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**Looking at the complex relationships between migration behavior and
conditional strategy based on energy metabolism in the European glass eel,
(*Anguilla anguilla*)**

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1 **1. Introduction**

2 The life cycle of the European eels has been described as a catadromous life history during which
3 they migrate between oceanic spawning areas and continental rearing habitats (Tesch, 2003). The
4 leptocephalus larvae drift with the Gulf Stream to join the continental shelf where they
5 metamorphose and are then referred to as glass eels. Then, glass eels migrate up estuaries to join
6 rivers using selective flood transport: during flood tide, glass eels move up in the water column
7 and migrate with the current while they go down and remain on or in the substratum during ebb
8 tide (Forward and Tankersley, 2001; Gascuel, 1986; Jellyman, 1979). However, a high degree of
9 geographical dispersion crossing marine and riverine water have been documented regarding to
10 different migratory patterns of settlement and river colonization (Daverat et al., 2006; Secor et al.,
11 1995; Tsukamoto and Arai, 2001; Tsukamoto et al., 1998; Tzeng, 1996). These different patterns
12 of migration could have a strong impact on the fate of the population because of the sex
13 determinism in eels, which is environmental (Geffroy and Bardonnnet, 2016; Krueger and Oliviera,
14 1999). Briefly, in European eels, males are generally observed to dominate in high-density
15 environments, often associated with estuarine or lower river reaches, whereas females tend to
16 become increasingly dominant with increasing distance from the sea (Adam et al., 2008; Davey
17 and Jellyman, 2005; Harrison et al., 2014; Laffaille et al., 2006; Parsons et al., 1977).

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

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

35 It is noteworthy that in most of the experiments supporting the conditional strategy, the energetic
36 status of individual was mainly evaluated using integrative proxies like body mass and size.
37 However, in fasting and migrating fish, energetic budgeting is a dynamic process mediated not
38 only by energy reserves but also associated with a highly interplaying network of energy
39 metabolisms. For example, we recently reported the existence of a non-random fluctuating
40 expression dynamics of autophagy- and lysosome-related genes during long term fasting in *A.*
41 *anguilla* glass eel and demonstrated a significant contribution of these transcripts production over
42 time to weight loss (Bolliet et al., 2017b). Besides its function in the removal of altered or
43 dysfunctional proteins and organelles, autophagy plays also a critical role in energy supply by
44 allowing starved cells to mobilize their own constituents including lipid and glycogen stores (Singh
45 et al., 2012). Another well-documented cellular energetic pivot is mitochondrial metabolic system
46 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).

67

68

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 ($12\pm 0.5^{\circ}\text{C}$) 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 ($0.2\text{-}0.3\ \mu\text{W}/\text{cm}^2$) and a constant UV light ($0.6\ \mu\text{W}/\text{cm}^2$). The water temperature
102 was kept at $12\pm 0.5^{\circ}\text{C}$ 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,

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

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

122 **2.4. Standard metabolic rate (SMR) assay**

123 22 active and 13 non-active glass eels and 12 active and 24 non-active glass eels in autumn and
124 spring, respectively, were randomly retrieved for oxygen consumption determination. Briefly,
125 glass eels were acclimated in a same tank with still water for three days to stay at rest and relative
126 quiet before analysis. Then, they were transferred to 12 respirometry chambers (diameter: 11.2mm,
127 length: 90mm) of an intermittent flow respirometer as described by Régnier et al. (2010), where
128 oxygen consumption was measured individually (one fish per chamber). Tagged glass eels were
129 introduced in the chambers at 3 p.m. and oxygen consumption was recorded continuously every
130 minute until 10 a.m. the next day. The closed/open phase of the system was 20min/20min and the
131 duration of the closed phase was determined in order that oxygen level in the chamber was always
132 kept above 80% O₂ saturation. After termination of the SMR measurements, the background
133 respiration, i.e. oxygen consumption within the respirometer due to microbial respiration, was
134 estimated by measuring the oxygen consumption rate in the respirometer without a fish (i.e. blank
135 run for two hours). Temperature and photoperiod used for the acclimatization phase and oxygen
136 measurement were similar to those used for the behavioral test. The first 15 hours were considered

137 as a period of acclimatization and the average oxygen consumption per eel was calculated using
138 the last four hours of recording. Measurements were conducted for three days in autumn and spring.
139 SMR ($\text{mm}^3 \text{O}_2/\text{h}$) was expressed in $\text{mm}^3 \text{O}_2$ consumed per hour. We then regressed the logarithm
140 of SMR on the logarithm of wet weight, and used the residuals of this model (*i.e.*, relative SMR)
141 for further analyses. Following oxygen consumption measurement, glass eels were anaesthetized,
142 individually measured for wet weight (Sartorius CP 153 balance, ± 1.0 mg) and length (± 1.0 mm)
143 and stocked at -80°C after immersion in liquid nitrogen.

144 **2.5. High throughput quantitative RT-PCR**

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

150 Primers specific to 59 genes involved in either energy metabolism or oxidative stress (Table 1)
151 were newly designed using Primer3 software (version 4.1.0) and based on the available genomic
152 resources for the European eel (GenBank assembly accession GCA_000695075.1). Primers were
153 validated on a Roche LightCycler 480 System (Roche Diagnostics, Neuilly sur Seine, France). The
154 assays were performed using a reaction mix of $6 \mu\text{l}$ per sample, each of which contained $2 \mu\text{l}$ of
155 diluted cDNA template, $0.24 \mu\text{l}$ of each primer ($10 \mu\text{M}$), $3 \mu\text{l}$ Light Cycler 480 SY Green Master
156 mix (Roche Diagnostics, 4887352001) and $0.52 \mu\text{l}$ DNase/RNase-free water (5 Prime GmbH,
157 2500020). The PCR protocol was initiated at 95°C for 10 min for initial denaturation of the cDNA
158 and hot-start Taq-polymerase activation, followed by 45 cycles of a 3-step amplification program
159 (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 $2^{\Delta C_{t_target}(\text{control-sample})}/2^{\Delta C_{t_reference}(\text{control-sample})}$ (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:

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

181 We assumed that $P(t, i)$ was a periodic function of time, since it has been previously shown that
182 glass eels display rhythmic swimming activity in response to current reversal (Bolliet and Labonne,
183 2008):

$$184 \text{logit}(P(t, i)) = a(i) \times \sin(t \times b(i) + c(i)) + d(i)$$

185 where $a(i)$ was the strength of the synchronized component of activity, $b(i)$ was related to period,
186 $c(i)$ was related to the trigonometric function phase, $d(i)$ was the non-synchronized component of
187 activity. Fish having a P value above the mean of P meanwhile having an activity periodicity close
188 to 12.4h were considered synchronized and active, others were considered non-active.

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

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

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

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

220

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

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

251 *Expressions of genes involved in energetic metabolism*

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

266 in spring glass eels compared to autumn ones, while genes involved in energy production and use
267 (mitochondrial metabolism and CMA) showed an opposite trend. In detail, four macroautophagy
268 genes (*atg5*, *atg7*, *atg12*, *ULK1*) and four mitophagy receptors (*fundc1*, *pink1*, *bnip3a*, *bnip3b*),
269 showed significantly higher transcript levels in spring than in autumn, reflecting advanced energy
270 distress in spring glass eels. In this regard, higher expression was also evidenced in spring glass
271 eels for the gene *drp1*, known as the main player in the process of mitochondrial fragmentation,
272 and three genes involved in mitochondrial biogenesis (*pgc1a1*, *pgc1a2*, *hsp60*), suggesting high
273 mitochondrial turnover to compensate to the loss of mitochondria through mitophagy. In contrast,
274 two genes (*mfn1* and *mfn2*) involved in mitochondrial fusion and cooperation were significantly
275 more expressed in autumn than in spring. Likewise, most studied mitochondrial metabolism related
276 genes, i.e., two genes coding for subunits of the mitochondrial membrane respiratory chain (*mt-*
277 *ATP6*, *mt-nd5*), two genes involved in fatty acid catabolism (*cpt1a1*, *hadh*) and five genes involved
278 in amino acid catabolism (*glud1*, *got1*, *got2*, *gpt2a*, *gpt2b*), exhibited higher expression in autumn
279 than in spring. Interestingly, a similar trend was also observed for the genes related to CMA
280 (*hsc70a*, *hsc70b*, *lamp2a*, *hsp90*, *phlpp1*), which has been demonstrated to play a major role in the
281 regulation of the hepatic intermediary metabolism. Similarly, five genes involved in the oxidative
282 stress defense (*sod1*, *sod2*, *mtl*, *gstp* and *catalase*) clustered in the group of genes overexpressed in
283 autumn glass eels, possibly as a consequence of higher mitochondrial activity in these animals
284 compared to their spring counterparts. Altogether, these results highlighted the existence of strong
285 differences of the metabolic status between autumn and spring glass eels. However, no clear
286 difference was evidenced between active and non-active glass eels whatever the season considered.

287

288 **3.3. Relationships of the swimming activity levels of each active glass eel with its energetic**
289 **status**

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

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

298 Among the five genomic functions of interest (autophagy-lysosome system, mitochondrial
299 metabolism, mitochondrial turnover, antioxidant system and cytosol catabolism), the expression
300 levels of mitochondrial metabolism genes and antioxidant system genes were significantly related
301 to swimming activity (Fig.7). We found a positive relationship between the first axis of the PCA
302 for both of the functions and swimming activity level (two-way ANOVA, $p=0.008$ for
303 mitochondrial metabolism and $p=0.007$ for antioxidant system), with no effect of season ($p=0.772$
304 and 0.074 , respectively) and no significant interaction ($p=0.560$ and 0.384 , respectively; See
305 Supplementary material 4). For both functions, the first axis of the PCA explained 51% of the total
306 gene expression variance. For mitochondrial metabolism function, this first axis was mainly
307 correlated to genes of fatty acid catabolism (*cpt1a2b* and *hadh*), amino acid catabolism (*got1*, *got2*,
308 *gpt2a*, *gpt2b* and *glud1*), mitochondrial respiratory chain complexes (*mt-nd5* and *mt-ATP6*) and
309 *12s rRNA*, while for antioxidant system, this first axis was mainly correlated to *sod1*, *sod2*, *catalase*,
310 *gstp* and *mtl* genes (See Supplementary material 5).

311 **4. Discussion**

312
313 The main objective of this study was to investigate the relationship between European glass eel
314 energetics and their pattern of migration behavior, in the framework of the conditional strategy.
315 For this purpose, we used autumn and spring marine glass eels known to present high and low
316 energetic stores, respectively. We did observe a higher propensity to migrate in autumn as predicted
317 by the conditional strategy, and individual swimming activity level was also higher in autumn.
318 However, when we compared the energy status of active and non-active glass eels within each
319 seasonal group, we found no support for the conditional strategy: both behavioral groups showed
320 similar wet weight and length. At the individual level, we also found little support for the
321 conditional strategy: energy stores were correlated to the level of swimming activity in active glass
322 eels only in spring, not in autumn. These results are further explained partly by variation in
323 metabolic rates between individuals, and partly by the transcriptome analyses, that indicate striking
324 contrasts in the metabolic states of autumn versus spring eels.

325 **4.1. Autumn and spring glass eels: Highly contrasting metabolic states**

326 Final wet weight was highly correlated to tryglycerides (see section 1.2) and wet weight was
327 considered in this study as a good proxy of energy stores. Here we show that autumn glass eels
328 displayed higher wet weight than spring ones, which is in close agreement to previous observations
329 made in *A. anguilla* glass eels (Charlon and Blanc, 1982; Claveau et al., 2015; De Casamajor et al.,
330 2000; Elie, 1979). Such seasonal differences in biomass and energetic stores have been suggested
331 to be due to the seasonal variations in oceanic ecosystems productivity affecting the growth of
332 leptocephalus larvae during transatlantic transport (Désaunay and Guerault, 1997).

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

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

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

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

364 **4.2. Active vs non-active glass eels**

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

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

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

476

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480

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482

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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 \pm
- 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.

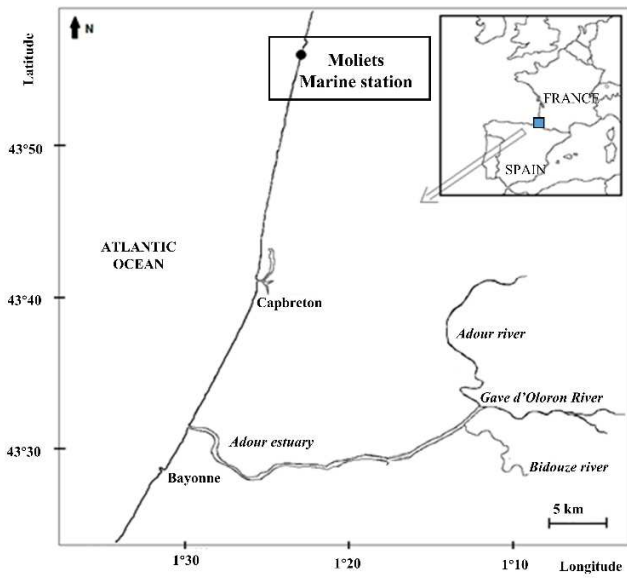


Fig. 1

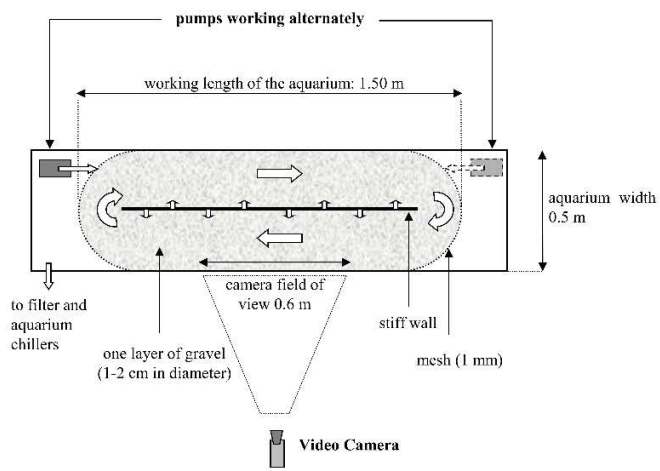


Fig. 2

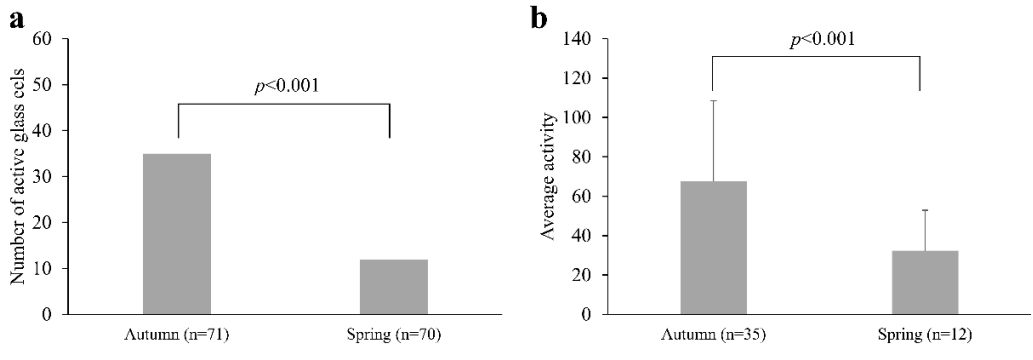


Fig. 3

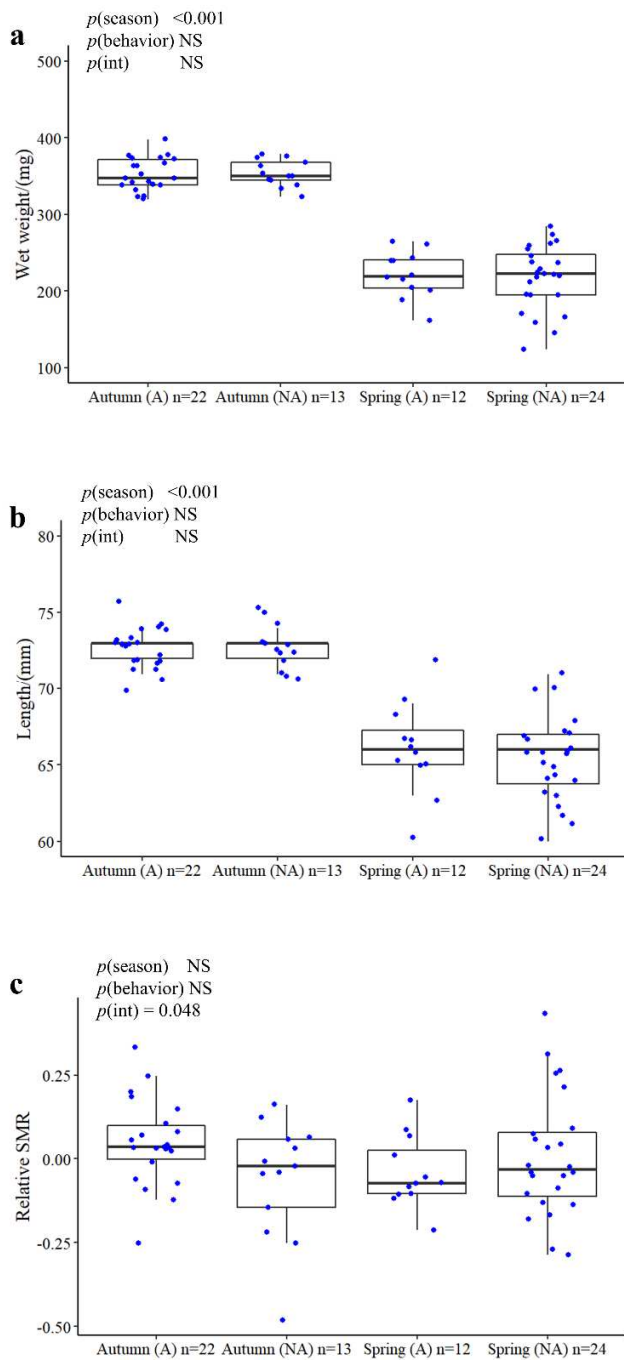


Fig. 4

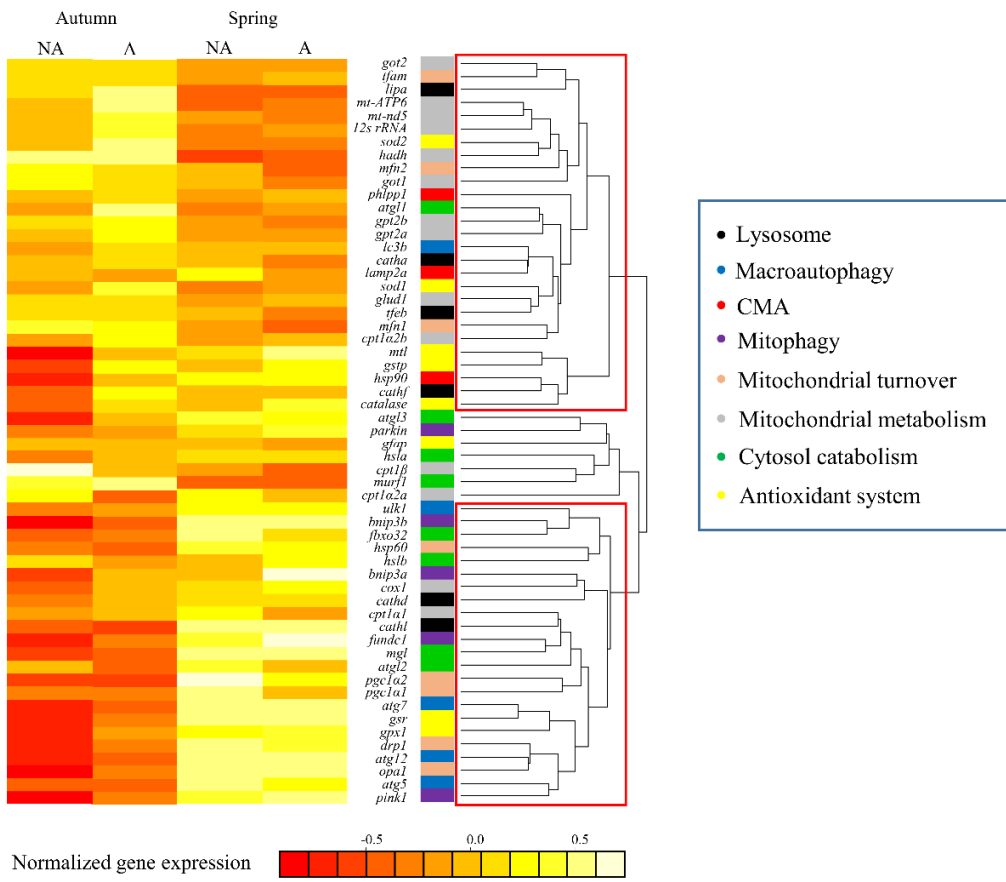


Fig. 5

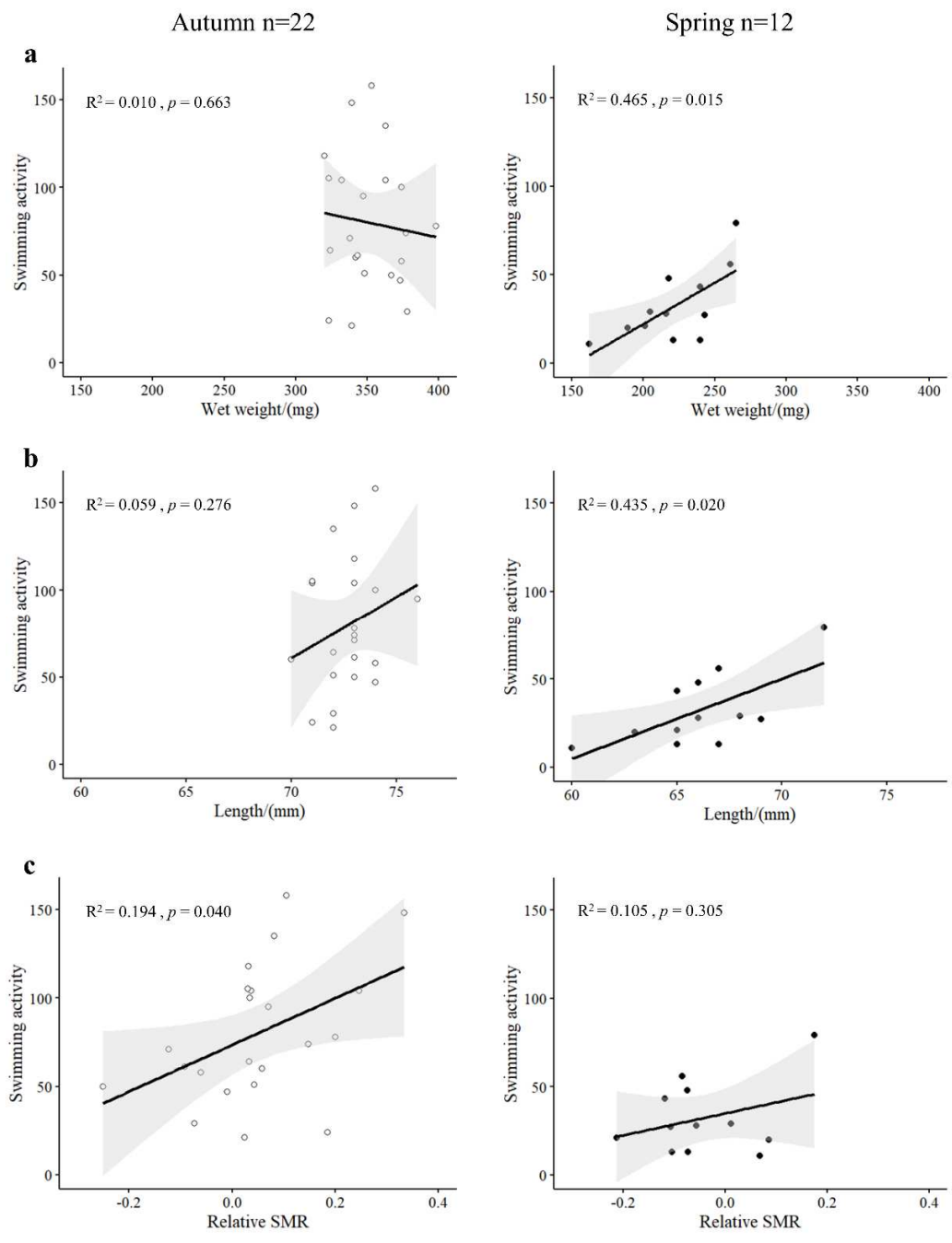


Fig. 6

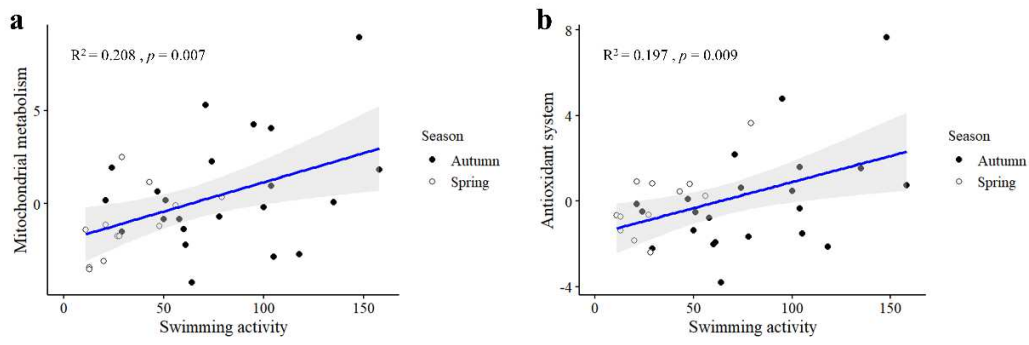


Fig. 7

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	<i>atg5</i> <i>atg7</i> <i>atg12</i> <i>lc3</i> <i>ulk1</i>	AGGGTCAGGTGGTCAATGAG CCTGAGCTCTCCCTGAACAC GCAGTAGGGGACACTCCTAT TACAGGACATAGGCCGCTAA GGACCTGTGGAGCATAGGAA	CTGTGCGCTCATCGTCTGGTA CAGATCCAAGGAAGGAACCA CACTGCCAAAACATTCAAATAAC ACTCGCTGTTCAAATGTCTT GGAAAAACTCATCGAAGTCCAT
	Lysosome	<i>catha</i> <i>cathd</i> <i>cathf</i> <i>cathl</i> <i>tfeb</i> <i>lipa</i>	GGGAACAAGCACCTGCATTA TCCAGGGAGAGTACATGGTTG GGGATATGGACATCGTAATGG TCAGTTCTACCAATCTGGAATCTAC TGTCCAGCAGTCACATGGAT GTGTGCGTTTGCTTGTGTCT	CGCCATCATCCTGAATTAGA ACATCTCCCAGAATCCACAG GCAGATGGGCGTTGTTTAAAT CTTCTGTCTTTGGCCATGT CTTCCGACAGCTCCTTCTTGA TTTTACAGTGGCTTCATGC
	CMA	<i>lamp2a</i> <i>phlpp1</i> <i>hsp90</i> <i>hsc70a</i> <i>hsc70b</i> <i>fundc1</i>	CTGAGGAATGCCAAGCTGAT GAGGAGGTGAAGAGGCACAG GAAGGCAGAGGCTGACAA ATGTCAAAGGGACCAGCAG AGCAGTTGGGATTGATCTGG TGGTGTGCAGGATATCTTTTTCA	TGAAATAGGCCACCAACACA CAGACTGCAGCATGACAGGT ATCAATCCCAGGCCAAGTT ATCAGCCTCTCGGTGTCAGT CAACTGTATTCTTCGCTGCAT GCTTTGTTACAGTCTCTTCTC
	Mitophagy	<i>pink1</i> <i>parkin</i> <i>bnip3a</i> <i>bnip3b</i>	TAGCTGCCAACATTCTGCAC CTGGACTGCTCCACACGTA GGGAAAATGAGTTGCACGTT TAGCCAGGGTAAATGCAAGG	CCAGGAAGCACCTCTGTAGC CGTACTGCTCCTCTCAAGG CCCTTGCAATTTGGGTAGAA TCCCAGCAGCAGGCTTATTT
Mitochondrial turnover	Mito Biogenesis	<i>pgc1a1</i> <i>pgc1a2</i> <i>tfam</i> <i>hsp60</i>	GATGAGAGACGGGTGGTGTGA AGGTGGCAAGACACAAAACC CACAATGCCGGCAAAGTTGA TGGACGCTGGAGACTTCTTT GCTGAGAGACGACCTGGTTC	GTGTAGCGGTAGGTGATGAAG CCGGACGTGAGGTACTTGAG CACAACGTCTCTCGTCCAA TCACTGTGAAGGATGGCAAA AAAGTGTTCCTCCGTGTTTCATC
	Mito Fusion	<i>mfn1</i> <i>mfn2</i> <i>opa1</i>	CCTGGTGCAATGTCTCTGGT CCACTACTACCAGAGGGGCT AAACTGGACCTGATGGATGC	TTTGATGTAGGCCGCAACT CCAGGACCTCCTTACGTTTC GGGAGGGGACTTCTTCTGC
	Mito Fission	<i>drp1</i>		
Mitochondrial metabolism	Fatty acid catabolism	<i>cpt1a1</i> <i>cpt1a2a</i> <i>cpt1a2b</i> <i>cpt1b</i> <i>hadh</i> <i>glud1</i>	AGAGACCTGTGACCCACAC TGATGTTCAAGGACTTGTGC AGGCAGGTGTACCTCTCTGG CCAGGCTGTGGATGAATCTT CCTTCGATGCTCTCCTTGAC CGAGGATCCGAACTTCTCA	TGAAGACATGTACCCACCA GTTCTGGCAAGTGCAACTCA GCGCACTGAGAGACTGACTG GCCGAGTGCTTTTGAAGTC GGCCACATCAATGTCTTCT GACAGGCTCAAACCGTGGTT
	Amino acid catabolism	<i>got1</i> <i>got2</i> <i>gpt2a</i> <i>gpt2b</i>	CAACAGATCGGCATGTTTCAG GGAGTGTACCCTCCAGAGC TTAAGAAGCCATTCAGTGGGT ACTCCACATCCAAGTGCTACAT	TTATTGGACCTTGGTGACAGC GTGTAGGCCCTGAGCTGCAT TGGGCATGTGCAGAGAGC CGACACCAGTTTGGTGAGC
	Mitochondrial respiratory chain complexes	<i>mt-ATP6</i> <i>mt-nd5</i> <i>cox1</i>	CTAGGCCTGCTCCATACAC CCTCTGTTCAAGGCTCCAT TAGAGGCCGGAGCTGG	CTGGTGTCTCTTGGCAGT GGGCTCAGGCGTTAAGGTA GGGGAGTTTGGTACTGTGTAAT GTGGCTGGCAGGAGTT
	Mito 12S rRNA	<i>12s rRNA</i>	ACCCGTGAGAATGCCCT	
Antioxidant system		<i>sod1</i> <i>sod2</i> <i>catalase</i> <i>mil</i> <i>gstp</i> <i>gsr</i> <i>gpx1</i> <i>gfap</i>	TCAAGGACAGAATGCTCAGC CACCACGCCACATATGTCAA AGCAACCGATCAAATGAAATTATGG TGCCTACGTGTAAGAAAAGCTG CACTGGGGATGTAGGCTGTT GCCCTATCGCTCTCAGTGAC CAAGTGATCATCTGGAGCC TGGAGATCGAGAGGGACAAC	TTTTCAGGCTTTCCTCGTTG AGCTCCCCTTGAGGTTCC CAGCTCCCCTTGGCGTG TTCATGTTCAAGGCAGGAATG GCCAGACTGATCAACTGCAA GAGGTCACAGACCCAGTGT GGGACGTTTTACTTCGCCAG CCTCTCCAACCTGGACACGAT
Cytosol catabolism	Lipid catabolism	<i>hsla</i> <i>hslb</i> <i>mgl</i> <i>atgl1</i> <i>atgl2</i> <i>atgl3</i>	GGTCACCTCCGGCATAAGTA CTGTCAAGAGACGGAGCTGAT TGCAGCACATAGACCAGATCA CACCAACACCAGCATTTCAGT GGTGATGGCAGAGATGTGTC GCGCGCAAAATTTAGTGTTC	AATGACCAACAGCAATGCAA AGATGAGGCGCATGTTGAC AACACCTTGACGGGTGAGAC CTCGATATTGCAGCAGTCCA CTTCTCAGGCACGGGTTG TCTCTTCACTCCCTTCAGC
	Protein catabolism	<i>fbxo32</i> <i>murfl</i>	TCCTCTTCTGGAAGGACACA CTGTGAAGTCTCGCCCTAC	TCCAGGAATCCATTGCACTA CAACTGCTTCTGACGTGAC
Reference gene		<i>luciferase</i>	CATTCTTCGCCAAAAGCACTCTG	AGCCCATATCCTTGTCTGATCCC

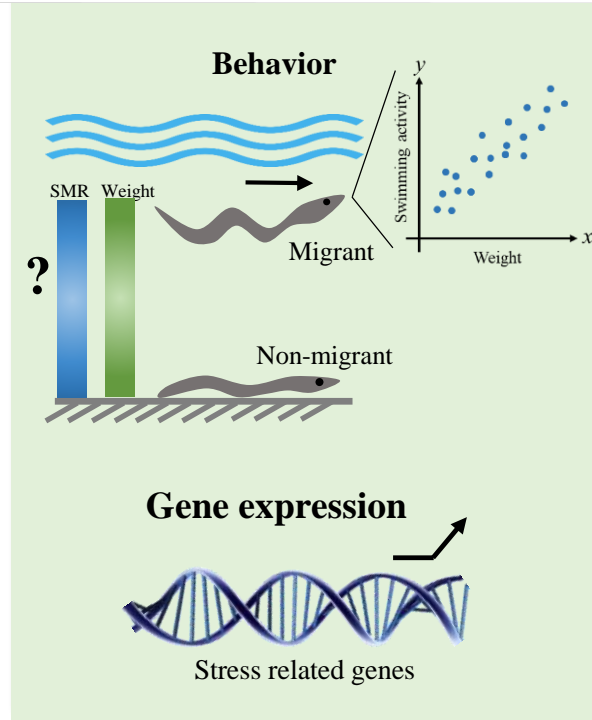
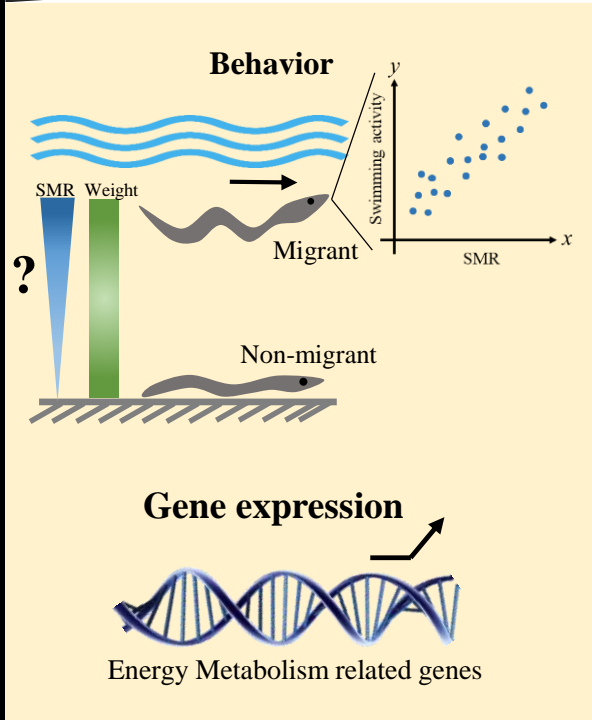
How does energetic status affect the probability to migrate in European glass eel?

Autumn



Spring

Energy stores
Probability to migrate



Vulnerability to stress ?