainly active at night in natural habitats. The number of glass eels in each flask was recorded at the end of each experiment, and individuals were classified according to their preferences: fresh water choosers (FWC), salt water choosers (SWC) and non-choosers (NCH). Locomotor activity was assessed as the percentage of glass eels having made a choice for either FW or SW. Charcoal-filtered dechlorinated tap water was used as FW while BW and SW were prepared by adding either FW or synthetic salts (Instant Ocean; www.instantocean.com) to sand-filtered St Lawrence Estuary water (salinity 20-25). NCHs were reused twice in order to obtain sufficient glass eels, thereby increasing the statistical power of subsequent growth experiments.

GROWTH EXPERIMENTS

Once subdivided according to their salinity preferences (FWC, SWC and NCH), glass eels were immediately transported to the Laboratoire de Recherche en Sciences Aquatiques (LARSA) at Laval University (Québec City) for growth experiments. All individuals were treated with a formaldehyde solution (0·011% v/v) for 30 min upon arrival to eliminate any potential parasites (Imada & Muroga, 1979; Chan & Wu, 1984). Growth experiments occurred
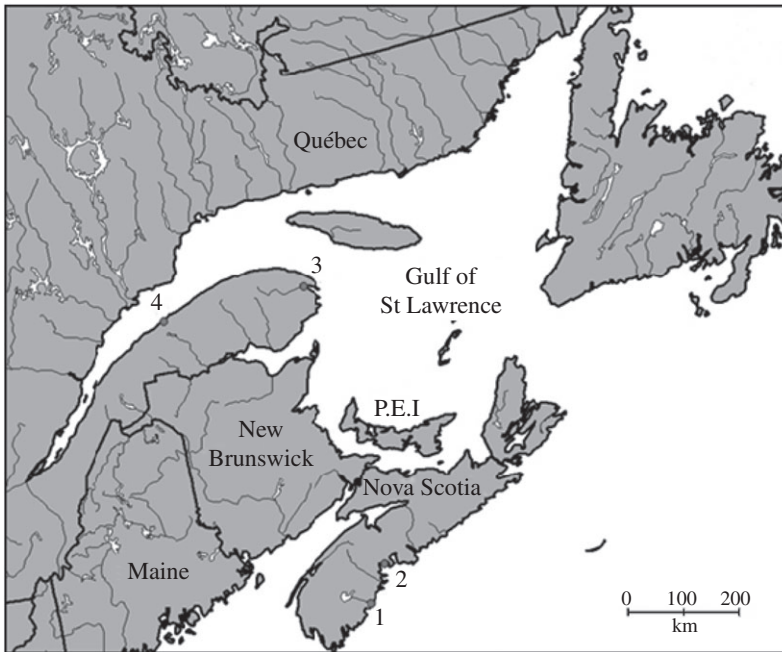
FIG. 1. Map of eastern Canada showing rivers sampled in 2011 and 2012: 1, Mersey River; 2, East River; 3, Saint-Jean River; 4, Grande-Rivière-Blanche.

in 2011 and 2012 but due to sampling difficulties resulting in smaller sample sizes only results from 2012 are presented and interpreted in this article. Nevertheless, results from 2011 are included in Appendix SI (Supporting Information).

All experimental contingents (FWC, SWC and NCH) from Grande-Rivière-Blanche and Mersey River were subdivided into triplicate groups of 40–50 individuals per 45 l tank into two independent series of FW (salinity 3, range ± 1) and BW (salinity 20, range ± 1) tanks with continuous recirculated filtered water. BW was chosen over SW as a growth medium because it is thought to be more representative of natural environments used by anguillids outside FW habitats (Daverat *et al.*, 2006). Mean initial density was $39\,\text{g}\,\text{m}^{-2}$, photoperiod was set at 12L:12D (35% light intensity, 60 W light bulbs), and the water temperature was $22 \cdot 0°$ C, range $\pm 0 \cdot 4°$ C. Total body length (L_T; ± 1 mm) and wet mass (M; $\pm 0 \cdot 02$ mg) of all glass eels were measured on days 0, 85, 154 and 210 over a 7 month period. Individuals were kept unfed 24 h beforehand and then anaesthetized with a mixture of eugenol dissolved in ethanol at a 1:10 ratio.

Tanks were randomly distributed on three-tiered shelves to minimize a possible effect of tank location on growth (Speare *et al.*, 1995). Oxygen content and temperature were monitored continuously, pH daily, and nitrite, ammonia and CO_2 contents biweekly; filters were backwashed weekly. Preventive malachite green treatments were conducted on all tanks simultaneously when increased mortalities were noticed. Individuals were fed *ad libitum* once a day, 6 days a week, alternating with frozen bloodworms *Chironomus* sp. and brine shrimp *Artemia* sp. cubes. Shelters were placed in each tank to minimize stress and agonistic behaviour. Uneaten food and faeces were removed daily.

DATA ANALYSIS

All analyses were performed with the SAS 9.2 statistical package (SAS Institute Inc.; www.sas.com). Statistical significance was accepted at $P < 0 \cdot 05$. Results are expressed as mean $\pm$ S.D.
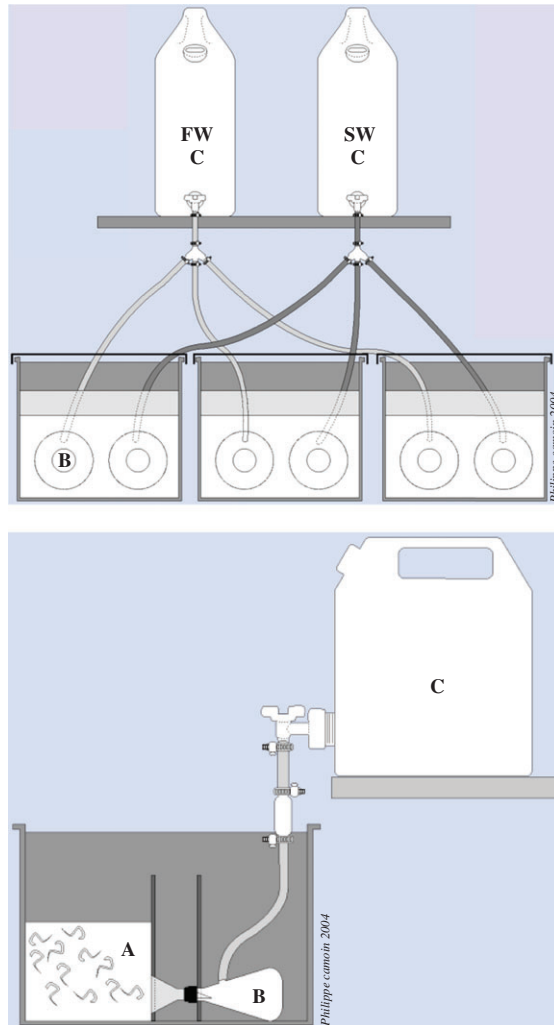
FIG. 2. Experimental setup used to evaluate both salinity preferences and locomotor activity of *Anguilla rostrata* glass eels. A, waiting chamber [brackish water (BW), salinity 18]; B, traps (500 ml filtering flasks); C, fresh (FW, salinity 0) and salt water (SW, salinity 33) tanks. Reproduced with permission from Edeline *et al.* (2005).

Behavioural experiments

A mixed logit model, $\text{logit}(p_{ijklm}) = \mu + R_i + B_j + RB_{ij} + T_l + \varepsilon_{m(ijkl)}$ (GLIMMIX procedure, SAS), was used to analyse differences in salinity preferences and locomotor activity where μ is the probability of either being active or preferring FW, i is the river (the origin), j is the batch (*i.e.* first or second sampling period) while R_i and B_j represent their respective effect. RB_{ij} represents the interaction between the river and batch, l is the tank with its effect T_l, k is the year, m the experiment and $\varepsilon_{m(ijkl)}$ is the random error. This model included the entire dataset in a single analysis (Table II), the unit of replication (sampling unit) was the tank and statistical analysis was performed using only glass eels that made a choice at the first opportunity. The river and the batch were included as fixed effects while the tanks and the experiments were defined as random factors. The influence of density ($P > 0.05$) and slight water temperature variations ($P > 0.05$)

TABLE II. Mean $\pm$ S.D. locomotor activity (percentage of glass eel making a salinity choice, *i.e.* active glass eels; A_{ctTOT}) and salinity preferences (percentage of active glass eels choosing fresh water; F_W) of *Anguilla rostrata* glass eels for 2011 and 2012 combined. Different superscript lower-case letters represent significant differences among rivers

River	n_{TOT}	A_{ctTOT} (%)	F_W (%)
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	$22 \cdot 07 \pm 0 \cdot 03^b$	$62 \cdot 6 \pm 5 \cdot 7$

n_{TOT}, total number of replicate tanks.

were not statistically significant and therefore not included in the model. Finally, control tests for experimental bias conducted with two flows of BW were also not significant ($P > 0 \cdot 05$) throughout the experiments.

Growth experiments

As L_T and M data ($\log_{10}$ transformed) were highly correlated ($y = 0 \cdot 254x + 1 \cdot 977$; $r^2 > 0 \cdot 95$), statistical analyses were performed on M only. The $\chi^{-3/4}$ transformation was applied prior to statistical analyses in order to achieve normality. Differences in M were investigated with a repeated analysis of variance (ANOVA) as a function of time ($n = 4$), rearing salinity ($n = 2$), river ($n = 3$) and salinity preferences ($n = 3$) using the MIXED procedure in SAS. Comparisons for each sampling event were made with the Tukey–Kramer multiple comparisons test. Differences in mortality rates between rivers were assessed with the Kruskal–Wallis test. The sampling unit for both repeated ANOVAs and mortality analyses was the tank.

Relative body condition and pigmentation

Relative body condition (K_n) was assessed using Le Cren's (1951) equation: $K_n = M(aL_T^n)^{-1}$, where M is in g and L_T in mm. The parameters a and n were estimated to be $5 \cdot 27 \times 10^{-5}$ and $1 \cdot 97$, respectively, by using the equation $\log_{10}M = \log_{10}a + n\log_{10}L_T$, where n is the slope of the least-square regression and a is a coefficient (10 raised to the power of the intercept).

Pigmentation and K_n were analysed separately as a function of the river, and the batch and previous salinity preference using multifactor analysis of variance (MANOVA). As ANOVA is robust to slight departures from normality (Maxwell & Delaney, 2004), and because kurtosis and skewness coefficients were very close to normality, these statistical tests were performed using untransformed data with the individual as the sampling unit. Pair-wise comparisons were made with Tukey–Kramer multiple comparisons test. These analyses were realized on a sub-set of 646 glass eels for which pigmentation data were available (Mersey: $n = 241$; Saint-Jean: $n = 177$; Grande-Rivière-Blanche: $n = 228$).

RESULTS

BEHAVIOUR EXPERIMENTS

Locomotor activity

The percentage of active glass eels (*i.e.* eels that made a choice between FW and SW) varied from 13 to 22% according to river (Table II). The influence of the river on locomotor activity was significant ($P < 0 \cdot 001$) with glass eels from Grande-Rivière-Blanche (St Lawrence Estuary) displaying a higher activity level than

glass eels from the other three rivers ($P < 0.05$; Table II). There was no significant difference in locomotor activity among the other rivers ($P > 0.05$). An overall decline in activity was noticed between the first ($16.2 \pm 2.3\%$) and second ($13.1 \pm 1.8\%$) sampling times, although this difference was not significant ($P > 0.05$).

Salinity preferences

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

GROWTH EXPERIMENTS

At T_0, glass eels from Grande-Rivière-Blanche were significantly longer than those from the Mersey River (65.0 ± 3.3 v. 60.0 ± 3.5; $P < 0.001$). Significant time and river effects ($P < 0.001$), as well as their interaction, were observed on M (Table III). While there was no difference in mean M between origins at T_0 and T_1 ($P > 0.05$), by T_2 glass eels from the Atlantic coast grew up to be significantly heavier than those from the St Lawrence Estuary. The significant river × time interaction indicates that M differences gradually increased between origins during the experiments. Indeed, by the end of the experiments, glass eels from the Mersey River were 2·46 times heavier than those from Grande-Rivière-Blanche (3.44 v. 1.40 g). Salinity did not significantly influence growth for either river throughout the experiments ($P > 0.05$). A significant salinity × time interaction was, however, observed (Table III). At T_4, glass eels from the Mersey River reared in FW tended to be larger than those in BW (3.73 ± 0.27 g v. 3.18 ± 0.21 g; Fig. 3), but there were no differences between FW and BW for Grande-Rivière-Blanche (1.41 ± 0.05 g v. 1.40 ± 0.05 g). The choice effect was not significant ($P > 0.05$), suggesting that the salinity preference displayed by glass eels in the first experiments did not translate into growth differences. The significant choice × time interaction, however, does suggest a variable influence throughout the experiments. A significant river × choice × salinity × time interaction was observed.

Mortality

The mortality rate was significantly lower ($P < 0.001$) for glass eels from the Mersey River ($15.8 \pm 10.7\%$) compared with those from Grande-Rivière-Blanche ($39.1 \pm 16.5\%$), although this was unrelated to salinity ($P > 0.05$). Escapement and cannibalism, although unquantifiable, contributed to mortality and no specific cause of death was found for glass eels that died of natural causes despite examination by an expert fish pathologist.

RELATIVE BODY CONDITION AND PIGMENTATION

Le Cren's K_n significantly decreased (Mersey: 1.13 ± 0.16 > Saint-Jean: 1.01 ± 0.12 > Grande-Rivière-Blanche: 0.90 ± 0.10, $P < 0.001$), between all rivers as glass eels continued their migration away from the Sargasso Sea. While the batch effect was

TABLE III. Main effects and interactions explaining mean wet mass differences between *Anguilla rostrata* glass eels from Grande-Rivière-Blanche and Mersey River in 2012

Effect	d.d.f.	n.d.f.	F	P
River	34	1	42·39	<0·001
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	<0·001
River × time	30	3	27·49	<0·001
Choice × time	29	6	2·88	<0·05
River × choice × time	27	6	1·10	>0·05
Salinity × time	30	3	4·96	<0·01
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

d.d.f., denominator degrees of freedom; n.d.f., numerator degrees of freedom.

TABLE IV. Main effects and interactions influencing Le Cren's body condition (K_n) and pigmentation of *Anguilla rostrata* glass eels from Grande-Rivière-Blanche, Mersey River and Saint-Jean Rivers in 2012

Effect	d.f.	K_n		Pigmentation	
		F	P	F	P
River	2	195·06	<0·001	151·00	<0·001
Batch	1	0·72	>0·05	90·88	<0·001
River × batch	2	31·41	<0·001	40·60	<0·001
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

not significant ($P > 0·05$), the river × batch interaction was significant ($P < 0·001$; Table IV). More specifically, K_n did not change significantly throughout the sampling season for glass eels from Grande-Rivière-Blanche ($0·92 ± 0·10 − 0·88 ± 0·10$, $P > 0·05$) but significantly decreased over time for glass eels from the Saint-Jean River ($1·06 ± 0·12 − 0·97 ± 0·10$, $P < 0·001$) and significantly increased for those from the Mersey River ($1·08 ± 0·14 − 1·18 ± 0·17$, $P < 0·001$). K_n did not vary significantly according to salinity preferences (FWC: $1·02 ± 0·16$; SWC: $1·02 ± 0·18$; NCH: $1·00 ± 0·15$, $P > 0·05$) and there were no significant river × choice interaction either ($P > 0·05$).

Unlike K_n, no clear pigmentation patterns were found as glass eels continued their migration, although there were significant pigmentation differences among all rivers
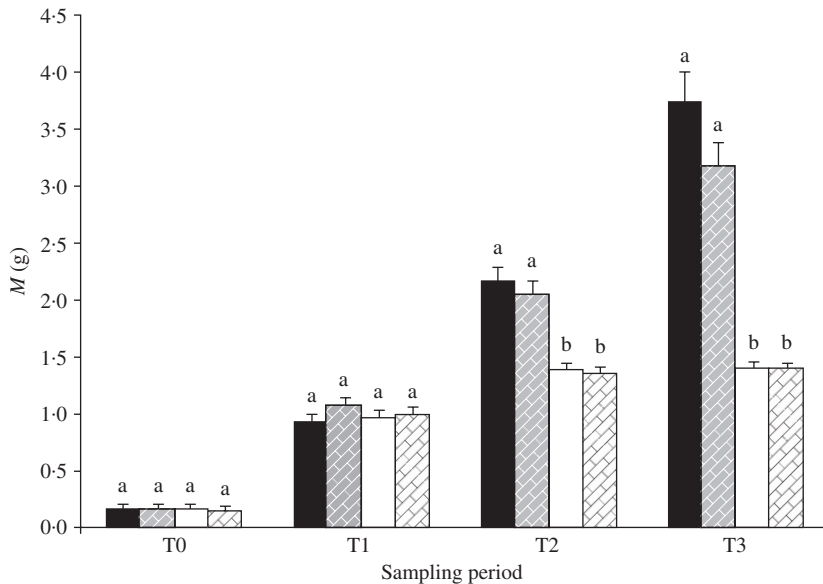
FIG. 3. Mean ± s.d. body mass (*M*) of *Anguilla rostrata* juvenile eels from Mersey River (■, ▨) and Grande-Rivière-Blanche (□, ▱) reared in fresh water (■, □) and brackish water (▨, ▱) over a 7 month period in 2012. Different lower-case letters indicate significant differences (*P* < 0·05) for a given sampling period.

(Mersey River: 2·58 ± 1·19; Saint-Jean River: 1·49 ± 1·06; Grande-Rivière-Blanche: 3·23 ± 0·72; *P* < 0·001). A significant pigmentation increase of 26% (2·06–2·79) was also noticed between batches of a given river during the course of the season (*P* < 0·001) with different rate of increase between rivers (*P* < 0·001) probably reflecting time differences between samplings. Furthermore, there were no differences in pigmentation between glass eels having exhibited different salinity preferences nor between active and inactive glass eels (*P* > 0·05).

DISCUSSION

SALINITY PREFERENCES

One of the main objectives of this study was to assess the salinity preferences of *A. rostrata* juveniles at four sampling sites in eastern Canada. Most glass eels were classified as inactive (making no choice) (78–89%) while most active glass eels significantly preferred FW (62–78%) over SW (22–38%). The salinity preferences of *A. rostrata* observed in this study are similar to those of *A. anguilla*, where the active FW-seeking eels varies between 50 and 70% (Tosi *et al.*, 1988, 1989, 1990; Edeline *et al.*, 2005). A FW preference is consistent with migration towards riverine habitats and suggests that the most active juvenile anguillids may seek out low salinity environments in the wild. Decreasing salinity gradients could therefore represent an important environmental cue, guiding active glass eels towards FW habitats. The use of salinity as an orienting sensory cue has been proposed in other fish species, such as *A. anguilla*

(Tosi *et al.*, 1988) and the green sturgeon *Acipenser medirostris* Ayres 1854 (Poletto *et al.*, 2013). Glass eels at both sampling times preferred FW, although this preference declined between the first and second sampling times (71·2 and 62·6%, respectively), suggesting that the propensity to colonize FW habitats decreases with time during the migratory season.

Active glass eels displayed similar FW preferences regardless of their geographic origin but glass eels from the St Lawrence Estuary, which had the lowest body condition, had a level of locomotor activity almost twice as high as glass eels from Nova Scotia (Table II), which also had the highest body condition. This difference in locomotor activity is surprising considering that the inverse result (*i.e.* increasing locomotor activity with body condition) has been demonstrated in *A. anguilla* glass eels (Bureau du Colombier *et al.*, 2007) and could possibly result from endocrine or genetic differences between glass eels of different species. The significant differences in pigmentation observed between rivers (Grande-Rivière-Blanche > Mersey > Saint-Jean) might also have played a role and while its association with salinity preferences has been shown in previous studies (Crean *et al.*, 2005), its effect on locomotor activity is unclear and often indirect (Bureau du Colombier *et al.*, 2007). A higher locomotor activity has also been linked to a higher migratory propensity in FW by *A. anguilla* glass eels (Edeline *et al.*, 2005; Bureau du Colombier *et al.*, 2009). Edeline *et al.* (2005) also found that glass eels that preferred SW to FW during the first behavioural experiments also significantly preferred FW to SW during the second trials. As in this study, locomotor activity represents the total percentage of glass eels that made a choice for either FW or SW, a higher locomotor activity (*i.e.* a positive rheotactic response) could then reflect an overall higher migratory propensity more accurately than salinity preference.

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

This study provides valuable insight into the behaviour exhibited by glass eels. The experimental design employed, however, could conceivably have influenced the results. For example, charcoal-filtered dechlorinated tap water was used as FW while BW and SW were prepared by adding either FW or synthetic salts (Instant Ocean) to sand-filtered St Lawrence Estuary water (salinity 20-25). As such, an influence of water source (tap and river water) on water odours and hence salinity preferences cannot be excluded, although any potential bias was consistent and could not have influenced comparisons between rivers. Furthermore, since the activity levels in the

experimental setup were comparable to what has been reported in nature (Daverat *et al.*, 2006), such bias is unlikely.

BODY CONDITION

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

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

PIGMENTATION

These results showed decreasing average FW preferences (71·2–62·6%) while pigmentation increased on average by 26% during the sampling season. This inverse relationship could partially explain why the less pigmented glass eels from the Saint-Jean River also exhibited the strongest preference for FW although the difference with other rivers was not significant. The results are in agreement with Edeline *et al.* (2005) who found that non-pigmented glass eels preferred FW, but are contrary to Crean *et al.* (2005), who showed an increasing preference for FW with increasing pigmentation in *A. anguilla*. The use of different methodologies could partially explain these conflicting results. Crean *et al.* (2005) compared average times spent in different salinities while this study and that of Edeline *et al.* (2005) measured the number of glass eels present in a given compartment at the end of the experiments. Another possibility is that the relationship between salinity preferences and pigmentation could be a mere correlation rather than a causal relationship. Pigmentation is a complex trait under the influence of several environmental factors, developing faster with higher water temperatures and more slowly with higher salinities (Dou *et al.*, 2003; Briand *et al.*, 2005). Glass eels from Saint-Jean River were less pigmented than those from Mersey River despite having been captured *c*. 4 weeks later, which could perhaps be explained by cold water temperatures (*c*. 5° C) associated with migration through the Gulf of St Lawrence in

May (Dutil *et al.*, 2009), or by an increased mortality of the more advanced stages during migration. Other variables, such as glass-eel arrival in different temporal waves or differences in time spent in the river's estuary before FW migration, might also have influenced pigmentation. Such factors might explain the higher pigmentation observed in glass eels from Grande-Rivière-Blanche.

INFLUENCE OF SALINITY ON GROWTH

Previous studies in controlled conditions involving *A. anguilla* and *A. rostrata* have shown higher growth rates in BW and SW compared with those reared in FW (Edeline *et al.*, 2005; Côté *et al.*, 2009). No such differences were found in this study between *A. rostrata* reared in FW (salinity 3) and BW (salinity 20) (Fig. 3) despite the significant salinity × time interaction, which probably reflects the mass differences observed between FW and BW elvers from the Mersey River at T_3. Such a difference, however, could be due to a difference in mortality between rivers. Nevertheless, the results on the effect of salinity on growth should be interpreted with caution. Salinity exerts a manifold influence in numerous species (Boeuf & Payan, 2001). Energetic costs associated with osmoregulation are frequently considered to be lower in isotonic conditions (Boeuf & Payan, 2001), although such costs probably represent only a small (<10%) fraction of the overall energy budget (Moyle & Cech, 2004; Evans, 2008). Furthermore, Bureau du Colombier *et al.* (2011) found no difference in the energetic cost of osmoregulation for glass eels kept in FW or SW. Salinity could nonetheless influence growth by its influence on food conversion, growth hormone production and feeding activity, which includes cannibalism in many species (Boeuf & Payan, 2001). When rearing *A. anguilla* glass eels under different salinities and diets, Rodríguez *et al.* (2005) found significant differences in growth related to salinity only when a lower quality diet was employed, suggesting that growth differences could be related to the interaction of diet and experimental salinities in controlled experiments. Nutritional requirements for glass eels and elvers are largely unknown, and many diets might be unsuitable (Rodríguez *et al.*, 2005). Better diet suitability might be sufficient to counterbalance the negative effects of less-than-optimal salinities and thus explain the absence of significant growth differences in the present experiments. The higher growth rates in BW and SW observed in natural habitats for a given region (Jessop *et al.*, 2008; Cairns *et al.*, 2009) may reflect the higher productivity of estuaries and coastal habitats and hence food availability compared with FW habitats in temperate latitudes (Gross *et al.*, 1988; Kaifu *et al.*, 2013). Differential growth associated with salinity reported in previous studies (Edeline *et al.*, 2005) could conceivably be the result of higher rearing salinities (salinity 34), although Côté *et al.* (2009) found that eels exhibited a faster growth rate in BW (salinity 22) than in FW.

FW and SW ecotypes

No difference in growth rate between eels having chosen different salinities during salinity preference experiments was observed. Edeline *et al.* (2005), however, found that *A. anguilla* glass eels that chose SW had higher growth rates than FWC or NCH regardless of rearing salinities and postulated that genetic factors might be involved. One possible reason for this discrepancy might lie in the methodology used. Edeline *et al.* (2005) sorted glass eels twice in two consecutive behavioural tests before growth

experiments and as such probably selected glass eels with the strongest salinity preferences. In the present experiments, glass eels used in the behavioural experiments were only sorted once, and therefore might not have been as selective as Edeline *et al.* (2005). The absence of differences in growth rate between eels that chose different salinities in this study suggests that FW and SW ecotypes, if present in *A. rostrata* as inferred by Castonguay *et al.* (1990), probably do not translate into growth differences. Instead, genetically based regional differences in growth appear more likely (Côté *et al.*, 2009; this study).

Interindividual differences in growth rate and mortality

A strong variability in size (0·18–33·1 g) among juvenile *A. rostrata* was observed by the end of the experiments, which might be indicative of hierarchical size effect or interindividual genetically based growth differences. Growth heterogeneity in glass eels raised in controlled conditions is a common feature in anguillid aquaculture (Angelidis *et al.*, 2005; Rodríguez *et al.*, 2005). Hierarchical size effect, where aggressive larger fish monopolize food, could partially explain some of the growth variation observed despite *ad libitum* feeding. Differences in growth rate, however, are observed in aquaculture even when anguillids are graded according to body size, suggesting the influence of underlying genetic factors or a sex-determined effect. Indeed, Côté *et al.* (in press) recently found evidence of marked growth rate differences between males and females, independent of geographic origin, whereby females showed a bimodal growth distribution (slow-growing and fast-growing), whereas male growth distribution was unimodal and intermediate between female modes. Significantly higher mortality rates were observed in glass eels from Grande-Rivière-Blanche (39·1 ± 16·5%) compared with those from Mersey River (15·8 ± 10·7%) in 2012. Because of the marked differences in size, high mortalities could possibly represent a source of bias in our experiments.

REGIONAL DIFFERENCES IN GROWTH

Despite being reared in identical conditions, glass eels from the Atlantic coast (Mersey River, NS) were 2·46 times heavier (3·44 *v.*1·40 g) than those from the St Lawrence Estuary (Grande-Rivière-Blanche, Québec) by the end of the experiment. Differences in juvenile *A. rostrata* growth rate between these two regions were previously reported by Côté *et al.* (2009), and new results showed that this growth difference was maintained after 3 years of common rearing (Côté *et al.*, in press). Recent population genetic analyses (Côté *et al.*, 2013) found no significant spatial or temporal genetic differentiation among *A. rostrata* collected between 30 and 48° N for 20 microsatellite loci, thus confirming the panmixia hypothesis in *A. rostrata*. The absence of genetic divergence in neutral markers, however, does not necessarily imply the absence of genetic differences in adaptive traits driven by natural selection (Côté *et al.*, 2009).

Recent studies have shown that selective environmental conditions result in differences in coding genes between glass eels of different geographic origins that also translate in different levels of gene transcription (Gagnaire *et al.*, 2012; Laflamme *et al.*, 2012; Côté *et al.*, 2014). *Anguilla rostrata* enter the Gulf of St Lawrence as glass eels (Dutil *et al.*, 2009) and must rely solely on their energetic reserves to complete their journey across several hundred km. Water temperatures in the Gulf in May are

cold ($\leq 5°$ C) and have been shown to drastically reduce swimming activity in glass eels (Linton *et al.*, 2007). Glass eels able to complete their migration despite limited energetic reserves and harsh conditions would also be genetically predisposed to lower growth rates. Glass eels predisposed to high growth rates on the other hand would be eliminated, possibly because high growth rate correlates with high metabolism (Burton *et al.*, 2011) and hence insufficient energetic reserves. Locally adaptive alleles could also help explain the *A. rostrat*'s ability to colonize such a wide variety of heterogeneous habitats (Gagnaire *et al.*, 2012). Furthermore, harsh environmental conditions and long distances from the spawning ground could also explain the much lower glass-eel abundance in the St Lawrence Estuary compared with the Atlantic coast of Canada (Dutil *et al.*, 2009) as well as the observed decreasing energy reserves in this study. Indeed, Gagnaire *et al.* (2012) clearly showed that sea-surface temperatures encountered by glass eels when they approach coastal areas from Florida to the St Lawrence Estuary areas impose selective pressures that are responsible for shaping allele frequency differences at functional coding genes. Regardless of the exact segregation mechanism, the differences in growth rate observed between *A. rostrata* of different origins in this study and in Côté *et al.* (2009) as well as the results of Gagnaire *et al.* (2012) strongly support the hypothesis of genetic or epigenetic differences among anguillids from different geographic origins associated with spatially varying selection within an otherwise panmictic context.

IMPLICATIONS FOR ANGUILLID ECOLOGY

No significant difference in salinity preference was observed among geographic origins. Despite the small number of sampled rivers, this may suggest that there are no geographic differences in distribution among the various continental habitats used by *A. rostrata* in Canada. Habitat selection, through its influence on population structure, has important implications for anguillid ecology. The colonization of estuarine habitats where eel densities are higher is associated with an increased proportion of males that complete their life cycle as soon as the required minimum size for successful migration is reached. In contrast, FW habitat colonization is associated with lower eel densities and a dominance of larger females maturing at older ages (Krueger & Oliveira, 1999; Goodwin & Angermeier, 2003). Residency in BW habitats increases growth (Morrison *et al.*, 2003; Cairns *et al.*, 2004; Jessop *et al.*, 2008; Lamson *et al.*, 2009), which in turn decreases predation risk and age at migration (Edeline & Elie, 2004; Davey & Jellyman, 2005; Tremblay, 2009). Edeline (2007) proposed that facultative catadromy could be understood in terms of fitness trade-offs. Residency in the more productive estuarine and BW habitats would provide increased resources at the cost of increased inter and intraspecific competition (including cannibalism) while the reduced growth rate associated with migration to FW habitats would be compensated by decreased competition. Anguillids would therefore find different but fitness-equivalent solutions by using opposite migratory behaviours. Lower anguillid densities driven by the current sharp stock decline could result in a population shift towards estuaries in response to lower intraspecific competition (Edeline, 2007). Moreover, the human-driven selective pressures of recent decades, notably hydroelectric dams and the commercial fishery for St Lawrence River *A. rostrata*, may have increased selection against upstream migrants and may have displaced the stable state of the conditional strategy, which resulted in a decline in the proportion of *A. rostrata* invading FW (McCleave & Edeline, 2009).

The *A. rostrata* decline would therefore appear larger in FW than for the population (species) as a whole.

Supporting Information

Supporting Information may be found in the online version of this paper:
Appendix SI. Growth experiments performed in 2011

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