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1 **Is there a link between acetylcholinesterase, behaviour and density populations**
2 **of the ragworm *Hediste diversicolor*?**

3 Isabelle Métais^{a*}, Amélie Châtel^a, Mohammed Mouloud^a, Hanane Perrein-Ettajani^a,
4 Mélanie Bruneau^a, Patrick Gillet^a, Nisrine Jrad^b, Catherine Mouneyrac^a

5 ^a Laboratoire Mer, Molécules, Santé (MMS, EA 2160), Université Catholique de
6 l'Ouest, Faculté des Sciences, Angers F-49000 France

7 ^b Laboratoire Angevin de Recherche en Ingénierie des Systèmes (LARIS, EA731),
8 Université Catholique de l'Ouest, Faculté des Sciences, Angers F-49000 France

9 * Corresponding author: imetais@uco.fr

10 **Abstract**

11 The main objective of the present study was to explore the potential link between
12 acetylcholinesterase (AChE) activity and burrowing behaviour of the ragworm *Hediste*
13 *diversicolor*, which may have consequences at higher levels of biological organisation.
14 Two complementary studies were conducted. AChE activity, at the sub-individual level,
15 and behavioural responses, at the individual level, were evaluated in worms from the
16 Loire estuary (France), whereas density and biomass of *H. diversicolor* were
17 determined at the population level. A Spearman positive correlation between both
18 biomarkers (AChE and burrowing) suggested that inhibition of AChE activity was linked
19 to behaviour impairments. At the population level, lower AChE and behaviour activities
20 were detected in worms corresponding to lower population density and biomass.
21 These results provide direct empirical field evidence demonstrating the sensitivity of
22 behaviour of *H. diversicolor* as a biomonitor of estuarine health status assessment.

23

24 **Key words:** *Hediste diversicolor*; acetylcholinesterase; population density;
25 **behaviour; Adverse Outcome Pathway.**

26

27 Estuaries, as transitional zones, provide a wide range of vital nesting and feeding
28 habitats and ecosystem services (Dallas and Jha, 2015), but since many decades,
29 they are affected by multiple anthropogenic activities, leading to the release of various
30 types of contaminants. To estimate the health status of ecosystems, biomonitoring
31 programs aimed to classically combine chemical and biological approaches (e.g.
32 multibiomarker approach) and ecological indicators. Conventional monitoring
33 techniques are mainly based on the determination of the contaminants' concentrations
34 in sediments and water. Evaluation of chemical contamination is a complex task, as
35 estuaries often present a mixture of contaminants and their metabolites, some of which
36 are costly or not yet accessible to analyze. It is widely recognised that chemical data
37 alone can only provide partial information; for example, the unpredictability of the
38 processes affecting the speciation of pollutants may alter their bioavailability and
39 therefore their toxicity (Birch, 2018). For this reason, the integration of biomarker
40 responses with chemical and ecological approaches on sentinel species allows
41 evaluation of the toxicity of mixtures of pollutants and sub-lethal effects even at low
42 concentrations (Amiard-Triquet et al., 2015). Biomarkers have been widely used to
43 assess contaminant exposure and its effects in marine species such as the ragworm
44 *Hediste diversicolor*, which plays an important role in the structure and functioning of
45 marine ecosystems. However, biomarkers are subjected to the influence biotic and
46 abiotic confounding factors (e.g. temperature, salinity, size/weight and reproductive
47 status). Thus, to use these biomarkers in biomonitoring programs, it is necessary to
48 previously determine baseline levels in species of interest, which is currently

49 challenging. Recently, Barrick et al. (2016; 2018) determined baseline assessment
50 criteria (BAC) and environmental assessment criteria (EAC) of biochemical biomarkers
51 (energy reserves, catalase: CAT, glutathione S transferases: GST,
52 acetylcholinesterase: AChE and thiobarbituric acid reactive substances: TBARs) in *H.*
53 *diversicolor* from a reference site (Durou et al., 2007). Regarding burrowing behaviour,
54 Kalman et al. (2010) showed no influence of confounding factors (weight, salinity and
55 grain size) on burrowing behaviour in *H. diversicolor*.

56 Field studies aiming at establishing a link between biomarkers at a low level of
57 organisation and possible effects at a higher level of organisation (e.g. population and
58 community) remain to be conducted. The adverse outcome pathway (AOP) approach
59 proposed by Ankley et al. (2010) aims to break through the gap linking direct molecular
60 initiating events with adverse outcomes at a higher level of organisation. AChE, a core
61 biomarker of neurotoxicity, is well recognised as a biomarker of ecological relevance,
62 as its activity inhibition observed in laboratory or field studies may lead to physiological
63 and behavioural impairments in organisms exposed to not only pesticides but also
64 metals, polycyclic aromatic hydrocarbons (PAHs), pharmaceutical products or algal
65 toxins (Lionetto et al., 2013; Sturve et al., 2016; Lehtonen et al., 2003; Kankaanpää et
66 al., 2007; Zhang et al., 2016).

67 Currently, the most robust of the developing pathway is the AOP linking AChE
68 inhibition to acute mortality (Russom et al., 2014). This AOP has been proposed to
69 support integrated approaches for testing and assessment
70 (<https://aopwiki.org/aops/16>). However, there are many biochemical, behavioural, and
71 physiological processes associated with the link between AChE inhibition and acute
72 mortality of organisms and, consequently, possible density depletion of the species
73 considered. The Loire estuary is a multi-contaminated estuary displaying a diffusive

74 pollution due to heavy metals, pesticides, PAHs and PCBs (Blanchet-Letrouvé et al.,
75 2013), and AChE inhibition in organisms has been often linked to the presence of these
76 contaminants. The main objective of the present study was to explore in the ragworm
77 *H. diversicolor* from the Loire estuary (France), the potential link between AChE activity
78 and burrowing behaviour that may have consequences at higher levels of biological
79 organisation.

80 The sampling site was located on the largest mudflat of the polyhaline domain of the
81 Loire estuary ('Les Brillantes') on the south shore downstream Paimboeuf (Fig. 1). Two
82 zones were selected: the first one (Site 1: 47°16'55.70"N; 2° 3'47.60"W) was located
83 in the upper intertidal area of the mudflat, 20 m from the shore, located close to an
84 active eroded cliff (Thibault de Chanvalon et al., 2016). The second one, (Site 2:
85 47°17'3.77"N; 2° 3'42.87"W) was located in the middle intertidal area. Two sampling
86 campaigns were conducted in May 2013 (after the flood period) and February 2014
87 (during the flood period), respectively.

88 For AChE activity determinations, 10 worms (*H. diversicolor*) in the same range of wet
89 weight (mean S1: 0.220±0.114 g; mean S2: 0.187±0.121 g) were gently collected by
90 hand, kept in wet sediment from their site of origin, immediately frozen at the laboratory
91 and then kept at -80°C until analysis. AChE activity was determined using the method
92 described in Buffet et al. (2011). Briefly, whole worm tissues were individually
93 homogenised in 80 mM Tris-HCl pH 7.2, 200 mM NaCl, 1 mM Dithiothreitol
94 supplemented with protease inhibitor cocktail according to the manufacturer's
95 instructions. The homogenates were centrifuged at 9000 *g* for 30 min at 4°C, and
96 supernatants were collected for protein (Bradford, 1976) and AChE activity
97 determinations. Ten microlitres of the supernatant was added in triplicate to 190 µl 10
98 mM DTNB and 100 µL 100 mM acetylthiocholine into a 96-well microplate. The

99 reactions were read at 412 nm for 3 minutes. Results were expressed as nmoles of
100 thiocholine produced per min and per mg protein (nmoles min⁻¹ mg⁻¹ protein).
101 Concerning behavioural experiments, burrowing tests were set up in a dark
102 temperature-controlled room (15°C at the time of collection). Worms (*H. diversicolor*)
103 were submitted to burrowing tests as previously described by Bonnard et al. (2009).
104 Sediments from each site were homogenised by hand one day before experimentation.
105 Briefly, burrowing behaviour was studied by placing individually 20 worms on
106 sediments from their site of origin and recording their positions every 2 min for 30 min.
107 Automated behavioural tests were carried out with the Multifreshwater Biomonitor®
108 (MFB) from LimCo International according to the methodology previously described
109 and validated by Buffet et al. (2013). *H. diversicolor* movements inducing signals within
110 the range of 0.5 Hz were identified as undulation, whereas signals induced between
111 1.0 and 2.0 Hz indicated head movements and feeding activity. One individual per
112 chamber (n=7) was placed on sediment surface and allowed to acclimate 1 h before
113 MFB recording during 7 h. One control chamber without any organisms was used for
114 measuring background noise signals. Concerning density measurements, four
115 sediment samples from both areas (Site 1 and Site 2) were randomly collected at low
116 tide using plastic cores (10 cm x 35 cm) at a distance of 10 m from each other.
117 Sediment was sieved through a 1 mm mesh to collect and count worms. Fresh weight
118 of *H. diversicolor* individuals was determined to establish biomass. An average of 4
119 biomass replicates was calculated as the sum of fresh weight (g FW) divided by the
120 sediment surface sampled (m²) and expressed as FWg m⁻². Statistical analysis for
121 burrowing kinetics was performed on R (R Core Team 2014) using a generalised non-
122 linear model with α as burrowing rate (h⁻¹). This generalised non-linear model with a
123 conditional binomial stochastic part was fitted to each data set by using Bayesian

124 inference as described in Buffet et al. (2014). For all the other parameters, results are
125 presented as mean \pm standard deviation, and statistical analyses were performed
126 using XLSTAT 2012. Normality was assessed using the Shapiro–Wilk test. To define
127 significant differences between sites for each sampling date, normally distributed data
128 were analyzed by one-way ANOVA (multiple comparisons of mean values) followed
129 by Tukey’s HSD post hoc method. When normality was not established, the non-
130 parametric Kruskal–Wallis and Mann–Whitney comparison tests were used. The level
131 of significance was established at $p < 0.05$. A Spearman's correlation (Daniel, 1990)
132 was computed to quantify the relationship between AChE activity and behavioural
133 toxicity parameter values. The Spearman's rank-order correlation is a non-parametric
134 correlation coefficient ρ , measuring strength and direction of the monotonic
135 relationship between AChE activity and MFB. The statistical significance test of the
136 Spearman correlation was performed to investigate whether the null hypothesis ‘H0:
137 There is no monotonic association between AChE and the behavioural toxicity
138 parameter MFB’ can be rejected.

139 Lower AChE activity was found in worms (*H. diversicolor*) sampled from Site 2
140 (10.07 ± 5.80 nmole.min⁻¹.mg⁻¹ proteins) than those in Site 1 (23.34 ± 8.90 nmole.min⁻¹.
141 mg⁻¹ proteins). AChE activities measured in *H. diversicolor* from Site 2 (compared to
142 Site 1) showed 40% and 79.5% of inhibition in May 2013 and February 2014,
143 respectively (Table 1). Results showing the positions of AChE activity measurements
144 in worms from both areas (Site 1 and Site 2) according to the four colour traffic light
145 previously identified by Barrick et al. (2018) (red: lethal effects expected; orange:
146 sublethal effects expected; yellow: range expected for non-impacted site; green:
147 evidence of good health) are illustrated in Figure 2. In February, AChE values were in
148 the range where sub-lethal effects (worms from Site 1) and lethal effects (worms from

149 Site 2) were expected. In May, AChE values for worms from Site 1 were in the yellow
150 range expected for non-impacted site, whereas those for worms from Site 2 were in
151 the range where sub-lethal effects were expected. Results of burrowing kinetics carried
152 out with *H. diversicolor* from both areas (Sites 1 and 2), allowed to burrow in sediment
153 from their site of origin, are shown in Table 2. Burrowing kinetics of worms from Site 2
154 were significantly impaired in February 2014 compared to those in Site 1. Behavioural
155 differences between worms from both sites measuring with the MFB are illustrated in
156 Table 2. Significant frequency decrease ($p=0.021$) corresponding to body undulation
157 (0.5 Hz) was observed for worms from Site 2 compared to those from Site 1. Decreases
158 of head movements and feeding activity (between 1.0 and 2.0 Hz) were also observed
159 for worms from Site 2 vs those from Site 1 ($p=0.066$). The mean Spearman's
160 correlation coefficient of 0.5404 revealed a positive correlation ($p=0.0686$) between
161 AChE activities and MFB values. Data of densities and biomass of *H. diversicolor* at
162 both sites are shown in Table 3. Densities and biomass of ragworms collected at Site
163 1 were significantly higher than those at Site 2 for both sampling dates (May 2013 and
164 February 2014).

165 The AOP approach initially proposed by Ankley et al. (2010) aims to link direct
166 molecular initiating events with adverse outcomes at a higher biological level of
167 organisation relevant to risk assessment. The usefulness of this approach for
168 biomarker-based environmental risk assessments has been recently demonstrated
169 using aquatic organisms (Wuk Lee et al., 2015). Effects observed at the individual and
170 subcellular level (defence and damage responses: oxidative stress, immunotoxicity,
171 neurotoxicity, genotoxicity and apoptosis) can be propagated upwards through
172 increasing levels of organisation. Behavioural damages could find their origin in
173 different biochemical and physiological impairments such as sensory system

174 disruption, endocrine disruption, neurotoxicity or energy disturbances (Amiard-Triquet
175 and Amiard, 2012) at a lower level and population effects at a higher level of
176 organisation. In the laboratory, numerous studies showed that decreased in AChE
177 activity induced behavioural disturbances in different species exposed to several
178 contaminants (Sismeiro-Vivas et al., 2007; Ren et al., 2015; Haverroth et al., 2015;
179 Geraudie et al., 2016). However, in the field, it is necessary to conduct studies able to
180 demonstrate a causal relationship between behavioural responses and effects at
181 higher levels of biological organisation such as population effects (Amiard-Triquet et
182 al., 2015). In the present study, we investigated, the potential links between three
183 different levels of biological organisation studied, precisely: sub-individual
184 (acetylcholinesterase activity), individual (burrowing behaviour) and population
185 (density and biomass) of worms. Results showed, at the biochemical level, a significant
186 lower value of AChE activity in worms (*H. diversicolor*) from Site 2 (decrease of 40%
187 in May 2013 and of 79% in February 2014 vs Site 1). Previously published studies
188 reported that a reduction of 20% in AChE activity in fish and invertebrates indicates
189 exposure to neurotoxic compounds (organophosphorus, pesticides), whereas
190 depletion of AChE activity by 20-50% indicates sublethal impact (Amiard-Triquet et al.,
191 2015). Mudflat sediments act as the final sink for various contaminants depending on
192 their properties. Sorption of organic contaminants on sediments is controlled by grain
193 size and organic matter content (Arslan-Alaton and Olmez-Hanci 2013). It has been
194 demonstrated that bioavailable metals and organic contaminant contents were low in
195 organic carbon enriched sites, tending to be adsorbed on fine-grained sediments
196 (Pelletier et al., 2011; Morrison et al., 2011). Site 1, compared to site 2, was composed
197 of slightly fine sediment and was slightly enriched in total organic carbon known to
198 have strong sorption capacities of contaminants (Luthy, 2004; Thibault de Chanvalon

199 et al., 2016). Moreover, Site 1 was located in an active eroded cliff where vegetation
200 and rhizosphere could develop synergies with microorganisms increasing pollutant
201 removal rates such as PAHs (Thibault de Chanvalon et al., 2016). At the individual
202 level, burrowing kinetics of worms from Site 2 were significantly impaired compared to
203 that in worms from Site 1. Schriks et al. (2006) used the MFB in *Xenopus laevis* as a
204 sensitive and useful tool to quantify behavioural effects induced by toxic compound. In
205 *H. diversicolor*, the use of MFB allows a more sensitive estimation of behavioural
206 activities of worms, as differentiation between body undulation, head movements and
207 feeding activity of worms can be assessed (Buffet et al., 2013). In this work, results
208 showed lower body undulation of worms from Site 2 (vs Site 1). Statistical analysis
209 (Spearman correlation) performed on the set results of AChE activities and behaviour
210 (MFB) showing a positive correlation between both biomarkers (biochemical and
211 behavioural) suggested that lower AChE activities were associated with lower
212 behaviour of worms (and inversely). Feeding behaviour disturbances have been
213 previously shown in *H. diversicolor* from the multipolluted Loire estuary as compared
214 to controls from a reference site (Fossi-Tankoua et al., 2012). Recently, Harayashiki
215 et al. (2016) demonstrated behavioural and AChE biochemical alterations in *Penaeus*
216 *monodon* post larvae diet-exposed to inorganic mercury, reducing swimming activity.
217 In the invertebrate *Gammarus fossarum*, Xuereb et al. (2009) showed that AChE
218 inhibition levels greater than 50% led to population depletion. At the population level,
219 worms exhibiting lower AChE activities and behaviour corresponded to those collected
220 from the site (Site 2) where lower population densities were found. Indeed, densities
221 of *H. diversicolor* showed a significantly contrasting status between both sites with
222 significantly higher densities (280.61 ± 325.69 to 634.57 ± 294.45 ind.m⁻²) in the upper
223 intertidal Site 1 and lower densities in the middle intertidal Site 2 (25.51 ± 23.29 to

224 66.96±28.28 ind.m⁻²). These observations cannot be relied on a patchy distribution of
225 worms on the mudflat, as the distribution of *H. diversicolor* is known to be rather
226 relatively uniform regarding the regularly distributed openings of burrows on the
227 sediment surface (Kristensen et al., 2013). However, it has been demonstrated in *H.*
228 *diversicolor* a double-way relationship between reworking behaviour and density as a
229 decrease in individual sediment reworking activity when density increase (Duport et al.
230 2006) and at high levels of algal enrichment (Murray et al. 2017). Moreover, at high
231 abundances, it has been shown that territorialism by this ragworm species is a
232 mechanism underplaying trophic competition, while both phenomena seem to
233 disappear at low population density (Kristensen et al. 2013). Site 1 was also enriched
234 in living benthic foraminifera with a total density of 2199.35 individuals per 100 cm⁻³
235 compared to that at site 2, where a density of only 48 individuals per 100 cm⁻³ was
236 found (Mojtahid et al., 2016). These authors correlated the microphytobenthic biofilm
237 development on 'Les Brillantes' mudflat with the foraminifera species abundance, as it
238 constitutes important quantities of food resources. Cardoso et al. (2004) showed that
239 *H. diversicolor* had greater abundance in areas where algae were available, as a food
240 source and a refuge from predators. Christensen et al. (2000) showed that an
241 abundant supply of nutritious food (phytoplankton) increased *H. diversicolor*
242 metabolism considerably. To cope, chemical stress is metabolically costly leading to a
243 shift in allocation of energy with implications for growth and reproduction processes
244 (Callow and Forbes, 1998). Thus, in the present study, it could be suggested that lower
245 food resources and energetic cost of defence mechanisms to cope with chemical
246 stress in worms from Site 2 vs Site 1 could lead to a reduction of the most energetic
247 expensive individual processes, growth and reproductive potential, resulting in
248 detrimental effects observed at the population level.

249 In conclusion, the contrasted status of *H. diversicolor* densities between both sites
250 could be partly explained by AChE activity decrease and behavioural impairments of
251 worms from the middle intertidal mudflat (Site 2) compared with the upper intertidal site
252 (Site 1), which could result in the detrimental population effects observed. This study
253 brings direct empirical field evidence supporting impact of AChE inhibition and
254 behaviour at the population level demonstrating the sensitivity of behaviour as a
255 biomonitor of estuarine health status assessment, as previously suggested by Roast
256 et al. (2001). This integrated methodology should be included in biomonitoring surveys
257 to evaluate habitat vulnerability and potential risk on higher trophic levels.

258

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261

262 **References**

263 Amiard-Triquet, C., Amiard, J.C., 2012. Behavioral ecotoxicology. In: Amiard-Triquet,
264 C., Amiard, J.C., Rainbow, P.S. (eds) Ecological biomarkers: indicators of
265 ecotoxicological effects. 10, 253-277.

266 Amiard-Triquet, C., Amiard, J.C., Mouneyrac, C., 2015. Predictive ecotoxicology and
267 environmental assessment. In: Amiard-Triquet, C., Amiard, J.C., Mouneyrac, C. (eds)
268 Aquatic Ecotoxicology: advancing tools for dealing with emerging risks. chap19, 463-
269 496.

270 Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D.,
271 Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrano, J.A., Tietge, J.E.,

272 Arslan-Alaton, I., Olmez-Hanci, T., 2013. Sources of Environmental Pollution:
273 Persistent Organic Pollutants. In: Suib, S.L. (ed) New and future developments in
274 catalysis. 12, 303-398.

275 Barrick, A., Châtel, A., Marion, J.M., Perrein, H., Bruneau, M., Mouneyrac, C., 2016. A
276 novel methodology for the determination of biomarker baseline levels in the marine
277 polychaete *Hediste diversicolor*. Mar. Pollut. Bull. 108.
278 10.1016/j.marpolbul.2016.04.056.

279 Barrick, A., Marion, J.M., Perrein, H., Châtel, A., Mouneyrac, C., 2018. Baseline levels
280 of biochemical biomarkers in the endobenthic ragworm *Hediste diversicolor* as useful
281 tools in biological monitoring of estuaries under anthropogenic pressure. Mar. Pollut.
282 Bull. 129, 81-85. 10.1016/j.marpolbul.2018.02.006.

283 Benyoucef, I., Blandin, E., Lerouxel, A., Jesus, B., Rosa, P., Méléder, V., Launeau, P.,
284 Barillé, L., 2014. Microphytobenthos interannual variations in a north-European
285 estuary (Loire estuary, France) detected by visible-infrared multispectral remote
286 sensing. Estuar. Coast. Shelf Sci. 136, 43-52.

287 Birch, G.F., 2018. A review of chemical-based sediment quality assessment
288 methodologies for the marine environment. Mar. Pollut. Bull. 133, 218-232.

289 Blanchet-Letrouvé, I., Lafont, A.G., Poirier, L., Baloche, S., Zalouck-Vergnoux, A.,
290 Dufour, S., Mouneyrac, C., 2013. Vg mRNA induction in an endangered fish species
291 (*Anguilla anguilla*) from the Loire estuary (France). Ecotoxicol. Environ. Saf. 97, 103-
292 113.

293 Bonnard, M., Roméo, M., Amiard-Triquet, C., 2009. Effects of copper on the burrowing
294 behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and
295 the bivalve *Scrobicularia plana*. Hum. Ecol. Risk Assess. 15, 11-26.

296 Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram
297 quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72,
298 248-254.

299 Buffet, P.E., Tankoua, O.F., Pan, J.F., Berhanu, D., Herrenknecht, C., Poirier, L.,
300 Amiard-Triquet, C., Amiard, J.C., Bérard, J.B., Risso, C., Guibbolini, M., Roméo, M.,
301 Reip, P., Valsami-Jones, E., Mouneyrac, C., 2011. Behavioural and biochemical
302 responses of two marine invertebrates *Scrobicularia plana* and *Hediste diversicolor* to
303 copper oxide nanoparticles. *Chemosphere.* 84(1), 166-74. doi:
304 10.1016/j.chemosphere.2011.02.003.

305 Buffet, P.E., Poirier, L., Zalouk-Vergnoux, A., Lopes, C., Amiard, J.C., Gaudin, P.,
306 Risso-de Faverney, C., Guibbolini, M., Gilliland, D., Perrein-Ettajani, H., Valsami-
307 Jones, E., Mouneyrac, C., 2014. Biochemical and behavioural responses of the marine
308 polychaete *Hediste diversicolor* to cadmium sulfide quantum dots (CdS QDs):
309 Waterborne and dietary exposure. *Chemosphere* 100, 53-70.

310 Buffet, P.E., Richard, M, Caupos, F., Vergnoux, A., Perrein-Ettajani, H., LunaAcosta,
311 A., Akcha, F., Amiard, J.C., Amiard-Triquet, C., Guibbolini, M., Risso-de Faverney, C.,
312 Thomas-Guyon, H., Reip, P., Dybowska, A., Berhanu, D., Valsami-Jones, E.,
313 Mouneyrac, C., 2013. Mesocosm study of fate and effects of CuO nanoparticles on
314 endobenthic species (*Scrobicularia plana*, *Hediste diversicolor*). *Environ. Sci. Technol.*
315 47, 1220-1228.

316 Calow, P., Forbes, V.E., 1998. How do physiological responses to stress translate into
317 ecological and evolutionary processes? *Comp. Biochem. Physiol. A* 120, 11–16.

318 Cardoso, P.G., Pardal, M.A., Raffaelli, D., Baeta, A., Marques, J.C., 2004.
319 Macroinvertebrate response to different species of macroalgal mats and the role of
320 disturbance history. *J. Exp. Mar. Biol. Ecol.* 308, 207-220.

321 Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in
322 sediment inhabited by suspension-feeding (*Nereis diversicolor*) and non-suspension-
323 feeding (*N. virens*) polychaetes. Mar. Ecol. Prog. Ser. 192, 203-217.

324 Dallas, L.J., Jha, A.N., 2015. Applications of biological tools or biomarkers in aquatic
325 biota: A case study of the Tamar estuary, South West England. Mar. Pollut. Bull. 95,
326 618-633.

327 Daniel, W.W., 1990. Spearman Rank Correlation Coefficient. Applied Nonparametric
328 Statistics (2nd ed., pp. 358-365). Boston, MA: PWS-Kent.

329 Duport, E., Stora, G., Tremblay, P., Gilbert, F., 2006. Effects of population density on
330 the sediment mixing induced by the gallery-diffusor *Hediste (Nereis) diversicolor* O.F.
331 Muller, 1776. J. Exp. Mar. Biol. Ecol. 336, 33-41.

332 Durou, C., Poirier, L., Amiard, J.C., Budzinski, H., Gnassia-Barelli, M., Lemenach, K.,
333 Peluhet, L., Mouneyrac, C., Roméo, M., Amiard-Triquet, C., 2007. Biomonitoring in a
334 clean and multi-contaminated estuary based on biomarkers and chemical analyses in
335 the endobenthic worm *Nereis diversicolor*. Environ. Pollut. 148, 445-458.

336 Fossi Tankoua, O., Buffet, P.E., Amiard, J.C., Amiard-Triquet, C., Méléder, V., Gillet,
337 P., Mouneyrac, C., Berthet, B., 2012. Intersite variations of a battery of biomarkers at
338 different levels of biological organisation in the estuarine endobenthic worm *Nereis*
339 *diversicolor* (Polychaeta, Nereididae) Aquat. Toxicol. 114-115, 96-103.

340 Geraudie, P., Bakkemo, R., Milinkovitch, T., Thomas-Guyon, H., 2016. First evidence
341 of marine diesel effects on biomarker responses in the Icelandic scallops, *Chlamys*
342 *islandica*. Environ. Sci. Pollut. Res. Int. 23, 16, 16504-16512.

343 Harayashiki, C.A.Y., Reichelt-Brushett, A.J., Liu, L., Butcher, P., 2016. Behavioural
344 and biochemical alterations in *Penaeus monodon* postlarvae diet-exposed to inorganic
345 mercury. Chemosphere 164, 241-247.

346 Haverroth, G.M., Welang, C., Mocelin, R.N., Postay, D., Bertoncello, K.T.,
347 Franscescon, F., Rosemberg, D.B., Dal Magro, J., Dalla Corte, C.L., 2015. Copper
348 acutely impairs behavioral function and muscle acetylcholinesterase activity in
349 zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf.* 122, 440-447.

350 Kalman, J., Buffet, P.E., Amiard, J.C., Denis, F., Mouneyrac, C., Amiard-Triquet, C.,
351 2010. Assessment of the influence of confounding factors (weight, salinity) on the
352 response of biomarkers in the estuarine polychaete *Nereis diversicolor*. *Biomarkers*
353 15, 5, 461-469.

354 Kankaanpää, H., Leiniö, S., Olin, M., Sjövall, O., Meriluoto, J., Lehtonen, K.K., 2007.
355 Accumulation and depuration of cyanobacterial toxin nodularin and biomarker
356 responses in the mussel *Mytilus edulis*. *Chemosphere* 68, 7, 1210-1217.

357 Kristensen, E., Delefosse, M., Quintana, C.O., Banta, G.T., Petersen, H.C., Jorgensen,
358 B., 2013. Distribution pattern of benthic invertebrates in Danish estuaries: The use of
359 Taylor's power law as a species-specific indicator of dispersion and behavior. *J. Sea*
360 *Res.* 77, 70-78.

361 Lehtonen, K.K., Kankaanpää, H., Leiniö, S., Sipiä, V.O., Pflugmacher, S., Sandberg-
362 Kilpi, E., 2003. Accumulation of nodularin-like compounds from the cyanobacterium
363 *Nodularia spumigena* and changes in acetylcholinesterase activity in the clam *Macoma*
364 *balthica* during short-term laboratory exposure. *Aquat. Toxicol.* 64, 461-476.

365 Lionetto, M.G., Caricato, R., Calisi, A., Giordano, M.E., Schettino, T., 2013.
366 Acetylcholinesterase as a biomarker in environmental and occupational medicine: New
367 insights and future perspectives. *Biomed. Res. Int.* ID 321213
368 <http://dx.doi.org/10.1155/2013/321213>.

369 Luthy, R.G., 2004. Organic contaminants in the environment: challenges for the
370 water/environmental engineering community. In: Norling, P., Wood-Black, F.,

371 Masciangioli, T.M. (eds). Water and Sustainable Development: Opportunities for the
372 Chemical Sciences: A Workshop Report to the Chemical Sciences Roundtable
373 Washington (DC): National Academies Press (US).

374 Mojtahid, M., Geslin, E., Coynel, A., Gorse, L., Vella, C., Davranche, A., Zozzolo, L.,
375 Blanchet, L., Bénéteau, E., Maillet, G., 2016. Spatial distribution of living (Rose Bengal
376 stained) benthic foraminifera in the Loire estuary (western France). J. Sea Res. 118,
377 1-16.

378 Morrison, G., Greening, H., Yates, K.K., 2011. Sediment contaminants and benthic
379 habitat quality. In: Morrison G, Greening H, Yates KK (eds) Integrating Science and
380 Resource Management in Tampa Bay, Florida. US Geological Survey Circular 1348,
381 7, 203-238.

382 Murray, F., Solan, M., Douglas, A., 2017. Effects of algal enrichment and salinity on
383 sediment particle reworking activity and associated nutrient generation mediated by
384 the intertidal polychaete *Hediste diversicolor*. J. Exp. Mar. Biol. Ecol. 495, 75-82.

385 Pelletier, M.C., Campbell, D.E., Ho, K.T., Burgess, R.M., Audette, C.T., Detenbeck,
386 N.E., 2011. Can sediment total organic carbon and grain size be used to diagnose
387 organic enrichment in estuaries? Environ. Toxicol. Chem. 30, 3, 538-547.

388 Ren, Z., Zhang, X., Wang, X., Qi, P., Zhang, B., Zeng, Y., Fu, R., Miao, M., 2015. AChE
389 inhibition: One dominant factor for swimming behavior changes of *Daphnia magna*
390 under DDVP exposure. Chemosphere. 120: 252-257.

391 Roast, S.D., Widdows, J., Jones, M.B., 2001. Impairment of mysid (*Neomysis integer*)
392 swimming ability: an environmentally realistic assessment of the impact of cadmium
393 exposure. Aquat. Toxicol. 52, 217-227.

394 Russom, C.L., Lalone, C.A., Villeneuve, D.L., Ankley, G.T., 2014. Development of an
395 adverse outcome pathway for acetylcholinesterase inhibition leading to acute mortality.
396 Environ. Toxicol. Chem. 33, 10, 2157-2169.

397 Schriks, M., van Hoorn, M.K., Faassen, E.J., van Dam, J.W., Murk, A.J., 2006. Real-
398 time automated measurement of *Xenopus leavis* tadpole behavior and behavioral
399 responses following triphenyltin exposure using the multispecies freshwater biomonitor
400 (MFB). Aquat. Toxicol. 77, 298-305.

401 Sismeiro-Vivas, J., Abrantes, N., Pereira, J.L., Castro, B.B., Gonçalves, F., 2007.
402 Short-term effects of Quirlan (chlorfenvinphos) on the behavior and
403 acetylcholinesterase activity of *Gambusia holbrooki*. Environ Toxicol. 22(2), 194-202.

404 Sturve, J., Scarlet, P., Halling, M., Kreuge, J., Macia, A., 2016. Environmental
405 monitoring of pesticide exposure and effects on mangrove aquatic organisms of
406 Mozambique. Mar. Environ. Res. 121, 9-19.

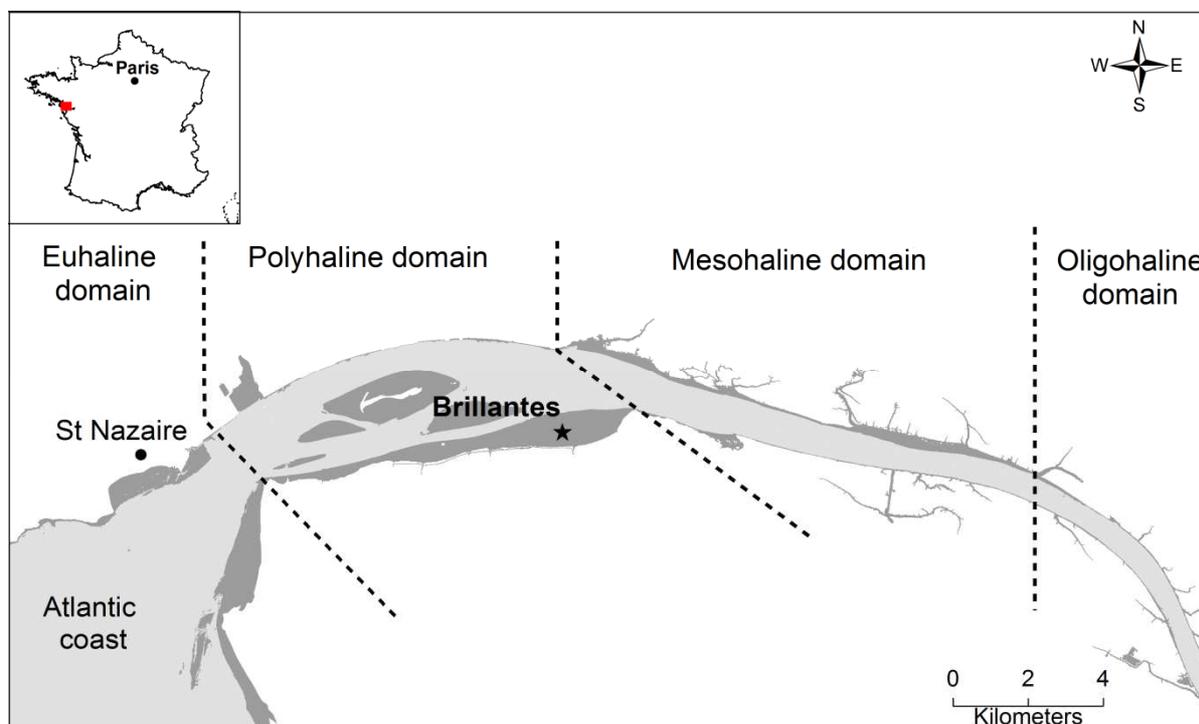
407 Thibault de Chanvalon, A., Mouret, A., Knoery, J., Geslin, E., Péron, O., Metzger, E.,
408 2016. Manganese, iron and phosphorus cycling in an estuarine mudflat sediment,
409 Loire, France. J. Sea Res. 118, 92-102.

410 Wuk Lee, J., Won, E.J., Raisuddin, S., Lee, J.S., 2015. Significance of adverse
411 outcome pathways in biomarker-based environmental risk assessment in aquatic
412 organisms. J. Environ. Sci. 35, 115-127.

413 Xuereb, B., Lefèvre, E., Garric, J., Geffard, O., 2009. Acetylcholinesterase activity in
414 *Gammarus fossarum* (Crustacea Amphipoda): Linking AChE inhibition and
415 behavioural alteration. Aquat. Toxicol. 94, 114-122.

416 Zhang, D.L., Liu, S.Y., Zhang, J., Zhang, J.K., Hu, C.X., Liu, Y.D., 2016. Respiratory
417 toxicity of cyanobacterial aphantoxins from *Aphanizomenon flos-aquae* DC-1 in the
418 zebrafish gill. Aquat. Toxicol. 176, 106-115.

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423 Fig. 1. Brillantes mudflat is localized in the polyhaline domain of the Loire estuary
424 (France), with its intertidal flats shown in dark gray (Benyoucef et al., 2014).

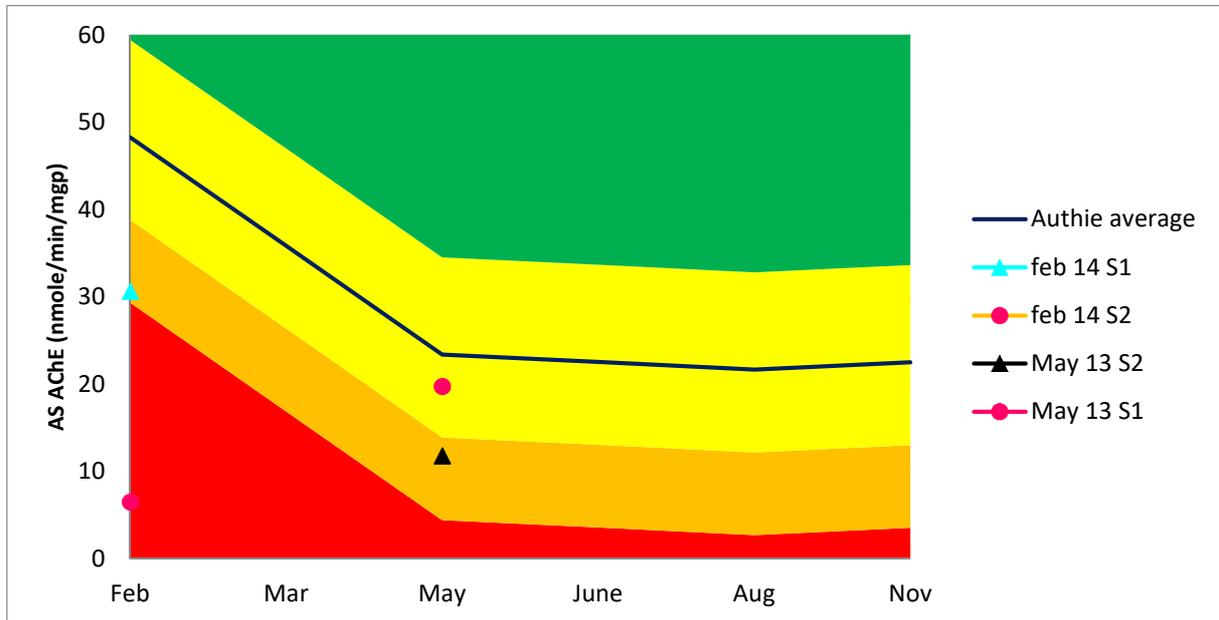
425
426 Table 1: AChE activity measured in *H. diversicolor* from site 1 and site 2 expressed in
427 nmole.min⁻¹.mg⁻¹ proteins. ^a significantly difference compared to ^b (p<0.05).

	AChE activity (nmole.min ⁻¹ .mg ⁻¹ proteins) Site1	AChE activity (nmole.min ⁻¹ .mg ⁻¹ proteins) Site2	P value
May 2013	19.75±5.01 ^a	11.83±5.70 ^b	0.008
February 2014	30.64±12.42 ^a	6.54±4.61 ^b	0.002

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433 Fig. 2 BAC (baseline assessment criteria) and EAC (environmental assessment
434 criteria) graph for AChE activity values (Barrick et al., 2018). The black line indicates
435 the mean values established for the low-impact site Authie, the yellow band indicates
436 the range of the BAC and the orange line indicates the range where sublethal effects
437 are expected to occur. AChE values for both sites (Site 1: S1; Site 2: S2) on May 2013
438 (May 13) and February 2014 (Feb 14) were plotted on the graph as well.

439

440 Table 2: Burrowing tests: Mean estimate α (h^{-1}) and the 95% credibility interval (CI)
441 obtained after *a posteriori* distribution for each site (site 1: S1, site 2: S2) and sampling
442 period (May 2013 and February 2014). MFB: Mean frequency occurrence (%) and
443 standard deviation (SD) of signals recorded with Multi Freshwater Biomonitor (MFB)
444 and corresponding to body undulation (0.5 Hz) and to head movements and feeding

445 activity (1 to 2 Hz) in *H. diversicolor*. * significant difference ($p < 0.05$). nd: not
 446 determined.

	Burrowing test α [CI] S1	Burrowing test α [CI] S2	MFB Frequency occurrence (mean% \pm SD) 0.5 Hz S1	MFB Frequency occurrence (mean% \pm SD) 0.5 Hz S2	MFB Frequency occurrence (mean% \pm SD) 1.2 Hz S1	MFB Frequency occurrence (mean% \pm SD) 1.2 Hz S2
May 2013	0.064 [0.036 - 0.099]	0.059 [0.033 - 0.091]	64.89 \pm 9.13*	43.95 \pm 16.15 * ($p=0.021$)	10.48 \pm 5.88	7.27 \pm 2.01 ($p=0.523$)
February 2014	0.103 [0.063 - 0.152]*	0.036 [0.020 - 0.059]*	58.00 \pm 4.43	49.51 \pm 7.70 ($p=0.066$)	6.44 \pm 0.97	5.61 \pm 0.92 ($p=0.173$)

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448 Table 3. Intersite variations of *H. diversicolor* density (number of individuals N m⁻² as
 449 mean \pm standard deviation) and biomass (Fresh weight FWg m⁻² as mean \pm standard
 450 deviation) for each sampling period (May 2013 and February 2014). nd: not determined

	Density (N.m ⁻²) Site 1	Density (N.m ⁻²) Site 2	Biomass (FWg.m ⁻²) Site 1	Biomass (FWg.m ⁻²) Site 2
May 2013	631.38 \pm 182.03	51.02 \pm 31.24	39.80 \pm 21.13	3.18 \pm 1.96
February 2014	280.61 \pm 325.69	66.96 \pm 28.28	19.84 \pm 23.26	4.46 \pm 4.64

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