

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

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1	New insights into methylmercury induced behavioral and energy-
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20 ABSTRACT

The effect of methylmercury (MeHg) was investigated in glass eel migration behavior and 21 metabolism. To migrate up estuary, glass eels synchronize their swimming activity to the flood 22 tide and remain on or in the substratum during ebb tide. Following seven days of exposure to 23 MeHg (100 ng L⁻¹), glass eels migration behavior was expressed by their swimming 24 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 that MeHg decreased the number of glass eels synchronized to the change in water current 28 direction and their swimming activity level. This last effect was more pronounced in non-29 synchronized fish than in synchronized ones, supporting the idea that non-synchronized glass 30 eels could be more vulnerable to stress. As regard the expression of energy-related genes, no 31 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. Finally, it is noteworthy that non-synchronized glass eels displayed lower expression level of 35 metabolism genes than their synchronized counterpart, but only in the head. Altogether, these 36 results support the interest of focusing on the head to investigate the facultative migration 37 behavior in glass eels and the effect of environmental stressors on this rhythmic behavior. 38

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

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42 **1. Introduction**

The status of European eel (Anguilla anguilla) remains critical since the recruitment of glass 43 eels strongly declined from 1980 to about 2010, and have remained at a low level since, which 44 has urged Euro-wide European eel regulation (council regulation (EC) no. 1100/2007) for the 45 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 currents more than 5000 km toward the European continent, after which they transform into a 50 juvenile stage, the glass eel. Then, glass eels migrate up estuary to reach freshwater habitats 51 where they grow and develop into yellow eels. At an age of 5 to 20 years they commence the 52 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).

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

environmental sex determinism in eels, (Geffroy and Bardonnet, 2016; Krueger and Oliveira,
1999). Briefly, in European eels, males are generally observed to dominate in high-density
environments, often associated with estuarine or lower river reaches, whereas individuals that
migrated upstream to the river tend to become mostly females (Adam et al., 2008; Davey and
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 condition (Bureau du Colombier et al., 2007; Edeline, 2007; Edeline et al., 2006; Liu et al., 72 2019). Indeed, most glass eels do not feed throughout estuarine migration and they depend on 73 energy reserves accumulated by the leptocephalus larvae during oceanic migration to reach the 74 river (Kawakami et al., 1999; Tesch, 2003; Bardonnet and Riera, 2005). Moreover, estuary is a 75 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 influence the energy metabolic processes of glass eels during migration. Due to its proximity to 78 urbanized and industrial areas, estuary represents a major sink for various contaminants 79 80 including various forms of mercury (Hg), both inorganic (Hg(II)) and methylated (MeHg), this latest form being recognized to adversely affect fish physiology, growth, health and behavior 81 (Cambier et al., 2009; Cambier et al., 2012; Lee et al., 2011; Murphy et al., 2008; Scheulhammer 82 et al., 2007). 83

Behavioral effects of MeHg exposure in fish have been largely documented, with a lot of concerns on prey capture ability (Weis and Khan, 1990; Zhou et al., 2001), predator avoidance (Webber and Haines, 2003), reproduction (Sandheinrich and Miller, 2006), habitat selection (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 through the processes of cellular repairment and detoxification which may thereby reduce glass 91 eels fitness and their migratory success through estuary. Claveau et al. (2015) reported that glass 92 eels exposed to MeHg (50 ng L⁻¹) for 11 days exhibited some perturbations of mitochondrial 93 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 propensity to migrate up estuaries being more affected than their 'migrant' counterpart. These 96 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

Procedures used in this study have been validated by the ethics committee N°073 (ref: 2017012015086652). The experiment was carried out in strict accordance with the EU legal frameworks, specifically those relating to the protection of animals used for scientific purposes (i.e., Directive 2010/63/EU), and under the French legislation governing the ethical treatment of animals (Decret no. 2013-118, February 1st, 2013). 110

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112 2.2 Primary test for MeHg accumulation kinetics over 30 days

113 2.2.1 Fish collection

The glass eels were collected at the mouth of a small estuary (courant d'Huchet) located 40 km north of the mouth of the Adour estuary, France $(43^{\circ} 55' \text{ N}, 1^{\circ} 23' \text{ W})$. They were sampled using a dip-net at night and during flood tide in February 2018, and were then transferred to the laboratory and maintained in a tank containing water from the fishing site. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water. Fish were kept under 12 °C and a photoperiod of 12 L / 12 D with a very low light intensity during the photophase (0.2-0.3 μ W/cm²).

121 2.2.2 MeHg exposure and kinetic sampling

After acclimation, glass eels were randomly selected and allocated by groups of three into a series of seven aquariums (1.5 L of aerated fresh water) with initial compartment concentration of 100 ng L⁻¹ of MeHg. Glass eels were exposed to this single spike of MeHg prepared as follow: The stock solution of MeHg at 1000 mg L⁻¹ was prepared by dissolving MeHg chloride obtained from Strem Chemicals (Newburyport, MA, USA) in methanol. The spiking solution at 100 μ g L⁻¹ ¹ was then prepared by diluting the stock solution in 1% hydrochloric acid.

Three glass eels were collected in the beginning of this test as controls. All the aquariums were sealed with a transparent cover to prevent water volatilization and to guarantee the photoperiod. The aeration, temperature and photoperiod were maintained as described for the acclimation period. During the exposure period, no mortality neither erratic swimming (abruptchange of direction or swimming speed) were observed.

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

138 2.2.3 Mercury speciation analysis

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

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

156 2.3.1 Fish collection and tagging

140 glass eels were collected in March 2018 at the same fishing site and using the same 157 158 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 morning following their capture, all glass eels were anesthetized (Benzocaine, 0.01 mg L⁻¹) and 160 161 individually tagged using Visible Implant Elastomer (VIE Tag) (combinations of one or two hypodermic spots of different colors as described by Imbert et al., 2008) in order to trace the 162 swimming behavior individually. Once tagged, glass eels were released to wake up in the water 163 164 from fishing site. During the next 48 h, the water was continuously aerated and progressively diluted with fresh water. 165

166 2.3.2 MeHg exposure

After acclimation, fish were randomly divided into two groups: control group without Hg addition and MeHg-group exposed to an initial MeHg concentration of 100 ng L⁻¹. Seven glass aquariums (5 L of aerated fresh water) were used for each group, with 10 fish in each aquarium. The exposure lasted for seven days. During the entire experimental period, both control and MeHg-exposed fish were kept under the same conditions as those during the acclimation period. No mortality neither erractic 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 analysis. Procedures of sample preparation and mercury measurement were the same as primary 176 test (see 2.2.3). All the other control and MeHg-exposed glass eels ($n = 6 \times 10$ glass eels in each 177 group) were transferred into two annular tanks (30 glass eels of both the control and MeHg 178 179 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 μ W/cm²) 181 in order to see the VIE Tag. The water temperature was kept at 12 ± 0.5 °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; Supplementary Fig. S1). The two pumps were programmed to alternately work to generate 184 clockwise or counterclockwise water flow every 6.2 h. In each tank, the swimming activity of 185 186 glass eels was traced continuously during 10 days by a camera programed to record 15 seconds every 40 min. The UV light allowed the identification of each glass eel during the light and dark 187 phases by its elastomer mark. During the 10 days, a total of 360 sessions of 15 seconds were 188 189 obtained.

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

197 2.3.4 Gene expression analysis

198 Sampling procedure

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

202 RNA extraction and quantitative real-time PCR

Frozen glass eels were cut in three sections, containing (i) the head including the gills, (ii) the heart, the liver, the spleen and the stomach and (iii) most of the muscle tissue with the intestine (hereafter referred to as head, viscera and muscle, respectively, Fig. 2). Then, each section was 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 Δ 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

To characterize the propensity of glass eels to migrate, we first investigated the synchronization of glass eel's swimming activity to the change in water current direction every 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 individual i at time t, P(t, i) and the periodicity of swimming occurrence of an individual i, 222 223 per(i), were derived from the model. Fish having a P value above the mean of P meanwhile having an activity periodicity close to 12.4 h were considered synchronized, others were 224 considered non-synchronized. Finally, the number of synchronized fish in the control and MeHg-225 exposed groups was compared by chi-squared test. 226

The second aspect to investigate the propensity of glass eels to migrate was the level of *swimming activity*, expressed as the total number of observations of glass eels swimming in the water column during the 360 sessions of 15 seconds, regardless of synchronization. The 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

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

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

All the statistical analyses relevant to gene transcriptions were examined using the first axis 243 of PCA for each genomic function. Two-way ANOVA was used to analyze the varying gene 244 245 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 Spearman's Rank Order Correlation test. The correlation was considered significant at p < 0.05248 249 level. Then, the slope of the correlations observed in control and MeHg-exposed groups were compared by a Fisher's Z transformation method. 250

251 **2. Results**

252 3.1 MeHg exposure and accumulation in glass eels

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

257 3.2 MeHg-induced behavioral changes

Two dead glass eels were found in both the control and the exposed groups after swimming 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 period of approximately 12.4 h were considered as synchronized and the others as nonsynchronized. As shown in Fig. 3a, the number of synchronized fish was lower in MeHgexposed group relative to the control (30 and 35 individuals, respectively corresponding to 52% and 60% of the total group, respectively), although the difference was not significant using a chisquared 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 significantly decreased the total number of observations when compared to control (chi-squared 269 test, $X^2 = 55.55$, p < 0.001, Fig. 3b). When analyzing separately the two behavioral phenotypes, 270 the decrease in the total swimming activity level was significant in non-synchronized glass eels 271 but not in synchronized ones (Table 1). In addition, the maximum number of observations per 272 fish decreased in the exposed group when compared to the control one (222 and 163 273 274 observations during the 360 sessions, respectively).

275 3.3 MeHg- and behavior-induced gene transcriptional responses

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

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 appear to have been significantly affected by the treatment of glass eels with MeHg, regardless 286 of the tissue examined. In contrast, significant different transcriptional responses were evidenced 287 in head tissues between synchronized and non-synchronized glass eels. Of the five functions 288 considered, only one (mitophagy) did not differ between the two behavioral groups. The 289 290 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 mask (or at least influence) the effect of MeHg, we then performed a Spearman's Rank Order 293 Correlation test between individual swimming activity level and the first axis of PCA for each 294 function in both control and MeHg-exposed fish. As locomotor activity is expected to increase 295 296 energy expenditure, correlations were tested using glass eels displaying similar range of activity. Although no significant difference could be evidenced between correlations observed in the 297 298 control and the contaminated groups (using a Fisher's Z transformation), Table 3 shows that all the functions considered, except mitophagy, presented in the head a significant positive 299 correlation to swimming activity after MeHg exposure but not in the control group. In the viscera, 300 except mitochondrial catabolism, all others functions displayed a positive correlation to 301 302 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 positively to the activity in both groups. 305

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.

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

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

Exposure to MeHg significatively decreased the total number of observations of glass eels 316 swimming in the water column and reduced the maximum number of observations per fish (222 317 318 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 activity after dietary exposure to MeHg at 10 mg.kg⁻¹ during 4 months and 8.7 µg.g⁻¹ during 320 seven days, respectively (Berntssen et al., 2003; Puga et al., 2016). Interstingly, when analyzing 321 separately the synchronized and non-synchronized glass eels, the decrease in swimming activity 322 level after MeHg exposure was only significant in the non-synchronized group, which supports 323 324 the idea that glass eels presenting a low propensity to migrate might be more vulnerable to stress than those displaying a high probability to migrate (Bolliet et al., 2017; Claveau et al., 2015). 325

A number of studies reported the toxic effects of MeHg, notably on the oxidative status, the mitochondrial function and the Calcium homeostasis in fish (Cambier et al., 2009; Cambier et al., 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 metabolism, antioxidant system or autophagy. Our results contrast with a previous study in glass 331 eels showing an activation of antioxidative defence system at the transcriptomic level after 332 eleven days of exposure to MeHg (50ng/l) (Claveau et al., 2015). However, in this last study, 333 gene expression was analyzed just after exposure while in the present one analyses were 334 335 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 studied, the levels of transcripts do not always correlate with the amount or the activity of the 339 corresponding proteins (Vogel and Marcotte, 2012; Yadetie et al., 2016). Future functional 340 341 studies will therefore be necessary to draw definitive conclusions on the effect of MeHg in the functions monitored in glass eels. 342

343 However, interestingly, we evidenced a significant positive correlation between individual swimming activity levels and the expression of the genes studied. The expression of genes 344 related to autophagy and mitophagy increased with activity in the muscle, both in the control and 345 exposed groups, probably reflecting an increase in energy requirement related to activity. 346 347 However, in the head and viscera, most of the genomic functions related to antioxidant system and metabolism showed a positive correlation in the contaminated group, but not in the control 348 349 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 gluthatione peroxydase involved in the anti-oxydant system, predisposing cell to oxidative stress 351

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; Yadieti et al., 2016). Thus, although our results must be taken with caution (because the 355 differences between correlations obtained for the control and contaminated groups were not 356 strong enough to be significant), they strongly suggest that contaminated glass eels were affected 357 358 by MeHg and needed to increase energy metabolism more than non-contaminated fish to cope 359 with increasing swimming activity.

It is also noteworthy that the head was the most affected section by MeHg, a positive 360 correlation being observed between swimming activity and all genomic functions. Although the 361 head section include not only the brain but also the gills, this result seems consistent with the 362 363 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 cross the blood-brain-barrier and accumulates in the brain having serious toxic effects including 365 366 proteome changes related to oxidative stress and mitochondrial dysfunction, morpho-structural changes and dysfunction in neurotransmission processes (Cariccio et al., 2019; for review see 367 Pereira et al., 2019; Pletz et al., 2016). Considering also that in both control and exposed glass 368 eels, the expression levels of metabolism-related genes were lower in non-synchronized glass 369 370 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, 1987). They are also known to avoid light and mainly migrate during the night, likely using a 377 circadian clock (Bolliet et al., 2007). In our experimental conditions, a very low light intensity 378 during photophase was used to avoid synchronization of glass eels activity to photoperiod that 379 380 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 was not statistically significant using a chi-squared test. Xenobiotics have been shown to disturb 384 the circadian system in different fish species (Prokkola and Nikinmaa, 2018) and a couple of 385 386 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; Styrishave and Depledge, 1996). In addition, Depledge (1984) evidenced an effect of mercury 388 exposure on tidal rhythmicity in the heart rate of the shorecrab Carcinus maenas. Though the 389 location of the circatidal clock is still unknown in fish, the pacemaker regulating circadian 390 rhythms has been located in the pineal gland. Interestingly, Korbas et al. (2012, 2013) reported 391 an accumulation of inorganic Hg in the pineal gland of zebrafish exposed to MeHg. Thus, the 392 393 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 glass eels settlement in estuary and consequently a decrease in population recruitment in upper 400 401 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 required to clearly assess the impact of this contaminant on their synchronization to 404 405 environmental cues and migration.

The results also support the interest of focusing on the head to investigate facultative migration behavior in glass eels and the effect of environmental stressors on this rhythmic behavior. However, as head samples include the entire brain tissue, eyes but also the gills, they 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.
- Fig. S4. Principal Component Analysis (PCA) on the expression levels of genes involved in each
 energy-related genomic function in muscle.
- Fig. S5. Box-plots (median, 25-75% CI, min-max) showing the comparison of genomic profiles
 to assess the effect of MeHg treatment and synchronization behavior using two-way ANOVA
 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.

			-	Pearson's chi-squared test			
Behavioral phenotype	Treatment	Number of fish	Number of observations	df	X^2	<i>p</i> -value	
Non-	Control	23	819	1	40.62	< 0.001	
synchronized	MeHg	28	731		40.05		
Symphesized	Control	35	2999	1	0.80	0.272	
Synchronized	MeHg	30	2516	1	0.80	0.572	

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	p (Treatment)	p (Behavior)	p (Int)
Antioxidant	Н	0.338	0.003	0.663
Antioxidant	V	0.637	0.344	0.239
system	М	0.268	0.636	0.650
Mitaahandrial	Н	0.585	0.003	0.107
	V	0.679	0.135	0.377
respiratory chain	М	0.242	0.489	0.945
Mitaahandrial	Н	0.776	0.006	0.206
ostabolism	V	0.762	0.329	0.626
Catabolishi	Μ	0.753	0.597	0.806
	Н	0.307	0.051	0.509
Mitophagy	V	0.784	0.499	0.903
	М	0.375	0.561	0.833
	Н	0.673	0.004	0.689
Macroautophagy	V	0.958	0.163	0.886
	М	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.

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

