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ARTICLE

Growth, Female Size, and Sex Ratio Variability in American Eel of Different Origins in Both Controlled Conditions and the Wild: Implications for Stocking Programs

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Abstract

Freshwater eels *Anguilla* spp. are declining worldwide, and a major challenge is understanding why these panmictic species show contrasting patterns of intraspecific phenotypic variation and recruitment. We present results on studies of the American Eel *A. rostrata* to understand and discriminate the effects of origin and plasticity on growth and sex determination. We considered two separate growth and one length-at-age data sets. The first growth data set originated from a 34-month rearing experiment starting from the glass eel life stage to test the effects of origin, salinity, and density on growth and sex determination. The second growth data set originated from a shorter rearing experiment of 18 months starting at the yellow eel stage (around 3 years old) and compared transplanted individuals in Lake Ontario (LO) with natural migrants to the LO area. The third data set compared transplanted individuals in LO sampled by electrofishing with naturally migrating individuals. Sex ratios were identical for all origins and treatments in the long-term growth experiment (34–35% females). While male size distribution had little variability, certain female groups had a large variation in growth and presented fast- and slow-growing clusters. On the other hand, both cases of natural migrants to the LO area were consistent with being only slow-growing females. We found that wild individuals rearing in the LO area were nearly exclusively transplanted individuals and that males, as well as fast-growing females, were present. Even though the entire species is panmictic, these results support a role for spatially varying selection in explaining the phenotypic variation observed among regions and among individuals of the same region, and such factors must be considered for any successful management strategies of American Eel.

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The economically important American Eel *Anguilla rostrata* poses a substantial puzzle for managers. Although the entire species is comprised of a single panmictic population (Côté et al. 2013), there is also extreme phenotypic variation among eels from different natural rearing environments in growth rate, sex ratio, and size at maturity (Jessop 2010). In particular, American Eels from the upper St. Lawrence River (USL) and Lake Ontario (LO; together abbreviated by USL_LO) are phenotypically distinct in that they are exclusively female and achieve larger ultimate size due to delayed sexual maturation compared with eels in more coastal rearing areas (Dutil et al. 1985; Tremblay 2009). Moreover, eel recruitment in the USL_LO has declined by 98% over the last 30 years; thus, this unique life history variant, found only in this portion of the species' range, is threatened. These declines are puzzling given the variable abundance trends that have been observed in Atlantic Canada (COSEWIC 2006, 2012; DFO 2010). Possible causes of the decline include fishing, pollution, habitat loss and alteration, barriers to migration, and mortality from hydroelectric turbine passage (Castonguay et al. 1994). However, despite panmixia, the population dynamics of this unique life history variant appears to be independent from the rest of the species, complicating conventional management methods determined by using genetically defined conservation units (Waples et al. 2008). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has recommended that American Eel status listing be changed from "Special Concern" (COSEWIC 2006) to "Threatened" (COSEWIC 2012). Ontario has declared it "Endangered" under Ontario's Endangered Species Act (MacGregor et al. 2010), and its status is under review for possible listing under the U.S. Endangered Species Act.

To mitigate the drastic decline in the USL_LO, glass eels (larval stage) were translocated from Nova Scotia and New Brunswick. Though these individuals did survive and grow in the USL_LO environment, they did not adopt the characteristic life history of eels in that area (slow-growing, large, maturing females), as they exhibited a strikingly different growth rate compared with eels that previously characterized the region, and a significant proportion of translocated eels were early sexually maturing males (Verreault et al. 2009, 2010; Pratt and Threader 2011). This suggested that environmentally driven plasticity alone is unlikely to explain regional phenotypic variations and that genetically based differences could also be involved. To test this hypothesis, we previously performed a 9-month experiment that revealed differences in growth and reaction norms between glass eels from the St. Lawrence Estuary in Québec and from Nova Scotia under controlled conditions (Côté et al. 2009).

The main goal of this study was to compare growth in controlled conditions and size variation of wild American Eel individuals from different rearing origins to help determine whether these important life history traits differ between geographic locations. To this end, we analyzed three separate data sets. First, a long-term growth experiment was performed representing an additional 25 months of growth (34 months total) as well as sex determination from a previously published experiment that was initiated with individuals at the glass eel stage (Côté et al. 2009). An additional treatment of high-density rearing conditions from the above experiment for one of the sampling locations was conducted to test the influence of density on sex ratio. Second, a separate, shorter (18 months) experiment was conducted with yellow eels (juvenile stage), which were reared in controlled conditions with samples collected from naturally migrating wild individuals at the fish ladder of the Beauharnois Dam (BH), Québec, and electrofished LO individuals that were likely the result of glass eel transplants from the Maritime provinces (Figure 1). Finally, in the third data set we extensively sampled wild individuals in the USL_LO to determine the extent to which transplanted eels compared with naturally migrating eels for the presence of males and growth rate. Based on the general expectation that American Eel life history is driven by their environmental rearing conditions, we would expect that eels captured from different locations and reared in identical conditions would have similar growth trajectories and sex ratios at the end of our experiment. However, if there is a genetically based difference to observed life history variation, we would expect to see these life history differences in eels when reared in a common garden experiment.

Methods

Long-term growth experiment.—Nonpigmented glass eels were obtained in 2007 from two sampling locations at river outlets, one in the St. Lawrence Estuary and one in Nova

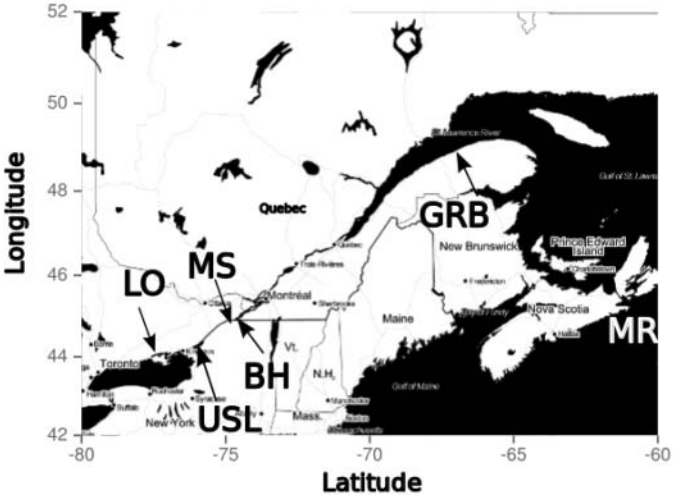

FIGURE 1. Sampling sites for American Eels in Lake Ontario, the upper St. Lawrence River, the St. Lawrence Estuary, and the Maritimes. The sampling sites are: Mira River (MR), Grande-Rivière-Blanche (GRB), Lake Ontario (LO), Beauharnois Dam (BH), upper St. Lawrence River (USL), and the Moses Saunders Generating Station (MS).

Scotia, just prior to the eels entering freshwater, therefore avoiding time spent in freshwater before the experiments. Grande-Rivière-Blanche (GRB) drains into the lower St. Lawrence Estuary, Québec. The Mira River (MR) is located in Cape Breton, Nova Scotia, and drains into the Atlantic Ocean (Figure 1). The GRB represents the most upriver location where glass eels are known to occur in the St. Lawrence watershed. Eels bound for the USL_LO undertake a protracted upstream migration in the St. Lawrence River; it takes eels at least 2 to 3 years to reach the upper St. Lawrence River (Castonguay et al. 1994; Zhu et al. 2013) as they transition from pigmented glass eels to yellow eels. Our intention was to collect glass eels in the St. Lawrence Estuary as close as possible to the St. Lawrence River, and the mouth of GRB is the farthest west in the St. Lawrence Estuary where glass eels are known to occur. Earlier experiments compared eels from these two origins after 9 months of rearing in contrasting salinity treatments (Côté et al. 2009), and in the present experiment we studied the continued long-term growth of these individuals. All controlled rearing was conducted at the Laboratoire de Recherche en Sciences Aquatiques (LARSA) at Université Laval, Québec. There were two salinity condition treatments: freshwater (salinity, 3 ± 1‰; hereafter FW) and brackish water (salinity, 22 ± 1‰; hereafter BW), and two tanks per treatment at an initial density of 100 individuals, or 45 g/m^2. Standard 75.7 liter aquaria were used; interior dimensions were 60.0 × 30.5 × 29.2 cm and water depth was 17.1 cm. With the MR glass eels, we established an additional high-density treatment of three times the density (135 g/m^2) for both FW and BW salinity treatments. After 9 months, eels from each of the four low-density groups (see Table 1 for sample sizes) were distributed by size (to reduce cannibalism and antagonistic behavior) in two, half-filled (to avoid escape), 1-m^3 tanks. The high-density groups were transferred into one 1-m^3 tank per treatment and all groups were reared for another 25 months for a total of 34 months. Temperature and salinity were 21 ±1°C and 2.5 ± 0.5‰, respectively. Physical–chemical conditions, including nitrites (NO_2^-, <0.1 mg/L), nitrates (NO_3^-, <200 mg/L), ammonia (NH_3, <0.004 mg/L), and pH (7–7.5, adjusted with Na_2CO_3), were monitored daily, and oxygen level monitoring was automated (YSI Oxyguard probe type 3: 90–100% saturation, 8.2 mg/L at 22°C to 9.1 mg/L at 20°C). For optimal growth, eels were fed twice a day with a mixture of fish roe, pellets, and Capelin *Mallotus villosus* to complete their dietary needs (De Silva et al. 2008). Eels were fed ad libitum, and feeding was monitored so that if some food did not remain (before daily cleaning), the ration was increased. This resulted in eels consuming 2–5% of their body weight per day. In all tanks and treatments, mortality always involved the small subordinate individuals. Eels were provided a heterogeneous environment (pipes in which to hide) that reduced agonistic behaviors (Knights 1987). Total length and weight were measured on all individuals every 4 months. In April 2010, all eels were euthanized with an overdose of eugenol. The majority had reached a TL of 30 cm or more; thus, sex could be determined based on visual inspection of the gonads (Beullens et al. 1997) and confirmed using the histological acetocarmine (1% staining solution; S70078, Fisher Scientific) squash method (Guerrero and Shelton 1974).

TABLE 1. Final size of American Eels from long-term (34 month) growth experiment. Total weight and length (mean ± SD) reached after 34 months of rearing for eels from the Mira River (MR) and Grande-Rivière-Blanche (GRB) after being initially reared in either freshwater (FW) or brackish water (BW) for 9 months prior to transfer to freshwater for another 25 months. *N* refers to the number of males (M) and females (F) (and percent) in each group at the end of the experiment.

Treatment	Sex	*N* (%)	TL (cm)	Weight (g)
Mira River				
BW	F	25 (35)	63 ± 15	658 ± 408
	M	47 (65)	43 ± 4	160 ± 56
FW	F	32 (36)	58 ± 16	522 ± 503
	M	57 (64)	41 ± 5	127 ± 47
Grande-Rivière-Blanche				
BW	F	40 (35)	48 ± 12	281 ± 288
	M	79 (64)	42 ± 5	135 ± 45
FW	F	24 (34)	52 ± 14	311 ± 296
	M	44 (66)	41 ± 5	126 ± 43

Short-term growth experiment.—In our second growth experiment, putative stocked yellow eels (see Results) were obtained by electrofishing in LO (Bay of Quinte; 44°8′N, 77°8′W). Natural upriver migrants were captured in the act of ascending the BH fish ladder (Figure 1). Both groups were transported to LARSA and reared only in FW conditions in 1-m3 tanks, as described above. Eels were fed blood worms and brine shrimp *Artemia* spp., ad libitum. Individuals were PIT-tagged and length and mass for each were measured every 3 months. As routinely done in any controlled studies of this type on eels, individuals were redistributed by size to prevent cannibalism and minimize strong dominance hierarchies that can prevent subordinate individuals from eating. At the end of 18 months a final measurement was taken and eels were sacrificed to determine sex by visual inspection of gonads.

Length at age in the wild.—In our third data set, individuals transplanted as glass eels into LO were electrofished from shoreline areas in the upper St. Lawrence River (44°25′N, 75°52′W) and the Bay of Quinte (44°8′N, 77°8′W), Ontario. Eels were sampled in May (2009–2013) and September (2009–2011). Sampling was conducted using boat electrofishing along 100-m shoreline transects at approximately 1 m deep at night (Pratt and Threader 2011). The eels were captured using dip nets and were euthanized with tricaine methanesulfonate (MS-222) for age and sex determination. When the glass eel transplants occurred, all transplanted individuals received an otolith mark with oxytetracycline hydrochloride (OTC) (Pratt and Threader 2011). Otoliths in this study were evaluated for this mark (except in the 2013 sample year) and

also used to determine age in all sampling years. In a targeted subset of individuals less than 40 cm in length (as males rarely attain lengths greater than 40 cm) gonads were analyzed for sexual differentiation. Gonads were fixed in Bouin's fixative, then dehydrated with 100% ethanol. Tissues were then embedded in wax, cut to 5 μm thickness, stained, and viewed under a microscope. Naturally migrating eels were collected from the eel ladder at the Moses Saunders Generating Station (MS) in Cornwall, Ontario, and included specimens ascending from Lac St. François into the upper St. Lawrence River (Figure 1). This provided a comparison group for the LO electrofishing samples as few naturally recruiting eels remain in USL_LO.

Statistical analysis of growth clustering.—For the two controlled growth experiments, we examined the TL size distribution for the final measurement of each sex separately by creating kernel density plots from the "lattice" R package. Kernel density plots are specifically designed to nonparametrically depict the population distribution from a sample. For each distribution, we used the "mclust" R package, which uses Akaike's information criterion (AIC) to determine the most likely number of clusters represented in the data, i.e., whether the growth best represents one or more clusters. Here, in instances where two clusters were found (see Results), we designated individuals as fast- or slow-growing based on the break in the distribution by visual inspection of the density plot.

Statistical analysis of growth and sex.—For the growth experiments, generalized linear models (GLMs) were performed with the $\log_e$-transformed final length as the dependent variable. In the case of the long-term growth experiment, the independent variables were treatment, sex, origin, and interactions. In the case of the short-term growth experiment, only origin was the independent variable as the BH origin contained only females. A logistic regression was used to determine whether origin or treatment (independent variables) affected sex (dependent variable). Also, to determine whether rearing density had an effect on sex, chi-square tests were performed on the high density treatment of MR separately for brackish and freshwater initial salinities.

RESULTS

Size-at-age differences between American Eels from MR and GRB observed during the first 9 months were still observed after their transfer to large tanks and until the end of the first (34 month) growth experiment (Figure 2). Eels from MR had a greater size at age (in both TL and weight) throughout the rearing experiment compared with GRB eels (Table 1). However, only MR eels retained the positive initial salinity effect on growth and development. The GLM indicated a significant effect of sex ($t = -5.56$, $P < 0.001$) and origin ($t = 5.50$, $P < 0.001$) and their interaction ($t = -3.92$, $P < 0.001$). In the previous step of model simplification, treatment was not significant ($t = -0.53$, $P = 0.597$) but the origin × treatment interaction was suggested, although it was also not significant ($t = 1.92$, $P = 0.0698$). For the second (short-term) growth experiment (Table 2), only female eels were present in BH, so only females were compared in this GLM, and origin had a significant effect on length ($t = 2.25$, $P = 0.025$).

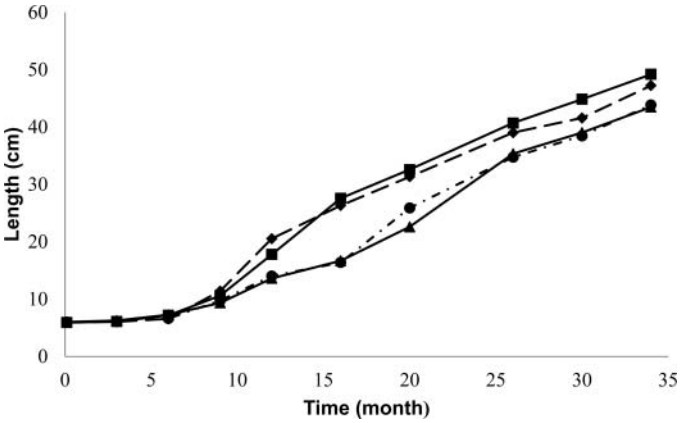

FIGURE 2. Growth in TL observed for American Eels from Mira River (MR) and Grande-Rivière-Blanche (GRB) that were initially reared in fresh or brackish water aquaria for 9 months prior to being transferred to freshwater tanks for another 25 months (34 months total). Mean TL of eels transferred from small aquaria to large tanks were measured from month 11 to month 34, that is from March 2008 to the end of the experiment in April 2010. Symbols correspond to the means of all individuals measured for each group. Symbols: diamonds = Mira River in freshwater; squares = Mira River in brackish water; circles = Grande-Rivière-Blanche in freshwater, and triangles = Grande-Rivière-Blanche in brackish water.

At the end of the 34-month growth experiment, the general pattern was that female length exhibited two clusters and males exhibited one cluster (Figures 3–5). The "mclust" procedure indicated two clusters in five out of six times for females with a large spread between the modes (Table 3). Two clusters were detected in both treatments of males from GRB only, but unlike in the females, the clusters were so close together that the density plot did not exhibit a clear bimodal pattern. The logistic regression revealed no significant effect of origin or salinity treatment (or their interaction) on sex

TABLE 2. Final sizes of American Eels from short-term (18 month) growth experiment. Total weight and length (mean ± SD) reached after 18 months of rearing for eels from Lake Ontario (LO) and Beauharnois Dam (BH). *N* refers to the number of individuals in each group at the end of the experiment. Since some individuals lost their PIT tag, their sex-specific starting length and weight could not be determined (and thus *N* for start measurement is lower than finish)

Origin	Sex	Start–Finish	*N*	TL (cm)	Weight (g)
BH	F	Start	140	28.7 ± 3.1	30±10
		Finish	156	29.7 ± 3.5	32 ± 13
LO	F	Start	86	28.8 ± 5.1	36 ± 23
		Finish	91	32.0 ± 7.7	51 ± 55
LO	M	Start	36	28.5 ± 4.8	39 ± 23
		Finish	36	32.4 ± 5.6	57 ± 38

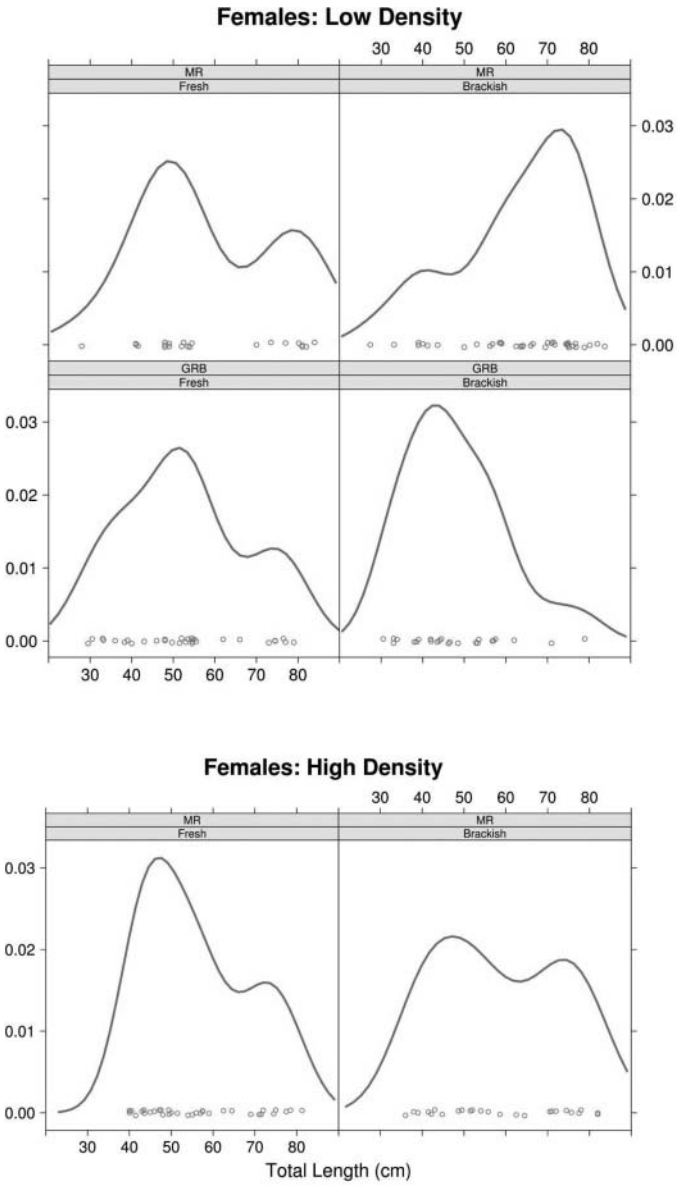

FIGURE 3. Density plots of final length for the American Eel females from the long-term (34 month) growth experiment including eels from both origins, Mira River (MR) and Grande-Rivière-Blanche (GRB), with fresh and brackish water and density treatments. The open circles along the *x*-axis represent the lengths of each individual. The distributions represent the kernel density estimation from the raw data.

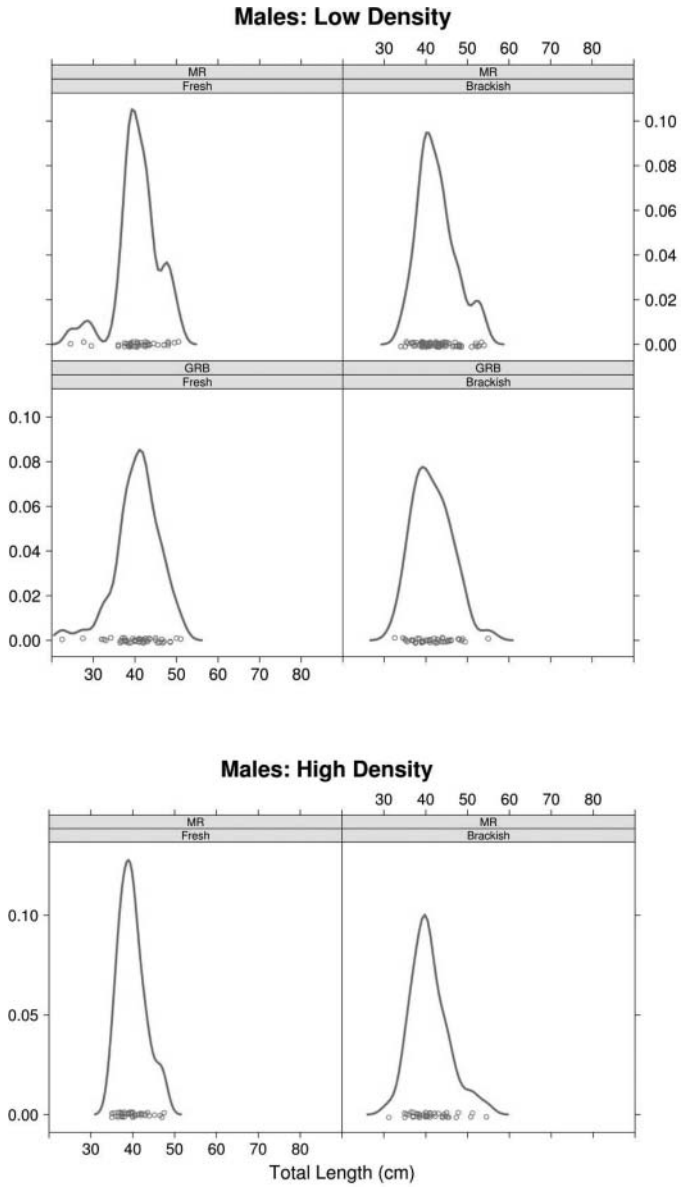

FIGURE 4. Density plots of final length for the American Eel males from the long-term (34 month) growth experiment including eels from both origins, Mira River (MR) and Grande-Rivière-Blanche (GRB), with fresh and brackish water and density treatments. The open circles along the *x*-axis represent the lengths of each individual. The distributions represent the kernel density estimation from the raw data.

differentiation, since the proportions of females were similar among eels from all origins and treatments (34% female overall; Table 1). The high density treatment had almost exactly the same sex ratio in both salinities and was not significantly different from the low density treatments (BW: 35% female, $P = 1$; FW: 35% female, $P = 0.343$). In all groups of the long-term experiment, males were smaller, on average, than females at the end of the experiment (Table 1). The mean TL and weight was 41.0 cm and 136 g, respectively, for males compared with 55.0 cm and 442 g for females.

Similarly, in the short-term growth experiment, the TL of females from LO represented two significant clusters, but the females from BH and the males from LO did not (Table 3; Figures 6, 7). Sex was skewed toward females in the short-term growth experiment (BH: 100% female; LO: 76% female).

Between 2009 and 2013, 510 individuals were captured via electrofishing; otoliths were extracted and assessed for age, and 433 of these (all except those in 2013) were assessed for the OTC mark. All individuals assessed with the exception of one had the OTC mark. That single nontransplanted individual

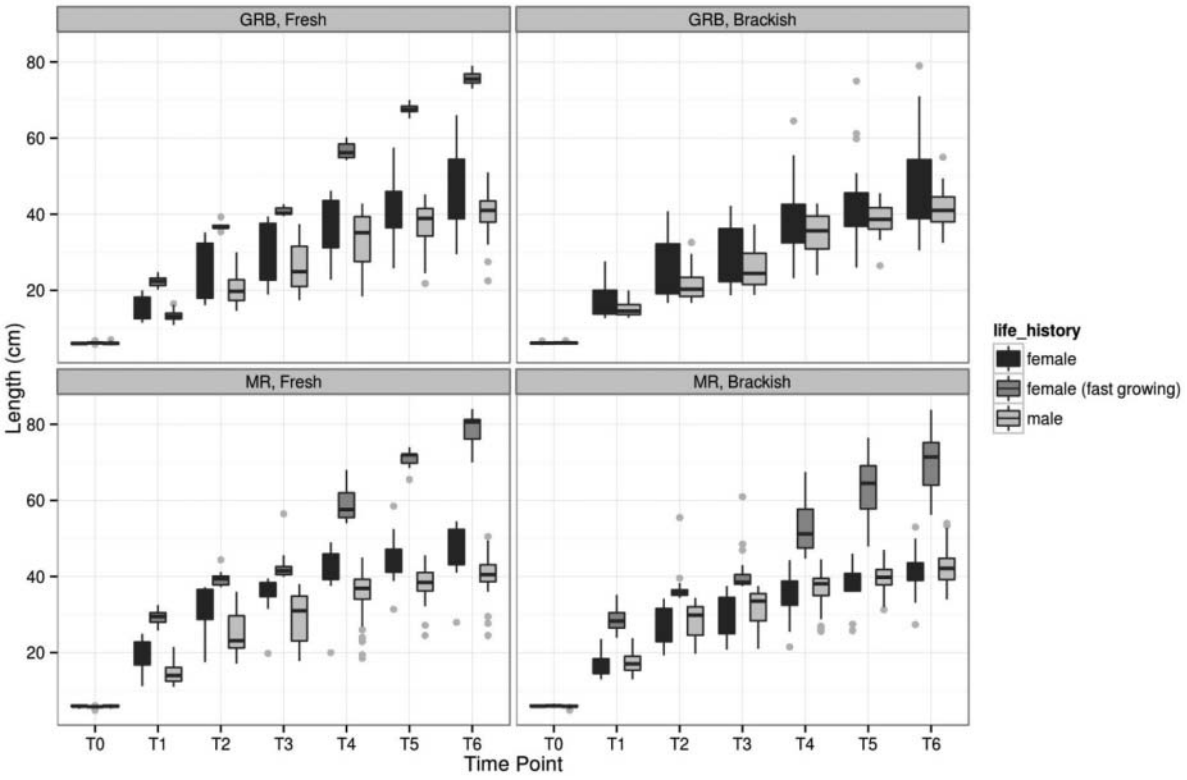

FIGURE 5. Distributions of TLs for each measurement period (ca. 4-month intervals) over the 25 months for the long-term growth experiment for eels from both origins, Mira River (MR) and Grande-Rivière-Blanche (GRB), with fresh (FR) and brackish water (BR) and density treatments. Females are separated into life history type (fast growing, dark grey boxes; slow growing, black boxes) based when the data represented two clusters. Males are represented by light grey boxes. Boxes represent the interquartile range (IQR) and whiskers extend to 1.5× IQR.

was an 11-year-old female that was 82.0 cm long. The rest of the individuals from USL_LO ranged between 2 and 7 years old, with age-7 eels represented by only a single individual (Table 4). Ages of the 96 individual eels sampled at the MS dam ranged from 3 to 9 years, with a single individual (TL, 52.3 cm) that was age 9. Of the 150 USL_LO individuals analyzed for sexual differentiation, 65 were female, 14 were male, and the remaining 71 were undifferentiated. Overall, there was a pattern of faster growth and higher variability in eels at USL_LO, whereas the natural upriver migrants at MS were slower growing with less variability (Table 4).

DISCUSSION

In this study, we combined three different and independent experiments: a long-term (34-month) growth experiment of glass eels from two different origins, a short-term (18-month) growth experiment starting with small yellow eels electrofished in LO that were compared with individuals naturally migrating upriver, and finally an analysis of length-at-age data of translocated individuals at LO and naturally migrating individuals at the MS dam. These data support three conclusions about American Eel life history. First, sex was not affected by salinity, origin, or density when fish were reared in controlled conditions from the glass eel stage. Thus, even the most upriver location of glass eel freshwater dispersal did not exhibit a greater proportion of females. Second, there was high variation in female growth rate that was not present in males, and females tended to cluster into slow- and fast-growing groups according to kernel density plots and AIC, which are influenced by origin and possibly the salinity environment. Third, results suggested that only individuals of the slow-growing female cluster undertook the long migration to the USL_LO. We discuss each of these results in turn, and then describe the management implications of our conclusions.

Sex Determination Is Not Affected by Salinity, Origin, or Density

We did not observe any significant difference in sex ratio between origins, treatments, or rearing densities of glass eels. Given that sex ratios differ between feeding locations under natural conditions, sex determination may be primarily environmentally determined in the American Eel through as yet unknown mechanisms (Holmgren and Mosegaard 1996; Davey and Jellyman 2005). Here, regardless of whether the eels were reared in brackish or freshwater for the first 9 months, the sex ratios were nearly identical; thus, our study

TABLE 3. Cluster analysis for final length distributions of American Eels from controlled rearing experiments. Results of the R package "mclust" indicating the number of clusters, one or two, is more likely (bold italic text) to describe each distribution (by origin × sex × treatment) as determined by the higher AIC value. GRB = Grande-Rivière-Blanche, MR = Mira River, BH = Beauharnois Dam, LO = Lake Ontario, FR = freshwater rearing, BR = brackish water rearing, M = males, F = females.

Origin	Treatment	Sex	Clusters	AIC value
Long term (34 months): low density				
GRB	FR	F	1	−252.5
			2	***−249.7***
GRB	BR	F	***1***	***−193.1***
			2	−196.5
MR	FR	F	1	−191.5
			2	***−183.6***
MR	BR	F	1	−319.2
			2	***−315.2***
GRB	FR	M	***1***	***−252.5***
			2	−249.7
GRB	BR	M	***1***	***−279.7***
			2	−285.3
MR	FR	M	1	−276.4
			2	***−274.5***
MR	BR	M	1	−477.8
			2	***−476.8***
Long term (34 months): high density				
MR	FR	F	1	−283.5
			2	***−278.8***
MR	BR	F	1	−212.3
			2	***−210.4***
MR	FR	M	***1***	***−283***
			2	−284.7
MR	BR	M	***1***	***−227***
			2	−228.7
Short term (18 months)				
BH	BR	F	***1***	***−1,566.3***
			2	−1,571.2
LO	BR	F	1	−1,057.7
			2	***−1,043.6***
LO	BR	M	***1***	***−398.1***
			2	−398.4

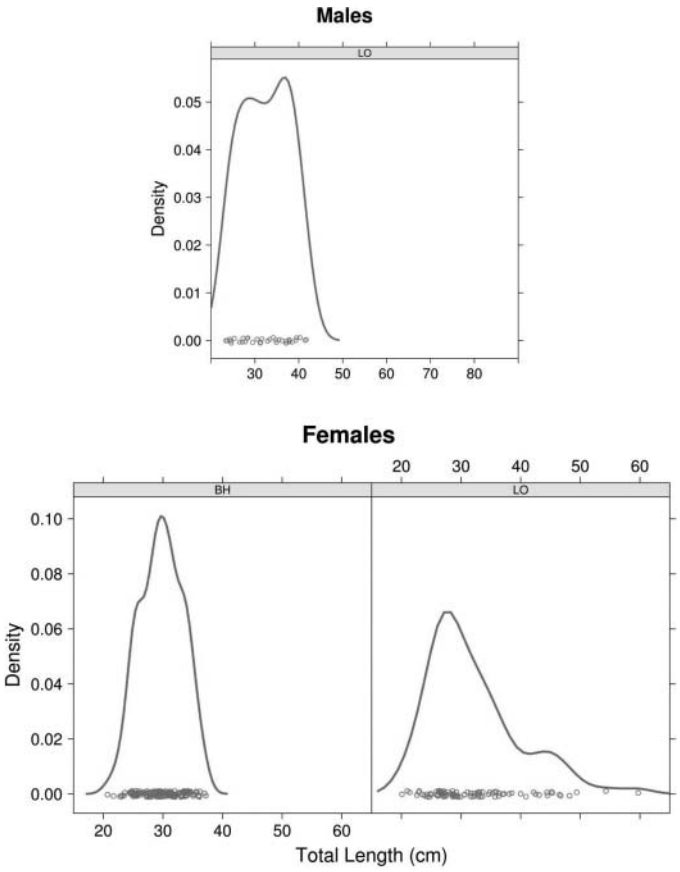

FIGURE 6. Density plots of final length of American Eels from the short-term (18 month) growth experiment including eels from two origins, individuals naturally migrating upstream at the Beauharnois Dam (BH), and transplanted individuals captured via electrofishing in Lake Ontario (LO). The open circles along the *x*-axis represent the lengths of each individual. The distributions represent the kernel density estimation from the raw data.

adds to the empirical evidence that salinity does not influence sex (Tesch 1977; Davey and Jellyman 2005).

The present study found different results from previous studies on the relationship between origin and sex ratio. Vladykov and Liew (1982) reared glass eels from two origins, similar to the present study (GRB and Didgeguash River [DR] in the Maritimes). In a single freshwater pond, they performed each experiment consecutively. Unlike our study, they found extremely different sex ratios in eels between the origins; only 18% were female from the DR origin and 65% were females from GRB. This result was logical as the GRB sampling location is the farthest upriver location of glass eels known, and would seemingly be the most likely to exhibit a female-biased sex ratio, but our study did not repeat this result. Vladykov and Liew (1982) collected their samples 4 years apart, thus temporal fluctuations (genetic or plastic) in sex were confounded with origin. This could have affected their results in several ways. First, the different cohorts of glass eels collected could have differed in sex ratio. Second, the environmental conditions for rearing could have differed between the growth periods. Third, since all individuals were in a single pond and not graded as they grew, cannibalism could have affected the sex ratios.

Density is thought to be the most important factor to influence sex determination due to suppression of growth rate (Davey and Jellyman 2005). Several studies reporting correlations of density and sex in different natural environments provide support for this prediction (Parsons et al. 1977; Krueger and Oliveira 1999; Beentjes and Jellyman 2003; Huertas and Cerda 2006; Melia et al. 2006). These studies provide observations in the natural environment that brackish areas tend to

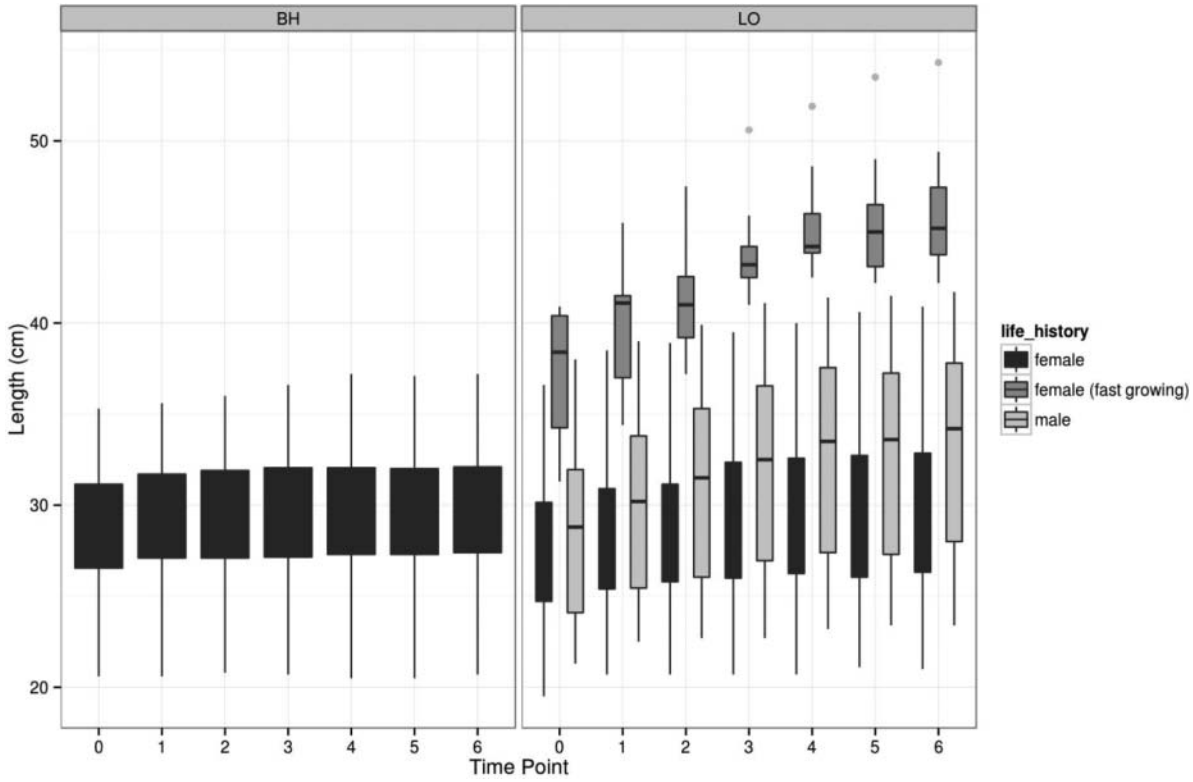

FIGURE 7. Total length of American Eels for each measurement period for the short-term (18 month) growth experiment. Females are separated into life history type (fast growing, dark grey boxes; slow growing, black boxes) based when the data represented two clusters. Males are represented by light grey boxes. Boxes represent the interquartile range (IQR) and whiskers extend to 1.5× IQR.

TABLE 4. Length (mean ± SD) for each age-class of natural migrants of American Eels in the "wild" experiment; sample size (*n*) is indicated in parentheses. Samples were collected via electrofishing at the upper St. Lawrence River and Lake Ontario and associated regions (USL_LO) and ascending the eel ladder at the Moses Saunders Generating Station (MS) in Cornwall, Ontario, between 2009 and 2013. Age was determined by analysis of otoliths..

Age (years)	Season	USL_LO	MS
0	Fall	13.3 ± 1.9 (7)	
1	Spring	13.4 ± 1.4 (17)	
	Fall	20.9 ± 3.7 (43)	
2	Spring	30.0 ± 5.1 (79)	
	Fall	29.4 ± 6.7 (76)	
3	Spring	31.9 ± 9.3 (112)	30.1 ± 4.8 (17)
	Fall	39.7 ± 9.9 (51)	
4	Spring	43.7 ± 13.4 (75)	33.6 ± 4.5 (27)
	Fall	52.7 ± 9.84 (11)	
5	Spring	54.5 ± 15.5 (33)	37.8 ± 5.7 (21)
	Fall	63.5 (1)	
6	Spring		40.3 ± 6.7 (17)
	Fall	69.6 ± 19.2 (3)	
7	Spring		40.1 ± 6.7 (10)
	Fall	28.7 (1)	
8	Spring		35.6 ± 0.86 (3)
9	Spring		52.3 (1)

have a higher density and a greater proportion of males. However, this observation could also be explained by nonrandom migration and/or locally varying selection (Edeline 2007; Edeline et al. 2007). Roncarati et al. (1997) performed the only other controlled experiment and used three densities of glass eels of European Eel *A. anguilla* from a single origin; they found that the proportion of males increased with density. The study by Roncarati et al. (1997) demonstrated a plastic response with density, but since they used eels from only a single origin, they could not assess reaction norm variability by origin. We found no effect of density on the sex ratio of MR eels. This could be because our density treatments were not in the range to influence sex or it suggests there are differences in sex determination plasticity in the American Eel compared with the European Eel.

Two Clusters in Female Growth

The overall pattern for the controlled rearing experiments is that females, not captured in the act of an upriver migration, exhibited high variability, and two size clusters and males did not. This was the case in females in five of six origin treatments in the long-term growth experiment and also in the short-term growth experiment from those individuals electrofished from USL_LO. In the full, wild-capture, length-at-age data, eels from USL_LO had higher variability than those

from MS. The long-term growth experiment also suggested an origin-by-treatment interaction effect for the MR females as the size distribution in the BW treatment was heavily skewed toward the fast-growing cluster, whereas the opposite skew was evident in the FW treatment. An origin-by-treatment effect on growth was also suggested by results obtained during the 9-month glass eel–elver growth experiment of Côté et al. (2009). Such origin × environment interactions determining growth are corroborated by studies in tilapias and other fishes, which showed that by promoting the production of growth hormones, osmoregulation also results in faster growth in individuals that are better adapted to a saline environment than in those better adapted to freshwater (Degani et al. 2003; Sakamoto and McCormick 2006). These differences are most parsimonious with quantitative genetic differences in geographically different groups of glass eels. An alternative hypothesis is that there are presently unknown environmental effects on female growth variation (but not sex determination) caused by the environment in the St. Lawrence Estuary that are not experienced by the MR individuals. Although this remains to be rigorously investigated, most of these observations suggest that geographic variations in growth result from gene × environment interactions and could reflect adaptive plasticity for maximizing fitness in the face of variable environmental constraints, not the least of which could be the length of the reproductive migration to the Sargasso Sea in the North Atlantic Ocean. It is also noteworthy that gene × environment interactions between a subset of eels from the same MR and GRB samples we used in the long-term experiment has also been documented at the level of gene expression, including for genes involved in growth metabolism (Côté et al. 2014). The observed patterns in growth over 34 months of common rearing support the hypothesis of a partial genetic basis for the differences in growth and growth reaction norms in eels from these two origins. Another recent experiment that used eels from the same regions starting from the glass eel stage also found differences in growth by origin (Boivin et al., in press). This is also supported by other indirect evidence. Namely, recent studies on glass eels have revealed contrasting growth rates between translocated eels from Nova Scotia and eels that naturally use Lake Ontario and the upper St. Lawrence River (Verreault et al. 2010; Pratt and Threader 2011). Those authors observed a much higher growth rate for translocated eels, which also began to sexually mature at a much younger age than previously observed in this region. This indicates that environmentally driven plasticity alone cannot explain regional phenotypic variations and that genetically based differences could also be involved.

If this is the case, what could be the possible explanations for genetically based differences in growth between sites? Given definite evidence for panmixia (Côté et al. 2013), plausible nonmutually exclusive hypotheses could be that genetically based phenotypic differences may reflect either nonrandom dispersal and/or differential mortality associated with individual genetic variation within a single panmictic population (Rousset 2000). For instance, Edeline et al. (2007) proposed that genetic differences among individuals could explain alternative dispersal tactics (Tsukamoto and Arai 2001; Daverat and Tomas 2006; Daverat et al. 2006; Thibault et al. 2007), whereby fast-growing eels would tend to remain in lower reaches and in brackish or salt water while those adopting a slow-growing strategy would be more likely to migrate farther inland and may have better survival. Higher mobility has recently been documented for GRB glass eels relative to those from Nova Scotia (Boivin et al., in press). Moreover, a pronounced clinal genetic variation in allozymes has been interpreted as evidence for a single-generation footprint of spatially varying selection (Williams et al. 1973; Koehn and Williams 1978). This was further supported by a recent study on the American Eel that revealed spatial variations in allele frequencies (based on the analysis of coding single-nucleotide-polymorphism markers) at many genes of known functions that covaried with sea surface temperature at sites of capture (Gagnaire et al. 2012). Also selection operating within a single generation has recently been demonstrated in the European Eel (Pujolar et al. 2014). These studies demonstrate that spatially varying selection generates genetic differences between eels from different locations. Along with the recent study of Côté et al. (2014) that revealed regional differences in patterns of gene expression and the results of the present study, this strongly suggests that regional variations in growth result from differential survival associated with variations in individual genetic characteristics related to contrasting coastal conditions when glass eels enter continental waters (Wang and Tzeng 1998). Both processes could result in regional genetic variations (and perhaps associated phenotypic variations) among individuals from the same cohort within an otherwise panmictic population.

Fast-Growing, Transplanted Individuals Dominate USL_LO, but Upriver Migrants are Slow-Growing Females

It is clear that the transplanted individual American Eels have survived and thrived at USL_LO, but they are not exhibiting the phenotypes and behaviors that characterize the region. Instead, the growth patterns of these transplanted individuals are similar to those from the controlled experiments, in which females exhibited larger size variance than males and many individuals exhibited fast growth. All individuals that were captured at the BH dam were females. In the laboratory, they grew the slowest of any other group in both growth experiments. From the size-at-age data the individuals caught at MS exhibited low length variability within year-classes, which is consistent with being slow-growing females, though they were not all sexed. We expect that these individuals would reach a larger size (but an old age) at maturity, which is the characteristic phenotype of the region. Reaching a larger size at maturity may allow females to accumulate ample fatty

acid reserves for undertaking and successfully completing the long migration towards the Sargasso Sea and fully developing gametes (Larsson et al. 1990; De Silva et al. 2002; Pierron et al. 2007; Van den Thillart et al. 2007). Such a female phenotype would best correspond to eels generally encountered in the upper reaches of the St. Lawrence River, including Lake Ontario (Tremblay 2009), which have among the longest migrations back to the Sargasso Sea across the species range.

Relevance for Management and Conservation: Future Research Avenues

Along with previous studies on eel population genetics, the relevance of these findings for the management and conservation of American Eel is twofold. On the one hand, definite evidence for panmixia (Côté et al. 2013) justifies the need for coordinated global actions towards improved management and conservation of anguillid eels. On the other hand, evidence for local and partially genetically based phenotypic differences also justifies the need for local management. In particular, these results indicate that unique phenotypic attributes of American Eels that use the upper parts of the St. Lawrence River basin for rearing habitat may be genetically distinct (from a functional standpoint) from those using the Maritimes region and, as such, could be irreplaceable. Management efforts should focus on promoting the natural migration of female eels to the upper St. Lawrence River, allowing them to reach full maturity, and promoting the natural migration to the Sargasso Sea. This also means that stocking the upper St. Lawrence River and Lake Ontario with glass eels from the Maritimes will not produce American Eels with the same phenotypic attributes as those naturally migrating to these waters, which has already been confirmed by the observation that stocked glass eels develop quickly into small silver eels (migrating adult stage) with a high proportion of males, a phenomenon never reported in this area before (Verreault et al. 2010).

While this study improves our knowledge of American Eel biology, the efficiency of eel management is still compromised by an insufficient understanding of the factors affecting its distribution and abundance in the various habitats it occupies. To this end, three future research avenues should be pursued: (1) characterize the availability of marine and estuarine habitats to see how important they are for eels relative to those in freshwater, which have been better documented, (2) test for the existence of glass eel and elver ecotypes in fresh and brackish–marine waters within the theoretical framework of conditional strategies, where coastal (brackish or salt water) and inland (freshwater) may be differentially colonized by such ecotypes, and (3) document the genomic, physiological, and behavioral bases controlling the expression of these ecotypes and their propensity to occupy different habitats. This would represent a major step towards improved management of the species, its sustained exploitation, and conservation. From a more fundamental point of view, this would also contribute to a better understanding of the mechanisms underlying the proximal and ultimate control of continental dispersion of eels and their consequences on eel adaptation to heterogeneous habitats.

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