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1 **New insights into methylmercury induced behavioral and energy-**  
2 **related gene transcriptional responses in European glass eel**  
3 **(*Anguilla anguilla*)**

4 Hengtong Liu<sup>1,2</sup>, Amaia Lamarins<sup>1</sup>, Jacques Labonne<sup>1</sup>, Mathilde Monperrus<sup>3</sup>, Pascale Coste<sup>1</sup>,  
5 Emmanuel Huchet<sup>1</sup>, Jacques Rives<sup>1</sup>, Iban Seiliez<sup>2,†</sup> and Valérie Bolliet<sup>1,†,\*</sup>

6 <sup>1</sup> *Université de Pau et des Pays de l'Adour, E2S UPPA, INRAE, ECOBIOP, Aquapôle INRAE,*  
7 *MIRA, F64310, Saint-Pée-sur-Nivelle, France*

8 <sup>2</sup> *INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, NuMéA, F64310, Saint-Pée-sur-*  
9 *Nivelle, France*

10 <sup>3</sup> *Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, Institut des Sciences Analytiques*  
11 *et de Physicochimie pour l'Environnement et les Matériaux – MIRA, UMR 5254, 64600, Anglet,*  
12 *France*

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15 <sup>†</sup>These authors contributed equally to this work

16 \* Corresponding author:

17 [valerie.bolliet@inrae.fr](mailto:valerie.bolliet@inrae.fr)

18 UMR 1224 Ecobiop, Aquapôle, 64310 Saint Pée sur Nivelle,

19 France. Tel.: +33 5 59 51 59 78

20 **ABSTRACT**

21 The effect of methylmercury (MeHg) was investigated in glass eel migration behavior and  
22 metabolism. To migrate up estuary, glass eels synchronize their swimming activity to the flood  
23 tide and remain on or in the substratum during ebb tide. Following seven days of exposure to  
24 MeHg (100 ng L<sup>-1</sup>), glass eels migration behavior was expressed by their swimming  
25 synchronization to the water current reversal every 6.2 h (mimicking the alternation of flood and  
26 ebb tides) and their swimming activity level. In relation to their behavior, we then analyzed the  
27 energy-related gene expression levels in individual head, viscera and muscle. Results showed  
28 that MeHg decreased the number of glass eels synchronized to the change in water current  
29 direction and their swimming activity level. This last effect was more pronounced in non-  
30 synchronized fish than in synchronized ones, supporting the idea that non-synchronized glass  
31 eels could be more vulnerable to stress. As regard the expression of energy-related genes, no  
32 significant difference was observed between control and MeHg-exposed fish. In contrast, when  
33 the swimming activity levels were plotted against transcriptional responses, positive correlations  
34 were evidenced in viscera and especially in the head of exposed glass eels but not in control.  
35 Finally, it is noteworthy that non-synchronized glass eels displayed lower expression level of  
36 metabolism genes than their synchronized counterpart, but only in the head. Altogether, these  
37 results support the interest of focusing on the head to investigate the facultative migration  
38 behavior in glass eels and the effect of environmental stressors on this rhythmic behavior.

39

40 **Keywords** : Glass eel; *Anguilla anguilla*; Migration behavior; Methylmercury; Metabolism

41

## 42 **1. Introduction**

43 The status of European eel (*Anguilla anguilla*) remains critical since the recruitment of glass  
44 eels strongly declined from 1980 to about 2010, and have remained at a low level since, which  
45 has urged Euro-wide European eel regulation (council regulation (EC) no. 1100/2007) for the  
46 protection and recovery of the stock (ICES, 2018). The European eel is a facultative catadromous  
47 species that crosses the Atlantic Ocean twice during its life. The present knowledge clearly  
48 indicate that the species reproduces in the Sargasso Sea, where the leptocephalus larval stage is  
49 first observed (Schmidt, 1923; van Ginneken and Maes, 2005). The larvae move with ocean  
50 currents more than 5000 km toward the European continent, after which they transform into a  
51 juvenile stage, the glass eel. Then, glass eels migrate up estuary to reach freshwater habitats  
52 where they grow and develop into yellow eels. At an age of 5 to 20 years they commence the  
53 silvering process and become silver eels at which point they begin their migration back to the  
54 Sargasso Sea to reproduce and probably die (Miller et al., 2015; Righton et al., 2016; Schmidt,  
55 1923; Tesch, 1980, 2003).

56 Glass eels migrate up estuaries to ascend rivers using selective flood transport: during flood  
57 tide, glass eels move up in the water column and migrate with the current while they go down  
58 and remain on or in the substratum during ebb tide (Forward and Tankersley, 2001; Gascuel,  
59 1986; Jellyman, 1979). They are also synchronized to the photoperiod, avoiding light and  
60 swimming mainly during the night when the water is clear. However, a high degree of  
61 geographical dispersion crossing marine and riverine water has been documented regarding to  
62 different migratory patterns of settlement and river colonization (Daverat et al., 2006; Secor et al.,  
63 1995; Tsukamoto and Arai, 2001; Tsukamoto et al., 1998; Tzeng, 1996). These different patterns  
64 of migration could have a strong impact on the fate of the population because of the

65 environmental sex determinism in eels, (Geffroy and Bardonnnet, 2016; Krueger and Oliveira,  
66 1999). Briefly, in European eels, males are generally observed to dominate in high-density  
67 environments, often associated with estuarine or lower river reaches, whereas individuals that  
68 migrated upstream to the river tend to become mostly females (Adam et al., 2008; Davey and  
69 Jellyman, 2005; Harrison et al., 2014; Laffaille et al., 2006; Parsons et al., 1977).

70 Although there is no consensus on the reason for the diverse migratory patterns, it has been  
71 suggested that early settlement in estuary or coast, if taking place, is partly due to low energy  
72 condition (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006; Liu et al.,  
73 2019). Indeed, most glass eels do not feed throughout estuarine migration and they depend on  
74 energy reserves accumulated by the leptocephalus larvae during oceanic migration to reach the  
75 river (Kawakami et al., 1999; Tesch, 2003; Bardonnnet and Riera, 2005). Moreover, estuary is a  
76 highly stressful ecosystem, where a combination of different stressors including large variations  
77 in the temperature, hydraulic conditions, salinities, oxygen availability or contaminants may  
78 influence the energy metabolic processes of glass eels during migration. Due to its proximity to  
79 urbanized and industrial areas, estuary represents a major sink for various contaminants  
80 including various forms of mercury (Hg), both inorganic (Hg(II)) and methylated (MeHg), this  
81 latest form being recognized to adversely affect fish physiology, growth, health and behavior  
82 (Cambier et al., 2009; Cambier et al., 2012; Lee et al., 2011; Murphy et al., 2008; Scheulhammer  
83 et al., 2007).

84 Behavioral effects of MeHg exposure in fish have been largely documented, with a lot of  
85 concerns on prey capture ability (Weis and Khan, 1990; Zhou et al., 2001), predator avoidance  
86 (Webber and Haines, 2003), reproduction (Sandheinrich and Miller, 2006), habitat selection  
87 (Sampaio et al., 2016) and more recently on memory and aggressiveness (Strungaru et al., 2018).

88 The underlying molecular mechanisms of MeHg toxicity are predominantly related with  
89 oxidative stress, which in turn may induce lipid peroxidation and DNA damages (Berntssen et al.,  
90 2003; Gonzalez et al., 2005). In glass eels, MeHg toxicity could increase energy expenditure  
91 through the processes of cellular repairment and detoxification which may thereby reduce glass  
92 eels fitness and their migratory success through estuary. Claveau et al. (2015) reported that glass  
93 eels exposed to MeHg (50 ng L<sup>-1</sup>) for 11 days exhibited some perturbations of mitochondrial  
94 structures and metabolism associated to an activation of antioxidative defence systems. However,  
95 the effect of MeHg differed between behavioral phenotypes of glass eels, those displaying a low  
96 propensity to migrate up estuaries being more affected than their ‘migrant’ counterpart. These  
97 findings suggested the existence of differences in sensitivity to MeHg exposure among glass eels  
98 that remained to be elucidated.

99 In the present study we aimed to identify the MeHg-induced repercussions in synchronization  
100 of glass eels to the water current reversal, their level of swimming activity and the transcriptomic  
101 profiles of energy-related genes. This was achieved by transcriptome analyses related to energy  
102 metabolism and direct observations of swimming behavior.

## 103 **Materials and methods**

### 104 *2.1 Ethics*

105 Procedures used in this study have been validated by the ethics committee N°073 (ref:  
106 2017012015086652). The experiment was carried out in strict accordance with the EU legal  
107 frameworks, specifically those relating to the protection of animals used for scientific purposes  
108 (i.e., Directive 2010/63/EU), and under the French legislation governing the ethical treatment of  
109 animals (Decret no. 2013-118, February 1st, 2013).

110

111

## 112 *2.2 Primary test for MeHg accumulation kinetics over 30 days*

### 113 *2.2.1 Fish collection*

114 The glass eels were collected at the mouth of a small estuary (courant d'Huchet) located 40  
115 km north of the mouth of the Adour estuary, France (43° 55' N, 1° 23' W). They were  
116 sampled using a dip-net at night and during flood tide in February 2018, and were then  
117 transferred to the laboratory and maintained in a tank containing water from the fishing site.  
118 During the next 48 h, the water was continuously aerated and progressively diluted with fresh  
119 water. Fish were kept under 12 °C and a photoperiod of 12 L / 12 D with a very low light  
120 intensity during the photophase (0.2-0.3  $\mu\text{W}/\text{cm}^2$ ).

### 121 *2.2.2 MeHg exposure and kinetic sampling*

122 After acclimation, glass eels were randomly selected and allocated by groups of three into a  
123 series of seven aquariums (1.5 L of aerated fresh water) with initial compartment concentration  
124 of 100 ng L<sup>-1</sup> of MeHg. Glass eels were exposed to this single spike of MeHg prepared as follow:  
125 The stock solution of MeHg at 1000 mg L<sup>-1</sup> was prepared by dissolving MeHg chloride obtained  
126 from Strem Chemicals (Newburyport, MA, USA) in methanol. The spiking solution at 100  $\mu\text{g L}^{-1}$   
127 was then prepared by diluting the stock solution in 1% hydrochloric acid.

128 Three glass eels were collected in the beginning of this test as controls. All the aquariums  
129 were sealed with a transparent cover to prevent water volatilization and to guarantee the  
130 photoperiod. The aeration, temperature and photoperiod were maintained as described for the

131 acclimation period. During the exposure period, no mortality neither erratic swimming (abrupt  
132 change of direction or swimming speed) were observed.

133 The MeHg-exposed fish were sampled at 1, 2, 3, 7, 11, 18 and 30 days. One aquarium was  
134 recovered on each sampling time point and the three fish in this aquarium were considered to be  
135 three replicates. Sampled fish were killed by anesthetic and quickly washed with distilled water.  
136 After biometry, all the fish were immediately frozen into liquid nitrogen and stored at -80 °C for  
137 future mercury speciation analysis.

### 138 *2.2.3 Mercury speciation analysis*

139 Each glass eel was lyophilized, mashed using an agate mortar and then submitted to  
140 microwave extraction according to the procedure previously published by Navarro et al. (2013).  
141 The supernatant was spiked with known amounts of standard solution of MeHg and Hg(II) and  
142 submitted to propylation. Mercury speciation analysis was performed by GC-ICPMS using the  
143 method with parameters, which are detailed by Navarro et al. (2013). Mercury species  
144 concentrations were determined by speciated isotope dilution (Monperrus et al., 2005).  
145 Analytical performances were evaluated using the Certified Reference Material DOLT- 4  
146 (Dogfish Liver, NRCC, Ottawa, Canada). Good agreement with certified values was obtained  
147 with recoveries of 105±8% and 95±7% for MeHg and Hg(II), respectively. Detection limits of  
148 0.1 and 0.2 ng.g<sup>-1</sup> were found for MeHg and Hg(II), respectively. All concentrations were  
149 expressed in ng Hg.g<sup>-1</sup> dry weight.

### 150 *2.3 Seven-day MeHg exposure*

151 The kinetics experiment ran over 30 days showed that glass eels exposed to MeHg presented  
152 an increase in MeHg concentration measured in whole body for up to seven days which then



153 reached a plateau (Fig. 1). The increase in Hg(II) concentration remained low and stabilized very  
154 quickly. According to these results, we chose 7 days as exposure duration to investigate the  
155 effect of MeHg on migration behavior and energy metabolism.

### 156 *2.3.1 Fish collection and tagging*

157 140 glass eels were collected in March 2018 at the same fishing site and using the same  
158 capture method described above (see 2.2.1). They were transferred to the laboratory and  
159 maintained at 12 °C overnight in an aerated tank containing water from the fishing site. In the  
160 morning following their capture, all glass eels were anesthetized (Benzocaine, 0.01 mg L<sup>-1</sup>) and  
161 individually tagged using Visible Implant Elastomer (VIE Tag) (combinations of one or two  
162 hypodermic spots of different colors as described by Imbert et al., 2008) in order to trace the  
163 swimming behavior individually. Once tagged, glass eels were released to wake up in the water  
164 from fishing site. During the next 48 h, the water was continuously aerated and progressively  
165 diluted with fresh water.

### 166 *2.3.2 MeHg exposure*

167 After acclimation, fish were randomly divided into two groups: control group without Hg  
168 addition and MeHg-group exposed to an initial MeHg concentration of 100 ng L<sup>-1</sup>. Seven glass  
169 aquariums (5 L of aerated fresh water) were used for each group, with 10 fish in each aquarium.  
170 The exposure lasted for seven days. During the entire experimental period, both control and  
171 MeHg-exposed fish were kept under the same conditions as those during the acclimation period.  
172 No mortality neither erratic swimming were observed during the experiment.

### 173 *2.3.3 Observations of the post-exposure swimming behavior*

174 After seven-day exposure, one aquarium of 10 glass eels from the control group and one  
175 aquarium of 10 specimens from the MeHg-exposed group were recovered for mercury speciation  
176 analysis. Procedures of sample preparation and mercury measurement were the same as primary  
177 test (see 2.2.3). All the other control and MeHg-exposed glass eels ( $n = 6 \times 10$  glass eels in each  
178 group) were transferred into two annular tanks (30 glass eels of both the control and MeHg  
179 groups in each tank) installed in two temperature-controlled rooms. The rooms were kept under  
180 the same conditions as describes above, except that we added a constant UV light ( $0.6 \mu\text{W}/\text{cm}^2$ )  
181 in order to see the VIE Tag. The water temperature was kept at  $12 \pm 0.5 \text{ }^\circ\text{C}$  and continuously  
182 recorded by thermistors placed in the tank. The annular tank system was specially designed to  
183 mimic tidal rhythm by being equipped with two pumps at its opposite ends (Liu et al., 2019;  
184 Supplementary Fig. S1). The two pumps were programmed to alternately work to generate  
185 clockwise or counterclockwise water flow every 6.2 h. In each tank, the swimming activity of  
186 glass eels was traced continuously during 10 days by a camera programed to record 15 seconds  
187 every 40 min. The UV light allowed the identification of each glass eel during the light and dark  
188 phases by its elastomer mark. During the 10 days, a total of 360 sessions of 15 seconds were  
189 obtained.

190 Glass eels use selective tidal-stream transport to migrate up estuary, wherein individuals  
191 synchronize their swimming activity to tidal current, but they also have to sustain the level of  
192 swimming activity. Thus, in our experimental conditions, the propensity of glass eels to migrate  
193 was firstly evaluated by their capacity to synchronize the swimming activity to the change in  
194 water current direction by a period of 12.4 h. Then, their activity levels were quantitatively  
195 analyzed by counting the total number of observations of each elastomer mark in the water  
196 column in the 360 sessions of 15 seconds videos.

197 *2.3.4 Gene expression analysis*

198 *Sampling procedure*

199 After swimming test, all the glass eels in annular tank were recovered, anaesthetized and then  
200 killed by a lethal bath of anaesthesia (Benzocaine, 0.05 mg L<sup>-1</sup>), flash-frozen in liquid nitrogen,  
201 and stored at -80 °C.

202 *RNA extraction and quantitative real-time PCR*

203 Frozen glass eels were cut in three sections, containing (i) the head including the gills, (ii) the  
204 heart, the liver, the spleen and the stomach and (iii) most of the muscle tissue with the intestine  
205 (hereafter referred to as head, viscera and muscle, respectively, Fig. 2). Then, each section was  
206 immediately stored in TRIzol reagent for total RNA extraction.

207 The protocol conditions for quantitative RT-PCR have been previously published (Lansard et  
208 al., 2010). The primers specific to 27 genes involved in antioxidant system, mitochondrial  
209 function and autophagy activity have been described in previous study by Liu et al. (2019). For  
210 the expression analysis, relative quantification of target gene expression was done using the  $\Delta$ CT  
211 method described by Pfaffl (2001). The relative expression of Luciferase was used for data  
212 normalization as described previously (Marandel et al., 2016).

213 *2.4 Statistical analyses*

214 *2.4.1 Propensity of glass eels to migrate*

215 To characterize the propensity of glass eels to migrate, we first investigated the  
216 synchronization of glass eel's swimming activity to the change in water current direction every  
217 6.2h (synchronized / non-synchronized). For this purpose, we used a modeling method to

218 categorize all the recovered glass eels into synchronized ones and non-synchronized ones, which  
219 has been previously described by Liu et al. (2019) (See Supplementary Text S1). Since it has  
220 been previously shown that glass eels display rhythmic swimming activity in response to current  
221 reversal (Bolliet and Labonne, 2008), two parameters, the probability of being swimming of an  
222 individual  $i$  at time  $t$ ,  $P(t, i)$  and the periodicity of swimming occurrence of an individual  $i$ ,  
223  $per(i)$ , were derived from the model. Fish having a  $P$  value above the mean of  $P$  meanwhile  
224 having an activity periodicity close to 12.4 h were considered *synchronized*, others were  
225 considered *non-synchronized*. Finally, the number of synchronized fish in the control and MeHg-  
226 exposed groups was compared by chi-squared test.

227 The second aspect to investigate the propensity of glass eels to migrate was the level of  
228 *swimming activity*, expressed as the total number of observations of glass eels swimming in the  
229 water column during the 360 sessions of 15 seconds, regardless of synchronization. The  
230 comparison between control and MeHg-exposed groups were conducted using chi-squared test.

#### 231 2.4.2. Principal Component Analysis (PCA) of gene transcriptional profiles

232 To determine the transcriptional profile of studied genes, PCA was used as a multivariate  
233 statistical approach to reduce the number of the variables considered. The data set of gene  
234 expression levels was compressed by PCA procedure without much loss of information. In detail,  
235 to evaluate the global transcriptional response in each pathway, we ran a PCA analysis for each  
236 cellular function, using a table providing the normalized gene expression levels for all  
237 individuals. The first axis of PCA performed on each function in each tissue explained 47% ~ 77%  
238 of the total variances of all the genes involved, making it an acceptable synthetic measure of  
239 gene expression level of each function (See Supplementary Table S1). Supplementary Fig. S2 ~  
240 Fig. S4 showed the relevance of all the genes involved in each function to the first axis of PCA.

241 We then retrieved the score of individuals on the first axis of the PCA, and used these  
242 coordinates as a synthetic indicator of the individual level of expression for the cellular function.

243 All the statistical analyses relevant to gene transcriptions were examined using the first axis  
244 of PCA for each genomic function. Two-way ANOVA was used to analyze the varying gene  
245 transcriptional profiles in response to MeHg exposure and synchronization behavior. The  
246 interactions in the responses were also evaluated. Differences were considered significant at  $p <$   
247 0.05. The relationships of swimming activity to gene transcriptional profiles were estimated by  
248 Spearman's Rank Order Correlation test. The correlation was considered significant at  $p < 0.05$   
249 level. Then, the slope of the correlations observed in control and MeHg-exposed groups were  
250 compared by a Fisher's Z transformation method.

## 251 **2. Results**

### 252 *3.1 MeHg exposure and accumulation in glass eels*

253 After seven days of exposure, concentrations of MeHg measured in the whole body of glass  
254 eels were 3.6 times higher than those measured in the control fish ( $476 \pm 58$  and  $132 \pm 31$  ng Hg  
255  $\text{g}^{-1}$  dry weight, respectively,  $p < 0.001$ ). Concentrations in Hg(II) remained similar with  $9 \pm 3$   
256 and  $7 \pm 2$  ng Hg  $\text{g}^{-1}$  dry weight in the control and exposed group, respectively.

### 257 *3.2 MeHg-induced behavioral changes*

258 Two dead glass eels were found in both the control and the exposed groups after swimming  
259 test, which left a total of 58 glass eels in each group.

260 The propensity of glass eels to migrate was first evaluated by their ability to synchronize to  
261 the change in water current direction every 6.2 h. Glass eels exhibiting swimming activity with a

262 period of approximately 12.4 h were considered as synchronized and the others as non-  
263 synchronized. As shown in Fig. 3a, the number of synchronized fish was lower in MeHg-  
264 exposed group relative to the control (30 and 35 individuals, respectively corresponding to 52%  
265 and 60% of the total group, respectively), although the difference was not significant using a chi-  
266 squared test ( $X^2 = 0.56$ ,  $p = 0.454$ ).

267 The level of swimming activity was then expressed by the total number of observations of  
268 glass eels swimming in the water column during the 360 sessions of 15 seconds. MeHg treatment  
269 significantly decreased the total number of observations when compared to control (chi-squared  
270 test,  $X^2 = 55.55$ ,  $p < 0.001$ , Fig. 3b). When analyzing separately the two behavioral phenotypes,  
271 the decrease in the total swimming activity level was significant in non-synchronized glass eels  
272 but not in synchronized ones (Table 1). In addition, the maximum number of observations per  
273 fish decreased in the exposed group when compared to the control one (222 and 163  
274 observations during the 360 sessions, respectively).

### 275 *3.3 MeHg- and behavior-induced gene transcriptional responses*

276 The transcriptional profiles of 27 genes involved in five metabolic functions were analyzed.  
277 Some of these genes code for proteins involved in the mitochondrial respiratory chain complexes,  
278 mitochondrial catabolism and the antioxidant system. The other genes code for proteins involved  
279 in macroautophagy (the best-characterized autophagy subclass) and mitophagy (a  
280 macroautophagy-dependent specific degradation of mitochondria). As outlined in Materials and  
281 Methods, the data set of gene expression levels was compressed by PCA procedure to reduce the  
282 number of the variables considered. All the statistical analyses were then examined using the  
283 score of individuals on the first axis of the PCA for each function considered.

284 We first analyzed the transcriptional responses of the five metabolic functions to both MeHg  
285 exposure and synchronization behavior. As shown in Table 2, none of the functions considered  
286 appear to have been significantly affected by the treatment of glass eels with MeHg, regardless  
287 of the tissue examined. In contrast, significant different transcriptional responses were evidenced  
288 in head tissues between synchronized and non-synchronized glass eels. Of the five functions  
289 considered, only one (mitophagy) did not differ between the two behavioral groups. The  
290 expression of genes from the other four functions was significantly higher in synchronized glass  
291 eels than the non-synchronized ones (Table 2 and Supplementary Fig. S5).

292 In order to consider in our analysis the effect of glass eels swimming activity, which could  
293 mask (or at least influence) the effect of MeHg, we then performed a Spearman's Rank Order  
294 Correlation test between individual swimming activity level and the first axis of PCA for each  
295 function in both control and MeHg-exposed fish. As locomotor activity is expected to increase  
296 energy expenditure, correlations were tested using glass eels displaying similar range of activity.  
297 Although no significant difference could be evidenced between correlations observed in the  
298 control and the contaminated groups (using a Fisher's Z transformation), Table 3 shows that all  
299 the functions considered, except mitophagy, presented in the head a significant positive  
300 correlation to swimming activity after MeHg exposure but not in the control group. In the viscera,  
301 except mitochondrial catabolism, all others functions displayed a positive correlation to  
302 swimming activity in the contaminated group but not in the control. Finally, in the muscle, no  
303 correlation could be observed for the antioxidant system, the mitochondrial chain and the  
304 mitochondrial catabolism, while genes involved in mitophagy and macroautophagy responded  
305 positively to the activity in both groups.

#### 306 **4. Discussion**

307 To clarify the adverse behavioral and metabolic responses due to MeHg exposure, changes in  
308 migration behavior and tissue-level transcriptions related to energetics were assessed with MeHg  
309 treatment in glass eels.

310 After a 7-day exposure, average MeHg concentration in whole body of glass eels was  
311  $476 \pm 58$  ng Hg g<sup>-1</sup> dry weight, revealing the ability of the whole body to accumulate MeHg in a  
312 short-term exposure. In contrast, the concentrations of Hg(II) remained low, supporting a  
313 previous study using isotopic tracers and showing a low potential of demethylation in glass eels  
314 after 11 days of exposure to MeHg (50 ng L<sup>-1</sup>) (Claveau et al., 2015).

#### 315 *4.1 Effect of MeHg on glass eels swimming activity and energy-related genes expression*

316 Exposure to MeHg significantly decreased the total number of observations of glass eels  
317 swimming in the water column and reduced the maximum number of observations per fish (222  
318 and 163 in the control and the exposed group, respectively). These results are consistent with  
319 previous studies conducted in *Salmo salar* and *Diplodus sargus* displaying lower swimming  
320 activity after dietary exposure to MeHg at 10 mg.kg<sup>-1</sup> during 4 months and 8.7 µg.g<sup>-1</sup> during  
321 seven days, respectively (Berntssen et al., 2003; Puga et al., 2016). Interestingly, when analyzing  
322 separately the synchronized and non-synchronized glass eels, the decrease in swimming activity  
323 level after MeHg exposure was only significant in the non-synchronized group, which supports  
324 the idea that glass eels presenting a low propensity to migrate might be more vulnerable to stress  
325 than those displaying a high probability to migrate (Bolliet et al., 2017; Claveau et al., 2015).

326 A number of studies reported the toxic effects of MeHg, notably on the oxidative status, the  
327 mitochondrial function and the Calcium homeostasis in fish (Cambier et al., 2009; Cambier et al.,  
328 2010; Claveau et al., 2015; Graves et al., 2017; Gonzalez et al., 2005; Nostbakken et al., 2012;



329 Rasinger et al., 2017; Richter et al., 2011; Yadetie et al., 2016). Surprisingly, we did not observe  
330 any effect of MeHg on transcriptional profiles of the studied genes involved in mitochondrial  
331 metabolism, antioxidant system or autophagy. Our results contrast with a previous study in glass  
332 eels showing an activation of antioxidative defence system at the transcriptomic level after  
333 eleven days of exposure to MeHg (50ng/l) (Claveau et al., 2015). However, in this last study,  
334 gene expression was analyzed just after exposure while in the present one analyses were  
335 conducted after the behavioral test, i.e., 10 days after the end of exposure. In addition, both  
336 studies focused on gene expression at a single time point that does not give a real picture of the  
337 dynamic aspect of the different events at play during complex and integrative processes such as  
338 antioxidant system, energy metabolism or autophagy. Furthermore, depending on the genes  
339 studied, the levels of transcripts do not always correlate with the amount or the activity of the  
340 corresponding proteins (Vogel and Marcotte, 2012; Yadetie et al., 2016). Future functional  
341 studies will therefore be necessary to draw definitive conclusions on the effect of MeHg in the  
342 functions monitored in glass eels.

343 However, interestingly, we evidenced a significant positive correlation between individual  
344 swimming activity levels and the expression of the genes studied. The expression of genes  
345 related to autophagy and mitophagy increased with activity in the muscle, both in the control and  
346 exposed groups, probably reflecting an increase in energy requirement related to activity.  
347 However, in the head and viscera, most of the genomic functions related to antioxidant system  
348 and metabolism showed a positive correlation in the contaminated group, but not in the control  
349 one. As mentioned above, oxydative stress and mitochondrial impairment are among the most  
350 studied effects of MeHg in fish. MeHg targets some specific thiol containing proteins such as  
351 glutathione peroxydase involved in the anti-oxydant system, predisposing cell to oxidative stress

352 and generation of Reactive Oxygen Species (known as ROS) (Farina et al., 2011). In addition,  
353 MeHg can also directly target specific thiol-containing enzymes of the respiratory chain complex  
354 and both effects may affect cellular energy pathways (Farina et al., 2011; Glaser et al., 2010;  
355 Yadieti et al., 2016). Thus, although our results must be taken with caution (because the  
356 differences between correlations obtained for the control and contaminated groups were not  
357 strong enough to be significant), they strongly suggest that contaminated glass eels were affected  
358 by MeHg and needed to increase energy metabolism more than non-contaminated fish to cope  
359 with increasing swimming activity.

360 It is also noteworthy that the head was the most affected section by MeHg, a positive  
361 correlation being observed between swimming activity and all genomic functions. Although the  
362 head section include not only the brain but also the gills, this result seems consistent with the  
363 literature reporting that the brain is a predominant target for MeHg in fish (Gonzalez et al., 2005;  
364 Graves et al., 2017; Pereira et al., 2014, for review 2019). Indeed, MeHg has been reported to  
365 cross the blood-brain-barrier and accumulates in the brain having serious toxic effects including  
366 proteome changes related to oxidative stress and mitochondrial dysfunction, morpho-structural  
367 changes and dysfunction in neurotransmission processes (Cariccio et al., 2019; for review see  
368 Pereira et al., 2019; Pletz et al., 2016). Considering also that in both control and exposed glass  
369 eels, the expression levels of metabolism-related genes were lower in non-synchronized glass  
370 eels than in synchronized ones in the head but not in the other sections, these results suggest that  
371 the head may represent the one to focus on for a better understanding of glass eel's migration and  
372 the effect of environmental stressors on this migration.

373 *4.2 Effect of MeHg on the rhythmic swimming activity in glass eels*

374 To migrate up estuary, glass eels use selective tidal stream transport and synchronize their  
375 swimming activity to the flood thanks to an endogenous clock (Bolliet et al., 2007; Forward and  
376 Tankersley, 2001; Hickman, 1981; McCleave and Kleckner, 1982; Wippelhauser and McCleave,  
377 1987). They are also known to avoid light and mainly migrate during the night, likely using a  
378 circadian clock (Bolliet et al., 2007). In our experimental conditions, a very low light intensity  
379 during photophase was used to avoid synchronization of glass eels activity to photoperiod that  
380 could have masked synchronization to the tidal cue. Thus, in the present study, synchronized  
381 glass eels corresponded to fish that synchronized their swimming activity to the change in water  
382 current direction with a period close to 12.4 h. A lower number of glass eels synchronizing to  
383 tidal period were observed in contaminated condition compared to control one, even though it  
384 was not statistically significant using a chi-squared test. Xenobiotics have been shown to disturb  
385 the circadian system in different fish species (Prokkola and Nikinmaa, 2018) and a couple of  
386 studies showed that MeHg disrupted circadian rhythms in rodents, the crayfish *Astacus astacus*  
387 and the freshwater crab *Potamon potamios* (Arito et al., 1983; Parmalee and Aschner, 2017;  
388 Styriehave and Depledge, 1996). In addition, Depledge (1984) evidenced an effect of mercury  
389 exposure on tidal rhythmicity in the heart rate of the shorecrab *Carcinus maenas*. Though the  
390 location of the circatidal clock is still unknown in fish, the pacemaker regulating circadian  
391 rhythms has been located in the pineal gland. Interestingly, Korbas et al. (2012, 2013) reported  
392 an accumulation of inorganic Hg in the pineal gland of zebrafish exposed to MeHg. Thus, the  
393 relationship between mercury species and the endogenous clock(s) driving the rhythmic  
394 swimming activity in glass eels appears as an interesting avenue to explore.

## 395 **5 Conclusion**

396 Our results suggest that MeHg may affect the estuarine migration of glass eels by reducing  
397 their ability to synchronize to the tide and their level of swimming activity. They also support the  
398 idea that non-synchronized fish may be more vulnerable to stress and the first affected by  
399 contamination. A decrease in the propensity to migrate *in natura* would lead to an increase in  
400 glass eels settlement in estuary and consequently a decrease in population recruitment in upper  
401 reaches. Non-migrant glass eels becoming mostly males, such effect would change the fate of the  
402 population by influencing the sex ratio in this species. A better understanding of the effect of  
403 MeHg on the maximum swimming activity in glass eels and their biological clocks is now  
404 required to clearly assess the impact of this contaminant on their synchronization to  
405 environmental cues and migration.

406 The results also support the interest of focusing on the head to investigate facultative  
407 migration behavior in glass eels and the effect of environmental stressors on this rhythmic  
408 behavior. However, as head samples include the entire brain tissue, eyes but also the gills, they  
409 also question the relationships between the ability to migrate and osmoregulatory functions.

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413

414 **Supplementary data**

415 **Text S1.** Openbugs code, the prior values for parameters, the Markov chain initial values and the  
416 data for the swimming activity and the inference model.

417 **Table S1.** Proportion of variance of PCA first axis.

418 **Fig. S1.** Diagrammatic top view of the annular flume.

419 **Fig. S2.** Principal Component Analysis (PCA) on the expression levels of genes involved in each  
420 energy-related genomic function in head.

421 **Fig. S3.** Principal Component Analysis (PCA) on the expression levels of genes involved in each  
422 energy-related genomic function in viscera.

423 **Fig. S4.** Principal Component Analysis (PCA) on the expression levels of genes involved in each  
424 energy-related genomic function in muscle.

425 **Fig. S5.** Box-plots (median, 25-75% CI, min-max) showing the comparison of genomic profiles  
426 to assess the effect of MeHg treatment and synchronization behavior using two-way ANOVA  
427 analysis.

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### Figure captions

**Fig. 1.** Kinetics of MeHg (blue circles) and Hg(II) (red triangles) concentrations (mean  $\pm$  sd) in glass eels along 0-30 days of exposure to MeHg. Data for 0 d are from control fish (n = 3).

**Fig. 2.** Sections of glass eel.

**Fig. 3.** Swimming behavior of control (blue bar, n = 58) and MeHg-exposed (orange bar, n = 58) glass eels over 10 days. (a) Bar chart showing the total number of glass eels which synchronized their swimming activity to the change in water current direction every 6.2 h, Pearson's chi-squared test,  $X^2=0.56$ ,  $p=0.454$  (b) Bar chart showing the total number of observations of all glass eels, Pearson's chi-squared test,  $X^2=0.56$ ,  $p<0.001$ .

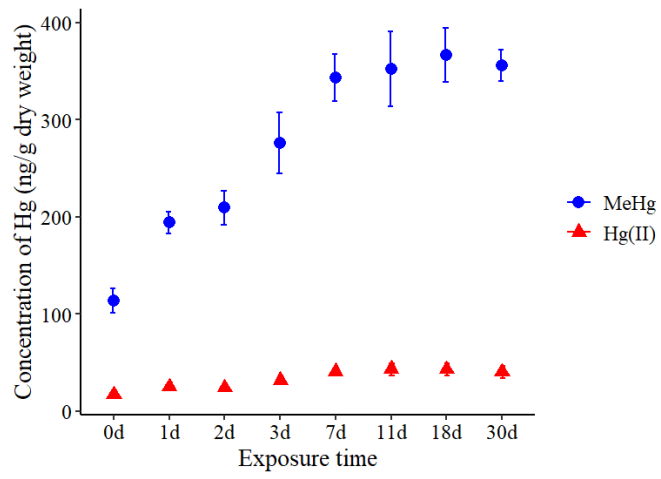


Figure 1

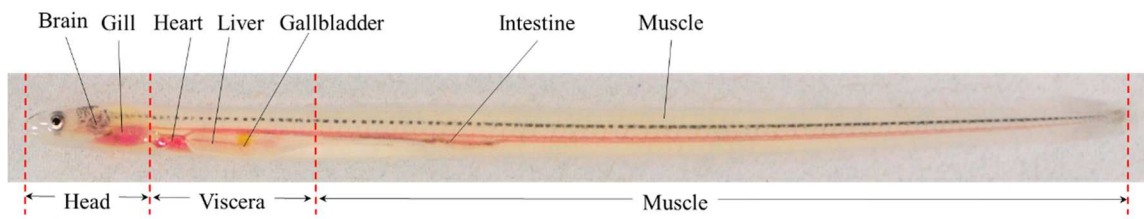


Figure 2

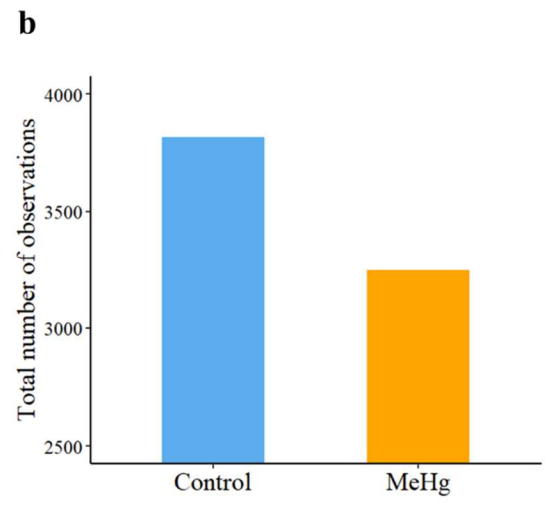
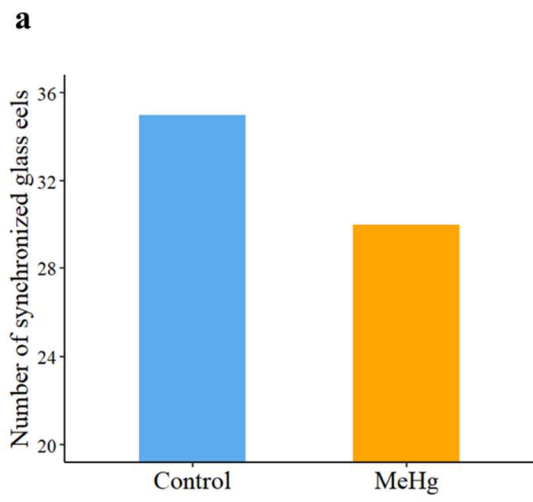


Figure 3

**Table 1.** Assessment of the MeHg-induced effects on swimming activity within two behavioral phenotypes. Synchronized glass eels: Fish which synchronized their swimming activity to the change in water current direction every 6.2 h. Number of fish: Total number of synchronized or non-synchronized glass eels observed during the behavioral test (for a total of 58 glass eels in both groups). Number of observations: Total number of observations of glass eels swimming in the water column during the 360 sessions recorded. A tagged glass eel can be observed only once during a recorded session. **Significant values are in bold.**

Behavioral phenotype	Treatment	Number of fish	Number of observations	Pearson's chi-squared test		
				df	X <sup>2</sup>	p-value
Non-synchronized	Control	23	819	1	40.63	<b>&lt; 0.001</b>
	MeHg	28	731			
Synchronized	Control	35	2999	1	0.80	0.372
	MeHg	30	2516			

**Table 2.** Statistical results of the two-way ANOVA for detecting transcriptional profiles of each genomic function in response to MeHg exposure and synchronization behavior. *P*-values of ANOVA are presented in the table. **Significant values are in bold.** Factor ‘Treatment’ is MeHg treatment (control vs MeHg exposure), factor ‘Behavior’ is behavioral phenotype (non-synchronized vs synchronized), ‘Int’ is interaction of both factors. H- head, V- viscera, M- muscle.

Genomic function	Tissue	<i>p</i> (Treatment)	<i>p</i> (Behavior)	<i>p</i> (Int)
Antioxidant system	H	0.338	<b>0.003</b>	0.663
	V	0.637	0.344	0.239
	M	0.268	0.636	0.650
Mitochondrial respiratory chain	H	0.585	<b>0.003</b>	0.107
	V	0.679	0.135	0.377
	M	0.242	0.489	0.945
Mitochondrial catabolism	H	0.776	<b>0.006</b>	0.206
	V	0.762	0.329	0.626
	M	0.753	0.597	0.806
Mitophagy	H	0.307	0.051	0.509
	V	0.784	0.499	0.903
	M	0.375	0.561	0.833
Macroautophagy	H	0.673	<b>0.004</b>	0.689
	V	0.958	0.163	0.886
	M	0.513	0.376	0.796

**Table 3.** Spearman's Rank Order Correlation test between individual swimming activity level and the first axis of PCA for each function in both control and MeHg-exposed fish. R and *p* values of Spearman's correlation test are presented in the table. **Significant values are in bold.** H- head, V- viscera, M- muscle.

Function	Tissue	Control		MeHg	
		r	<i>p</i> -value	r	<i>p</i> -value
Antioxidant system	H	0.32	0.169	0.65	<b>&lt;0.001</b>
	V	0.05	0.826	0.53	<b>0.014</b>
	M	0.33	0.156	0.32	0.135
Mitochondrial respiratory chain	H	0.22	0.346	0.57	<b>0.005</b>
	V	0.30	0.193	0.43	<b>0.049</b>
	M	0.27	0.251	0.27	0.205
Mitochondrial catabolism	H	0.23	0.326	0.68	<b>&lt;0.001</b>
	V	-0.10	0.686	0.28	0.223
	M	-0.07	0.772	0.06	0.774
Mitophagy	H	0.63	<b>0.003</b>	0.76	<b>&lt;0.001</b>
	V	0.40	0.078	0.51	<b>0.019</b>
	M	0.67	<b>0.001</b>	0.75	<b>&lt;0.001</b>
Macroautophagy	H	0.39	0.091	0.72	<b>&lt;0.001</b>
	V	0.38	0.099	0.62	<b>0.002</b>
	M	0.75	<b>&lt;0.001</b>	0.68	<b>&lt;0.001</b>



