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1. Introduction

The life cycle of the European eels has been described as a catadromous life history during which they migrate between oceanic spawning areas and continental rearing habitats (Tesch, 2003). The leptocephalus larvae drift with the Gulf Stream to join the continental shelf where they metamorphose and are then referred to as glass eels. Then, glass eels migrate up estuaries to join rivers using selective flood transport: during flood tide, glass eels move up in the water column and migrate with the current while they go down and remain on or in the substratum during ebb tide (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979). However, a high degree of geographical dispersion crossing marine and riverine water have been documented regarding to different migratory patterns of settlement and river colonization (Daverat et al., 2006; Secor et al., 1995; Tsukamoto and Arai, 2001; Tsukamoto et al., 1998; Tzeng, 1996). These different patterns of migration could have a strong impact on the fate of the population because of the sex determinism in eels, which is environmental (Geffroy and Bardonnet, 2016; Krueger and Oliviera, 1999). Briefly, in European eels, males are generally observed to dominate in high-density environments, often associated with estuarine or lower river reaches, whereas females tend to become increasingly dominant with increasing distance from the sea (Adam et al., 2008; Davey and Jellyman, 2005; Harrison et al., 2014; Laffaille et al., 2006; Parsons et al., 1977).

The underlying mechanisms of this facultative migration are far from being elucidated but some studies proposed a conditional strategy based on individual's energetic status in the European eels (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006). Indeed, most glass eels do not feed during migration (Bardonnet and Riera, 2005) and energy reserves accumulated by the leptocephalus larvae during oceanic migration are used to sustain activity and reach fresh water (Kawakami et al., 1999; Tesch, 2003). According to the conditional strategy, glass eels presenting

high wet weight (Edeline, 2007; Edeline et al., 2006) or high dry weight (Bureau du Colombier et al., 2007) should have a higher propensity to migrate than those showing low energy stores. However, some results appear to contradict the conditional strategy hypothesis, either in experimental conditions (Bolliet et al., 2017a) or in natural environments (Gaillard et al., 2015). The European eel is listed in the IUCN red list as 'critically endangered' and while the reasons for this tremendous decline of the eel population are still not fully understood, anthropogenic causes are often pointed out. In glass eels, global changes (pollution, increase in water temperature) may lead to an increase in energy expenditure because of a higher metabolism or detoxication processes. The role of the energetic status of glass eels on their ability to migrate has to be elucidated, particularly in a context where managers do not account for variation in energetic condition in their population exploitation and conservation policy.

It is noteworthy that in most of the experiments supporting the conditional strategy, the energetic status of individual was mainly evaluated using integrative proxies like body mass and size. However, in fasting and migrating fish, energetic budgeting is a dynamic process mediated not only by energy reserves but also associated with a highly interplaying network of energy metabolisms. For example, we recently reported the existence of a non-random fluctuating expression dynamics of autophagy- and lysosome-related genes during long term fasting in A. *anguilla* glass eel and demonstrated a significant contribution of these transcripts production over time to weight loss (Bolliet et al., 2017b). Besides its function in the removal of altered or dysfunctional proteins and organelles, autophagy plays also a critical role in energy supply by allowing starved cells to mobilize their own constituents including lipid and glycogen stores (Singh et al., 2012). Another well-documented cellular energetic pivot is mitochondrial metabolic system mediated through ATP production and antioxidant activity (Bermejo-Nogales et al., 2015), but the

47 detailed role of mitochondrial functions as autophagy processes on mediating glass eel's
48 locomotion has been seldom studied.

49 Here, we sought to clarify the relationship between European glass eel energetics and their pattern
50 of migration behavior, in the framework of the conditional strategy. For this purpose, we
51 characterized the individual's energetic status of glass eels in terms of wet weight (as a proxy of
52 energy stores), standard metabolic rate (SMR) and transcriptomic profile of metabolism-related
53 genes and determined the relationship of these energy- and metabolism-related factors with the
54 propensity to migrate of glass eels, as evaluated in experimental condition. In order to strengthen
55 the possible link between energetic status of glass eels and their propensity to migrate, we used
56 glass eels arriving at the mouth estuary in autumn and spring known to present high and low
57 energetic stores, respectively (Charlon and Blanc, 1982; Claveau et al., 2015; De Casamajor et al.,
58 2000; Elie, 1979).

59

60 **2. Materials and methods**

61 **2.1. Ethics**

62 Procedures used in this study have been validated by the ethics committee N°073 (ref:
63 2017012015086652). The experiment was carried out in strict accordance with the EU legal
64 frameworks, specifically those relating to the protection of animals used for scientific purposes
65 (i.e., Directive 2010/63/EU), and under the French legislation governing the ethical treatment of
66 animals (Decret no. 2013-118, February 1st, 2013).

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69 **2.2. Origin and handling of fish**

70 The main migration season of glass eel in South-West France coastal area lasts from November to
71 April. In this study, one group of 72 marine glass eels was sampled using a dip-net at night and
72 during flood tide on November 3rd 2016 and the same number of glass eels were sampled in the
73 same way on November 17th (hereafter autumn glass eels). Similarly, 72 glass eels were sampled
74 on April 13th 2017 and the same number of glass eels were sampled again on April 28th (hereafter
75 spring glass eels). The sampling marine site is located at Moliets (43° 55′N, 1° 23′W, located 40
76 km north of the mouth of the Adour estuary, Fig.1). After each collection, glass eels were
77 transferred to the laboratory and maintained at 12°C overnight in a tank containing aerated water
78 from the fishing site. In the next morning, all glass eels were anesthetized and individually
79 measured for initial wet weight (Sartorius CP 153 balance, ±1.0 mg) and length (±1.0 mm).
80 Measurements of tryglycerides (TG) at the end of the experiment allowed us to highlight a
81 significant correlation between the final wet weight of individuals and their TG levels in both
82 seasons (Pearson's correlation test, p=0.0002 and p=0.00001, respectively). According to these
83 results, wet weight was considered in this study as a good proxy of energy stores. Pigment stages
84 were determined according to the criteria of Elie et al. (1982), who described eight stages: VA, VB,
85 VIA0, VIA1, VIA2, VIA3, VIA4 and VIB, in order of increasing pigmentation. 100% of marine
86 glass eels presented a VB stage, regardless of the season.

87 36 of the 72 individuals were randomly selected and tagged using Visible Implant Elastomer (VIE
88 Tag) (combinations of one or two hypodermic spots of different colors as described by Imbert et
89 al., 2008) in order to follow the swimming activity individually. Once tagged, glass eels were
90 released to wake up in the water from fishing site with the untagged fish. During the next 48 h, the

91 water was continuously aerated and progressively diluted with fresh water. Water temperature
92 (12±0.5°C) was regulated using an air conditioner.

93 **2.3. Experimental design and protocol**

94 For each experiment, 36 tagged animals were mixed with the same number of untagged ones to
95 facilitate synchronization of swimming activity to the change in water current direction by
96 increasing density (Bolliet and Labonne, 2008; Bolliet et al., 2007). This group of 72 glass eels
97 were released to an annular tank (Fig.2) installed in a temperature-controlled room. To mimic the
98 tides, the tank was equipped with two pumps - located at its opposite ends - that alternately
99 generated clockwise or counterclockwise water flow every 6.2 h as described in Bolliet et al. (2007).
100 The room was maintained under a photoperiod of 12 L/ 12 D with a very low light intensity during
101 the photophase (0.2-0.3 $\mu W/cm^2$) and a constant UV light (0.6 $\mu W/cm^2$). The water temperature
102 was kept at 12± 0.5°C and continuously recorded by thermistors placed in the tank.

103 The swimming activity of glass eels was traced individually during seven days by a camera
104 programed to record 15 seconds every 40 min. The UV light allowed the identification of each
105 glass eel during the light and dark phases by its elastomer mark. The sampling session duration
106 was chosen to allow fish to pass once through the camera's field of view when swimming in the
107 water column (see Bolliet and Labonne, 2008). A total of 177 sessions of 15 seconds were obtained
108 for autumn glass eels and the same number of sessions for spring ones.

109 To migrate up estuary, glass eels must synchronize their swimming activity to the tide to use
110 selective flood transport but they also have to sustain swimming activity. So, to evaluate the
111 propensity of glass eels to migrate in our experimental conditions, we first analyzed the
112 synchronization of their swimming activity to the change in water current direction (every 6.2h).
113 When the swimming activity was synchronized to the water current reversal with a period of 12.4h,

glass eels were considered as having a high propensity to migrate and were called ‘active’. In contrast, fish that did not synchronize and stayed in the substratum most of the time were called ‘non-active’. Then, the level of swimming activity in active glass eels was quantitatively analyzed by counting the total number of observations of each elastomer mark for the 177 sessions of 15sec recorded in each experiment.

After the swimming test, we obtained 35 active glass eels in autumn (19 and 16 in the two experiments, respectively) and 12 active glass eels in spring (5 and 7 in the two experiments, respectively). Active and non active glass eels were pooled per season for analyses.

2.4. Standard metabolic rate (SMR) assay

22 active and 13 non-active glass eels and 12 active and 24 non-active glass eels in autumn and spring, respectively, were randomly retrieved for oxygen consumption determination. Briefly, glass eels were acclimated in a same tank with still water for three days to stay at rest and relative quiet before analysis. Then, they were transferred to 12 respirometry chambers (diameter: 11.2mm, length: 90mm) of an intermittent flow respirometer as described by Régnier et al. (2010), where oxygen consumption was measured individually (one fish per chamber). Tagged glass eels were introduced in the chambers at 3 p.m. and oxygen consumption was recorded continuously every minute until 10 a.m. the next day. The closed/open phase of the system was 20min/20min and the duration of the closed phase was determined in order that oxygen level in the chamber was always kept above 80% O_2 saturation. After termination of the SMR measurements, the background respiration, i.e. oxygen consumption within the respirometer due to microbial respiration, was estimated by measuring the oxygen consumption rate in the respirometer without a fish (i.e. blank run for two hours). Temperature and photoperiod used for the acclimatization phase and oxygen measurement were similar to those used for the behavioral test. The first 15 hours were considered

as a period of acclimatization and the average oxygen consumption per eel was calculated using the last four hours of recording. Measurements were conducted for three days in autumn and spring. SMR (mm^3 O_2/h) was expressed in mm^3 O_2 consumed per hour. We then regressed the logarithm of SMR on the logarithm of wet weight, and used the residuals of this model (*i.e.*, relative SMR) for further analyses. Following oxygen consumption measurement, glass eels were anaesthetized, individually measured for wet weight (Sartorius CP 153 balance, ±1.0 mg) and length (±1.0 mm) and stocked at -80°C after immersion in liquid nitrogen.

2.5. High throughput quantitative RT-PCR

The extraction of total RNA from each glass eel analyzed for its oxygen consumption was performed using TRIzol reagent (Invitrogen, 15596018) according to the manufacturer's recommendations. One microgram of the resulting total RNA was reverse transcribed into cDNA using the SuperScript III Reverse Transcriptase kit (Invitrogen, 18080085) with random primers (Promega, Charbonniéres, France) according to the manufacturer's instructions.

Primers specific to 59 genes involved in either energy metabolism or oxidative stress (Table 1) were newly designed using Primer3 software (version 4.1.0) and based on the available genomic resources for the European eel (GenBank assembly accession GCA_000695075.1). Primers were validated on a Roche LightCycler 480 System (Roche Diagnostics, Neuilly sur Seine, France). The assays were performed using a reaction mix of 6 µl per sample, each of which contained 2 µl of diluted cDNA template, 0.24 µl of each primer (10 µM), 3 µl Light Cycler 480 SY Green Master mix (Roche Diagnostics, 4887352001) and 0.52 µl DNAse/RNAse-free water (5 Prime GmbH, 2500020). The PCR protocol was initiated at 95 °C for 10 min for initial denaturation of the cDNA and hot-start Taq-polymerase activation, followed by 45 cycles of a 3-step amplification program (15 s at 95 °C; 10 s at 60 °C and 15 s at 72 °C). Melting curves were systematically monitored

160 (temperature gradient at 1.1 °C/10 s from 65 to 94 °C) at the end of the last amplification cycle to
161 confirm the specificity of the amplification reaction. Each PCR assay included negative controls
162 (reverse transcriptase- and cDNA template-free samples, respectively). Finally, to confirm
163 specificity of the designed primers, the amplicons were purified and sequenced (Beckman-Coulter
164 Genomics, Takeley, UK). The validated primers are listed in Table 1.

165 High-throughput qRT–PCR was performed by the Genotoul service (https://get.genotoul.fr) in
166 Toulouse (France) using the BioMark 96:96 Dynamic Array integrated fluidic circuits (Fluidigm
167 Corporation, San Francisco, USA) described in Cassan-Wang et al. (2012). The specificity of the
168 PCR products was confirmed by analyzing melting curves. Only primers that produced a linear
169 amplification and qPCR products with a single-peak melting curves were used for further analyses.
170 The efficiency of each pair of primers was determined from the data of amplification Ct value plot
171 with a serial dilution of mixture cDNA. $2^{-\Delta\Delta C_T}$ method was used to calculate relative mRNA fold
172 change using formula $2^{\Delta Ct\_target\ (control-sample)}/2^{\Delta Ct\_reference\ (control-sample)}$ (Livak and Schmittgen, 2001).
173 The relative expression of Luciferase was used for data normalization as described previously
174 (Marandel et al., 2016).

175 **2.6. Statistical analyses**

176 To characterize the propensity of glass eels to migrate, we first investigate the synchronization of
177 glass eel's swimming activity to the change in water current direction (active/non-active). We
178 assumed that the swimming activity of an individual i at time t $AC(t,i)$ followed a Bernoulli
179 distribution of probability $P(t,i)$ such as:

180 $$AC(t,i) \sim dbern\big(P(t,i)\big)$$

We assumed that $P(t,i)$ was a periodic function of time, since it has been previously shown that glass eels display rhythmic swimming activity in response to current reversal (Bolliet and Labonne, 2008):

$$logit\big(P(t,i)\big) = a(i) \times \sin\big(t \times b(i) + c(i)\big) + d(i)$$

where $a(i)$ was the strength of the synchronized component of activity, $b(i)$ was related to period, $c(i)$ was related to the trigonometric function phase, $d(i)$ was the non-synchronized component of activity. Fish having a *P* value above the mean of *P* meanwhile having an activity periodicity close to 12.4h were considered synchronized and active, others were considered non-active.

A Markov-chain Monte-Carlo (MCMC) sampling approach with Gibbs algorithm in the Bayesian framework (Spiegelhalter et al., 2000, Openbugs software, Version 3.2.3) was used to estimate parameters a, b, c, d. Convergence of estimates was reached during a first set of 10 000 iterations. Another consecutive set of 5000 iterations was run to approximate the posterior distribution of parameter estimates (see Supplementary material 1).

The second parameter used to evaluate the propensity of glass eels to migrate was the level of swimming activity in active glass eels, expressed as the mean number of times each individual was seen swimming in the water column during 7 days.

All other statistical analyses were performed using the R software (v.3.3.1)/R Commander Package. Data were presented as means ± standard deviation. The comparison of number of active fish in the two seasons was analyzed by chi-square test based on the counts of active/ non-active fish and the average swimming activity in the two seasons was compared by Student's t test. Normalized genes expressions were first analyzed using a hierarchical clustering analysis based on correlation between genes (R Pvclust package). We used the Approximately Unbiased bootstrapping approach

to detect clusters of genes (with an accuracy p-value > 0.95) that displayed the same overall patterns throughout the experimental conditions (see Supplementary materials 2 and 3). Two-way ANOVA was used to analyze the varying wet weight, length and relative SMR in response to season and migration behavior. The interactions in the responses were also evaluated. Differences were considered statistically significant at $p<0.05$. For synchronized fish, the relationships of swimming activity to wet weight, length and relative SMR were estimated by simple linear regression model. The regression model was considered significant at $p<0.05$ level. Finally, we evaluated whether genes expression level was correlated with swimming activity (for synchronized fish) for both seasons. To do so, we first grouped genes into five metabolism-related functions: autophagy-lysosome system, mitochondrial turnover, mitochondrial metabolism, antioxidant system and cytosol catabolism (see Table 1). For each group of genes, we ran a Principal Component Analysis (PCA), using a table providing the gene expression levels for all individuals. We then retrieved the score of individuals on the first axis of the PCA, and used these coordinates as a synthetic indicator of the individual level of expression for the genomic function. We then assessed the effects of the swimming activity, the season, and the interaction of both on this synthetic indicator of the genomic function, using a linear regression model. Bonferroni's adjustment for multiple comparisons (here five) lowered the statistical significance to $p<0.01$ level.

221 ## 3. Results

222 ### 3.1. Seasonal variation in glass eel swimming activity

223 One dead glass eel was found in autumn glass eels and two in springtime glass eels after the
224 swimming test, which left a total of 71 and 70 glass eels in autumn and spring, respectively. The
225 propensity of glass eels to migrate was investigated by their ability to synchronize their swimming
226 activity to the change in water current direction every 6.2h. Glass eels presenting a swimming
227 activity with a period of 12.4h were considered as active and the others as non-active. Results
228 showed that the number of active glass eels was higher in autumn than in spring (chi-square test,
229 X^2=15.0, p<0.001; Fig.3a). Then, the level of swimming activity was analyzed in active glass eels
230 using the mean number of times each individual was seen swimming in the water column during 7
231 days. Swimming activity level was higher in autumn than in spring with a mean of 68±41 and
232 32±21 observations in autumn and spring, respectively (Student's t test, p<0.001; Fig.3b). The
233 mean number of observations in inactive glass eels (not synchronized to the change in water current
234 direction) was 3±4 in autumn and 2±3 in spring.

235 ### 3.2. Energetic status of glass eels depending on season and migration behavior

236 The energetic status (wet weight, length, standard metabolic rate (SMR) and transcriptomic profile
237 of metabolism-related genes) was investigated in 35 autumn glass eels (22 active and 13 non-active)
238 and in 36 spring glass eels (12 active and 24 non-active).

239 #### *Wet weight, length and relative SMR*

240 The initial wet weight of autumn glass eels measured before the swimming test ranged from 315
241 to 398 mg and was higher than that measured in spring glass eels (124 to 285 mg, Fig. 4a, two-way
242 ANOVA, p<0.001). Similar results were observed for length ranging from 70 to 76 mm in autumn

and from 60 to 72 mm in spring (Fig. 4b, two-way ANOVA, $p<0.001$). No significant difference in length or weight was observed between active and non-active fish in autumn or spring (Fig. 4a, b, two-way ANOVA, $p=0.902$ in wet weight, $p=0.565$ in length). There was also no interaction between migration behavior and season for these two parameters. Relative SMR, showed no significant difference depending on season (Fig. 4c, two-way ANOVA, $p=0.666$) or migration behavior ($p=0.441$). However, a weakly significant interaction between these two factors was observed ($p=0.048$), wherein active glass eels presented a slightly higher relative SMR than non-active ones in autumn but not in spring.

Expressions of genes involved in energetic metabolism

We analyzed mRNA levels of 59 genes and showed the results by a heat map in a red-white scale (from lower to higher mRNA-expression level, Fig. 5). Some of these genes code for proteins involved in the autophagy/lysosome-related functions, including Macroautophagy (the best characterized sub-class of autophagy involving the formation of double-membrane organelles, or autophagosomes, which engulf portions of cytoplasm for subsequent degradation via lysosomes), Mitophagy (a macroautophagy-dependent specific degradation of mitochondria) and Chaperone-mediated autophagy (a specific autophagic route, known as CMA, that involves the direct delivery of cytosolic proteins targeted for degradation to the lysosomes). The other genes code for proteins involved in mitochondrial turnover and metabolism, the cytosol catabolism and finally the antioxidant system. mRNA levels of these genes were compared in response to season and migration behavior. Using clustering analyses and bootstrapping on normalized individual gene expression data we detected two clusters of genes (and some genes outside) that displayed the same overall patterns throughout the experimental conditions. Overall, the genes involved in energy stress resistance (macroautophagy and mitophagy) clustered in the group of genes overexpressed

in spring glass eels compared to autumn ones, while genes involved in energy production and use (mitochondrial metabolism and CMA) showed an opposite trend. In detail, four macroautophagy genes (*atg5, atg7, atg12, ulk1*) and four mitophagy receptors (*fundc1, pink1, bnip3a, bnip3b*), showed significantly higher transcript levels in spring than in autumn, reflecting advanced energy distress in spring glass eels. In this regard, higher expression was also evidenced in spring glass eels for the gene *drp1*, known as the main player in the process of mitochondrial fragmentation, and three genes involved in mitochondrial biogenesis (*pgc1α1*, *pgc1α2*, *hsp60*), suggesting high mitochondrial turnover to compensate to the loss of mitochondria through mitophagy. In contrast, two genes (*mfn1* and *mfn2*) involved in mitochondrial fusion and cooperation were significantly more expressed in autumn than in spring. Likewise, most studied mitochondrial metabolism related genes, i.e., two genes coding for subunits of the mitochondrial membrane respiratory chain (*mt-ATP6*, *mt-nd5*), two genes involved in fatty acid catabolism (*cpt1α1*, *hadh*) and five genes involved in amino acid catabolism (*glud1*, *got1*, *got2*, *gpt2a*, *gpt2b*), exhibited higher expression in autumn than in spring. Interestingly, a similar trend was also observed for the genes related to CMA (*hsc70a*, *hsc70b*, *lamp2a*, *hsp90*, *phlpp1*), which has been demonstrated to play a major role in the regulation of the hepatic intermediary metabolism. Similarly, five genes involved in the oxidative stress defense (*sod1, sod2, mtl, gstp* and *catalase*) clustered in the group of genes overexpressed in autumn glass eels, possibly as a consequence of higher mitochondrial activity in these animals compared to their spring counterparts. Altogether, these results highlighted the existence of strong differences of the metabolic status between autumn and spring glass eels. However, no clear difference was evidenced between active and non-active glass eels whatever the season considered.

3.3. Relationships of the swimming activity levels of each active glass eel with its energetic status

The individual swimming activity level, expressed as the number of observations for each active glass eel swimming in the water column, ranged from 11 to 161 in autumn and from 11 to 79 in spring.

Linear regression models showing the correlations between the level of swimming activity in active glass eels and the individual wet weight, length and relative SMR are presented in Fig. 6. In autumn glass eels, activity levels were positively correlated to relative SMR (Fig. 6c) but not with wet weight or length (Fig. 6a, b), while spring glass eels displayed a positive correlation between activity and body weight and length, but not with relative SMR (Fig. 6a, b, c).

Among the five genomic functions of interest (autophagy-lysosome system, mitochondrial metabolism, mitochondrial turnover, antioxidant system and cytosol catabolism), the expression levels of mitochondrial metabolism genes and antioxidant system genes were significantly related to swimming activity (Fig.7). We found a positive relationship between the first axis of the PCA for both of the functions and swimming activity level (two-way ANOVA, p=0.008 for mitochondrial metabolism and p=0.007 for antioxidant system), with no effect of season (p=0.772 and 0.074, respectively) and no significant interaction (p=0.560 and 0.384, respectively; See Supplementary material 4). For both functions, the first axis of the PCA explained 51% of the total gene expression variance. For mitochondrial metabolism function, this first axis was mainly correlated to genes of fatty acid catabolism (*cpt1α2b* and *hadh*), amino acid catabolism (*got1*, *got2*, *gpt2a*, *gpt2b* and *glud1*), mitochondrial respiratory chain complexes (*mt-nd5* and *mt-ATP6*) and *12s rRNA*, while for antioxidant system, this first axis was mainly correlated to *sod1*, *sod2*, *catalase*, *gstp* and *mtl* genes (See Supplementary material 5).

4. Discussion

The main objective of this study was to investigate the relationship between European glass eel energetics and their pattern of migration behavior, in the framework of the conditional strategy. For this purpose, we used autumn and spring marine glass eels known to present high and low energetic stores, respectively. We did observe a higher propensity to migrate in autumn as predicted by the conditional strategy, and individual swimming activity level was also higher in autumn. However, when we compared the energy status of active and non-active glass eels within each seasonal group, we found no support for the conditional strategy: both behavioral groups showed similar wet weight and length. At the individual level, we also found little support for the conditional strategy: energy stores were correlated to the level of swimming activity in active glass eels only in spring, not in autumn. These results are further explained partly by variation in metabolic rates between individuals, and partly by the transcriptome analyses, that indicate striking contrasts in the metabolic states of autumn versus spring eels.

4.1. Autumn and spring glass eels: Highly contrasting metabolic states

Final wet weight was highly correlated to tryglycerides (see section 1.2) and wet weight was considered in this study as a good proxy of energy stores. Here we show that autumn glass eels displayed higher wet weight than spring ones, which is in close agreement to previous observations made in *A. anguilla* glass eels (Charlon and Blanc, 1982; Claveau et al., 2015; De Casamajor et al., 2000; Elie, 1979). Such seasonal differences in biomass and energetic stores have been suggested to be due to the seasonal variations in oceanic ecosystems productivity affecting the growth of leptocephalus larvae during transatlantic transport (Désaunay and Guerault, 1997).

To further characterize the glass eels studied here, we measured their relative standard metabolism but no significant difference was observed between seasons. A high-throughput qRT-PCR analysis was then performed allowing the simultaneous assessment of the expression of 59 genes involved in energy metabolism and oxidative stress. They include genes of the autophagy-related pathways (including macroautophagy, mitophagy and CMA), genes involved in mitochondrial turnover and metabolism and genes of the cellular antioxidant system. Our results show that most of the monitored mitochondrial metabolism-related genes clustered in the group of genes overexpressed in glass eels caught in autumn compared to those sampled in spring. Similarly, the two genes *mfn1* and *mfn2*, known to promote mitochondrial fusion and therefore the cooperation between these organelles (Chen et al., 2003) also grouped to this cluster. These data strongly support that autumn glass eels displayed higher energy production capacity and use than the spring ones. Interestingly, autumn glass eels also presented higher expression of genes related to CMA, known as a master regulator of intermediary metabolism (Tasset and Cuervo, 2016), which support this idea.

In contrast, genes involved in energy stress resistance (macroautophagy and mitophagy) grouped in the cluster of genes overexpressed in spring glass eels compared to autumn glass eels. Macroautophagy is now widely recognized to play a major role in mobilizing diverse cellular energy and nutrient stores (including proteins, carbohydrates and lipids) as well as cellular organelles (including mitochondria, peroxisome, endoplasmic reticulum, the nucleus and ribosomes) during starvation in a large panel of taxa (Kaur and Debnath, 2015). This process is described as a key adaptive response to modulate the metabolism and provides energy when nutrients are scarce. Macroautophagy increases during periods of cellular stress in many eukaryotic species (from yeast to mammals) in order to conserve energy and promote survival. In this regard, we recently reported a significant contribution of macroautophagy-related transcripts production

during long-term fasting to weight loss in glass eels (Bolliet et al., 2017b). The over-representation of genes involved in macroautophagy but also in mitophagy in the cluster of genes highly expressed in spring glass eels compared to their autumn counterpart would therefore reflect a higher catabolic state and an energy distress in spring glass eels.

Overall, the data clearly show that autumn and spring marine glass eels exhibit strong differences in term of both energetic stores and transcriptomic profile of metabolism-related genes, making them relevant models for a better understanding of the relationship between glass eel's organism energetics and their pattern of migration behavior.

4.2. Active *vs* non-active glass eels

To migrate up estuary, glass eels synchronize to the flood and mobilize energy stores accumulated by the leptocephalus stage to sustain activity (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979; McCleave and Kleckner, 1982; Wippelhauser and McCleave, 1987). In our experimental conditions, fish that synchronized their swimming activity to the change in water current direction with a period close to 12.4h were considered as having a high probability to migrate. They were called active glass eels. In contrast, non-active glass eels did not synchronized to the water current reversal and were considered as having a low propensity to migrate.

As most glass eels do not feed during migration (Bardonnet and Riera, 2005), energy stores have been considered as a physiological driver in fueling the landward colonization (Edeline, 2007; Edeline et al., 2006). Using salinity preference experiment, it has been suggested that European glass eels choosing fresh water should present a higher energetic status and migration activity than those choosing salt water (Edeline et al., 2005). According to these studies, it was expected here that active glass eels would have a higher wet weight that non-active ones. However, although the higher number of active glass eels and activity level observed in autumn glass eels were associated

379 to a higher initial wet weight when compared to spring fish, active and non-active glass eels did
380 not show any difference in wet weight in autumn nor in spring. This suggests that energy stores
381 might possibly enhance migration but also that a conditional strategy based on an individual's
382 energetic status cannot fully explain the European glass eel migration behavior observed in our
383 experimental conditions.

384 Another factor that may be involved in the underlying mechanism of migration concerns relative
385 SMR that represents an integrative measure of the energy expenditure and the celerity at which
386 energy stores are consumed. In salmonids, it is known to fluctuate among individuals in correlation
387 to dominance, aggression, growth rate or starvation (Findstad et al., 2007; see Eliason et al., 2016;
388 Metcalfe et al., 2016 for reviews). Studies investigating the relationships between SMR and
389 propensity to migrate in fish remain scarce but there is some evidence that low energy condition
390 and excessive energy depletion are important factors determining successful upriver migration in
391 salmonids (Eliason and Farell, 2016 and references herein). In the present study, active glass eels
392 presented a slightly higher relative SMR than non-active ones in autumn but not in spring. Links
393 between metabolic rate and fish behavior show a high intra-specific plasticity and are far from
394 being understood (Auer et al., 2016; Careau et al., 2008; Metcalfe et al., 2016). During fasting,
395 fish with a high SMR may have less energy available to allocate to activity but SMR may also
396 reflect their capacity to perform activity (Biro and Stamps, 2010; Careau et al., 2008). In addition,
397 fasting fish with a high SMR will deplete their energy stores more quickly that could decide them
398 to migrate in order to switch their habitat and reach growth area. On the other hand, relative SMR
399 was measured after the swimming test and although fish were kept in a tank during three days
400 before oxygen measurement, one cannot exclude the fact that it was not long enough for active fish
401 to fully recover to a resting metabolic rate after 7 days of activity. Zhang et al. (2018) evidenced

402 that exercise training increased excess post-exercise oxygen consumption in exhausted *Salmo salar*
403 when compared to control exhausted fish. Duration of recovering reached 15h which was longer
404 than previously found in the literature (Zhang et al., 2018) but still below the resting period fixed
405 in our experiment before oxygen measurement. Further experiment comparing oxygen
406 consumption before and after behavioral test are now required to validate the link between relative
407 SMR and decision to synchronize to the change in water current direction in glass eels.

408 To further investigate the difference between migration patterns, we then monitored the
409 transcriptomic profile of active and non-active glass eels within and between seasonal groups.
410 However, no clear difference was observed between active and non-active glass eels whatever the
411 considered season. Altogether, our data challenged the conditional strategy based on individual's
412 energetic status for the European glass eels. The relationship between relative SMR and migration
413 behavior remains to be elucidated and although no clear molecular evidence was observed between
414 active and non-active fish, it is not excluded that the genes analyzed and the associated metabolic
415 pathways may be involved in the different behavior observed. Indeed, we focused on gene
416 expression at a single time point that does not give a real picture of the dynamic aspect of the
417 different events at play during complex and integrative metabolic processes such as energy
418 expenditure at rest, which include not only gene transcription but also protein translation and
419 enzyme activity.

420 **4.3. Variability in swimming activity level**

421 Although the swimming activity of all active glass eels was synchronized to the change in water
422 current direction, we observed a high inter-individual variability in activity level as expressed by
423 the total number of observations of each individual swimming in the water column. When the
424 activity level was plotted to the relative SMR, a positive correlation was found in autumn glass eels

425 but not in spring ones, which may support hypothesis proposed in the previous section concerning
426 SMR in active and non-active autumn fish. In contrast, when the activity level was individually
427 plotted to the weight, a positive correlation was found in spring but not in autumn. Interestingly,
428 the wet weight was higher in autumn glass eels than in spring ones and no overlapping was
429 observed in their distributions. This could suggests a threshold in energy stores below which energy
430 becomes a limiting factor for swimming efficiency. In regarding to gene expression analysis,
431 whatever the season, the genes related to mitochondrial metabolism and antioxidant system
432 positively correlated to swimming activity level. During endurance exercise, the increase of
433 mitochondrial respiration induces the production of reactive oxygen species (ROS), which can lead
434 to the accumulation of cellular damage, unless it can be counterbalanced by antioxidants that act
435 to quench ROS and prevent the oxidation of other important biological molecules (Powers et al.,
436 2011). This finding is also supported by a recent report on birds, where the authors summarized an
437 upregulated antioxidant system coping with the oxidative challenges associated with migratory
438 flight (Cooper-Mullin and McWilliams, 2016).

439 **4.4. Consequences for eel management**

440 As we demonstrated, there is strong seasonal effect on both condition of eels and their behavior.
441 Whereas autumn eels displayed high wet weight and activity, spring eels were strongly constrained
442 energy wise, and displayed reduced migration activity. Population monitorings and management
443 actions should therefore be evaluated in the light of these results. For instance, because of being in
444 energy distress, spring glass eels might be more vulnerable to stress, whereas autumn glass eels
445 could provide a higher plasticity to cope with variation in environmental conditions. In the context
446 of global change, wherein fluctuations in temperatures, hydraulic conditions or pollutants may
447 increase energy expenditure, the spring component of populations should be carefully monitored.

448 Measures for the recovery of the stock of European eel as presented by the EU (Council Regulation
449 (EC) 1100/2007) proposes stocking of eels i.e. a transfer of glass eels from their fishing area to
450 another place more suitable to their survival and/or growth. Such management actions should
451 however be planned by carefully balancing their costs and benefits: the spring eels should probably
452 be avoided, for they might have limited ability to adapt to the new environments and also because
453 they represent a distinct yet threatened part of the phenotypic variation in the population. Autumn
454 glass eels might adapt with more efficiency to new environments. In any case, if any restocking
455 action is to be implemented, the managers would be well inspired to track the effect of glass eels
456 origins (i.e., season) on the success of their action.

457

458 **5. Conclusion**

459 Autumn and spring glass eels displayed different energy status that may affect migration behavior
460 in different ways. Autumn fish displayed high energy reserves and capacity for energy production
461 that may have triggered migration behavior in our experimental conditions. However, in both
462 seasons, a conditional strategy based on individual's energy status could not explain why some fish
463 decided to synchronize to the water current direction while some others stayed sheltering in the
464 substratum. The relative SMR may be a possible candidate to explain the different patterns of
465 behavior in autumn glass eels but further studies are now needed to clearly elucidate this point.
466 Spring glass eels presented a higher energy distress than the autumn ones and molecular results
467 also evidenced a higher expression of genes involved in fission, macroautophagy and mitophagy.
468 These processes may have helped glass eels to maintain standard metabolism for vital functions
469 but may be not sufficient to allow a high swimming activity. Indeed, migration requires energy in
470 addition to others physiological tasks and when energy stores dwindle, the ability to migrate may

be directly related to the fish's maximum capacity to increase oxygen consumption. Investigating the metabolic scope in active and non-active fish could be another interesting avenue to explore. Finally, it is also paramount for stocks and biodiversity managers to recognize that the physiological status of glass eels arriving on the oceanic shelf change drastically throughout the seasons, making them nonrandom parts of the whole phenotypic variation at the species level.

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649

650 **Figure legends**
651

652 Fig.1 Sampling station of glass eels.

653 Fig.2 Diagrammatic top view of the annular flume.

654 Fig.3 Swimming behavior of autumn and spring glass eels. (a) Number of active glass eels, (b)
655 Level of swimming activity of active glass eels expressed as the mean number of times each
656 individual was seen swimming in the water column during 7 days. Data is presented by means ±
657 standard deviation. Statistical significance *p*-values are indicated at 0.05% level. Numbers in the
658 x-axis text represent sample sizes for each group.

659 Fig.4 Box-plots (median, 25–75% CI, min-max) of the wet weight (a), length (b) and relative SMR
660 (c) of glass eels in response to season (autumn and spring) and differentiated migration behavior
661 (A= active, NA= non-active). Statistical significance *p*-values and the interactions in the responses
662 are indicated. Numbers in the x-axis text represent sample sizes for each group.

663 Fig.5 Heatmap of mean gene expressions calculated per season and migration behavior (A= active,
664 NA= non-active). The heatmap is organized through hierarchical clustering based on correlation
665 of expression between genes among individuals. Two clusters were detected using bootstrapping
666 resampling to estimate approximated unbiased values. They were statistically supported with
667 accuracy *p*-values > 0.95. Six other genes did not fit in any of the two clusters.

668 Fig.6 Scatter plot for the observed individual swimming activity of active autumn and spring glass
669 eels against the wet weight (a), length (b) and relative SMR (c). Empty circle= autumn, full circle=
670 spring.

671 Fig. 7 Relationship between the first axis of the PCA for genes related to mitochondrial metabolism
672 (a) and antioxidant system (b) and the swimming activity level. Empty circle: autumn, full circle:
673 spring. The prediction plotted on the figure correspond to a simple linear regression between the
674 first PCA axis and the swimming activity level (considering no effect of season was detected) with
675 associated R^2 and *p*-value.

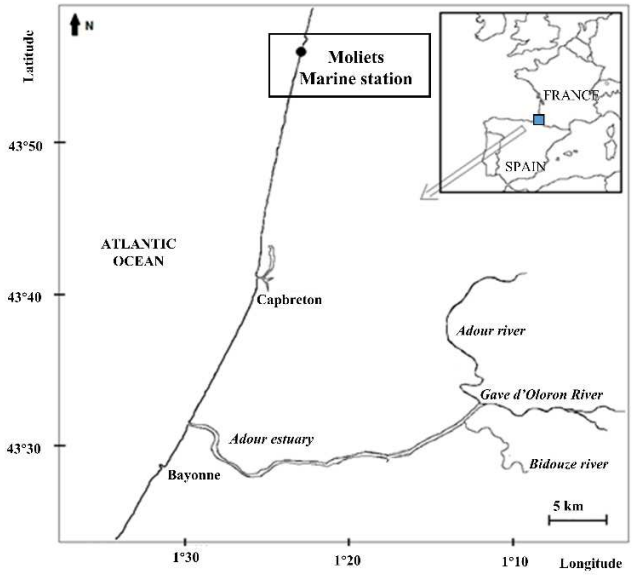
N
Latitude
Moliets
Marine station
FRANCE
SPAIN
43°50
ATLANTIC
OCEAN
43°40
Capbreton
Adour river
Gave d'Oloron River
43°30
Adour estuary
Bayonne
Bidouze river
5 km
1°30
1°20
1°10
Longitude

Fig. 1

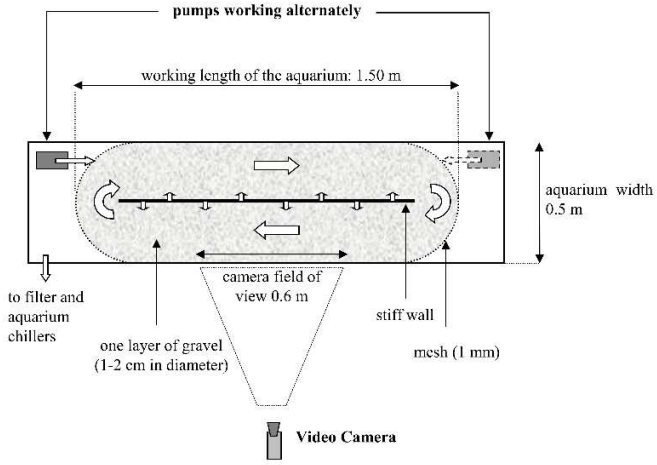
pumps working alternately
working length of the aquarium: 1.50 m
aquarium width
0.5 m
to filter and
aquarium
chillers
camera field of
view 0.6 m
stiff wall
one layer of gravel
(1-2 cm in diameter)
mesh (1 mm)
Video Camera

Fig. 2

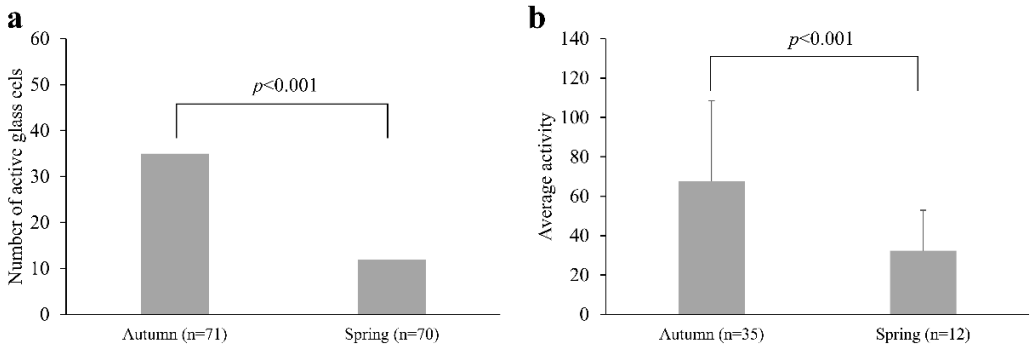
a
60
50
40
30
20
10
0
Number of active glass eels
p<0.001
Autumn (n=71)
Spring (n=70)
b
140
120
100
80
60
40
20
0
Average activity
p<0.001
Autumn (n=35)
Spring (n=12)

Fig. 3

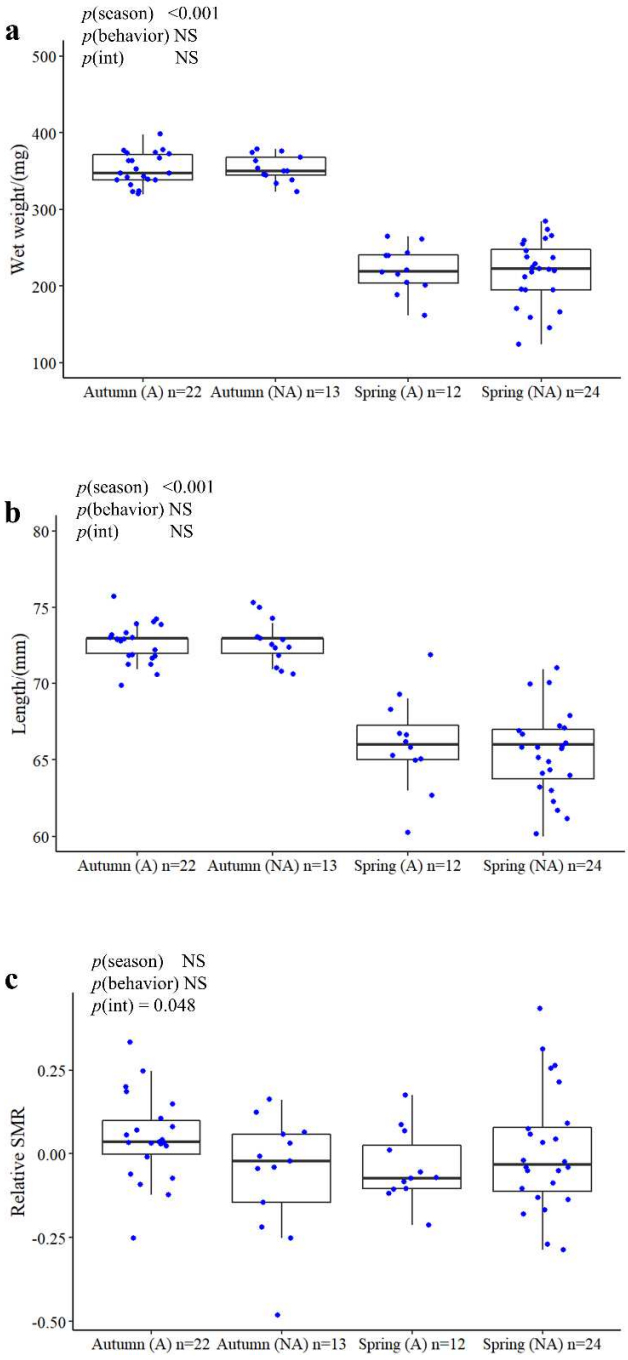
a
p(season) <0.001
p(behavior) NS
p(int) NS
Wet weight/(mg)
500
400
300
200
100
Autumn (A) n=22
Autumn (NA) n=13
Spring (A) n=12
Spring (NA) n=24
b
p(season) <0.001
p(behavior) NS
p(int) NS
Length/(mm)
80
75
70
65
60
Autumn (A) n=22
Autumn (NA) n=13
Spring (A) n=12
Spring (NA) n=24
c
p(season) NS
p(behavior) NS
p(int) = 0.048
Relative SMR
0.25
0.00
-0.25
-0.50
Autumn (A) n=22
Autumn (NA) n=13
Spring (A) n=12
Spring (NA) n=24

Fig. 4

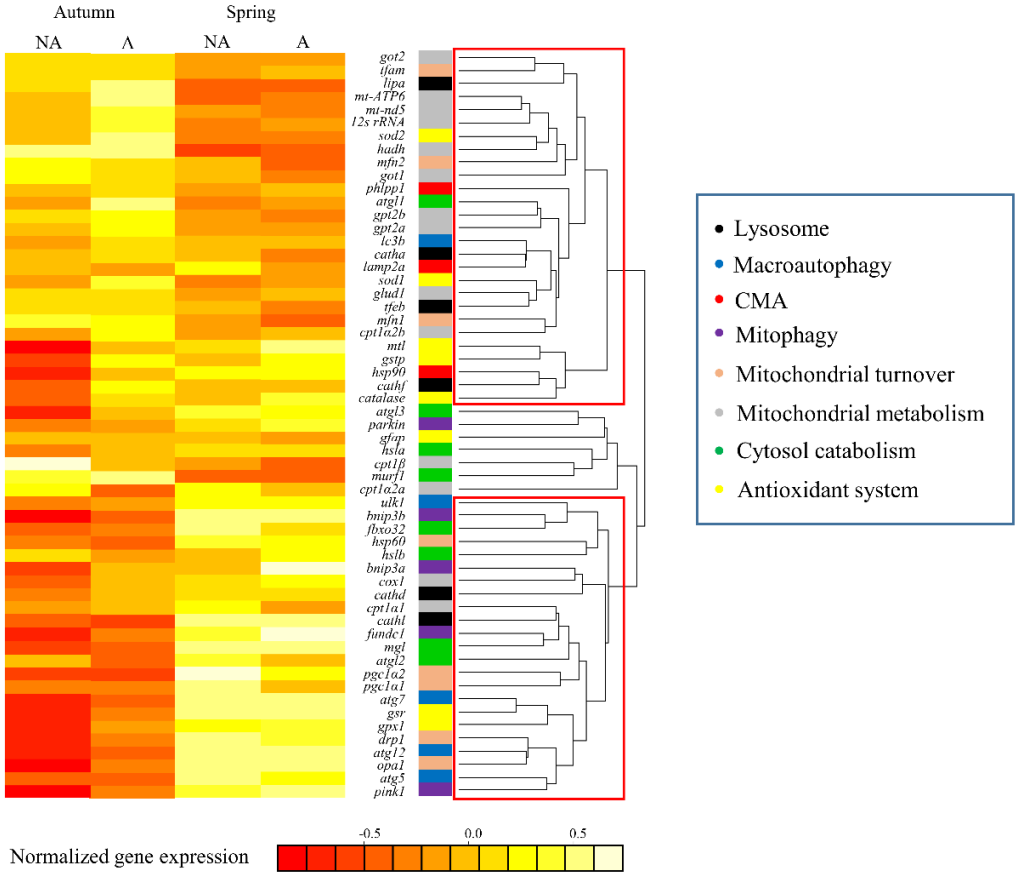
Autumn
Spring
NA
A
NA
A
got2
tfam
lipa
mt-ATP6
mt-nd5
12s rRNA
sod2
hadh
mfn2
got1
phlpp1
atgl1
gpt2b
gpt2a
lc3b
catha
lamp2a
sod1
glud1
tfeb
mfn1
cpt1a2b
mtl
gstp
hsp90
cathf
catalase
atgl3
parkin
gfap
hsl a
cpt1β
murf1
cpt1a2a
ulk1
bnip3b
fbxo32
hsp60
hslb
bnip3a
cox1
cathd
cpt1a1
cath1
fundc1
mgl
atgl2
pgc1a2
pgc1a1
atg7
gsr
gpx1
drp1
atg12
opa1
atg5
pink1
Lysosome
Macroautophagy
CMA
Mitophagy
Mitochondrial turnover
Mitochondrial metabolism
Cytosol catabolism
Antioxidant system
-0.5
0.0
0.5
Normalized gene expression

Fig. 5

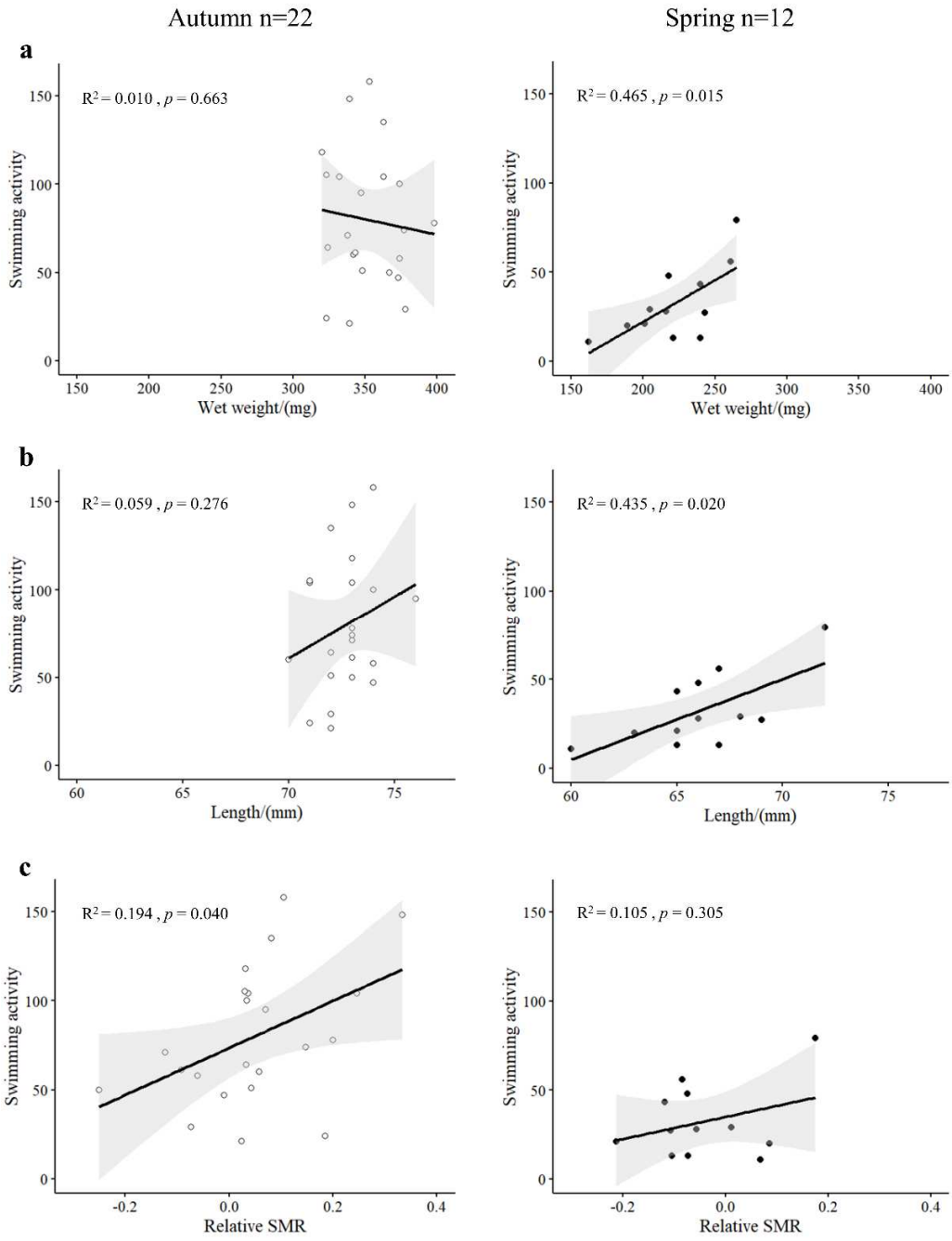
Autumn n=22
Spring n=12
a
b
c
R² = 0.010 , p = 0.663
R² = 0.465 , p = 0.015
R² = 0.059 , p = 0.276
R² = 0.435 , p = 0.020
R² = 0.194 , p = 0.040
R² = 0.105 , p = 0.305
Swimming activity
Wet weight/(mg)
Length/(mm)
Relative SMR

Fig. 6

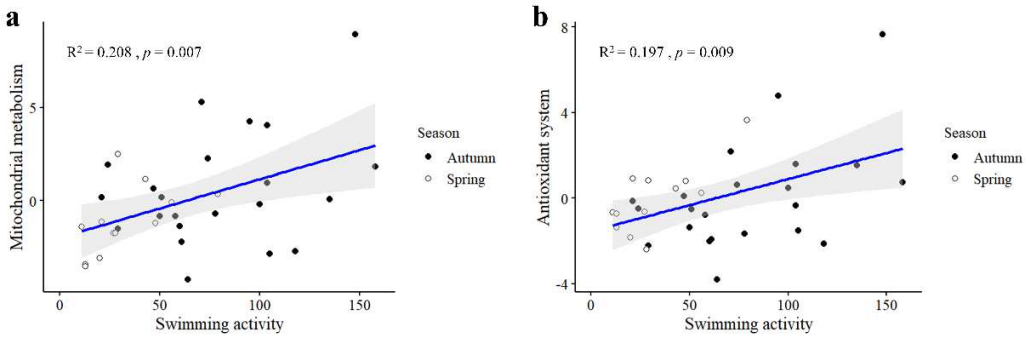
a
R² = 0.208 , p = 0.007
Mitochondrial metabolism
5
0
0
50
100
150
Swimming activity
Season
Autumn
Spring
b
R² = 0.197 , p = 0.009
Antioxidant system
8
4
0
-4
0
50
100
150
Swimming activity
Season
Autumn
Spring

Fig. 7

Table 1. Genes involved in each metabolism-related function and sequences of the primer pairs used for real-time quantitative RT-PCR.

Function	Sub-function	Gene *abb.*	5'/3' Forward primer	5'/3' Reverse primer
Autophagy-lysosome system	Macroautophagy	*atg5*	AGGGTCAGGTGGTCAATGAG	CTGTCGCTCATCGTCTGGTA
		atg7	CCTGAGCTCTCCCTGAACAC	CAGATCCAAGGAAGGAACCA
		atg12	GCAGTAGGGGACACTCCTAT	CACTGCCAAAACATTCAAATAAC
		lc3	TACAGGACATAGGCCGCTAA	ACTCGCTGTTCAAATGTCCT
		ulk1	GGACCTGTGGAGCATAGGAA	GGAAAAACTCATCGAAGTCCAT
	Lysosome	*catha*	GGGAACAAGCACCTGCATTA	CGCCATCATCCTGAATTAGA
		cathd	TCCAGGGAGAGTACATGGTTG	ACATCTCCCAGAATCCACAG
		cathf	GGGATATGGACATCGTAATGG	GCAGATGGGCGTTGTTTAAT
		cathl	TCAGTTCTACCAATCTGGAATCTAC	CTTCCTGTCTTTGGCCATGT
		tfeb	TGTCCAGCAGTCACATGGAT	CTTCCGACAGCTCCTTCTTGA
		lipa	GTGTGCGTTTGCTTGTGTCT	TTTTCACAGTGGCTTCATGC
	CMA	*lamp2a*	CTGAGGAATGCCAAGCTGAT	TGAAATAGGCCACCAACACA
		phlpp1	GAGGAGGTGAAGAGGCACAG	CAGACTGCAGCATGACAGGT
		hsp90	GAAGGCAGAGGCTGACAA	ATCAATTCCCAGGCCAAGTT
		hsc70a	ATGTCAAAGGGACCAGCAG	ATCAGCCTCTCGGTGTCAGT
		hsc70b	AGCAGTTGGGATTGATCTGG	CAACTTGATTCTTCGCTGCAT
	Mitophagy	*fundc1*	TGGTGTGCAGGATATCTTTTTCA	GCTTTGTTCACGTCCTTCTC
		pink1	TAGCTGCCAACATTCTGCAC	CCAGGAAGCACCTCTGTAGC
		parkin	CTGGACTGCTTCCACACGTA	CGTACTGCTCCTCTCCAAGG
		bnip3a	GGGAAAATGAGTTGCACGTT	CCCTTGCATTTTGGGTAGAA
		bnip3b	TAGCCAGGGTAAATGCAAGG	TCCCAGCAGCAGGTCTATTT
Mitochondrial turnover	Mito Biogenesis	*pgc1α1*	GATGAGAGACGGGTGGTGTA	GTGTAGCGGTAGGTGATGAAG
		pgc1α2	AGGTGGCAAGACACAAAACC	CCGGACGTGAGGTACTTGAG
		tfam	CACAATGCCGGCAAAGTTGA	CACAACGTCCTCTCGTCCAA
		hsp60	TGGACGCTGGAGATCTTCTT	TCACTGTGAAGGATGGCAAA
	Mito Fusion	*mfn1*	GCTGAGAGACGACCTGGTTC	AAAGTGTTTCTCCGTGTTCATC
		mfn2	CCTGGTGCAATGTCTCTGGT	TTTGATGTAGGCCGCCAACT
		opa1	CCACTACTACCAGAGGGGCT	CCAGGACCTCCTTCACGTTC
	Mito Fission	*drp1*	AAACTGGACCTGATGGATGC	GGGAGGGGTACTTCTTCTGC
Mitochondrial metabolism	Fatty acid catabolism	*cpt1α1*	AGAGACCTGTGACCCCACAC	TGAAGACATGTACCCCACCA
		cpt1α2a	TGATGTTCAGGAGCTTGTGC	GTTCTGGCAAGTGCAACTCA
		cpt1α2b	AGGCAGGTGTACCTCTCTGG	GCGCACTGAGAGACTGACTG
		cpt1β	CCAGGCTGTGGATGAATCTT	GCCGAGTGCTTTTTGAAGTC
		hadh	CCTTCGATGCTCTCCTTGAC	GGCCACATCAATGTCTTCCT
	Amino acid catabolism	*glud1*	CGAGGATCCGAACTTCTTCA	GACAGGCTCAAAACGTGGTT
		got1	CAACAGATCGGCATGTTCAG	TTATTGGACCTTGGTGACAGC
		got2	GGAGTGTTACCGTCCAGAGC	GTGTAGGCCTTGAGCTGCAT
		gpt2a	TTAAGAAGCCATTCACTGAGGT	TGGGCATGTGCAGAGAGC
		gpt2b	ACTCCACATCCAAGTGCTACAT	CGACACCAGTTTGGTGAGC
	Mitochondrial respiratory chain complexes	*mt-ATP6*	CTAGGCCTGCTCCCATACAC	CTGGTGTTCCTTCTGGCAGT
		mt-nd5	CCTCTGTTCAGGCTCCAT	GGGCTCAGGCGTTAAGGTA
		cox1	TAGAGGCCGGAGCTGG	GGGGAGTTTGGTACTGTGTAAT
	Mito 12S rRNA	*12s rRNA*	ACCCGTGAGAATGCCCT	GTGGCTGGCACGAGTT
Antioxidant system		*sod1*	TCAAGGACAGAATGCTCACG	TTTTCAGGCTTTCCTCGTTG
		sod2	CACCACGCCACATATGTCAA	AGCTCCCCTTGAGGTTCC
		catalase	AGCAACCGATCAAATGAAATTATGG	CAGCTCCCTTGGCGTG
		mtl	TGCACTACGTGTAAGAAAAGCTG	TTCATGTTCAGGCAGGAATG
		gstp	CACTGGGGATGTAGGCTGTT	GCCAGACTGATCAACTGCAA
		gsr	GCCCTATCGCTCTCAGTGAC	GAGGTCACAGACCCCAGTGT
		gpx1	CAAGTGCATCATCTGGAGCC	GGGACGTTTTACTTCGCCAG
		gfap	TGGAGATCGAGAGGGACAAC	CCTCTCCAACTGGACACGAT
Cytosol catabolism	Lipid catabolism	*hsla*	GGTCACCTCCGGCATAAGTA	AATGACCAACAGCAATGCAA
		hslb	CTGTCAGAGACGGAGCTGAT	AGATGAGGCGCATGTTGAC
		mgl	TGCAGCACATAGACCAGATCA	AACACCTTGACGGGTGAGAC
		atgl1	CACCAACACCAGCATTCAGT	CTCGATATTGCAGCAGTCCA
		atgl2	GGTGATGGCAGAGATGTGTC	CTTCTCAGGCACGGGTTG
		atgl3	GCGCGCAAAATTTAGTGTTT	TCCTCTTCACTCCCTTCAGC
	Protein catabolism	*fbxo32*	TCCTCTTCTGGAAGGACACA	TCCAGGAATCCATTGCACTA
		murf1	CTGTGAAGTCTCGCCCCTAC	CAACTGCTTCTGACGCTGAC
Reference gene		*luciferase*	CATTCTTCGCCAAAAGCACTCTG	AGCCCATATCCTTGTCGTATCCC

How does energetic status affect the probability to migrate in European glass eel?