



Regional variation in energy storage strategies in American glass eels from Eastern Canada



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ABSTRACT

Energy status was analyzed in glass eels captured during two early waves of arrival at the mouths of the Mersey River, Nova Scotia, Canada (MR), and Grande-Rivière-Blanche, Québec, Canada (GRB), and according to their salinity preference (freshwater, brackish, or saltwater). Glass eels captured in the GRB estuary were larger, more pigmented, and exhibited higher whole-body glycogen, phospholipid, and sterol and wax ester contents. Those from MR had a higher condition index and a higher whole-body triacylglycerol content, suggesting different patterns of storage and/or use of energy reserves. Within a river, a delay of two weeks in estuarine arrival was characterized by significantly lower energy reserves. No differences in energy storage were observed according to salinity preference. Thus, the results revealed the occurrence of different energy storage strategies according to glass eel migration distance and duration, but not according to salinity preference.

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1. Introduction

Numerous biological studies have postulated that bioenergetic constraints have shaped migratory strategies for a wide variety of taxa including fishes (Bernatchez and Dodson, 1987; Schultz and Conover, 1997; Jonsson and Jonsson, 1998; Slotte, 1999; Stockwell and Johnson, 1999; Morinville and Rasmussen, 2003; Bureau Du Colombier et al., 2007; Busch et al., 2011; Hasler et al., 2012), birds (Johnston and McFarlane, 1967; Wiens and Innis, 1974), and insects (Roff, 1991; Rankin and Burchsted, 1992). In euryhaline fishes, migration is one of the most energetically demanding physiological processes (Gross et al., 1988).

American eel (*Anguilla rostrata*, Lesueur 1817) must perform extensive migrations during their life cycle. The leptocephalus larvae are carried by Gulf Stream currents for more than 3800 km from the spawning area in Sargasso Sea to the northern portion of their distribution range in coastal regions of Canada (McCleave, 2001; Tesch, 2003). At an overall mean age of 7–9 months, American eels metamorphose into glass eels, which are considered to be the recruitment stage. This major biological transformation triggers the estuarine migration (e.g., Tesch, 2003). Once they reach estuarine areas, glass eels may migrate upstream in rivers (migratory) or settle in salt or brackish water (residents) for feeding (Lamson et al., 2006; Jessop et al., 2008).

While American eel migration has been the subject of numerous studies, clear evidence for facultative catadromy (non-obligatory

trophic migration to fresh water) has only recently been documented. Tsukamoto et al. (1998) were the first to describe a “sea eel” ecophenotype. Daverat et al. (2006) later reported six different patterns of habitat use in temperate eel species, i.e., *A. rostrata*, *Anguilla anguilla*, and *Anguilla japonica*. In eastern Canada, many studies have also demonstrated the presence of different migratory patterns in *A. rostrata* (Cairns et al., 2004; Lamson et al., 2006; Thibeault et al., 2007; Jessop et al., 2012; Clément et al., 2014).

The occurrence of facultative catadromy means that eels may exhibit intra-specific variation in physiological capacities to cope with the different environmental conditions that are encountered. In European eel, facultative catadromy has been partly explained by variation in the threshold reaction norm to individual energetic status (Edeline et al., 2006; Edeline, 2007; Bureau Du Colombier et al., 2011). Thus, individuals most likely to settle in a saltwater habitat (hereafter saltwater ecotype) are characterized by a low condition factor and low thyroid activity but a high level of growth hormone secretion. Such an endocrine profile results in low locomotor activity, decreased sensitivity to odors, low negative rheotaxis, a preference for saltwater, faster growth rate, and settlement in saltwater (Edeline et al., 2005a, 2005b, 2006; Edeline, 2007). In contrast, individuals most likely to settle in fresh water (hereafter freshwater ecotype) are characterized by a high energetic status and high thyroid activity but a low level of growth hormone secretion, which leads to high locomotor activity, high sensitivity to odors, high negative rheotaxis, a preference for fresh water, and a lower growth rate.

Energy availability can be a limiting factor in migration, particularly in species that do not feed during migration or subsist on energetic

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reserves, like lipids, accumulated by the preceding stage (e.g., *Alosa sapidissima*: Leonard and McCormick, 1999). Glass eels may not feed until their entry into estuaries (Charlon and Blanc, 1983; Desaunay and Guerault, 1997). Thus, to sustain their energetic demand, glass eels will catabolize the energy stored by the leptocephali during their ocean migration (*A. japonica*: Kawakami et al., 1999; *A. rostrata*: Tesch, 2003). Leptocephali feed on particulate organic matter such as marine snow, zooplankton fecal pellets, gelatinous zooplankton, larvaceans, and discarded appendicularian houses (Pfeiler, 1999; Riemann et al., 2010; Miller et al., 2013). The nutritional condition of leptocephali, which is affected by food availability, global warming trends, and local continental factors, will affect glass eel survival and development (*A. rostrata*, *A. anguilla*: Desaunay and Guerault, 1997; *A. japonica*: Kawakami et al., 1999; *A. rostrata*, *A. anguilla*, *A. japonica*: Munk et al., 2010; Knights, 2003).

In Canada, American eel is a threatened species (COSEWIC, 2012). Furthermore, the recruitment decline in the St. Lawrence system is far more drastic than on the Atlantic coast, with a reduction of more than 99% from 1986 to 2012 in the St. Lawrence system compared to 39% from 1993 to 2009 in Scotia-Fundy (Cairns et al., 2014). This is of major concern because this portion of the species, which is panmictic (Côté et al., 2013), is believed to have been the major source of female reproductive output before this decline (Castonguay et al., 1994; Cairns et al., 2007; Dutil et al., 2009). Edeline (2007) developed a theoretical model based on the “conditional evolutionarily stable strategy” model, which predicts that the proportion of migrants in the population would decrease with decreased overall recruitment.

As stated above, different migratory patterns have been observed in Atlantic Canada. In the Maritimes, the presence of a saltwater ecotype has been described (Cairns et al., 2004; Jessop et al., 2012; Clément et al., 2014), while the presence of different ecotypes has not yet been investigated in the St. Lawrence estuary. One hypothesis would be that sample origin defines the presence of freshwater vs. saltwater ecotypes. Alternatively, based on Edeline (2005), it could be that ecotypes are represented in both samples but are only revealed by salinity preference experiments. Boivin et al. (2015) compared salinity preference among glass eels captured in four different rivers (two in Nova Scotia and two in Québec) and showed that, among those that showed salinity preference, 60 to 75% of glass eels displayed similar preference for fresh water regardless of their geographic origin. However, controlled experiments have revealed the occurrence of growth variations and gene expression as a function of salinity conditions among regions, supporting the hypothesis of spatial variation in selection between glass and yellow eels from different origins even though the species is panmictic (Côté et al., 2009, 2014, 2015; Boivin et al., 2015). Moreover, a recent population genomics study by Pavey et al. (2015) recently provided strong evidence for genetic differentiation between yellow eels occupying brackish vs. eels occupying freshwater.

In this context, the objectives of this study were to determine how the energetic profile would influence migration distance (Nova Scotia vs. St. Lawrence estuary). We also tested the hypothesis that differences in condition and energy status would determine salinity preference, with high energy reserves being associated with a preference for fresh water. To do so, we examined glycogen and lipid profiles, two biochemical sources of energy used by different stages of fish larvae (*Sciaenops ocellata*: Vetter et al., 1983; *A. sapidissima*: Leonard and McCormick, 1999; *Onchorhynchus kisutch* and *Onchorhynchus tshawytscha*: Trudel et al., 2005; *Pseudopleuronectes americanus*: Fraboulet et al., 2010, 2011). Lipid class characterization is a powerful tool to identify energy reserves when energetic macromolecules are not clearly identified. It has been widely demonstrated that triacylglycerol (TAG), which is made up of three fatty acids that esterify to a glycerol backbone, is a common storage lipid in fishes, but other neutral lipids like wax ester, which have only one fatty acid that esterifies to a fatty alcohol, could play a role (Budge et al., 2006). Such information will improve our understanding of diadromous behavior and the migration strategy used

by American glass eels. This will allow appropriate management strategies to be developed that—it is hoped—will lead to stock recovery.

2. Material and methods

2.1. Fish collection

Glass eels were captured ($n = 4822$) in the estuaries of two east coast Canadian rivers: from a commercial elver fishery in the Mersey River, Nova Scotia, on 26–28 March and 20–21 April 2012 and from Grande-Rivière-Blanche, Québec, on 2–6 and 18–21 June 2012 (Figs. 1 and 2). These periods represent the early arrival of glass eels in this area (Côté et al., 2013). Glass eel captures began two hours before the nighttime high tide and lasted for three hours. Samplers waded into river mouths and captured eels using dip-nets and headlamps. Glass eels were transferred by car to the wet-lab facility at Maurice-Lamontagne Institute (IML; Fisheries and Oceans Canada) in buckets containing water from the estuary. The introduction and transfer of glass eels between provinces were done under conditions specified in the license obtained from Fisheries and Oceans Canada. Salinity preference tests were done upon arrival at IML and individuals tested by Boivin et al. (2015) were used in the present study.

Following salinity preference determination (see Boivin et al., 2015 for a complete description of the methodology), a total of 120 glass eels were sampled for analyses: 30 glass eels from each sampling site and sample date (total of 60 for each river) including 10 with a preference for fresh water, 10 with a preference for brackish water, and 10 with a preference for salt water for each river and each sample date (Fig. 2). Fish were individually anesthetized in an aqueous solution of MS-222 (0.68 mM l^{-1} of ethyl 3-aminobenzoate methanesulfonate; Sigma-Aldrich) in a Petri dish. Total body length (from the tip of the snout to the tip of the caudal fin; $\pm 1 \text{ mm}$) and wet mass ($\pm 1 \text{ mg}$) were measured. Pigmentation stage was identified according to Haro and Krueger (1988). Glass eels were rinsed with brackish water, gently blotted dry, and transferred to 1.5 ml Eppendorf tubes that were immediately placed on dry ice. Samples were kept frozen ($-80 \text{ }^\circ\text{C}$) until analysis.

2.2. Homogenates

For each sample, the whole glass eel was cryogenically ground using a stainless 12 mm \varnothing grinding bead in a Mixer Mill MM 400 (Retsh, Germany). The grinding bead was immersed for 30 s in liquid nitrogen before being transferred to the Mixer Mill for 1 min at a frequency of 12 Hz; each individual was ground twice. The homogenization equipment was cleaned with ethanol and rinsed with MilliQ water between samples. Ground tissue was transferred to Eppendorf tubes containing 0.8 ml ice-cold 10 mM phosphate buffer (pH 7.4) and stored at $-80 \text{ }^\circ\text{C}$.

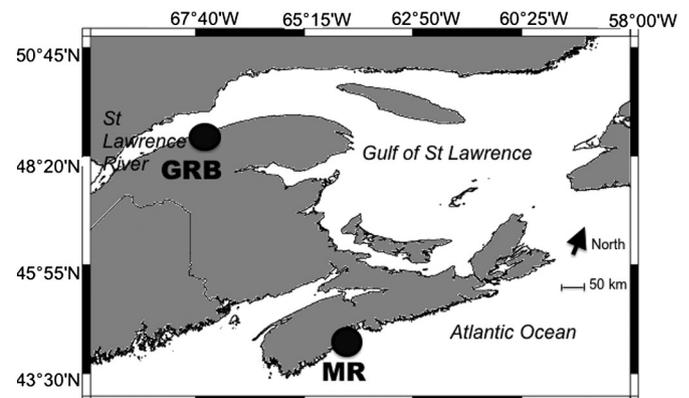


Fig. 1. River estuaries where glass eel were sampled for this study. Grande-Rivière-Blanche (GRB), $48^{\circ}47' \text{ N}$, $67^{\circ}41' \text{ W}$; Mersey River (MR), $44^{\circ}02' \text{ N}$, $64^{\circ}42' \text{ W}$.

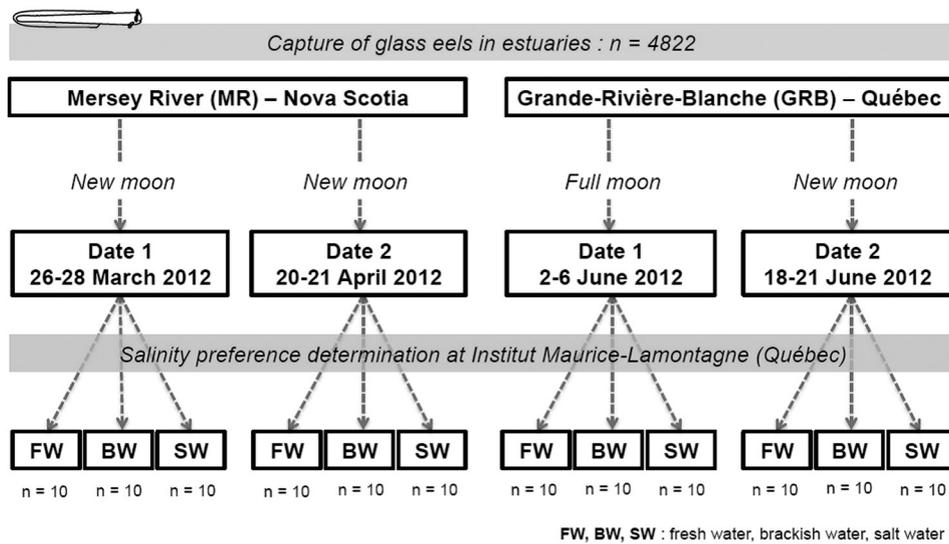


Fig. 2. Experimental design.

2.3. Analyses

The Le Cren condition index (Kn), which is independent of size (Le Cren, 1951), was used because American glass eel growth is not isometric ($a_c \neq 3$) (Fig. 3). The index is calculated as follows:

$$Kn = W_m (aL^b)^{-1}$$

where W_m is wet mass, L is total length, and a and b are empirically determined constants. The a and b constants were obtained by fitting a linear regression through \log_{10} transformed length and mass data, which resulted in the following equation:

$$\log_{10} W_m = -4.95 + 2.33 \log_{10} L; r^2 = 0.47; n = 195.$$

Glycogen was measured using the quantitative enzyme assay described by Carr and Neff (1984) using a microplate reader (VMAX, Molecular Devices) at 414 nm. Lipids were extracted according to the Folch et al. (1957) procedure modified by Parrish (1999). The relative proportions of the different lipid classes (hydrocarbons [HC], sterol [SE] and wax esters [WE], ketones [KET], triacylglycerols [TAG], free fatty acids [FFA], acetone-mobile polar lipids [AMPL], and phospholipids [PL]) were determined using an Iatrosan Mark-VI analyzer (Iatron

Laboratories Inc., Tokyo, Japan) and were developed in a four-solvent system (Parrish, 1987, 1999). Lipids were extracted from 0.6 ml of homogenate with 4 ml of a chloroform-methanol (2:1) solution in a glass Dounce tissue homogenizer followed by the addition of 1.5 ml of KCl. The organic phase was collected after each of two centrifugations (2 min at 2000 rpm), evaporated under nitrogen flux at 35 °C, resuspended in 0.250 ml of chloroform, and stored at -80 °C. Extracts and the standard were spotted onto silica gel-coated chromarods (SIII; Shell USA), and lipid classes were separated using four different solvents and then quantified by thin-layer chromatography using flame ionization detection (Iatrosan MK-6, Shell USA). Lipid class peaks were quantified with PeakSimple software version 3.21 (SRI, Inc.), and lipid classes were identified and quantified using standard calibration curves obtained for each standard (Sigma Chemicals, Inc.). In addition, each analysis run included one composite standard in one of the 10 rods available, as suggested by Parrish (1987). Lipid classes were measured as $\mu\text{g}/\text{mg}$ of wet mass, summed to obtain total lipids, and expressed as percentage of total lipids.

2.4. Statistical analyses

The effect of river and date of capture on wet mass, length, Kn, glycogen concentration, and total lipid concentration were analyzed with two-way ANOVAs ($\alpha = 0.05$) using STATISTICA v6.0 software (www.statsoft.com). Significant differences were identified with Tukey's multiple comparison tests ($p < 0.05$). Normality and homoscedasticity of data were verified with the Kolmogorov-Smirnov and Levene tests, respectively. The effect of salinity (experimental data) was analyzed using one-way ANOVA for glass eels originating from the same river and same date of capture to isolate the effect of salinity. Three-way ANOVAs could not be used because of capture differences from site to site. Quantitative pigmentation index data were analyzed with the nonparametric Kruskal-Wallis test. Lipid classes were analyzed separately using three-way PERMANOVA ($p < 0.05$) with 9999 permutations based on a Bray-Curtis matrix (river, date of capture, salinity preference). A posteriori comparisons were done using a PERMANOVA pairwise test. To analyze the similarity between profiles, non-metric multi-dimensional scaling (n-MDS) and Simper analyses were performed with Primer 6.1.1.12 and PERMANOVA + 1.0.2. Percentage data (lipid classes) were arcsine transformed (Sokal and Rohlf, 1995). When significant effects were found, variations of these effects were illustrated by two-way ANOVAs on arcsine-transformed data. Relationships between Kn and four proxies of energy content (glycogen, total lipids, triacylglycerols, and sterol and wax

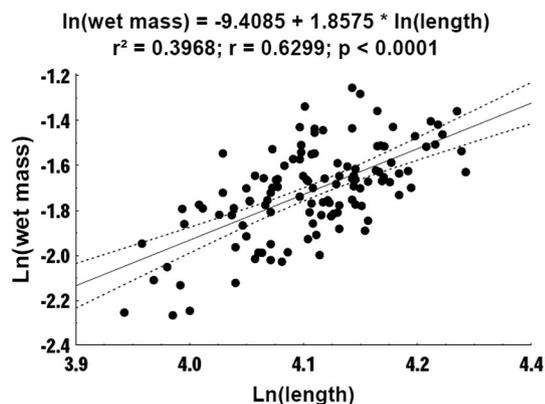


Fig. 3. Linear regression of the biometric relationship in American glass eel between length (mm) and wet mass (g) on an ln–ln axis. The figure shows the fitted regression line and 95% confidence intervals (dashed lines); the regression equation, coefficient of determination (r^2), correlation coefficient (r), and p -value are also given.

esters; expressed in μg per mg of wet mass) were analyzed by linear regression ($\alpha = 0.05$).

3. Results

3.1. Comparison between rivers and dates of capture

Date of capture or origin did not influence the wet mass or total lipid content of individuals (Table 1). However, those that arrived later were more pigmented (Fig. 4A; $p < 0.001$), and glass eels from GRB were longer (Table 1) and more pigmented (Fig. 4B; $p = 0.027$). Moreover, the Kn of glass eels entering MR was higher than that of eels entering GRB. Kn increased with time of capture in MR but not in GRB (Table 1).

The glycogen content of MR glass eels was similar between capture dates. However GRB glass eels captured during the first sampling period had significantly more glycogen than those captured two weeks later (Table 1), and their glycogen content was significantly higher than MR for both dates.

PL and TAG were the two main lipid classes present in *A. rostrata* glass eels followed by ST and SE-WE (Table 1). TAG, PL, and SE-WE altogether explained more than 75% of the dissimilarities between river and date of capture (Table 2); TAG alone explained near 40%. Indeed, TAG were significantly higher in glass eels from MR than in those from GRB, with correspondingly lower PL and SE-WE contents since the content of total lipids was similar between origins (Table 1). For both rivers, glass eels that entered the estuary earlier in the season had significantly more TAG and SE-WE than those arriving later (Table 2).

3.2. Comparison among glass eels exhibiting different salinity preferences

Few differences were observed among glass eels exhibiting different salinity preferences. For each river and for each date of capture, wet mass and total lipid content were similar for glass eels with different salinity preferences (Table 3). Significant differences in length for glass eels with different salinity preferences were only observed in glass eels from MR during the second sampling session, with glass eels exhibiting a preference for freshwater being longer than those with a preference for brackish water. In glass eels from MR arriving earlier, those that preferred salt water had a higher Kn than those preferring brackish water. Moreover, those preferring fresh water had higher glycogen content than those preferring brackish water (Table 3). No differences in lipid class profiles were observed (Table 2).

3.3. Condition index and energy reserves

Overall, Kn was significantly correlated with different proxies of energy content, but correlation coefficients were low (Fig. 5). Surprisingly,

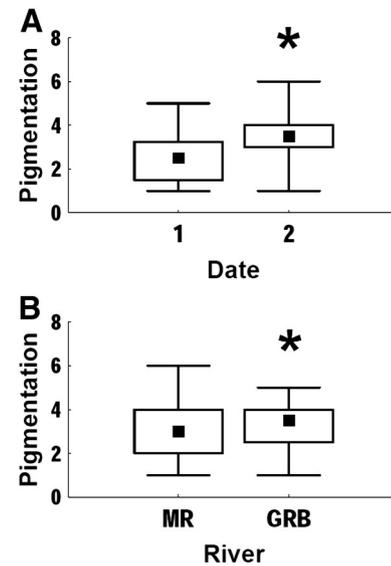


Fig. 4. Kruskal–Wallis results on pigmentation stage for each date (A) and each river (B) represented by boxplot figures. Asterisks indicate significant differences between rivers or dates of capture. Boxplots show minimum and maximum values, 25–75% rectangles, and the median. GRB: Grande-Rivière-Blanche; MR: Mersey River.

Kn was negatively correlated with glycogen and SE-WE contents (Fig. 5A; 5D). There was no relationship between total lipid content and Kn (Fig. 5B). However, Kn was positively correlated with TAG content (Fig. 5C).

4. Discussion

The aim of this study was 1) to verify whether differences in energetic status might be related to differences in migration distance, migration duration, or salinity preference of glass eels and 2) to determine whether the energetic status can reveal information on the physiological processes underlying the differentiation of marine or freshwater ecotypes. The results revealed the occurrence of different energy storage strategies according to migration distance and duration, but not according to salinity preference.

4.1. Comparisons between rivers and dates of capture

Within a river, wet mass was similar between capture dates, suggesting that glass eels arriving later in the river estuary did not experience greater migration costs; this was true for both MR and GRB glass eels. Dutil et al. (2009) estimated that one to two months were required for glass eels to transit from Cabot Strait to the St. Lawrence estuary, and

Table 1
Results of two-way ANOVA (River, Date, River \times Date) on wet mass (g), length (mm), Le Cren condition index (Kn), glycogen content ($\mu\text{g mg}^{-1}$ of wet mass), total lipids ($\mu\text{g mg}^{-1}$ of wet mass), and relative proportions (% of total lipids) of triacylglycerols (TAG), phospholipids (PL), sterol and wax esters (SE-WE), and sterols (ST) in glass eels captured in two rivers (Mersey River: MR, Grande-Rivière-Blanche: GRB) at first arrival (MR 1, GRB 1) and at the next spring tide (MR 2, GRB 2). Mean \pm SE. Bold characters indicate significant differences between rivers, bold italic characters indicate significant differences between dates of capture, and different superscript letters indicate significant interactions between factors were present. ns: no significant difference.

	MR 1	MR 2	GRB 1	GRB 2	Effect River, Date, or River \times Date
	N = 30	N = 29	N = 30	N = 29	
Wet mass	0.18 \pm 0.01	0.20 \pm 0.01	0.18 \pm 0.01	0.18 \pm 0.01	ns
Length	60.10 \pm 0.70	61.28 \pm 0.64	65.49 \pm 0.58	65.56 \pm 0.72	GRB > MR, $p < 0.0001$
Kn	1.04 \pm 0.03 ^b	1.13 \pm 0.03 ^a	0.92 \pm 0.02 ^c	0.89 \pm 0.02 ^c	River \times Date, $p < 0.05$
Glycogen	0.23 \pm 0.04 ^c	0.28 \pm 0.05 ^c	0.88 \pm 0.07 ^a	0.70 \pm 0.07 ^b	River \times Date, $p < 0.05$
Total lipids	7.91 \pm 1.93	6.32 \pm 0.46	5.85 \pm 0.36	4.86 \pm 0.33	ns
TAG (%)	45.17 \pm 1.98	42.19 \pm 1.83	15.03 \pm 1.96	7.88 \pm 1.81	MR > GRB, $p < 0.0001$; 1 > 2, $p < 0.01$
PL (%)	28.80 \pm 1.11	28.08 \pm 1.58	48.43 \pm 1.24	50.48 \pm 1.42	GRB > MR, $p < 0.0001$
SE-WE (%)	6.53 \pm 1.59	10.39 \pm 0.62	13.29 \pm 0.63	15.75 \pm 0.55	GRB > MR, $p < 0.0001$; 2 > 1, $p < 0.05$

Table 2

Results of three-way PERMANOVA, average similarity, average dissimilarity, and dissimilarity contributions greater than 10% in lipid profiles. River: Mersey (MR), Grande-Rivière-Blanche (GRB); Date of capture: first week of arrival and two weeks later; salinity preference: fresh, salt or brackish water. TAG: triacylglycerols; PL: phospholipids; SE-WE: sterol and wax esters. Bold: significant differences.

Source	Df	Pseudo-F	P (perm)	Average similarity (%)	Average dissimilarity (%)	Dissimilarity contribution (>10%)
River	1	260.60	0.0001	MR (82.61) GRB (84.47)	40.43	TAG (40.30) PL (26.46) SE-WE (12.61)
Date	1	8.84	0.0005	Date 1 (73.05) Date 2 (70.14)	28.73	TAG (39.00) PL (25.66) SE-WE (14.39)
Salinity	2	2.00	0.0922	–	–	–
River × Date	1	0.64	0.5598	–	–	–
River × Salinity	2	0.38	0.8438	–	–	–
Date × Salinity	2	0.33	0.8748	–	–	–
River × Date × Salinity	2	1.51	0.1916	–	–	–
Residuals	106	75.31				

this is exactly the delay observed between captures in MR and GRB. However, glass eels at GRB were longer and more pigmented than those from MR, indicating that they were nearer the elver stage and perhaps beginning the transformation to the yellow eel stage. It has been shown that American glass eel length increases with migration distance (Haro and Krueger, 1988; Laflamme et al., 2012) and that upstream migration is more costly for smaller individual (Weihs, 1977). The results from the present study agree with those obtained in the more southern part of this species' distribution area, where a very low pigmentation index was found in glass eels entering river estuaries in Florida (Sullivan et al., 2009). This is not specific to American eel: numerous studies have shown that glass eels of different species are older and longer at recruitment relative to distance from the breeding site (European glass eels: Naismith and Knights, 1988; Japanese glass eels: Tsukamoto and Umezawa, 1990; American and European glass eels: Wang and Tzeng, 2000).

The results indicate that wet masses were similar between capture dates. Similarly, Bureau du Colombier et al. (2011) observed no differences in wet mass in recently captured and starved European glass eels following 28 days spent at different salinities. Moreover, there is a general pattern in migratory fishes that those species (and populations within species) that make difficult and long migrations are larger and use their energy reserves more efficiently than those that make short migrations (Bernatchez and Dodson, 1987). This could possibly explain the observed differences between GRB and MR glass eels. The other explanation would be that feeding resumed with the development of pigmentation (Tesch, 2003).

In European silver eels, migration success depends on the amount of lipids stored during the growth phase (Boëtius and Boëtius, 1985). However, while we found no significant difference in total lipids, there were significant differences in lipid class composition depending on the river of origin, notably in the relative proportions of TAG followed by PL. Neutral lipids, and especially TAG, are generally the preferred source of metabolic energy in marine fishes for growth, reproduction, and swimming, particularly for the first ontogenic stages (Tocher et al., 2008). Lipids can be obtained from either external or internal body sources, and they may or may not be correlated to body mass. In the present study, neither total lipids nor mass varied with the river of origin, so glass eels were either eating during their migration to river estuaries or they had sufficient reserves to sustain their migration to the sampling sites. However, since our results are expressed relative to wet mass, they cannot take into account the possible effects of changes in water content. Another hypothesis is that glass eels captured in GRB could have had more lipids at the beginning of migration that would have been used during their transit from the Sargasso Sea to the St. Lawrence estuary. Indeed, GRB glass eels arrived one month later than the MR eels.

If it is assumed that glass eels do not eat during the estuarine migration (Charlon and Blanc, 1983; Tesch, 2003), lower energy reserves in GRB glass eels would be expected. In fact, there was a lower proportion of TAG, the main lipid reserve. This is consistent with previous studies done on unfed larvae that showed either lipid depletion or specific TAG depletion with time (e.g., Glencross, 2009). TAG constitutes a pool of energy reserves in marine fishes and is considered as the most

Table 3

ANOVA results for salinity preference for each river and date of capture on wet mass (g), length (mm), Le Cren condition index (Kn), glycogen content ($\mu\text{g mg}^{-1}$ of wet mass), and total lipids ($\mu\text{g mg}^{-1}$ of wet mass). Mean \pm SE. Different letters indicate significant differences among salinities. FW: Freshwater preference; SW: Saltwater preference; BW: Brackish water preference; ns: no significant difference.

	Mersey River—Date 1				Mersey River—Date 2			
	FW	SW	BW		FW	SW	BW	
	N = 10	N = 10	N = 10		N = 9	N = 10	N = 10	
Wet mass	0.17 \pm 0.01	0.19 \pm 0.01	0.17 \pm 0.01	ns	0.22 \pm 0.01	0.20 \pm 0.01	0.18 \pm 0.01	ns
Length	60.1 \pm 1.31	59.8 \pm 0.91	60.39 \pm 1.49	ns	63.1 \pm 0.76 ^a	61.6 \pm 1.23 ^{ab}	59.3 \pm 1.0 ^b	p < 0.05
Kn	1.02 \pm 0.03 ^{ab}	1.13 \pm 0.05 ^a	0.96 \pm 0.05 ^b	p < 0.05	1.19 \pm 0.04	1.13 \pm 0.06	1.07 \pm 0.05	ns
Glycogen	0.38 \pm 0.07 ^a	0.18 \pm 0.06 ^{ab}	0.12 \pm 0.06 ^b	p < 0.05	0.31 \pm 0.09	0.33 \pm 0.09	0.22 \pm 0.06	ns
Total lipids	6.75 \pm 0.81	5.59 \pm 0.79	11.41 \pm 5.72	ns	7.20 \pm 0.65	6.05 \pm 0.72	5.81 \pm 0.96	ns
	Grande-Rivière-Blanche—Date 1				Grande-Rivière-Blanche—Date 2			
	FW	SW	BW		FW	SW	BW	
	N = 10	N = 10	N = 10		N = 10	N = 10	N = 10	
Wet mass	0.19 \pm 0.01	0.18 \pm 0.01	0.18 \pm 0.01	ns	0.18 \pm 0.01	0.18 \pm 0.01	0.19 \pm 0.01	ns
Length	65.5 \pm 0.80	65.9 \pm 1.19	65.1 \pm 1.09	ns	65.6 \pm 1.30	65.8 \pm 1.25	65.3 \pm 1.31	ns
Kn	0.95 \pm 0.04	0.90 \pm 0.02	0.90 \pm 0.03	ns	0.88 \pm 0.03	0.87 \pm 0.03	0.92 \pm 0.03	ns
Glycogen	0.86 \pm 0.08	0.89 \pm 0.19	0.88 \pm 0.08	ns	0.81 \pm 0.17	0.67 \pm 0.10	0.62 \pm 0.09	ns
Total lipids	6.18 \pm 0.38	5.85 \pm 0.92	5.53 \pm 0.50	ns	4.13 \pm 0.40	5.56 \pm 0.64	4.81 \pm 0.58	ns

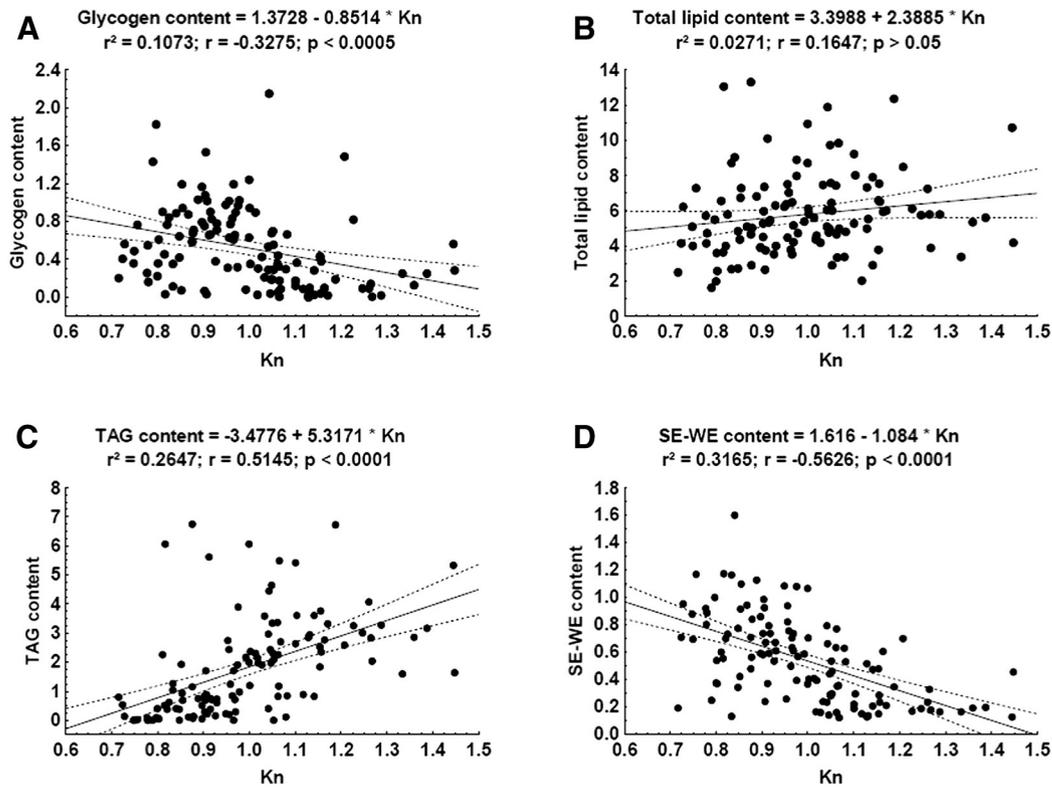


Fig. 5. Glycogen (A), total lipid (B), tryacylglycerol (TAG) (C), and sterol and wax ester (SE-WE) (D) contents in relation to the Le Cren condition index (Kn). Data are expressed as μg of mg of wet mass. The coefficient of determination (r^2), correlation coefficient (r), and p -values are shown.

efficient nutrient for maximizing energy production (e.g., Glencross, 2009). Bernatchez and Dodson (1987) showed that energy efficiency increases with increased migratory distance, thus the preferential use of TAG at GRB could be explained by a greater efficiency of energy use in those glass eels migrating further north.

The relative percentages of the different lipid fractions vary greatly in starved larvae depending on species, life stage, and environmental conditions (e.g., fishes: Rainuzzo et al., 1997; Turchini et al., 2009). In unfed *Solea senegalensis* larvae, weight loss is due to lipid catabolism and lipid depletion since these larvae preferentially consume neutral lipids during development (Mourente and Vázquez, 1996). Unfed larvae of Atlantic bonito, *Sarda sarda*, gained dry mass and lost lipid content, mainly TAG and SE, during development (Ortega and Mourente, 2010). In turbot, *Scophthalmus maximus*, a rapid decrease in lipids with simultaneous reduction in the dry weight occurred in unfed larvae, and SE and TAG fractions were preferentially catabolized (Rainuzzo et al., 1997). Lipid depletion with specific catabolism of TAG was also observed during the migration of starved lamprey larvae, *Petromyzon marinus* (Kao et al., 1997).

Since TAG were preferentially used, it is somewhat surprising that there was no change in wet mass. In Japanese glass eels, wet weight was shown to be correlated to the lipid content of the peritoneal cavity, and this relationship was suggested as a useful way to estimate nutritional status (Kawakami et al., 1999). The same authors also observed that glass eels that arrived first at river mouths had higher mass than those that arrived two months later. A correlation between the percentage of body fat and eel size was also found in adult American eels (Gallagher et al., 1984), and lipid percentage was higher in larger European eels than in smaller ones (Degani, 1986). In the present study, the replacement of storage lipids by structural ones may explain the absence of wet mass differences.

The PL and SE-WE fractions were higher in GRB glass eels. In early juvenile fish, PL improve growth as well as survival rate and stress resistance (Glencross, 2009; Tocher et al., 2008). PL are mainly used as

structural elements of biological membranes, so this could explain why this fraction is more important in more developed and longer glass eels. In copepods, reef corals, and several fishes, WE can be used as metabolic energy reserves (Lee et al., 1971; Rahn et al., 1973; Figueiredo et al., 2012), and WE metabolism may be linked with TAG metabolism since tryacylglycerol lipases act on WE (e.g., Tocher, 2003). SE fractions have not been extensively studied in fishes, but they could be catabolized as energy reserves in the same way as TAG or WE (e.g., Ortega and Mourente, 2010) while also being structural components of the cell architecture. Similar trends for SE-WE and PL fractions were observed, i.e., a greater proportion in more developed GRB glass eels along with a decrease in TAG, thus it is suggested that the changes in proportions observed in the present study would probably be more related to the structural role of SE.

Glycogen content was more than twice as high in GRB glass eels, suggesting that they preferentially oriented their metabolism to glucose conservation. In European glass eels, Degani et al. (1986) showed that lipids are preferred to carbohydrates to sustain metabolic needs. In adults, Larsson and Lewander (1973) revealed the utilization of liver and muscle triglycerides as energy sources and for the stimulation of gluconeogenesis, both of which increased in later phases of starvation. Moon (1983) suggested a minor role of carbohydrates in the fasting period of immature American eels, as shown by a decline in glycogen phosphorylase activity. Jedryczkowski (1979) and Degani et al. (1986) also showed that glycolysis efficiency in European eel was lower in freshwater during early development based on changes in aldolase activity. Differences in the relative proportion of palmitic acid in fatty acids were identified between freshwater and marine fishes (Ackman, 1967), thus glass eels from GRB may have a strategy close to freshwater fishes. However, fatty acid analyses are needed to confirm this.

Glucose is essential to sustain oxidative metabolism in specific cells such as nervous tissue. TAG metabolism may help maintain glucose levels through gluconeogenesis and glycogen synthesis pathways, or glucose stocks may be preserved through energy production sustained

by fatty acids or ketones to the β oxidation pathway (e.g., Tocher, 2003; McCue, 2010). Thus, having high glycogen storage coupled with a reduced TAG proportion seems plausible. As reviewed by McCue (2010), the ability to recover glycogen storages could differ as starvation progresses or be linked to a difference in the ability to endure a greater period of starvation and to prioritize metabolic costs in specific organs and tissues.

The presence of differences in energy stores deserves further investigation. Indeed, despite panmixia, a latitudinal cline in allele frequencies was observed in genes encoding for enzymes related to energetic metabolism, including sorbitol dehydrogenase, alcohol dehydrogenase, and phosphohexose isomerase, in American glass eels captured from Florida to Newfoundland (Koehn and Williams, 1978). More recently, Gagnaire et al. (2012) identified several genes that had spatially varying selection associated with habitat heterogeneity (three genes associated with lipid metabolism, two with saccharide metabolism, three with protein biosynthesis, three with defense response, and one with molecular function). This observation suggests that glass eels colonizing different areas of the geographical range, which are characterized by different physico-chemical characteristics, are exposed to differential patterns of selection. Moreover, adaptation to the water temperature gradient encountered in river estuaries from south to north would be relevant in variants of genes implicated in metabolism (Gagnaire et al., 2012). More recently, Pavay et al. (2015) performed a genome-wide association study that demonstrated a polygenic basis that discriminates American eels from freshwater and brackish water habitats. They found that 331 co-varying loci out of 42,424 were associated with the divergent ecotypes. These 331 SNPs are associated with 101 genes that represent vascular and morphological development, calcium ion regulation, growth and transcription factors, and olfactory receptors. Finally Côté et al. (2014) also showed that gene \times environment interactions may explain growth differences between MR and GRB yellow eels since differences were found in the expression of genes related to energy metabolism, energy respiration, growth, differentiation, and development.

Within a river, a delay of two weeks in estuarine arrival was characterized by significantly lower energy reserves. In GRB, TAG and glycogen contents were lower in fish captured later in the season while SE–WE increased and body condition, total lipid content, and wet mass remained constant. This again supports the hypothesis of the use of TAG and carbohydrates to sustain metabolism and a structural role for the lipids found in the SE–WE fraction in this particular region.

MR glass eels arriving later also showed lower proportions of TAG and higher SE–WE contents, but their glycogen content was similar and Kn was higher than those in GRB glass eels. This indicates a difference in the use of metabolic reserves between the two areas. The patterns of Kn are difficult to explain in the absence of changes in total lipids and a decrease of storage lipids. The use of dry mass to express total lipids could have circumvented this.

4.2. Comparison between glass eels exhibiting different salinity preferences

One of the main objectives of this study was to verify if energy status could be associated with habitat selection. A worldwide decline in freshwater eel recruitment is occurring, and settlement in saltwater environments is apparently increasing in American and European eels (e.g., Lambert, 2005; McCleave and Edeline, 2009). Behavior experiments using MR and GRB glass eels allowed the identification of active glass eels, which had a preference either for freshwater or saltwater, and inactive eels, which had a preference for brackish water (Boivin et al., 2015). Here, it was tested whether different salinity preferences could be correlated with specific energy status. Indeed, fatty acid requirements (e.g., Glencross, 2009), digestibility, transport, uptake, elongation and desaturation processes, and β -oxidation of fatty acids (e.g., Turchini et al., 2009) should be considered when looking at body lipid composition, but it may also be affected by abiotic factors including

water salinity, temperature, and light (e.g., Dantagnan et al., 2013). Thus, salinity affects fish metabolism (Sampekalo et al., 1992), and differences in energy stores in glass eels could explain the occurrence of different metabolic strategies between the ecotypes considered.

Based on the conditional evolutionarily stable strategy suggested for European eel, in which migration in freshwater or saltwater at recruitment depends on the individual's energetic and thyroid status, freshwater glass eels should have a high energetic status and high thyroid activity, which would result in freshwater preference, low growth rate, and high migratory activity in contrast with saltwater glass eels (American yellow eel: Castonguay et al., 1990; European glass eel: Edeline et al., 2004, 2005a, 2005b; Edeline, 2007; European elver and yellow eel: Imbert et al., 2008). Then lower energy reserves and larger size in glass eels with saltwater preference would have been expected compared to those preferring fresh water. Not only there was no difference based on salinity preference, but the river differences also did not support this hypothesis for American glass eels since the freshwater ecotype would be expected to be more frequent in GRB and the marine ecotype more frequent on the Atlantic coast (i.e., MR). It should be remembered that energetic status differences in European eel were suggested from condition factor data (Edeline et al., 2006; Bureau Du Colombier et al., 2011). It is plausible that the dichotomy between freshwater and marine ecotype in our system would be better reflected by geographical differences rather than salinity preferences. Because condition factor did not differ between rivers, it is very difficult to make such comparisons with data on European eel. However, the present results are consistent with those obtained by Boivin et al. (2015), who observed no relationship between salinity preference and body condition in American eel, but observed differences in growth between origins under controlled conditions.

5. Conclusion

These results on American eel did not support the hypothesis of conditional strategy, i.e., that migration in freshwater or saltwater at recruitment depends on the individual's energetic status. Instead, the presence of higher carbohydrate content and differences in lipid storage and/or use of different lipid classes corroborate the occurrence of genetic differences between habitats and related to sites colonized by glass eels. How differences observed between rivers and dates of capture may affect glass eel survival and recruitment is unknown, but it certainly deserves further attention.

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References

- Ackman, R.G., 1967. Characteristics of the fatty acid composition and biochemistry of some fresh-water fish oils and lipids in comparison with marine oils and lipids. *Comp. Biochem. Physiol.* 22, 907–922.
- Bernatchez, L., Dodson, J.J., 1987. Relationship between bioenergetics and behavior in anadromous fish migrations. *Can. J. Fish. Aquat. Sci.* 44, 399–407.
- Boëtius, I., Boëtius, J., 1985. Lipid and protein content in *Anguilla anguilla* during growth and starvation. *Dana* 4, 1–17.

- Boivin, B., Castonguay, M., Audet, C., Pavey, S., Dionne, M., Bernatchez, L., 2015. How does salinity influence habitat selection and growth in juvenile American eels *Anguilla rostrata*? *J. Fish Biol.* 86, 765–784.
- Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar. Mamm. Sci.* 22, 759–801.
- Bureau Du Colombier, S., Bolliet, V., Lambert, P., Bardonnet, A., 2007. Energy and migratory behavior in glass eels (*Anguilla anguilla*). *Physiol. Behav.* 92, 684–690.
- Bureau Du Colombier, S., Bolliet, V., Lambert, P., Bardonnet, A., 2011. Metabolic loss of mass in glass eels at different salinities according to their propensity to migrate. *Estuar. Coast. Shelf Sci.* 93, 1–6.
- Busch, S., Johnson, B.M., Mehner, T., 2011. Energetic costs and benefits of cyclic habitat switching: a bioenergetics model analysis of diel vertical migration in coregonids. *Can. J. Fish. Aquat. Sci.* 68, 706–717.
- Cairns, D.K., Chaput, G., Poirier, L.A., Avery, T.S., Castonguay, M., Mathers, A., Casselman, J.M., Bradford, R.G., Pratt, T., Verreault, G., Clarke, K., Veinott, G., Bernatchez, L., 2014. Recovery potential assessment for the American Eel (*Anguilla rostrata*) for eastern Canada: life history, distribution, reported landings, status indicators, and demographic parameters. *DFO Can. Sci. Adv. Sec. Res. Doc.* 2013/134 (xiv + 157 pp.).
- Cairns, D.K., Omilusik, D.L., Leblanc, P.H., Atkinson, E.G., Moore, D.S., McDonald, N., 2007. American eel abundance indicators in the southern Gulf of St. Lawrence. *Can. Data Rep. Fish. Aquat. Sci.* 1192 (iv + 119 pp.).
- Cairns, D.K., Shiao, J.C., Iizuka, Y., Tzeng, W.N., MacPherson, C.D., 2004. Movement patterns of American eels in an impounded watercourse, as indicated by otolith microchemistry. *N. Am. Fish. Manag.* 24, 452–458.
- Carr, R.S., Neff, J.M., 1984. Quantitative semi-automated enzymatic assay for tissue glycogen. *Comp. Biochem. Physiol. B Comp. Biochem.* 77, 447–449.
- Castonguay, M., Dutil, J.-D., Audet, C., Miller, R., 1990. Locomotor activity and concentration of thyroid hormones in migratory and sedentary juvenile American eels. *Trans. Am. Fish. Soc.* 119, 946–956.
- Castonguay, M., Hodson, P.V., Couillard, C.M., Eckersley, M.J., Dutil, J.-D., Verreault, G., 1994. Why is recruitment of the American Eel, *Anguilla rostrata*, declining in the St. Lawrence River and Gulf? *Can. J. Fish. Aquat. Sci.* 51, 479–488.
- Charlon, N., Blanc, J.M., 1983. A study of the elvers (*Anguilla anguilla* L.) in the Adour Basin area. 2. Food intake and variations of biochemical characteristics from the outset of migration. *Arch. Hydrobiol.* 98, 240–249.
- Clément, M., Chiasson, A.G., Veinott, G., Cairns, D.K., 2014. What otolith microchemistry and stable isotope analysis reveal and conceal about anguillid eel movements across salinity boundaries. *Oecologia* 175, 1143–1153.
- COSEWIC, 2012. Committee on the Status of Endangered Wildlife in Canada assessment and status report on the American Eel *Anguilla rostrata* in Canada. Fisheries and Oceans Canada, Ottawa (xii + 109 pp.).
- Côté, C.L., Castonguay, M., Verreault, G., Bernatchez, L., 2009. Differential effects of origin and salinity rearing conditions on growth of glass eels of the American eel *Anguilla rostrata*: implications for stocking programmes. *J. Fish Biol.* 74, 1934–1948.
- Côté, C.L., Gagnaire, P.-A., Bourret, V., Verreault, G., Castonguay, M., Bernatchez, L., 2013. Population genetics of the American eel (*Anguilla rostrata*): FST = 0 and North Atlantic Oscillation effects on demographic fluctuations of a panmictic species. *Mol. Ecol.* 22, 1763–1776.
- Côté, C.L., Castonguay, M., Svetlana McWilliam, K., Cramb, G., Bernatchez, 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* 15, 403–418.
- Côté, C.L., Pavey, S., Stacey, J.A., Pratt, T., Castonguay, M., Audet, C., Bernatchez, L., 2015. Growth, female bimodality and sex ratio variability in American Eel (*Anguilla rostrata*) of different origins in both controlled conditions and the wild: implications for stocking programs. *Trans. Am. Fish. Soc.* 44, 246–257.
- Le Cren, E.D.L., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J. Anim. Ecol.* 20, 201–219.
- Dantagnan, P., Bórquez, A., Pavez, C., Hernández, A., 2013. Feeding ω -3 PUFA enriched rotifers to *Galaxias maculatus* (Jenyns, 1842) larvae reared at different salinity conditions: effects on growth parameters, survival and fatty acids profile. *Lat. Am. J. Aquat. Res.* 41, 404–411.
- Daverat, F., Limburg, K.E., Thibault, I., Shiao, J.C., Dodson, J.J., Caron, F., Tzeng, W.N., Iizuka, Y., Wickström, H., 2006. Phenotypic plasticity of habitat use by three temperate eel species, *Anguilla anguilla*, *A. japonica* and *A. rostrata*. *Mar. Ecol. Prog. Ser.* 308, 231–241.
- Degani, G., 1986. Dietary effects of lipid source, lipid level and temperature on growth of glass eel (*Anguilla anguilla*). *Aquaculture* 56, 207–214.
- Degani, G., Hahamu, H., Levanon, D., 1986. The relationship of eel *Anguilla anguilla* (L.) body size, lipid, protein, glucose, ash, moisture composition and enzyme activity (aldolase). *Comp. Biochem. Physiol. A Physiol.* 84, 739–745.
- Desaunay, Y., Gueraud, D., 1997. Seasonal and long-term changes in biometrics of eel larvae: a possible relationship between recruitment variation and North Atlantic ecosystem productivity. *J. Fish Biol.* 51 (Supplement A), 317–339.
- Dutil, J.D., Dumont, P., Cairns, D.K., Galbraith, P.S., Verreault, G., Castonguay, M., Proulx, S., 2009. *Anguilla rostrata* glass eel migration and recruitment in the estuary and Gulf of St. Lawrence. *J. Fish Biol.* 74, 1970–1984.
- Edeline, E., 2005. Facteurs du contrôle de la dispersion continentale chez l'anguille (Ph.D. thesis), Paul Sabatier Toulouse III (144 pp.).
- Edeline, E., 2007. Adaptive phenotypic plasticity of eel diadromy. *Mar. Ecol. Prog. Ser.* 341, 229–232.
- Edeline, E., Bardonnet, A., Bolliet, V., Dufour, S., Elie, P., 2005a. Endocrine control of *Anguilla anguilla* glass eel dispersal: effect of thyroid hormones on locomotor activity and rheotactic behavior. *Horm. Behav.* 48, 53–63.
- Edeline, E., Dufour, S., Briand, C., Fatin, D., Elie, P., 2004. Thyroid status is related to migratory behavior in *Anguilla anguilla* glass eels. *Mar. Ecol. Prog. Ser.* 282, 261–270.
- Edeline, E., Dufour, S., Elie, P., 2005b. Role of glass eel salinity preference in the control of habitat selection and growth plasticity in *Anguilla anguilla*. *Mar. Ecol. Prog. Ser.* 304, 191–199.
- Edeline, E., Lambert, P., Rigaud, C., Elie, P., 2006. Effects of body condition and water temperature on *Anguilla anguilla* glass eel migratory behaviour. *J. Exp. Mar. Biol. Ecol.* 331, 217–225.
- Figueiredo, J., Baird, A.H., Cohen, M.F., Flot, J.F., Kamiki, T., Meziane, T., Tsuchiya, M., Yamasaki, H., 2012. Ontogenetic change in the lipid and fatty acid composition of scleractinian coral larvae. *Coral Reefs* 31, 613–619.
- Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Fraboulet, E., Lambert, Y., Tremblay, R., Audet, C., 2010. Assessment of paternal effect and physiological cost of metamorphosis on growth of young winter flounder *Pseudopleuronectes americanus* juveniles in a cold environment. *J. Fish Biol.* 76, 930–948.
- Fraboulet, E., Lambert, Y., Tremblay, R., Audet, C., 2011. Growth and lipid composition of winter flounder juveniles reared under natural and fixed photoperiod and temperature conditions. *N. Am. J. Aquac.* 73, 89–96.
- Gagnaire, P.-A., Normandeau, E., Côté, C., Møller Hansen, M., Bernatchez, L., 2012. The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). *Genetics* 190, 725–736.
- Gallagher, M.L., Kane, E., Beringer, R., 1984. Effect of size on composition of the American eel, *Anguilla rostrata*. *Comp. Biochem. Physiol. A Physiol.* 78, 533–536.
- Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. *Rev. Aquac.* 1, 71–124.
- Gross, M.R., Coleman, M.R., McDowall, R.M., 1988. Aquatic productivity and the evolution of diadromous fish migration. *Science* 239, 1291–1293.
- Haro, A.J., Krueger, W.H., 1988. Pigmentation, size, and migration of elvers (*Anguilla rostrata* (Lesueur)) in a coastal Rhode Island stream. *Can. J. Zool.* 66, 2528–2533.
- Hasler, C.T., Cooke, S.J., Hinch, S.G., Guimond, E., Donaldson, M.R., Mossop, B., Patterson, D.A., 2012. Thermal biology and bioenergetics of different upriver migration strategies in a stock of summer-run Chinook salmon. *J. Therm. Biol.* 37, 265–272.
- Imbert, H., Arrowsmith, R., Dufour, S., Elie, P., 2008. Relationships between locomotor behavior, morphometric characters and thyroid hormone levels give evidence of stage-dependent mechanisms in European eel upstream migration. *Horm. Behav.* 53, 69–81.
- Jedryczkowski, W., 1979. Elements of energy balance of eel *Anguilla anguilla* (L.). *Pol. Arch. Hydrobiol.* 29, 159–172.
- Jessop, B.M., Cairns, D.K., Thibault, I., Tzeng, W.N., 2008. Life history of American eel *Anguilla rostrata*: new insights from otolith microchemistry. *Aquat. Biol.* 1, 205–216.
- Jessop, B., Wang, C.-H., Tzeng, W.-N., You, C.-F., Shiao, J.-C., Lin, S.-H., 2012. Otolith Sr:Ca and Ba:Ca may give inconsistent indications of estuarine habitat use for American eels (*Anguilla rostrata*). *Environ. Biol. Fish.* 93, 193–207.
- Johnston, D.W., McFarlane, R.W., 1967. Migration and bioenergetics of flight in the Pacific golden plover. *Condor* 69, 156–168.
- Jonsson, N., Jonsson, B., 1998. Body composition and energy allocation in life-history stages of brown trout. *J. Fish Biol.* 53, 1306–1316.
- Kao, Y.-H., Youson, J., Sheridan, M., 1997. Differences in the total lipid and lipid class composition of larvae and metamorphosing sea lampreys, *Petromyzon marinus*. *Fish Physiol. Biochem.* 16, 281–290.
- Kawakami, Y., Mochioka, N., Kimura, R., Nakazono, A., 1999. Seasonal changes of the RNA/DNA ratio, size and lipid contents and immigration adaptability of Japanese glass-eels, *Anguilla japonica*, collected in northern Kyushu, Japan. *J. Exp. Mar. Biol. Ecol.* 238, 1–19.
- Knights, B., 2003. A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *Sci. Total Environ.* 310, 237–244.
- Koehn, R.K., Williams, G.C., 1978. Genetic differentiation without isolation in the American eel, *Anguilla rostrata*. II. Temporal stability of geographic patterns. *Evolution* 32, 624–637.
- Laflamme, S., Côté, C., Gagnaire, P.-A., Castonguay, M., Bernatchez, L., 2012. RNA/DNA ratios in American glass eels (*Anguilla rostrata*): evidence for latitudinal variation in physiological status and constraints to oceanic migration? *Ecol. Evol.* 2, 875–884.
- Lambert, P., 2005. Exploration multiscalaire des paradigmes de la dynamique de population d'anguilles européennes à l'aide d'outils de simulation Ph.D. thesis, 1. Université Bordeaux (219 pp.).
- Lamson, H., Shiao, J.C., Iizuka, Y., Tzeng, W.N., Cairns, D., 2006. Movement patterns of American eels (*Anguilla rostrata*) between salt- and freshwater in a coastal watershed, based on otolith microchemistry. *Mar. Biol.* 149, 1567–1576.
- Larsson, A., Lewander, K., 1973. Metabolic effects of starvation in the eel, *Anguilla anguilla* L. *Comp. Biochem. Physiol. A Physiol.* 44, 367–374.
- Lee, R.F., Nevenzel, J.C., Paffenhofer, G.A., 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Mar. Biol.* 9, 99–108.
- Leonard, J.B.K., McCormick, S.D., 1999. Effects of migration distance on whole-body and tissue-specific energy use in American shad (*Alosa sapidissima*). *Can. J. Fish. Aquat. Sci.* 56, 1159–1171.
- McCleave, J.D. 2001. Eels. *Encyclopedia of Ocean Sciences* (Elsevier Ltd., 2001) and *Encyclopedia of Ocean Sciences*, 2nd Edition (Elsevier Ltd. 2009). Editors-in-Chief: Steele, J. H., Thorpe, S. A. and Turekian, K. K. Oxford. 262–272. Academic Press.
- McCleave, J.D., Edeline, E., 2009. Diadromy as a conditional strategy: patterns and drivers of eel movements in continental habitats. *Am. Fish. Soc. Symp.* 69, 97–119.
- McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 156, 1–18.

- Miller, M.J., Chikaraishi, Y., Ogawa, N.O., Yamada, Y., Tsukamoto, K., Ohkouchi, N., 2013. A low trophic position of Japanese eel larvae indicates feeding on marine snow. *Biol. Lett.* 9, 20120826.
- Moon, T.W., 1983. Metabolic reserves and enzyme activities with food deprivation in immature American eels, *Anguilla rostrata* (LeSueur). *Can. J. Zool.* 61, 802–811.
- Morinville, G.R., Rasmussen, J.B., 2003. Early juvenile bioenergetic differences between anadromous and resident brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* 60, 401–410.
- Mourente, G., Vázquez, R., 1996. Changes in the content of total lipid, lipid classes and their fatty acids of developing eggs and unfed larvae of the Senegal sole, *Solea senegalensis* Kaup. *Fish. Physiol. Biochem.* 15, 221–235.
- Munk, P., Hansen, M.M., Maes, G.E., Nielsen, T.G., Castonguay, M., Riemann, L., Sparholt, H., Als, T.D., Aarestrup, K., Andersen, N.G., Bachler, M., 2010. Oceanic fronts in the Sargasso Sea control the early life and drift of Atlantic eels. *Proc. Biol. Sci.* 277, 3593–3599.
- Naismith, I.A., Knights, B., 1988. Migrations of elvers and juvenile European eels, *Anguilla anguilla* L., in the River Thames. *J. Fish Biol.* 33 SA, 161–175.
- Ortega, A., Mourente, G., 2010. Comparison of the lipid profiles from wild caught eggs and unfed larvae of two scombroid fish: northern bluefin tuna (*Thunnus thynnus* L., 1758) and Atlantic bonito (*Sarda sarda* Bloch, 1793). *Fish. Physiol. Biochem.* 36, 461–471.
- Parrish, C.C., 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by latroscan flame ionization detection. *Can. J. Fish. Aquat. Sci.* 44, 722–731.
- Parrish, C.C., 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts, M., Wainman, B. (Eds.), *Lipids in Freshwater Ecosystems*. Springer, New York, pp. 4–20.
- Pavey, S.A., Gaudin, J., Normandeau, E., Dionne, M., Castonguay, M., Audet, C., Bernatchez, L., 2015. RAD Sequencing highlights polygenic discrimination of habitat ecotypes in the panmictic American eel. *Cur. Biol.* 25, 1666–1671.
- Pfeiler, E., 1999. Developmental physiology of elopomorph leptocephali. *Comp. Biochem. Physiol. A* 123, 113–128.
- Rahn, C.H., Sand, D.M., Schlenk, H., 1973. Wax esters in fish. Metabolism of dietary palmityl palmitate in the gourami (*Trichogaster cosbyi*). *J. Nutr.* 103, 1441–1447.
- Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. *Aquaculture* 155, 103–115.
- Rankin, M.A., Burchsted, J.C.A., 1992. The cost of migration in insects. *Annu. Rev. Entomol.* 37, 533–559.
- Riemann, L., Alfredsson, H., Hansen, M.M., Als, T.D., Nielsen, T.G., Munk, P., Aarestrup, K., Maes, G.E., Sparholt, H., Petersen, M.I., Bachler, M., Castonguay, M., 2010. Qualitative assessment of the diet of European eel larvae in the Sargasso Sea resolved by DNA barcoding. *Biol. Lett.* 6, 819–822.
- Roff, D.A., 1991. Life history consequences of bioenergetic and biomechanical constraints on migration. *Am. Zool.* 31, 205–216.
- Sampekalo, J., Takeuchi, T., Watanabe, T., 1992. Comparison of gill lipids between fresh water fish. *J. Tokyo Univ. Fish.* 79, 71–76.
- Schultz, E.T., Conover, D.O., 1997. Latitudinal differences in somatic energy storage: adaptive responses to seasonality in an estuarine fish (Atherinidae: *Menidia menidia*). *Oecologia* 109, 516–529.
- Slotte, A., 1999. Effects of fish length and condition on spawning migration in Norwegian spring spawning herring (*Clupea harengus* L.). *Sarsia* 84, 111–127.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. fourth ed. WH Freeman and Co., New York.
- Stockwell, J.D., Johnson, B.M., 1999. Field evaluation of a bioenergetics-based foraging model for kokanee (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* 56 (S), 140–151.
- Sullivan, M.C., Wuenschel, M.J., Able, K.W., 2009. Inter and intra-estuary variability in ingress, condition and settlement of the American eel *Anguilla rostrata*: implications for estimating and understanding recruitment. *J. Fish Biol.* 74, 1949–1969.
- Tesch, F.-W., 2003. In: Thorpe, J.E. (Ed.), *The Eel*, 3rd edition Blackwell Science, Oxford.
- Thibeault, I., Dodson, J.J., Caron, F., Tzeng, W.-N., Iizuka, Y., Shiao, J.C., 2007. Facultative catadromy in American eels: testing the conditional strategy hypothesis. *Mar. Ecol. Prog. Ser.* 344, 219–229.
- Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* 11, 107–184.
- Tocher, D.R., Bendiksen, E.A., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture* 280, 21–34.
- Trudel, M., Tucker, S., Morris, J.F.T., Higgs, D.A., Welch, D.W., 2005. Indicators of energetic status in juvenile coho salmon and chinook salmon. *N. Am. J. Fish. Manag.* 25, 374–390.
- Tsukamoto, K., Nakai, I., Tesch, W.V., 1998. Do all freshwater eels migrate? *Nature* 396, 635–636.
- Tsukamoto, K., Umezawa, A., 1990. Early life history and oceanic migration of the eel, *Anguilla japonica*. *La Mer* 28, 188–198.
- Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. *Rev. Aquac.* 1, 10–57.
- Vetter, R.D., Hodson, R.E., Arnold, C., 1983. Energy metabolism in a rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). *Can. J. Fish. Aquat. Sci.* 40, 627–634.
- Wang, C.H., Tzeng, W.N., 2000. The timing of metamorphosis and growth rates of American and European eel leptocephali: a mechanism of larval segregative migration. *Fish. Res.* 46, 191–205.
- Weih, D., 1977. Effects of size on sustained swimming speeds of aquatic organisms. In: Pedley, T.J. (Ed.), *Scale Effects in Animal Locomotion*. Academic Press, New York, pp. 333–338.
- Wiens, J.A., Innis, G.S., 1974. Estimation of energy flow in bird communities: a population bioenergetics model. *Ecology* 55, 730–746.