Growth, female size and sex ratio variability in American Eel (*Anguilla rostrata*) of different origins in both controlled conditions and the wild: Implications for stocking programs

Caroline L. Côté [¤§], Scott A. Pavey [¤§*], Joshua A. Stacey[¥], Thomas C. Pratt[¥], Martin Castonguay [£], Céline Audet[δ], and Louis Bernatchez [§]

[§] *Institut de Biologie Intégrative et des Systèmes (IBIS), Pavillon Charles-Eugène-Marchand 1030, Avenue de la Médecine, Université Laval, Québec, Québec, Canada, G1V 0A6, Canada*

[¥] *Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 1219 Queen St. E., Sault Ste. Marie, Ontario, Canada, P6A 2E5*

£ *Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, 850 Route de la Mer, Mont-Joli, Québec, Canada, G5H 3Z4*

[δ] *Institut des sciences de la mer de Rimouski, Université du Québec à Rimouski,310 allée des Ursulines, Rimouski, Québec, Canada, G5L 3A1*

*Author to whom correspondence should be addressed. Tel.: 418 265-3456; fax: 1 418 656-7176; email: scott.pavey.1@ulaval.ca

[¤]CLC and SAP are equal first authors of this paper.

Running title: Growth variation by sex and origin in American Eel

ABSTRACT

Freshwater eels (*Anguilla spp.*) are declining worldwide and a major challenge is understanding why these panmictic species show contrasting patterns of intra-specific phenotypic variation and recruitment. Here we present results on 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 dataset. The first growth dataset originated from a long-term rearing experiment starting from the glass eel life stage for 34 months to test the effects of origin, salinity and density on growth and sex determination. The second growth dataset 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 dataset compared individuals from electrofishing sampling of transplanted individuals in LO 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 variance, certain female groups had large variance 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 only slow growth 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. Despite the fact that 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, which must be considered for any successful management strategies of American Eel.

<A>Introduction

The economically important American Eel (*Anguilla rostrata*) poses a substantial puzzle for managers. Although it has been firmly established that the entire species is comprised of a single panmictic population (Côté et al. 2013), there is also extreme phenotypic variation among natural rearing environments in growth rate, sex ratio and size at maturity (Jessop 2010). In particular, 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 to more coastal rearing areas (Dutil et al. 1985; Tremblay 2009). Moreover, recruitment in the USL_LO has declined by 98% over the last 30 years threatening this unique life history variant found only in this portion of the species range. These declines are puzzling given the variable abundance trends that have been observed in Atlantic Canada (COSEWIC 2006; DFO 2010; COSEWIC 2012). Possible causes of the decline include fishing, pollution, habitat loss and alteration, barriers to migration, and hydroelectric turbine mortality (Castonguay et al. 1994). However, despite panmixia, the population dynamics of this unique life history appears to be independent from the rest of the species, complicating the conventional wisdom of management by 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.

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

81 The main goal of this study was to compare growth in controlled conditions and
82 size variation of wild individuals from different rearing origins to help to determine if
83 these important life history traits differ between geographic locations. To this end, we
84 analyzed three separate datasets. First, a long-term growth experiment was performed
85 representing an additional 25 months of growth (34 months total) as well as sex
86 determination from a previously published experiment that was initiated with individuals
87 at the glass eel stage (Côté et al. 2009). An additional treatment of high density rearing
88 conditions from the above experiment for one of the sampling locations was conducted to
89 test the influence of density on sex ratio. Second, a separate, shorter (18 months)
90 experiment was conducted with yellow eels, which were reared in controlled conditions
91 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 Maritimes (Figure 1). Finally, in the third dataset we extensively sampled wild individuals in the USL_LO to determine the extent to which transplanted eels compared to naturally migrating eels for the presence of males and growth rate. Based on the general expectations that 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 when reared in a common garden experiment.

<A>Materials and methods

Long-term growth experiment.--Non-pigmented glass eels were obtained in 2007 from two sampling locations at river outlets, one in the St. Lawrence Estuary and one in Nova Scotia, just prior to entering freshwater, therefore potentially avoiding time spent in freshwater before experiments. Grande-Rivière-Blanche (GRB) drains into the lower St. Lawrence Estuary, Québec. Mira River (MR) is located in Cape Breton, Nova Scotia and drains into the Atlantic Ocean (Figure 1). The GRB glass eel represents the most upriver location where glass eels are known to occur in the St. Lawrence watershed. Eels bound for the LO_USL undertake a protracted upstream migration in the St. Lawrence River; as they transition from pigmented glass eels to yellow eels it takes them at least two to three years to reach the upper St. Lawrence River (Castonguay et al. 1994; Zhu et al. 2013).

Our intention was to collect glass eels in the St. Lawrence Esturary as close as possible to the St. Lawrence River, and the mouth of GRB is the furthest west in the St Lawrence Estuary where glass eels are known to occur. Earlier experiments compared eels from these two origins after nine months of rearing in contrasting salinity treatments (Côté et al. 2009). In the present experiment, we report 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. There were two salinity condition treatments: freshwater (salinity 3 ± 1 ppt; hereafter FW) and brackish water (salinity 22 ± 1 ppt, hereafter BW) and 2 tanks per treatment at an initial density of 100 individuals or 45g m^{-2}. Standard 20 gallon aquaria were used with interior dimensions of (60.0 cm X 30.5 cm 29.2 cm) and height of water was 17.1 cm. With the MR glass eels, we established an additional high density treatment of 3x the density (135 g m^{-2}) for both FW and BW. After nine months, eels from each of the four low density groups (sample size in Table 1) 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°C ±1 and 2.5 ± 0.5 ppt, respectively. Physical-chemical parameters, including nitrites (NO_2 < 0.1 mg L^{-1}), nitrates (NO_3 < 200 mg L^{-1}), ammonia (NH_4 < 0.004 mg L^{-1}), 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^{-1} at 22°C to 9.1 mg L^{-1} at 20°C). For optimal growth, eels were fed twice a day with a mixture of fish roe, pellets, and capelin 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 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 (TL) and weight (W) were measured on all individuals every four months. In April 2010, all eels were euthanized with an overdose of eugenol. The majority had reached a TL of 30 cm or more, and 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).

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 the LARSA and reared only in FW conditions in 1 m^3 tanks as above. The food for this experiment was blood worms and brine shrimp *ad libitum*. Eels were individually PIT tagged and length and mass for each individual were measured every three 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 individuals were sacrificed to determine sex by visual inspection of gonads.

158 *Length-at -age in the wild.*--In our third dataset, individuals transplanted as glass eels into
159 LO were electrofished from shoreline areas in the upper St. Lawrence River (44°25'N
160 75°52'W) and the Bay of Quinte (44°8'N 77°8'W), Ontario. Eels were sampled in May
161 (2009-2013) and September (2009-2011). Sampling was conducted with boat-
162 electrofishing along 100 m shoreline transects at approximately 1 m depth at night (Pratt
163 and Threader 2011). The eels were captured using dip nets, and were euthanized with
164 MS-222 for age and sex determination. When the glass eel transplants occurred, all
165 transplanted individuals received an otolith mark with oxytetracycline hydrochloride
166 (OTC) (Pratt and Threader 2011). Otoliths in this study were evaluated for this mark
167 (except 2013 sample year) and also used to determine age in all sampling years. In a
168 targeted subset of individuals focused below 40 cm (as males rarely attain greater length
169 than 40 cm) gonads were analyzed for sexual differentiation. Gonads were fixed in
170 Bouin's fixative, then dehydrated with 100% EtOH. Tissues were then embedded in wax,
171 cut to 5 µm thickness, stained, and viewed under a microscope. Naturally migrating eels
172 were collected from the eel ladder at the Moses Saunders Generating Station in Cornwall,
173 Ontario, and included specimens ascending from Lac St. François into the upper St.
174 Lawrence River (Figure 1). This provided a comparison group for the LO electrofishing
175 as few naturally recruiting eels remain in USL_LO.

176 *Statistical analysis of growth clustering.*--For the two controlled growth experiments, we
177 examined the total length size distribution for the final measurement of each sex
178 separately by creating kernel density plots from the "lattice" R package. Kernel density
179 plots are specifically designed to non-parametrically depict the population distribution
180 from a sample. For each distribution, we used the "mclust" R package which uses AIC to

181 determine the most likely number of clusters represented in the data, i.e. whether the
182 growth best represents one or more clusters. Here, in instances where two clusters were
183 found (see Results), we designated individuals as fast or slow growing based on the break
184 in the distribution by visual inspection of the density plot.

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

194

195 <A>Results

196 Size-at-age differences between eels from MR and GRB observed during the first
197 nine months were still observed after the transfer to large tanks and until the end of the
198 first (34-month) growth experiment (Figure 2). Eels from MR had a greater size at age
199 (both TL and W) throughout the rearing experiment compared to GRB eels (Table 1).
200 However, only MR eels retained the positive initial salinity effect on growth and
201 development. The generalized linear model indicated a significant effect of sex (t=-5.56,

202 $P < 0.001$) and origin (t=5.50, $P < 0.001$) and their interaction (t= -3.92, $P < 0.001$). In
203 the previous step of model simplification, treatment was not significant (t=-0.53 $P =$
204 0.597) but the origin*treatment interaction was suggested although it was not significant
205 (t=1.92 $P = 0.0698$). For the second (short-term) growth experiment (Table 2), only
206 females were present in BH, so only females were compared in this GLM, and origin had
207 a significant effect on length (t=2.25, $P = 0.025$).

208 At the end of the 34-month growth experiment, the general pattern was that
209 female length exhibited two clusters and males exhibited one cluster (Figs. 3-5). The
210 "mclust" procedure indicated two clusters in five out of six times for females with a large
211 spread between the modes (Table 3). Two clusters were detected in both treatments of
212 males of GRB only, but unlike in the females, the clusters were so close together that the
213 density plot did not exhibit a clear bimodal pattern. The logistic regression revealed no
214 significant effect of origin or salinity treatment (or their interaction) on sex
215 differentiation, since the proportions of females were similar among all origins and
216 treatments (34% female overall; Table 1). The high density treatment had nearly exactly
217 the same sex ratio in both salinities and was not significantly different (BW 35% female,
218 P = 1 FW 35% female, $P = 0.343$). In all groups of the long-term experiment, males were
219 on average smaller than females at the end of the experiment (Table 1). The mean size
220 and weight was 41.0 cm and 136 g for males compared to 55.0 cm and 442 g for females.

221 Similarly, in the short-term growth experiment, the female length from LO
222 represented two significant clusters, but the females from BH and the males from LO did

223 not (Table 3, Figure 6 and Figure 7). Sex was skewed toward female in the short-term
224 growth experiment (BH: 100% female; LO: 76% female).

225 Between 2009 and 2013, 510 individuals were captured via electrofishing and
226 otoliths were extracted and assessed for age and 433 of these (all except 2013) were
227 assessed for the OTC mark. All individuals assessed with the exception of one had the
228 OTC mark. That one non-transplanted individual was an 11 year old female that was 82.0
229 cm long. The rest of the individuals from LO_USL ages ranged between 2-7 years old,
230 with age class 7 represented by only a single individual (Table 4). With the 96 individuals
231 sampled at Moses Saunders, ages ranged from 3-9, with a single individual (length 52.3
232 cm) that was age 9. Of the 150 LO_USL individuals analyzed for sexual differentiation,
233 65 were female, 14 were male, and the remaining 71 were undifferentiated. Overall, there
234 was a pattern of faster growth and higher variance at LO_USL, whereas the natural
235 upriver migrants at MS were slower growing with less variance (Table 4).

236

237 <A>Discussion

238 In this paper, we combined three different and independent experiments: a long-
239 term (34-month) growth experiment of glass eels from two different origins, a short-term
240 (18-month) growth experiment starting with small yellow eels electro-fished in LO
241 compared with individuals naturally migrating upriver, and finally length-at-age data of
242 translocated individuals at LO and naturally migrating individuals at the MS dam. These
243 data support three conclusions about American eel life history. First, sex was not affected
244 by salinity, origin or density when reared in controlled conditions from the glass eel

stage. Thus, even the most upriver location of glass eel freshwater dispersal does not exhibit a greater proportion of females. Second, there is high variance in female growth rate that is not present in males, whereby females tend to group into slow- and fast-growing according to kernel density plots and AIC criterion, which are influenced by origin and possibly the salinity environment. Third, results suggest that only individuals of the slow-growing female cluster undertake the long migration to the USL_LO. Below we discuss each of these in turn, and then the management implications of these conclusions.

<B>Sex determination 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 in natural conditions, it has been suggested that sex determination is primarily environmentally determined in American Eel through as yet unknown mechanisms (Holmgren and Mosegaard 1996; Davey and Jellyman 2005). Here, regardless whether the eels were reared in brackish or freshwater for the first nine months, the sex ratios were nearly identical, thus our study 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 between the origins; only 18% female

267 from the DR origin and 65% females from GRB. This result was logical as the GRB
268 sampling location is the furthest upriver location of glass eels known, and would
269 seemingly be the most likely to exhibit a female biased sex ratio, but our study did not
270 repeat this result. Vladykov and Liew (1982) collected their samples 4 years apart, thus
271 temporal fluctuations (genetic or plastic) in sex were confounded with origin. This could
272 have affected their results in several ways. First, the different cohorts of glass eels
273 collected could have differed in sex ratio. Second, the environmental conditions for
274 rearing could have differed between the growth periods. Third, since all individuals were
275 in a single pond and not graded as they grew, cannibalism could have affected the sex
276 ratios.

277 Density is thought to be the most important parameter in sex determination due to
278 suppression of growth rate (Davey and Jellyman 2005). Several studies reporting
279 correlations of density and sex in different natural environments provided support for this
280 prediction (Parsons et al. 1977; Krueger and Oliveira 1999; Beentjes and Jellyman 2003;
281 Huertas and Cerda 2006; Melia et al. 2006). These studies provide observation in the
282 natural environment that brackish areas tend to have higher density and a greater
283 proportion of males. However, this observation could also be explained by non-random
284 migration and/or locally varying selection (Edeline 2007; Edeline et al. 2007). Roncarati
285 et al. (1997) performed the only other controlled experiment having three densities at the
286 glass eel stage of European Eel (*A. anguilla*) from a single origin and they found that the
287 proportion of males increased with density. That study demonstrated a plastic response
288 with density, but since they used only a single origin, they could not assess reaction norm
289 variability by origin. In our side experiment, we found no effect of density on sex ratio of

MR eels. This could be because our density treatments were not in the range to influence sex or it may suggest differences in sex determination plasticity in the American Eel compared with the European Eel.

<B>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 of 5/6 origin treatments in the long-term growth experiment and also in the short-term growth experiment from those individuals electrofished from LO_USL. In the full wild capture length-at-age data, USL_LO had higher variability than MS. The long-term growth experiment also suggested an origin by treatment interaction effect for the MR females as the size distribution in the brackish water treatment was heavily skewed toward the fast growing cluster with the opposite skew in the freshwater 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 is corroborated by studies in tilapia and other fishes, which reported that by promoting the production of growth hormones, osmoregulation also results in faster growth in individuals that are better adapted to saline environment compared to 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 as of yet unknown environmental effects on female growth variation (but not sex

312 determination) caused by the environment in the St. Lawrence Estuary that are not
313 experienced by the MR individuals. Although this remains to be rigorously investigated,
314 most of these observations suggest that geographic variations in growth result from
315 gene*environment interactions and could reflect adaptive plasticity for maximizing
316 fitness in the face of variable environmental constraints, not the least of which could be
317 the length of the reproductive migration to the Sargasso Sea. It is also noteworthy that
318 gene*environment interactions between a subset of eels from the same MR and GRB
319 samples used here in the long-term experiment has also been document at the level of
320 gene expression, including for genes involved in growth metabolism (Côté et al. 2014).
321 The observed patterns in growth over 34 months of common rearing support the
322 hypothesis of a partial genetic basis for the differences in growth and growth reaction
323 norms in eels from these two origins. Another recent experiment that used eels from the
324 same regions starting from the glass eel stage also found differences in growth by origin
325 (Boivin et al. In Review). This is also supported by other indirect evidence. Namely,
326 recent studies on glass eels have revealed contrasting growth rates between translocated
327 eels from Nova Scotia and eels that naturally use Lake Ontario and the upper St.
328 Lawrence River (Verreault et al. 2010; Pratt and Threader 2011). These authors observed
329 a much higher growth rate for translocated eels, which also began to sexually mature at a
330 much younger age than previously observed in this region. This indicates that
331 environmentally driven plasticity alone cannot explain regional phenotypic variations and
332 that genetically based differences could also be involved.

333 If so, what could be the possible explanations for genetically based differences in
334 growth between sites? Given definite evidence for panmixia (Côté et al. 2013), plausible

335 non-mutually exclusive hypotheses could be that genetically based phenotypic
336 differences may reflect either non-random dispersal and/or differential mortality
337 associated with individual genetic variation within a single panmictic population (Rousset
338 2000). For instance, Edeline et al. (2007) proposed that genetic differences among
339 individuals could explain alternative dispersal tactics (Tsukamoto and Arai 2001; Daverat
340 et al. 2006; Daverat and Tomas 2006; Thibault et al. 2007), whereby fast-growing eels
341 would tend to remain in lower reaches and brackish/saltwater while those adopting a
342 slow-growing strategy would be more likely to migrate further inland and may have
343 better survival. Higher mobility has recently been documented for GRB glass eels relative
344 to those from Nova Scotia (Boivin et al. In Review). Moreover, a pronounced clinal
345 genetic variation in allozymes has been interpreted as evidence for a single-generation
346 footprint of spatially varying selection (Williams et al. 1973; Koehn and Williams 1978).
347 This was further supported by a recent study that revealed spatial variations in allele
348 frequencies (based on the analysis of coding SNP markers) at many genes of known
349 functions that covaried with sea surface temperature at sites of capture (Gagnaire et al.
350 2012). Also selection operating within a single generation has recently been
351 demonstrated in European eel also (Pujolar et al. 2014). These studies demonstrate that
352 spatially varying selection generates genetic differences between eels from different
353 locations. Along with the recent study of Côté et al. (2014) that revealed regional
354 differences in patterns of gene expression and the results of this study, this strongly
355 suggests that regional variations in growth could result from differential survival
356 associated with variations in individual genetic characteristics related to contrasting
357 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 a same cohort within an otherwise panmictic population.

<B> Fast growing, transplanted individuals dominate USL_LO but upriver migrants are slow growing females

It is clear that the transplanted individuals have survived and thrived at LO_USL, but they are not exhibiting the phenotypes and behaviors that characterize the region. Instead, the growth patterns of these transplanted individuals are similar to the controlled experiments, with females exhibiting larger size variance than males, with many individuals exhibiting fast growth. All individuals that were captured at the BH dam were females. In the lab, they grew the slowest of any other group in either growth experiment. The size-at-age data from individuals caught at MS exhibited low length variability within year class, and consistent with being slow growing females, though they were not all sexed. We expect that these individuals would reach the larger size at maturity (but an old age at maturity), which is the characteristic phenotype of the region. It has been hypothesized that reaching a larger size at maturity may allow females to attain 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 migration back to the Sargasso across the species range.

<B>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 two-fold. On the one hand, definite evidence for panmixia (Côté et al. 2013) justifies the need for global coordinated actions towards improved management and conservation of eel. On the other hand, evidence for local and partially genetically based phenotypic differences also justifies the need for local management. In particular, these results suggest that unique phenotypic attributes of eels using 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, allow them to reach full maturity, and promote 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 eels with same phenotypic attributes as those naturally migrating to these waters, as already confirmed by the observation that stocked glass eels migrate as young and small silver eels with a high proportion of males, a phenomenon never reported before (Verreault et al. 2010).

While this study improves our knowledge of eel biology, the efficiency of its 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: i) characterize the availability of marine and estuarine habitats to see how important they are relative to those in freshwater, which have been better documented, ii) test the existence of glass eel/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 iii) 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 eel and their consequences on eel adaptation to heterogeneous habitats.

<A>Acknowledgements

We are grateful to Denis Talbot (U. Laval) for statistical assistance; to Lucie Papillon (U. Laval), Laure Devine (DFO), Nathalie Brodeur (U. Laval), Guy Verreault (MRNF Québec), and Hacène Tamdrari (U. Québec Rimouski) for field assistance and useful discussions; to Yvonne Carey (Atlantic Elver Fishery) for generously providing fishing tips, dip nets, and glass eel samples; Hydro-Québec and Ontario Power Generation for supplying naturally recruiting eels from their respective eel ladders; and to Mike Campbell (South Shore Trading Co.) for providing samples and advice on elver rearing. The assistance of Serge Higgins, Jean-Christophe Therrien and Jade Larivière, Marie-Christine T. Dion, Isabelle Langlois-Parisé, Anne St-Pierre, Éric Boucher, Audrey Jobin-Piché, Émile Warren (LARSA-U. Laval) was invaluable throughout the rearing experiments. Laure Devine's comments as well as two anonymous

referees improved an earlier draft. Funding for this study was obtained from the Great Lakes Fishery Commission and SARCEP (DFO, Canada). We are also grateful to Québec-Océan and the Biology Department of Laval University for scholarships. SAP was supported by Ressources Aquatique Québec (RAQ). This study is a contribution to the research program of Québec-Océan.

<A>References

Beentjes, M. P., and D. J. Jellyman. 2003. Enhanced growth of longfin eels, *Anguilla dieffenbachii*, transplanted into Lake Hawea, a high country lake in South Island, New Zealand. New Zealand Journal of Marine and Freshwater Research 37(1):1-11.

Beullens, K., E. H. Eding, P. Gilson, F. Ollevier, J. Komen, and C. J. J. Richter. 1997. Gonadal differentiation, intersexuality and sex ratios of European eel (*Anguilla anguilla* L) maintained in captivity. Aquaculture 153(1-2):135-150.

Boivin, B., M. Castonguay, C. Audet, S. A. Pavey, M. Dionne, and B. L. In Review. How does salinity influence habitat selection and growth in juvenile American eels *Anguilla rostrata*? Journal of Fish Biology.

Castonguay, M., P. V. Hodson, C. M. Couillard, M. J. Eckersley, J. D. Dutil, and G. Verreault. 1994. Why Is Recruitment of the American Eel, *Anguilla rostrata*, Declining in the St-Lawrence-River and Gulf. Canadian Journal of Fisheries and Aquatic Sciences 51(2):479-488.

COSEWIC. 2006. COSEWIC Assessment and status report on the American Eel *Anguilla rostrata* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa.

COSEWIC. 2012. COSEWIC assessment and status report on the American Eel *Anguilla rostrata* in Canada. Ottawa.

Côté, C. L., M. Castonguay, K. Svetlana, G. McWilliam, G. Cramb, and B. L. 2014. In absence of local adaptation, plasticity and spatially varying selection rule: a view from genomic reaction norms in a panmictic species (*Anguilla rostrata*). BMC Genomics (online early).

Côté, C. L., M. Castonguay, G. Verreault, and L. Bernatchez. 2009. Differential effects of origin and salinity rearing conditions on growth of glass eels of the American eel *Anguilla rostrata*: implications for stocking programmes. Journal of Fish Biology 74(9):1934-1948.

Côté, C. L., P.-A. Gagnaire, V. Bourret, G. Verreault, M. Castonguay, and L. Bernatchez. 2013. Population genetics of the American eel (*Anguilla rostrata*): F(ST) = 0 and North Atlantic Oscillation effects on demographic fluctuations of a panmictic species. Molecular Ecology 22(7):1763-1776.

Daverat, F., K. E. Limburg, I. Thibault, J. C. Shiao, J. J. Dodson, F. O. Caron, W. N. Tzeng, Y. Iizuka, and H. Wickstrom. 2006. Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A. japonica* and *A. rostrata*. Marine Ecology-Progress Series 308:231-241.

467 Daverat, F., and J. Tomas. 2006. Tactics and demographic attributes in the European eel
468 *Anguilla anguilla* in the Gironde watershed, SW France. Marine Ecology-
469 Progress Series 307:247-257.
470 Davey, A. J. H., and D. J. Jellyman. 2005. Sex determination in freshwater eels and
471 management options for manipulation of sex. Reviews in Fish Biology and
472 Fisheries 15(1-2):37-52.
473 De Silva, S. S., R. M. Gunasekera, and R. O. Collins. 2002. Some morphometric and
474 biochemical features of ready-to-migrate silver and pre-migratory yellow stages
475 of the shortfin eel of south-eastern Australian waters. Journal of Fish Biology
476 61(4):915-928.
477 De Silva, S. S., R. M. Gunasekera, G. Gooley, and B. A. Ingram. 2008. Growth of
478 Australian shortfin eel (*Anguilla australis*) elvers given different dietary protein
479 and lipid levels. Aquaculture Nutrition 7(1):53-56.
480 Degani, G., I. Tzchori, S. Yom-Din, D. Goldberg, and K. Jackson. 2003. Growth
481 differences and growth hormone expression in male and female European eels
482 [*Anguilla anguilla* (L.)]. General and Comparative Endocrinology 134(1):88-93.
483 DFO. 2010. Status of American Eel and progress on achieving management goals.
484 Fisheries and Oceans Canada, Ottawa.
485 Dutil, J. D., B. Legare, and C. Desjardins. 1985. Discrimination of a fish stock, the eel
486 (*Anguilla rostrata*), based on the presense of mirex, a chemical synthesis product.
487 Canadian Journal of Fisheries and Aquatic Sciences 42(3):455-458.
488 Edeline, E. 2007. Adaptive phenotypic plasticity of eel diadromy. Marine Ecology-
489 Progress Series 341:229-232.

Edeline, E., L. Beaulaton, R. Le Barh, and P. Elie. 2007. Dispersal in metamorphosing juvenile eel *Anguilla anguilla*. Marine Ecology-Progress Series 344:213-218.

Gagnaire, P.-A., E. Normandeau, C. Côté, M. M. Hansen, and L. Bernatchez. 2012. The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). Genetics 190:725-735.

Guerrero, R. D., and W. L. Shelton. 1974. An aceto-carmine squash method for sexing juvenile fishes. The Progressive Fish Culturist 36(1):56-AASMFS.

Holmgren, K., and H. Mosegaard. 1996. Implications of individual growth status on the future sex of the European eel. Journal of Fish Biology 49(5):910-925.

Huertas, M., and J. Cerda. 2006. Stocking density at early developmental stages affects growth and sex ratio in the European eel (*Anguilla anguilla*). Biological Bulletin 211(3):286-296.

Jessop, B. M. 2010. Geographic effects on American eel (*Anguilla rostrata*) life history characteristics and strategies. Canadian Journal of Fisheries and Aquatic Sciences 67(2):326-346.

Knights, B. 1987. Agonistic behavior and growth in the European Eel, *Anguilla anguilla* L, in relation to warm-water aquaculture. Journal of Fish Biology 31(2):265-276.

Koehn, R. K., and G. C. Williams. 1978. Genetic differentiation without isolation in American Eel, *Anguilla rostrata* 2: Temporal stability of geographic patterns. Evolution 32(3):624-637.

Krueger, W. H., and K. Oliveira. 1999. Evidence for environmental sex determination in the American eel, *Anguilla rostrata*. Environmental Biology of Fishes 55(4):381-389.

513 Larsson, P., S. Hamrin, and L. Okla. 1990. Fat content as a factor inducing migratory
514 behavior in the eel (*Anguilla anguilla* L.) to the Sargasso Sea.
515 Naturwissenschaften 77(10):488-490.
516 MacGregor, R., J. Casselman, L. Greig, W. A. Allen, L. McDermott, and T. Haxton.
517 2010. DRAFT recovery strategy for the American eel (*Anguilla rostrata*) in
518 Ontario. Prepared for Ontario Ministry of Natural Resources, Peterborough,
519 Ontario.
520 Melia, P., D. Bevacqua, A. J. Crivelli, J. Panfili, G. A. De Leo, and M. Gatto. 2006. Sex
521 differentiation of the European eel in brackish and freshwater environments: a
522 comparative analysis. Journal of Fish Biology 69(4):1228-1235.
523 Parsons, J., K. U. Vickers, and Y. Warden. 1977. Relationship between elver recruitment
524 and changes in sex-ratio of silver eels *Anguilla anguilla*-L migrating from Lough
525 Neagh, Northern-Ireland. Journal of Fish Biology 10(3):211-229.
526 Pierron, F., M. Baudrimont, A. Bossy, J. P. Bourdineaud, D. Brethes, P. Elie, and J. C.
527 Massabuau. 2007. Impairment of lipid storage by cadmium in the European eel
528 (*Anguilla anguilla*). Aquatic Toxicology 81(3):304-311.
529 Pratt, T. C., and R. W. Threader. 2011. Preliminary evaluation of a large-scale American
530 eel conservation stocking experiment. North American Journal of Fisheries
531 Management 31(4):619-627.
532 Pujolar, J. M., M. W. Jacobsen, T. D. Als, J. Frydenberg, K. Munch, B. Jónsson, J. B.
533 Jian, L. Cheng, G. E. Maes, L. Bernatchez, and M. M. Hansen. 2014. Genome-
534 wide single-generation signatures of local selection in the panmictic European eel.
535 Molecular Ecology 23(10):2514-2528.

536 Roncarati, A., P. Melotti, O. Mordenti, and L. Gennari. 1997. Influence of stocking
537 density of European eel (*Anguilla anguilla*, L.) elvers on sex differentiation and
538 zootechnical performances. Journal of Applied Ichthyology-Zeitschrift Fur
539 Angewandte Ichthyologie 13(3):131-136.
540 Rousset, F. 2000. Genetic differentiation between individuals. Journal of Evolutionary
541 Biology 13(1):58-62.
542 Sakamoto, T., and S. D. McCormick. 2006. Prolactin and growth hormone in fish
543 osmoregulation. General and Comparative Endocrinology 147(1):24-30.
544 Tesch, F. W. 1977. The Eel. Biology and Management of Anguillid Eels. Chapman &
545 Hall, London.
546 Thibault, I., J. J. Dodson, F. Caron, W. N. Tzeng, Y. Iizuka, and J. C. Shiao. 2007.
547 Facultative catadromy in American eels: testing the conditional strategy
548 hypothesis. Marine Ecology-Progress Series 344:219-229.
549 Tremblay, V. 2009. Reproductive strategy of female American eels among five
550 subpopulations in the St. Lawrence River Watershed. Pages 85-102 *in* J. M.
551 Casselman, and D. K. Cairns, editors. Eels at the edge: science, status, and
552 conservation concerns., Symposium 58. American Fisheries Society, Bethesda.
553 Tsukamoto, K., and T. Arai. 2001. Facultative catadromy of the eel *Anguilla japonica*
554 between freshwater and seawater habitats. Marine Ecology Progress Series
555 220:265-275.
556 Van den Thillart, G., A. Palstra, and V. Van Ginneken. 2007. Simulated migration of
557 European silver eel; Swim capacity and cost of transport. Journal of Marine
558 Science and Technology-Taiwan 15:1-16.

559 Verreault, G., W. Dargere, and R. Tardif. 2009. American eel movements, growth, and
560 sex ratio following translocation. American Fisheries Society Symposium 58:129-
561 345.
562 Verreault, G., P. Dumont, J. Dussureault, and R. Tardif. 2010. First record of migrating
563 silver American eels (*Anguilla rostrata*) in the St. Lawrence Estuary originating
564 from a stocking program. Journal of Great Lakes Research 36(4):794-797.
565 Vladykov, V. D., and P. K. L. Liew. 1982. Sex of adult American eels (*Anguilla rostrata*)
566 collected as elvers in two different streams along the eastern shore of Canada, and
567 raised in the same freshwater pond in Ontario. Proceedings ot the 1980 North
568 American eel Conference; Ontario Fishries Technical Report Series 4:88-93.
569 Wang, C. H., and W. N. Tzeng. 1998. Interpretation of geographic variation in size of
570 American eel *Anguilla rostrata* elvers on the Atlantic coast of North America
571 using their life history and otolith ageing. Marine Ecology-Progress Series
572 168:35-43.
573 Waples, R. S., A. E. Punt, and J. M. Cope. 2008. Integrating genetic data into
574 management of marine resources: how can we do it better? Fish and Fisheries
575 9(4):423-449.
576 Williams, G. C., R. K. Koehn, and J. B. Mitton. 1973. Genetic differenciation without
577 isolation in the American eel, *Anguilla rostrata*. Evolution 27(2):192-204.
578 Zhu, X. H., Y. M. Zhao, A. Mathers, and L. D. Corkum. 2013. Length frequency age
579 estimations of American Eel recruiting to the Upper St. Lawrence River and Lake
580 Ontario. Transactions of the American Fisheries Society 142(2):333-344.

581

582

583 <A>Figure Captions

584 **Figure 1:** Map of sampling sites. The sampling sites are Mira River (MR), and Grande-
585 Rivière-Blanche (GRB), Lake Ontario (LO), and Beauharnois Dam (BH), Upper St.
586 Lawrence (USL) and the Moses Saunders Generating Station (MS).

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

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

601 **Figure 4:** Density plots of final length for the males of the long-term (34-month) growth
602 experiment including both origins, Mira River (MR) and Grande Rivière Blanche (GRB)
603 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 5: Distributions of total lengths for each measurement period (approx. 4 month intervals) over the 25 months for the long-term growth experiment for 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 (fast growing, dark grey and slow growing, black) based when the data represented two clusters. Males are represented in light grey. Boxes represent the inter-quartile range (IQR) and whiskers extend to 1.5*IQR.

Figure 6: Density plots of final length for the short-term (18-month) growth experiment including 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.

Figure 7: Total length for each measurement for the short-term (18-month) growth experiment Females are separated into life history (fast growing, dark grey and slow growing, black) based when the data represented two clusters. Males are represented in light grey. Boxes represent the inter-quartile range (IQR) and whiskers extend to 1.5*IQR.

<A>Tables

Table 1: Final size for long-term (34-month) growth experiment. Mean total weight and length (mean ± SD) reached after 34 months of rearing for eels from the Mira River (MR) and Grande-Rivière-Blanche (GRB) initially reared in either fresh water (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.

Origin	Treatment	Sex	N (%)	Length (cm)	Weigh (g)
MR	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
GRB	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

Table 2: Final sizes for short-term (18-month) growth experiment. Mean 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 weight could not be determined (and thus N for start measurement is lower than finish)

Origin	Sex	Start/Finish	N	Length(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

Table 3: Cluster analysis for final length distributions of controlled rearing experiments. Results of the R package "mclust" indicating the number of clusters, one or two, is more likely (bolded) to describe each distribution (by origin*sex*treatment) as determined by the higher AIC value.

Origin	Treatment	Sex	Clusters	AIC
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**	**-1566.3**
			2	-1571.2
LO	BR	F	1	-1057.7
			2	**-1043.6**
LO	BR	M	**1**	**-398.1**
			2	-398.4

643

644

645

646

647

648

649

650 **Table 4:** Mean length for each age class of American Eel in the wild experiment.
651 Samples were collected via electrofishing at the Lake Ontario and Upper St. Lawrence
652 and regions (LO_USL) and ascending the eel ladder at the Moses Saunders Generating
653 Station in Cornwall, Ontario (MS) between 2009 and 2013. Age was determined by
654 otoliths. Length and sample sized are in the following format: (cm±SD (n)).

Otolith Age	Season	LO_USL	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)

655

656

Figure 1:

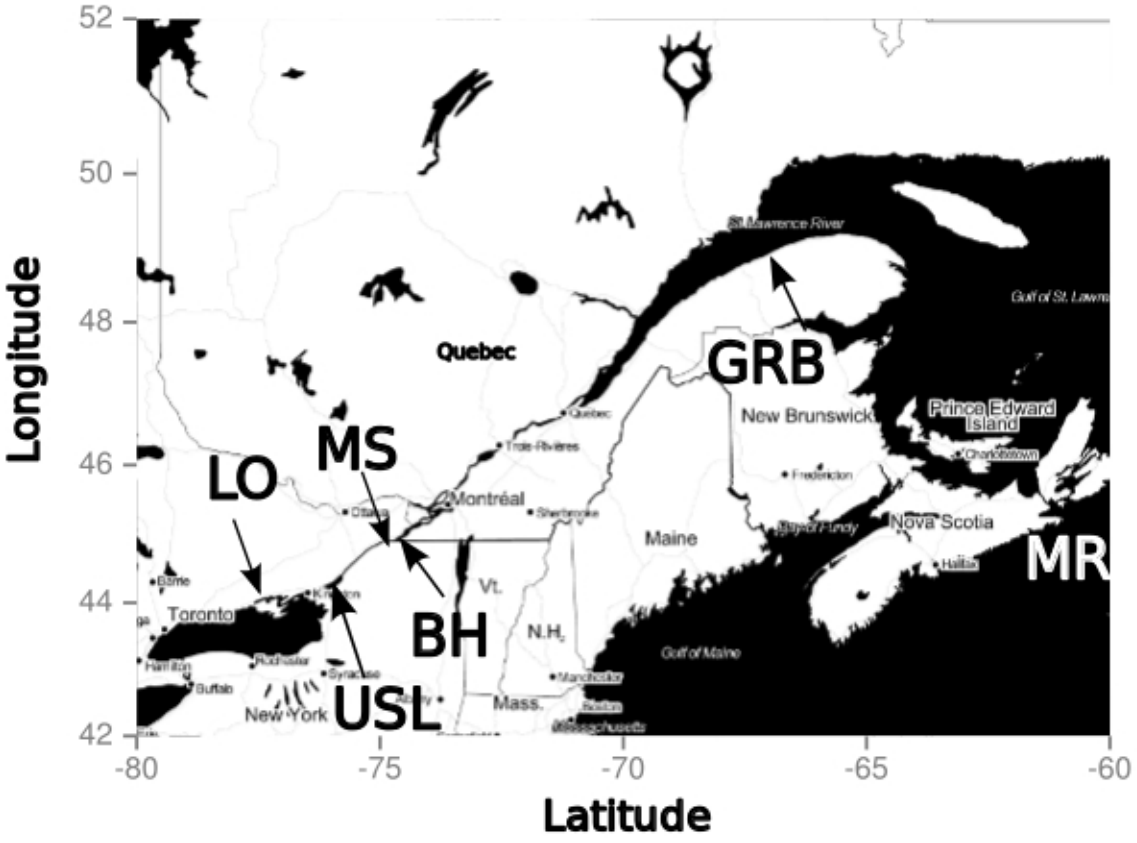

Longitude
Latitude
52
50
48
46
44
42
-80
-75
-70
-65
-60
Quebec
GRB
MS
LO
BH
USL
MR
New Brunswick
Prince Edward Island
Nova Scotia
Maine
Montréal
Toronto
New York
Vt.
N.H.
Mass.
Gulf of Maine
St. Lawrence River

Figure 2:

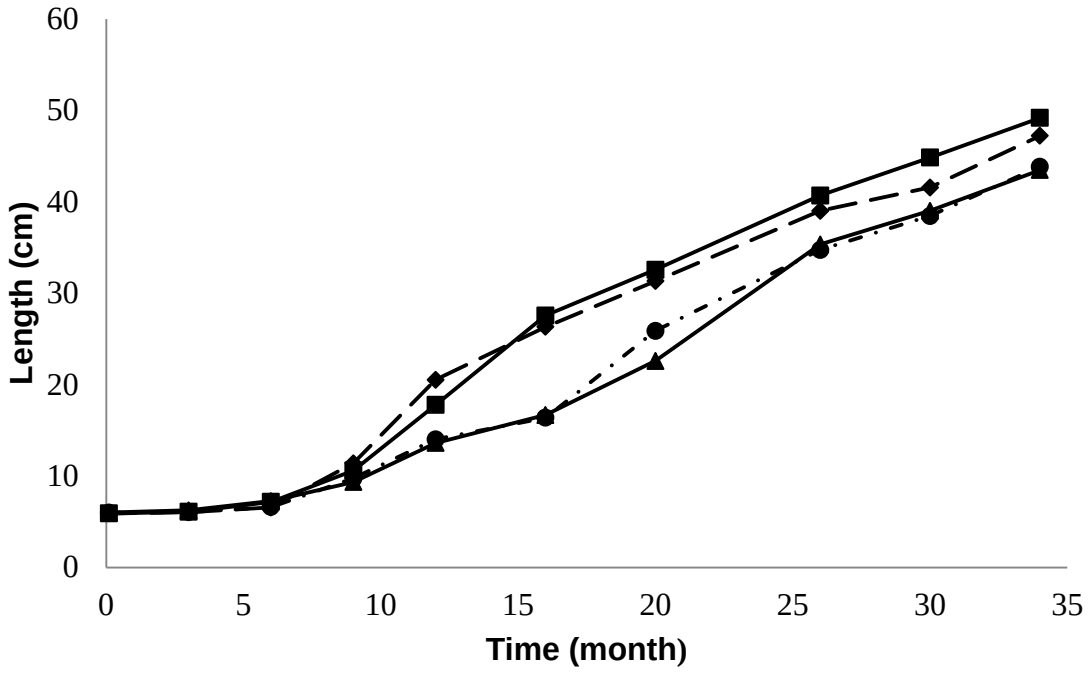


Figure 3:

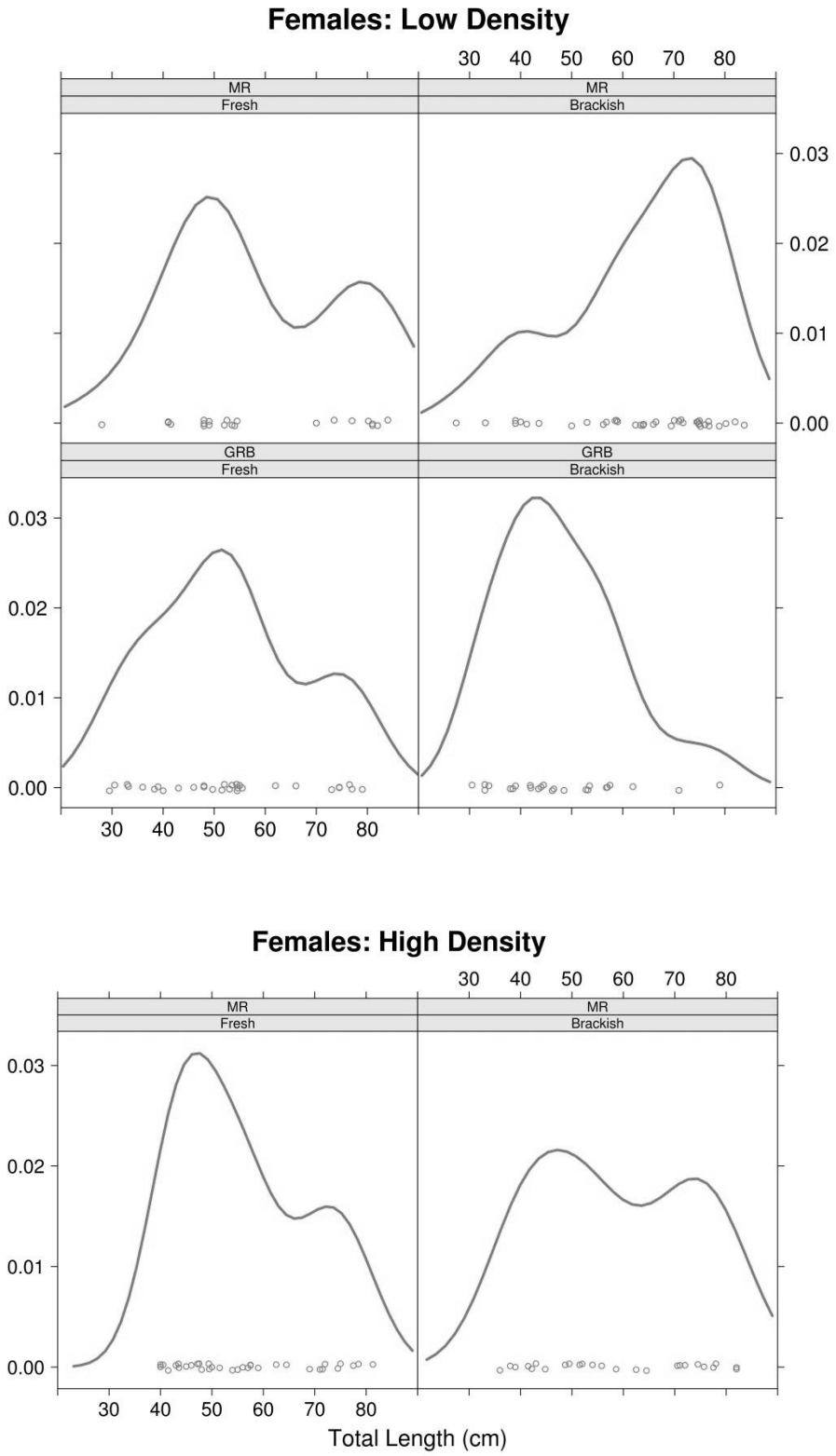

Females: Low Density
MR
Fresh
MR
Brackish
GRB
Fresh
GRB
Brackish
0.00
0.01
0.02
0.03
30
40
50
60
70
80
Females: High Density
Total Length (cm)

Figure 4:

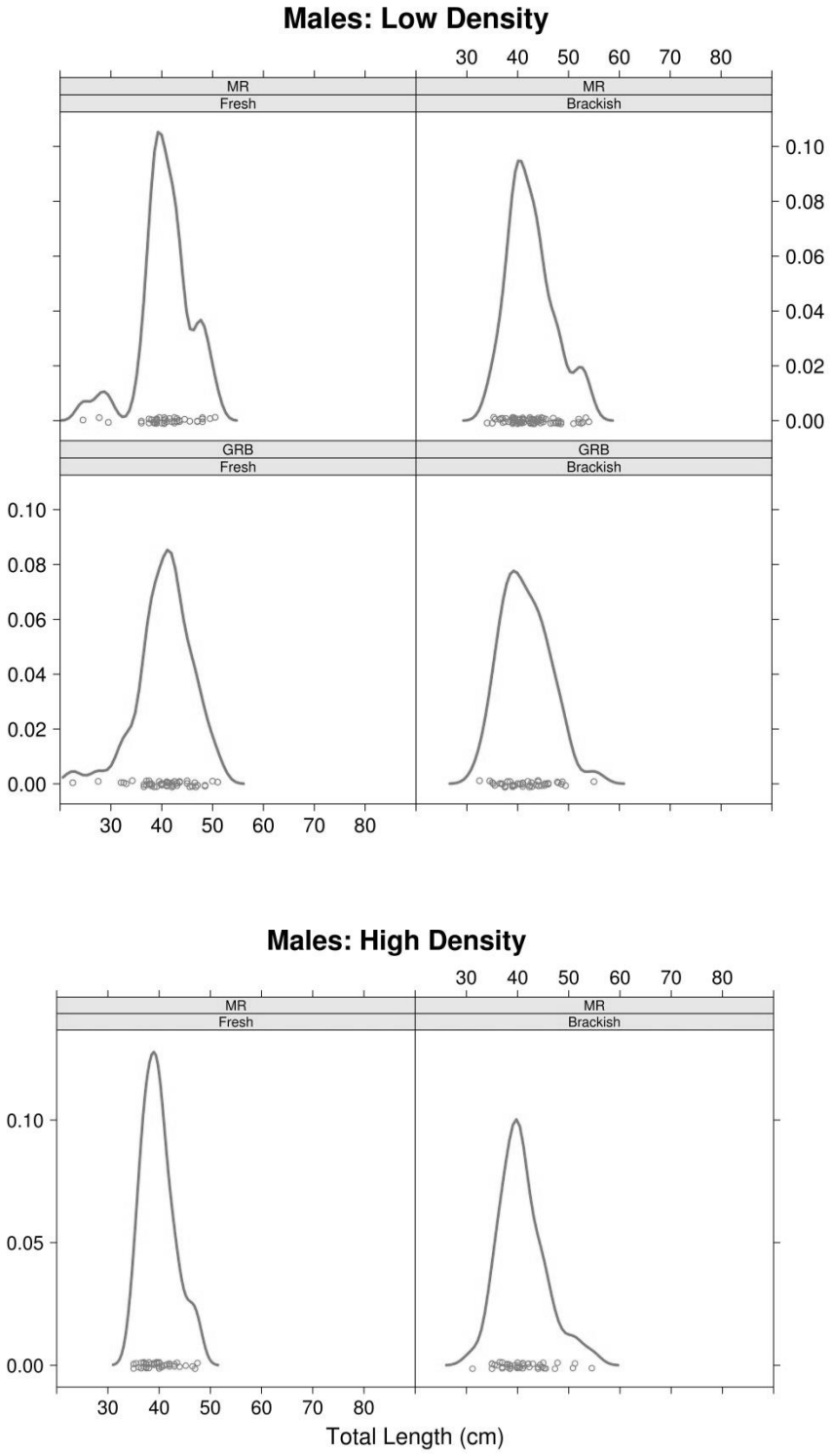

Males: Low Density
MR
Fresh
MR
Brackish
GRB
Fresh
GRB
Brackish
0.00
0.02
0.04
0.06
0.08
0.10
30
40
50
60
70
80
Males: High Density
MR
Fresh
MR
Brackish
0.00
0.05
0.10
Total Length (cm)

Figure 5:

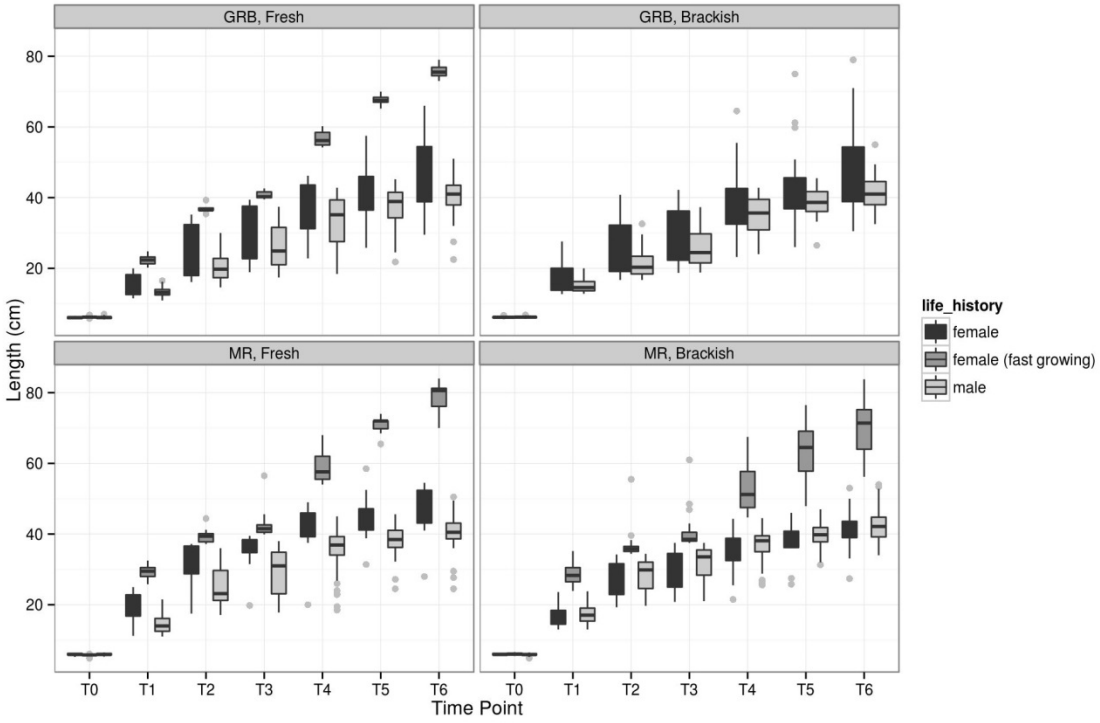

GRB, Fresh
GRB, Brackish
MR, Fresh
MR, Brackish
Length (cm)
80
60
40
20
T0
T1
T2
T3
T4
T5
T6
Time Point
life_history
female
female (fast growing)
male

Figure 6:

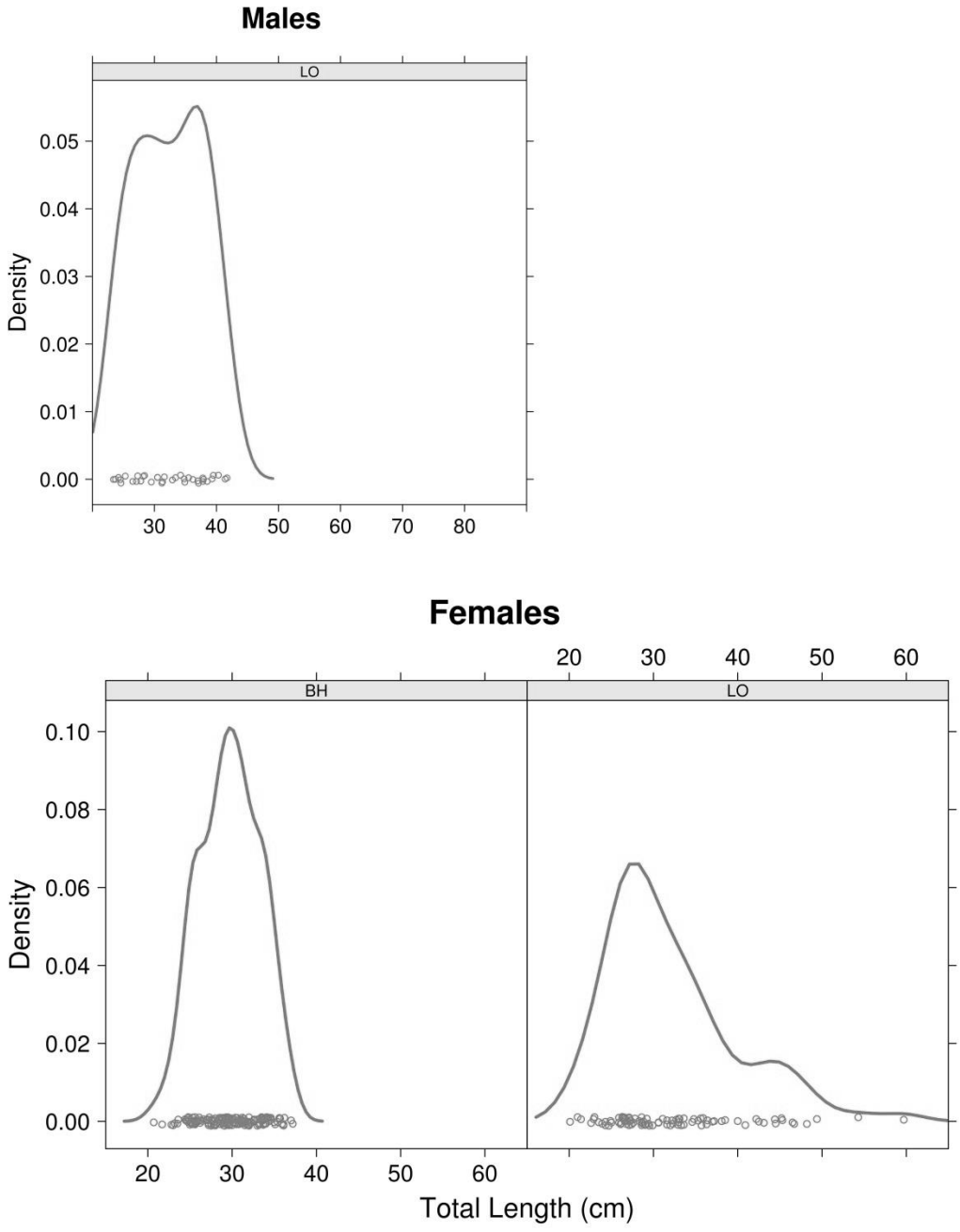
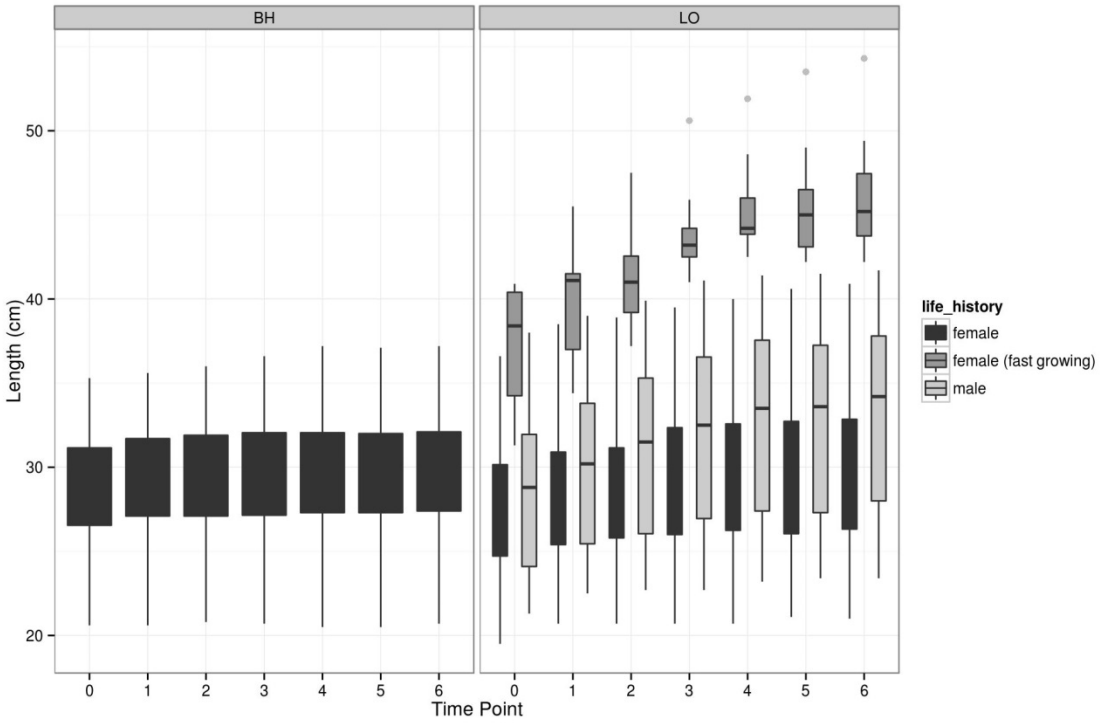

Males
LO
Density
0.05
0.04
0.03
0.02
0.01
0.00
30
40
50
60
70
80
Females
20
30
40
50
60
BH
LO
0.10
0.08
0.06
0.04
0.02
0.00
Density
20
30
40
50
60
Total Length (cm)

Figure 7


BH
LO
Length (cm)
50
40
30
20
0
1
2
3
4
5
6
Time Point
life_history
female
female (fast growing)
male