



HAL
open science

Physiological and comparative proteomic analyzes reveal immune defense response of the king scallop *Pecten maximus* in presence of paralytic shellfish toxin (PST) from *Alexandrium minutum*

Yasmine Even, Emilien Pousse, Coraline Chapperon, Sébastien Artigaud, Hélène Hégaret, Benoit Bernay, Vianney Pichereau, Jonathan Flye-Sainte-Marie, Fred Jean

► To cite this version:

Yasmine Even, Emilien Pousse, Coraline Chapperon, Sébastien Artigaud, Hélène Hégaret, et al.. Physiological and comparative proteomic analyzes reveal immune defense response of the king scallop *Pecten maximus* in presence of paralytic shellfish toxin (PST) from *Alexandrium minutum*. *Harmful Algae*, 2022, 115, pp.102231. 10.1016/j.hal.2022.102231 . hal-03723474

HAL Id: hal-03723474

<https://hal.univ-brest.fr/hal-03723474>

Submitted on 14 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Physiological and comparative proteomic analyzes reveal immune defense response of the**
2 **king scallop *Pecten maximus* in presence of paralytic shellfish toxin (PST) from *Alexandrium***
3 ***minutum*.**

4
5
6 *Authors:* Yasmine Even^{1*}, Emilien Pousse¹, Coraline Chapperon¹, Sébastien Artigaud¹, Hélène
7 Hégaret¹, Benoit Bernay², Vianney Pichereau¹, Jonathan Flye-Sainte-Marie¹, Fred Jean¹.

8
9 1: Laboratoire des Sciences de l'Environnement Marin, UMR 6539 CNRS/UBO – Institut
10 Universitaire Européen de la Mer, Technopôle Brest-Iroise, 29280 Plouzané, France

11 2 : Plateforme Proteogen, US EMerode, Université de Caen Normandie, Esplanade de la paix,
12 14032 Caen, France

13
14 * *Corresponding author:* Dr Yasmine Even, PhD, LEMAR – IUEM, Technopôle Brest-Iroise,

15 29280 Plouzané, France; yasmine.even@univ-brest.fr; Tel: 33+298 498 670; Fax: 33+298 498 645

16 **Abstract**

17 The king scallop, *Pecten maximus* is a highly valuable seafood in Europe. Over the last few years, its
18 culture has been threatened by toxic microalgae during harmful algal blooms, inducing public health
19 concerns. Indeed, phycotoxins accumulated in bivalves can be harmful for human, especially para-
20 lytic shellfish toxins (PST) synthesized by the microalgae *Alexandrium minutum*. Deleterious effects
21 of these toxic algae on bivalves have also been reported. However, its impact on bivalves such as
22 king scallop is far from being completely understood. This study combined ecophysiological and
23 proteomic analyzes to investigate the early response of juvenile king scallops to a short term exposure
24 to PST producing *A. minutum*. Our data showed that all along the 2-days exposure to *A. minutum*,
25 king scallops exhibited transient lower filtration and respiration rates and accumulated PST. Signifi-
26 cant inter-individual variability of toxin accumulation potential was observed among individuals.
27 Furthermore, we found that ingestion of toxic algae, correlated to toxin accumulation was driven by
28 two factors: 1/ the time it takes king scallop to recover from filtration inhibition and starts to filtrate
29 again, 2/ the filtration level to which king scallop starts again to filtrate after inhibition. Furthermore,
30 at the end of the 2-day exposure to *A. minutum*, proteomic analyzes revealed an increase of the killer
31 cell lectin-like receptor B1, involved in adaptative immune response. Proteins involved in detoxifi-
32 cation and in metabolism were found in lower amount in *A. minutum* exposed king scallops. Proteo-
33 mic data also showed differential accumulation in several structure proteins such as β -actin, paramy-
34 osin and filamin A, suggesting a remodeling of the mantle tissue when king scallops are subjected to
35 an *A. minutum* exposure.

36

37 *Key words:* Paralytic shellfish toxins, *Alexandrium minutum*, *Pecten maximus*, ecotoxicology,
38 proteomics.

39

40 1. Introduction

41 King scallop, *Pecten maximus* is a highly valued seafood product and is of major commercial
42 importance in Europe with global capture production of 60,000 tons in 2019 (FAO Fisheries and
43 Aquaculture Department). This marine bivalve lives in coastal waters at the surface of, or slightly
44 buried in the sediment (Baird, 1958). Like many shellfishes feeding on microalgae, king scallops are
45 exposed to harmful algal blooms (HAB) (Chauvaud et al., 2001, 1998). HAB occur worldwide and
46 can negatively affect king scallops including hatchery seeds and juveniles (Anderson et al., 2012;
47 Borcier et al., 2017; Li et al., 2002), thus causing significant economic and social impacts. A better
48 understanding of the different effects caused by harmful algae exposures on the king scallop is
49 therefore essential to improve the management of fisheries or aquaculture practices.

50 Toxic microalgae of the genus *Alexandrium*, are responsible for a large proportion of HAB
51 events worldwide. Indeed, the dinoflagellates *Alexandrium* spp. can produce saxitoxin and its
52 derivatives that are paralytic shellfish toxins (PST), responsible for the paralytic shellfish poisoning
53 (PSP) syndrome in human following consumption of contaminated seafood. The PST can be lethal
54 for mammals by inducing paralysis via binding to voltage-dependent sodium channels involved in
55 the nervous influx, thus inhibiting membrane depolarization and blocking proliferation of action
56 potentials (Narahashi and Moore, 1968; Ritchie and Rogart, 1977). Although less toxic for
57 invertebrates such as bivalves, the consumption of toxic algae such as *Alexandrium minutum* that
58 synthesizes PST, which accumulate within the digestive gland and soft tissues, can results in several
59 deleterious effects (Borcier et al., 2017; Castrec et al., 2018; Contreras et al., 2012a, 2012b; Fabioux
60 et al., 2015; Li et al., 2002; Pousse et al., 2018). Assessing the effect of toxic dinoflagellates upon
61 bivalves is of great importance, as it first represents a hazard for human after consumption of
62 contaminated seafood, but also for other marine organisms exposed to these toxic dinoflagellates,
63 therefore impacting socioeconomic activity, especially associated with the closure of fishing areas,
64 when toxin concentration exceeds authorized values (Anderson et al., 2012; Geraci et al., 1989;
65 Hégaret et al., 2009; Reyero et al., 1999). Among the *A. minutum* species, several strains have been

66 identified with variable amounts of PST produced, as well as variable levels of cytotoxic potency,
67 associated to the production of some uncharacterized bioactive extracellular components (BEC),
68 independent from PST (Borcier et al., 2017; Castrec et al., 2018; Long et al., 2018). In the latter
69 studies, clear damages of BEC on bivalves or other marine organisms have been demonstrated,
70 whereas toxic effects of PST on bivalves are less well established (Mat et al., 2013; Payton et al.,
71 2017).

72 The effects of *A. minutum* and its toxins on mussels and oysters have been well studied
73 (Bougrier et al., 2003; Castrec et al., 2019; Fabioux et al., 2015; Haberkorn et al., 2010b, 2010a; Mat
74 et al., 2018; Payton et al., 2017), however very little is known about its physiological impact on the
75 king scallop. Coquereau et al (2016) and Borcier et al (2017) respectively demonstrated that an
76 exposure to *A. minutum*, depending on the strain, could lead to modified valve movement, but also a
77 decreasing filtration rate and shell growth as well as histological damages and altered escape
78 response. No studies have, however, investigated the ecophysiological and associated proteomic
79 responses of the king scallop following an exposure to this toxic microalgae. In order to better
80 understand protective mechanisms activated in bivalves when exposed to toxic algae, we assessed the
81 response of king scallop juveniles to an environmental stress, corresponding to a short term exposure
82 to a PST producing strain of *A. minutum*, by characterizing physiological and proteome modifications
83 in king scallops. More specifically, we measured toxin accumulation in king scallops and its
84 consequences on physiological parameters such as filtration and respiration. We further performed
85 proteomic analyzes on mantle tissues to evaluate the responses of juvenile king scallops to *A. minutum*
86 exposure at the protein levels.

87

88 **2. Material and methods**

89 *2.1. Animals and sample collection*

90 King scallop (*P. maximus*) juveniles were obtained from the Tinduff hatchery (Plougastel-Daoulas,
91 France). Average individual length was 35.9 ± 2.1 mm (*mean* \pm *SD*, $n = 35$). In order to make sure
92 that toxin accumulation in king scallops is not due to contaminations prior to our experiment,
93 individuals were acclimated a week prior experiments into seawater (35 PSU) flow-through tanks and
94 fed *ad libitum* with a standard 50/50 mix of algae *Tisochrysis lutea* (T-iso) and *Chaetoceros muelleri*
95 which are common aquaculture feed.

96

97 2.2. Algal cultures

98 *T. lutea* (strain CCAP 927/14) and *C. muelleri* (strain CCAP1010/3) were produced in continuous (10
99 L bioreactor) and batch cultures (2 L and 6 L glass bottles) in Conway medium (Walne, 1966) made
100 with UV sterilized 1- μ m filtered seawater (35 PSU) that was aerated with a mix of air and CO₂
101 (Walne, 1970).

102 Cultures of *A. minutum* (strain Daoulas 1257) were grown in 2 L and 6 L glass bottles with L1
103 medium (Guillard and Hargraves, 1993) made with 1- μ m filtered seawater (35 PSU). Air but no CO₂
104 was added to the cultures. Room temperature was maintained at 18°C with a 12:12 photoperiod. The
105 mean toxicity of *A. minutum* strain Daoulas 1257 was 52.8 fg STX equivalent cell⁻¹ (equivalent to
106 0.63 fmol cell⁻¹; Pousse et al., 2018) at the end of the exponential growth phase. This strain was
107 reported to not produce any bioactive extracellular compounds responsible for toxic effects in bi-
108 valves (Castrec et al., 2018) or allelopathic effects (Long et al., 2018).

109

110 2.3. Exposure to *A. minutum* and sampling

111 In order to examine the potential physiological effects of *A. minutum* strain Daoulas 1257 and its PST
112 upon king scallops, experimental trials were divided into 2 phases. Each phase lasted 2 days.

113 During the first phase, each group of king scallops ($n=25$) were fed with a normal diet characterized
114 by an equal algal mix of non-toxic algae (50/50, *T. lutea* and *C. muelleri*, for algal concentrations see
115 Fig. 1).

116 During the second phase, king scallop individuals were exposed to 2 different conditions:

117 1) absence of food: no algae (NA); experiment named TC-NA hereafter (n=7).

118 2) toxic diet: *A. minutum* strain Daoulas 1257 (A); experiment repeated 3 times and named
119 TC-A1, TC-A2, TC-A3 (n=18); the concentration of *A. minutum* distributed to king scallops
120 was respectively 560, 440 and 500 cells mL⁻¹ (Fig. 1).

121 During the 4 days, the flow rate in each chamber was adjusted to 30 mL min⁻¹. At the end of the 4
122 days of experiment, juvenile king scallops were individually measured and weighted (total, shell and
123 humid flesh mass) and mantles were collected and stored at -80°C for further proteomic analyzes. For
124 specimen exposed to *A. minutum* the digestive gland was collected, weighted and stored at -80°C to
125 perform a PST accumulation quantification (Fig. 1).

126

127 2.4. Experimental set up for physiological measurements

128 Experiments were run through a physiological measurement system designed to allow the simultane-
129 ous measurements of king scallops respiration and clearance rates (see experimental device described
130 in Flye-Sainte-Marie et al., 2007; Savina and Pouvreau, 2004). Seven king scallop juveniles were
131 each placed into a transparent open-flow 540 mL chamber (n=7) connected to the system. An 8th
132 chamber remained empty, i.e. without any individual, and was used as a control. Chambers were
133 supplied with thermoregulated (ca. 15.7°C) filtered (1 µm) seawater at 35 PSU. Concentrated algal
134 culture was constantly added to the system at the inlet of the "mixing tank" to reach concentrations
135 between 24 000 and 40 000 cells mL⁻¹ and between 440 and 560 cells mL⁻¹ for T-iso/Chaeto and *A.*
136 *minutum* respectively. These concentrations were adjusted as a function of the fluorescence in the
137 control chamber as in Pousse et al., 2018. The seawater inflow was controlled within each chamber
138 by 2 peristaltic pumps placed upstream (each peristaltic pump controlled the seawater flow of 4 cham-
139 bers) and maintained at a rate of ca. 30 mL min⁻¹. Algal concentrations were measured in the control
140 chamber for each experiment. The 8-tank experimental device is equipped with a multiparameter

141 probe (WTW MultiLine 3430, Fisher Scientific, Suwanee, GA, USA) measuring both dissolved ox-
 142 ygen and temperature, and a fluorometer (WETstar chlorophyll a, WET Labs, Philomat, USA) quan-
 143 tifying fluorescence in seawater outflow coming from each chamber successively (by alternating con-
 144 trol and experimental chambers). Measures lasted 15 min within each chamber with a record every
 145 10 sec. A full set of measurements was thus conducted over 210 min. Real time data could be visual-
 146 ized using a graphical user-interface developed specifically for this system and recorded data were
 147 sent to the controller and stored continuously. In order to prevent the development of biofilm that
 148 could cause a bias in respiration measurements, the full system was emptied and cleaned with perace-
 149 tic acid and hydrogen peroxide, rinsed with hot freshwater and then with filtered seawater between
 150 the 2 phases of each experimental trial. Juvenile king scallops were kept in filtered seawater during
 151 this procedure.

152

153 2.5. Physiological parameters

154 2.5.1. Respiration rate (*RR*)

155 The average respiration rate (*RR*, mg O₂ h⁻¹) of juvenile king scallops was assessed using the follow-
 156 ing equation:

$$157 \quad RR = -\left(0_2(\textit{control}) - 0_2(\textit{scallop})\right) \times FR$$

158 where:

159 $-0_2(\textit{control})$ corresponds to the average oxygen concentration (mg O₂ L⁻¹) in the control chamber
 160 recorded prior and after the measurements made in the experimental chamber, and

161 $-0_2(\textit{scallop})$ is the average oxygen concentration (mg O₂ L⁻¹) measured in the experimental cham-
 162 ber and

163 *FR* is the seawater flow rate (L h⁻¹) through the chambers.

164

165 2.5.2. Clearance rate (*CR*)

166 Clearance rate (CR in L h⁻¹) can be defined as the volume of water cleared of particle per unit of time
 167 by an individual. Throughout the experiment, seawater outflow from the different chambers was sam-
 168 pled and algal concentration was measured using a Coulter Counter Multisizer. Fluorescence values
 169 were converted to cell concentration (cells L⁻¹). CR was calculated as follows:

$$170 \quad CR = \frac{-(FC(control) - FC(scallop))}{FC(control)} \times FR$$

171 where $FC(control)$ is the average cell concentration (cells L⁻¹) recorded in the control chamber be-
 172 fore and after the measurements made in experimental chambers, $FC(scallop)$ corresponds to the
 173 average cell concentration (cells L⁻¹) measured in the experimental chamber and FR is the seawater
 174 flow rate (L h⁻¹) through the chambers.

175

176 Both respiration and clearance rates were standardized using the following equation (Bayne et al.,
 177 1987):

$$178 \quad Y_s = (W_s/W_e)^b \times Y_e.$$

179 Where Y_s is the physiological rate (respiration or clearance rate in this study) for the standard total
 180 mass W_s , Y_e is the physiological rate of the individual that occupied the experimental chamber with a
 181 total mass of W_e and b is the allometric coefficient for the clearance (0.67) and respiration (0.75) rates
 182 (Bayne et al., 1987). Physiological rates were standardized for a juvenile king scallop with a total
 183 mass of 7 g.

184 Type II linear regressions with range major axis method were applied to adjust linear relationships
 185 between the number of consumed algal cells and toxin concentration by using the R package
 186 “Lmodel2” (Legendre et al., 2014).

187

188 2.5.3. Clearance rate inhibition index (CRII)

189 The clearance rate inhibition index (CRII) was calculated for each individual, using standardized CR
 190 of day 2 (fed with non-toxic algae) and standardized CR of day 4 (exposed to toxic algae). The CRII

191 was used to quantify CR inhibition due to *A. minutum* and was calculated as follows (Pousse et al.,
192 2018):

$$193 \quad CRII = 1 - \frac{CR(day2)}{CR(day4)}$$

194 *2.5.4. Time for filtration recovery*

195 The time for filtration recovery was measured from the beginning of *A. minutum* exposure to the time
196 when individual clearance rate reached 0.5 L h⁻¹. This threshold was chosen arbitrarily to detect low
197 levels of filtration activity, without considering erratic filtration behaviors. When individuals CR did
198 not reach this threshold, a time of 40h was applied.

199

200 *2.6. Toxin accumulation*

201 The PST accumulation was estimated individually using juvenile king scallop digestive gland. HCl
202 was added to digestive gland samples (1:1 w:w) that were then ground using a beadblaster, boiled for
203 5 min at 104°C and centrifuged for 10 min. PSTs were subsequently estimated using a Saxitoxin PSP
204 ELISA kit (Abraxis), following instructions from the manufacturer as described in Lassudrie et al.
205 (2014). The acid hydrolysis can induce chemical conversion of some PST analogues to STX (Vale et
206 al., 2008). This Abraxis PSP ELISA assay recognizes mostly STX, and other PSTs only to varying
207 degrees (cross-reactivities of 100% for STX and from 29% to 0% for other PSTs). Thus, toxicity of
208 the digestive glands was expressed as µg of PST per 100 g of wet DG weight.

209

210 *2.7. Other ecophysiological parameters measured*

211 *2.7.1. Toxin accumulation potential*

212 The concentration of toxic algae delivered to king scallops slightly differed in the 3 assays (Fig. 1).
213 Therefore, in order to be able to compare toxin accumulation in all individuals exposed to *A. minutum*,
214 we calculated the toxin accumulation potential of each individual by dividing the toxin concentration

215 accumulated in digestive glands (DG) at the end of day 4 by the number of *A. minutum* cells
216 distributed during days 3&4 for each individual (μg of PST per 100 g of DG per toxic algal cell
217 delivered).

218

219 2.7.2. Number of *A. minutum* cells consumed ($\times 10^6$ per 100g of DG) and toxins ingested –TI)

220 From the individual unstandardized CR obtained and the algal concentrations measured in the
221 experimental chambers, the number of *A. minutum* cells consumed can be estimated. To do so, the
222 mean algal concentration (cell L^{-1}) to which a king scallop has been exposed to during a recording
223 cycle (3.5 hours) was multiplied to the unstandardized clearance rate (L h^{-1}) calculated for the
224 corresponding cycle and by 3.5 (h), the duration of each recording cycle. All recording cycles were
225 then added together and divided by the DG mass (g) to obtain the whole quantity of *A. minutum* cells
226 consumed per DG mass during the experiment.

227 To calculate the overall toxins ingested (TI, μg of STX per 100 g of DG), the number of cells
228 consumed was multiplied by the cellular toxins concentration corresponding to the *A. minutum* strain
229 used in this experiment ($52.8 \times 10^{-9} \mu\text{g STX cell}^{-1}$).

230

231 2.7.3. Toxin accumulation efficiency (TAE)

232 The toxin accumulation efficiency (TAE) reflects the balance between toxin uptake and elimination
233 processes. It corresponds to the proportion of toxins accumulated (TA, i.e. measured) in king scallop
234 DG during days 3&4 relative to that ingested (TI), and is calculated as follows (Bougrier et al., 2003;
235 Mafra et al., 2010; Pousse et al., 2018):

$$236 \quad TAE = 100 \times \frac{TA}{TI}$$

237

238 2.8. Protein extraction

239 Frozen mantle tissue was crushed with a mixer mill (MM400; RETSCH, Haan, Germany) and kept
240 frozen using liquid nitrogen. For each animal, 100 mg of the obtained mantle tissue powder was
241 homogenized in 100 mM Tris–HCl (pH 6.8) with 1% of protease inhibitor mix (GE Healthcare, Little
242 Chalfont, UK) and centrifuged (50 000 g, 5 min, 4°C). Samples were precipitated overnight at 4°C
243 using TCA 20% (1/1:v/v). After centrifugation (20 000 g, 30 min, 4°C), pellets were washed with
244 70% acetone and re-suspended in thiourea/urea/CHAPS buffer (2 M urea, 7 M thiourea, 4% CHAPS,
245 1% DTT) containing 1% ampholytes (IPG Buffer, pH 4–7; GE Healthcare, Little Chalfont, UK).
246 Protein concentrations were determined using the Bradford derived method and all samples were
247 adjusted to 800 µg of proteins in 250µL.

248

249 *2.9. Two-dimensional electrophoresis (2-DE)*

250 Prior to isoelectric focusing, IPG (immobilized pH gradient) strips (pH 4–7, 13 cm; GE Healthcare,
251 Little Chalfont, UK) were passively rehydrated with 250 µL of protein solution in wells for 14h.
252 Isoelectric focusing was conducted using the following protocol: 250 V for 15 min, 500 V for 2 h,
253 gradient voltage increased to 1000 V for 1 h, gradient voltage increased to 8000 V for 2.5 h, 8000 V
254 for 2 h and finally reduced to 500 V (Ettan IPGphor3; GE Healthcare, Little Chalfont, UK). After
255 isoelectrofocalisation, strips were incubated in equilibration buffer (50 mM Tris–HCl pH 8.8, 6 M
256 urea, 30% glycerol, 2% SDS and 0.002% Bromophenol Blue) for two periods of 15 min, the first one
257 completed with 1 g L⁻¹ dithiothreitol and the second time with 48 g L⁻¹ iodoacetamide. The IPG strips
258 were then placed on top of 12% polyacrylamide gels (SDS-PAGE) and were run in a 10°C thermo-
259 regulated electrophoresis unit (SE 600 Ruby; Amersham Biosciences, Amersham, UK) at 10 mA per
260 gel for 1 h and then 30 mA per gel until complete migration. Gels were subsequently stained with
261 Coomassie Blue (PhastGel R350, GE Healthcare) and unspecific coloration was destained with an
262 aqueous solution containing 30% methanol and 7% acetic acid. The resulting gels were scanned with
263 a transparency scanner (G:BoxChemi XL 1.4; SynGene) in gray scale with 16-bit depth and a
264 resolution of 100 dpi.

265

266 *2.10. Gel image and statistical analyzes for protein abundance*

267 Images were aligned and spots were detected and quantified using the Progenesis SameSpots software
268 (version 3.3, Nonlinear Dynamics) with manual alignment completed by automated algorithm. All
269 detected spots were manually checked and artifact spots were removed. Data were exported as raw
270 values and statistical analyzes were conducted in R (R Core Team, 2020) using the prot2D (Artigaud
271 et al., 2013) and limma packages (Ritchie et al., 2015) from the Bioconductor suite (Gentleman et al.,
272 2004). Data were normalized (quantile normalization) and the samples were paired compared be-
273 tween exposed and non-exposed to *A. minutum* conditions using moderated t-test with 7 replicates
274 per condition. For comparisons, we used a moderated t-test, a modified t-test for which the standard
275 errors have been moderated across spots, increasing the reliability of the test (Artigaud et al., 2013).
276 Once the values of moderated t-test were calculated, a global correction by false discovery rate (fdr)
277 was applied, in order to take into account multiple comparisons issues and paired-comparison correc-
278 tion. Spots with a fdr threshold lower than 0.1 and an absolute fold change higher than 1.5 were
279 considered as differentially expressed.

280

281 *2.11. Mass spectrometry*

282 Proteins that changed significantly in abundance in response to *A. minutum* exposure were excised
283 from gels and prepared for analyzes by mass spectrometry (MS) as described in Artigaud et al.,
284 (2015). Gel pieces were washed with 50 mM ammonium bicarbonate (BICAM), dehydrated in 100%
285 acetonitrile (ACN) and vacuum dried. Gel pieces were rehydrated with BICAM containing 0.5 µg of
286 porcine recombinant trypsin (sequencing grade; Promega, Madison, Wisconsin, USA) and incubated
287 overnight at 37°C. Peptides were extracted from the gels by alternative washing with 50 mM BICAM
288 and ACN, and with 5% formic acid and ACN. Between each step, the supernatants were pooled, and
289 finally concentrated by evaporation using a centrifugal evaporator (Concentrator 5301; Eppendorf,
290 Hamburg, Germany).

291 MS experiments were carried out on an AB Sciex 5800 proteomics analyzer equipped with TOF-TOF
292 ion optics and OptiBeam™ on-axis laser irradiation with 1000 Hz repetition rate. The system was
293 calibrated before analysis with a mixture of des-Arg-bradykinin, angiotensin I, Glu1-fibrinopeptide
294 B, ACTH (18–39) and ACTH (7–38), and mass precision was better than 50 ppm in reflectron mode.
295 A laser intensity of 3400 was typically employed for ionizing. MS spectra were acquired in the
296 positive reflector mode by summarizing 1000 single spectra (5×200) in the 700–4000 Da mass range.
297 MS/MS spectra from the twenty most intense ions were acquired in the positive MS/MS reflector
298 mode by summarizing a maximum of 2500 single spectra (10×250) with a laser intensity of 4300.
299 For tandem MS experiments, the acceleration voltage was 1 kV, and air was used as the collision gas.
300 Gas pressure medium was selected as settings.

301 The fragmentation pattern based on the occurrence of y, b and a ions was used to determine peptide
302 sequences. Database searching was performed using the Mascot 2.5.1 program (Matrix Science). A
303 custom EST database was used by combining *P. maximus* sequences from Illumina RNAseq
304 sequenced from mantle tissues (Artigaud et al., 2014) and from hemocyte cells (Pauletto et al., 2014).
305 The variable modifications allowed were as follows: methionine oxidation and dioxidation, acetyl
306 (K) and carbamidomethyl (C). Trypsin was selected as enzyme and 3 missed cleavages were allowed.
307 Mass accuracy was set to 300 ppm and 0.6 Da for the MS and MS/MS modes, respectively.

308

309 *2.12. Statistical analyzes for toxin accumulation clusters*

310 According to their accumulation potential, individuals were segregated into groups by applying an
311 hierarchical clustering function with the Ward's method (Pousse et al., 2018). The function 'hclust'
312 available in the R package 'stats' (R Core team, 2020) was applied on the accumulation potential
313 calculated for each individual. Three clusters were defined from the clustering function accordingly
314 to Pousse et al. (2018), and Mat et al. (2018) who described phenotypic and genotypic differences in
315 oysters divided into 3 accumulation clusters. This method defined 3 clusters of king scallops

316 differentially accumulating toxins and named high, medium and low according to their high,
317 intermediate and low accumulation potential, respectively (Fig. 2b).

318

319 **3. Results**

320 *3.1. Inter-individual variability in toxin accumulated and toxin accumulation potential in king*
321 *scallops exposed to *A. minutum*.*

322 All king scallops exposed to *A. minutum* for 2 days accumulated toxins and no mortality was
323 observed. For the 3 assays TC-A1, TC-A2 & TC-A3 juvenile king scallops were exposed to different
324 concentrations of *A. minutum* (Fig. 1) allowing us to observe a variation in the toxin loads
325 accumulated ranging from 7 to 220 µg STX per 100 g of DG.

326 After a 2-day exposure to *A. minutum* toxic algae, our data showed a high variability in toxin
327 accumulation potential between individuals.

328 Furthermore, according to their accumulation potential, and after applying a hierarchical clustering
329 function with the Ward's method, individuals were segregated into 3 clusters of king scallops
330 differentially accumulating toxins: high (n=5: TC-A1=1, TC-A2=4), intermediate (n=8: TC-A1=3,
331 TC-A2=1, TC-A3=4) and low (n=5: TC-A1=2, TC-A2=2, TC-A3=1) accumulation potential groups
332 (Fig. 2). It is noteworthy that the 3 clusters are irrespective of the 3 replicates.

333 Within only a 2-day exposure to *A. minutum*, the inter-individual variability in toxin accumulation
334 was high, with an average twice higher in the high accumulation cluster than in the low one.

335

336 *3.2. Links between feeding behavior and toxin accumulation*

337 Our results demonstrate a significant linear correlation ($R^2=0.65$) between toxin accumulation in DG
338 and the total numbers of *A. minutum* cells ingested by each king scallop for the 2 days of toxic algae
339 exposure (Fig. 3a).

340 In all 3 accumulation potential clusters, clearance activity rapidly dropped to almost stop at day 3,
341 when king scallops were exposed to *A. minutum*, and this low clearance rate persisted at day 4 for the

342 3 clusters (Fig. 3b). Therefore, for all 3 clusters the clearance rates observed during days 3&4 in the
343 presence of *A. minutum* were significantly lower compared to the ones in the presence of non-toxic
344 algae on days 1&2 (p-value<0.005). However, no significant inter-individual difference in clearance
345 rate was observed in the 3 accumulation potential clusters when exposed to toxic algae.

346 A significant inverse relationship between CRII and the concentration of toxins in king scallop DG
347 (p-value=0.014, Fig. 4a) could be observed. Similar observation was made between CRII and toxin
348 accumulation potential (p-value=0.038, Fig. 4c). No significant correlation could be observed be-
349 tween the CRII and the three different clusters, although a positive trend was visible and should be
350 further analyzed using a higher number of individuals.

351 Data showed that the time for filtration recovery was positively correlated with the toxin concentra-
352 tions (p-value=0.014, Fig. 4b), but showed significant inverse relationship with the potential of king
353 scallop to accumulate toxins (p-value=0.002, Fig. 4d). However, King scallop toxin accumulation
354 efficiency (TAE) did not correlate with accumulation potential (Fig. 5).

355

356 3.4. Transient inhibition in king scallop respiration rates when exposed to toxic algae *A. minutum*.

357 A significantly lower respiration rate was observed on day 3 at the beginning of *A. minutum* exposure
358 compared to *T-iso/C.muelleri* feeding days (days1&2), in correlation to clearance rate results.
359 However, on day 4, in presence of *A. minutum*, the respiration rate came back to its initial level
360 (observed in presence of non-toxic algae during the first 2 days of experiment) (p-value<0.04 ; figure
361 6a&b). On days 1&2, in presence of non-toxic algae, the respiration rate was not significantly
362 different for the 3 toxin accumulation potential clusters. Similarly, all 3 clusters of king scallops
363 displayed decreased respiration activity on day 3 right after contact with *A. minutum* (less than 4h),
364 and recovered on day 4 (Fig. 6b).

365

366 3.5. Proteomic analyzes: differentially accumulated protein spots between king scallops exposed 367 and non-exposed to *A. minutum*.

368 In order to complete the above ecophysiological data, the proteomic response was studied by
369 comparing TC-NA (n=7) and TC-A groups (n=7). In total, 13 protein spots were found to be
370 differentially accumulated in the 2-DE gels between *A. minutum* exposed and non-exposed king
371 scallop mantle tissues (Fig. 7). Twelve of the 13 differentially regulated protein spots were
372 successfully identified. They corresponded to 6 different proteins (Table 1). Over the 6 identified
373 proteins, 2 of them displayed higher accumulation levels and 4 were less accumulated in exposed
374 samples, as compared to non-exposed ones. The differentially expressed proteins are involved in cell
375 or tissue structure (beta-actin, filamin A & myosin), in immune response (killer cell lectin-like
376 receptor), energetic metabolism (fructose-bisphosphate aldolase) and detoxification (major vault
377 protein). Several of the identified protein spots appeared as a characteristic horizontal line of spots
378 on the 2-DE electrophoregrams (corresponding to a change in iso-electric point of proteins) as
379 observed for β -actin (spots 8, 9, 10 & 12), paramyosin (spots 2 & 11) and killer cell lectin-like
380 receptor (spots 5 & 6). This may correspond to different phosphorylation states of the proteins (Fig.
381 7).

382

383 **4. Discussion**

384 In the present study, both ecophysiological and proteomic approaches were used to study the
385 ecotoxicological response of juvenile king scallops, *P. maximus*, to the toxic microalgae *A. minutum*.
386 High inter-individual variability in toxin accumulation due to feeding behavior has previously been
387 suggested in oysters (Bougrier et al., 2003; Haberkorn et al., 2011; Mat et al., 2013; Pousse et al.,
388 2018). The first aim of this study was to test whether this hypothesis could be expanded to other
389 bivalve species such as *P. maximus*. Here we show that a 2-day exposure to *A. minutum* affects the
390 physiology (behavior and biochemistry) of king scallops. Ecophysiological data highlight 1/ the inter-
391 individual variability in PST accumulation between individual king scallops, linked to feeding
392 behavior; 2/ the *A. minutum* induced inhibition of clearance and of respiration rates, followed by a

393 recovery; 3/ the influence of level and time for filtration recovery on toxin accumulation. Finally,
394 proteomic data revealed that *A. minutum* exposure caused a differential accumulation in proteins
395 involved in cell/tissue structure, metabolism, detoxification and immune response.

396

397 *4.1. Physiological effects*

398 *4.1.1. Inter-individual variability in king scallops toxin accumulation depends on filter-feeding*
399 *behavior when exposed to A. minutum.*

400 First of all, we found a significant correlation between toxin accumulation in DG and the total number
401 of *A. minutum* cells ingested by each king scallop. This result suggests that the cells consumed by
402 king scallops during the 2 days of *A. minutum* exposure contributed to the majority of toxin accumu-
403 lation measured in king scallop DG. Therefore we are confident that toxin accumulation in king scal-
404 lops is not due to contaminations prior to our experiment. Furthermore, despite their common cohort
405 origin and their identical rearing conditions, a high inter-individual variability in toxin accumulation
406 was found in king scallop juveniles. We cannot exclude that such feature could be due to low numbers
407 of king scallop individuals. However, a high inter-individual variability in toxin accumulation has
408 also been observed in *C. gigas* under controlled laboratory conditions (Mat et al., 2013; Pousse et al.,
409 2018). As for oysters (Boullot et al., 2017; Mat et al., 2018; Pousse et al., 2018), the 3 different
410 profiles in toxin accumulation potential obtained from the Ward's method showed different ecophys-
411 iological responses within tested king scallop juveniles. Furthermore, as for oysters and some other
412 tested bivalves (Bougrier et al., 2003; Contreras et al., 2012b; Pousse et al., 2018), we found a corre-
413 lation between the numbers of toxic algal cells consumed by king scallops and the final toxin con-
414 centrations in DG. Our findings also demonstrate that in king scallops, inter-individual differences in
415 toxin accumulation are mainly due to filter-feeding behavior, as observed in several studies on oysters
416 *C. gigas* (Bougrier et al., 2003; Haberkorn et al., 2011; Mat et al., 2018; Pousse et al., 2018). Indeed,
417 when exposed to *A. minutum*, king scallop exhibited inter-individual variability in the time for filtra-
418 tion recovery and the inhibition of clearance rate (CRII) affecting PST accumulation. Such filter-

419 feeding behavior variability among king scallop individuals toward toxic algae has been observed in
420 several bivalve species (Hégaret et al., 2009; Leverone et al., 2007). As for oysters, we could hypoth-
421 esize that the high inter-individual variability found among king scallop individuals might be linked
422 to differences in toxin sensitivity (Pousse et al., 2018). More precisely, king scallop toxin accumula-
423 tion efficiency (TAE), which is another explanatory parameter allowing to understand the inter-indi-
424 vidual variability in toxin accumulation was analyzed. Therefore, TAE represents the balance between
425 toxin uptake (i.e. the amount of toxins incorporated) and elimination processes and can depend on
426 pre-ingestion selection, toxin depuration or assimilation (Bougrier et al., 2003; Mafra et al., 2010;
427 Pousse et al., 2018). The TAE values we found for *A. minutum* exposed king scallops (16-59%, av-
428 erage: 32%) are comparable to those calculated for mussels (30-60%, Mafra et al., 2010) and oysters
429 (10-40%, Mafra et al., 2010 and 35%, Pousse et al., 2018) when exposed to *Pseudo-nitzschia* or *A.*
430 *minutum* for at least 2 days. Species exhibiting high TAE have been suggested to be less sensitive to
431 STX and tend to accumulate it more (For review see Bricelj and Shumway, 1998).

432 In contrast, this study demonstrated opposite results for *P. maximus* compare to *C. gigas* (Pousse et
433 al., 2018). Indeed, toxin accumulation potential was not influenced by king scallop size/weight or by
434 TAE, which suggests differences in some of the factors influencing toxin accumulation potential
435 among bivalve species. In king scallop, the only factor found to drive toxin accumulation is feeding
436 behavior. Also, in our study, the animal size does not seem to be a factor influencing toxin accumu-
437 lation. Whereas inter-individual differences in toxin accumulation related to body size have been ob-
438 served among bivalve species, results similar to our findings have been reported for domoic acid in
439 king scallops (Mafra et al., 2010; Morono et al., 2001).

440

441 4.1.2. Low clearance and respiration activities of king scallops when exposed to toxic *A. minutum*

442 As soon as they were exposed to *A. minutum*, all tested king scallops nearly stopped their feeding
443 activity. This reaction toward toxin producing algae has already been highlighted in some, but not all,
444 shellfish species (Contreras et al., 2012b; Hégaret et al., 2009b; Leverone et al., 2007; Pousse et al.,

445 2018; Seger et al., 2020). Although most of the king scallops re-started filtration again within the 5h
446 following the beginning of *A. minutum* exposure, filtration recovery remained very low in comparison
447 to oysters subjected to similar conditions (Pousse et al., 2018). This result suggests a higher sensitivity
448 of king scallops toward this toxic algae. Coquereau et al (2016) demonstrated indeed that a 2-hour
449 exposure of king scallop to *A. minutum* caused an increase in valve movements, especially closure
450 and expulsion. Similarly, Borcier et al (2017) recorded less filtration and shell growth after immediate
451 exposure to *A. minutum*. Furthermore, Leverone et al. (2007) have shown that among all tested
452 bivalves including eastern oyster (*C. virginica*), northern quahog (*M. mercenaria*) and green mussel
453 (*P. viridis*), the bay scallop *Argopecten irradians*, which is the closest species to king scallop *P.*
454 *maximus* from this study, was the most sensitive species toward the toxic algae *Karenia brevis*
455 (Leverone et al., 2007). Modification of king scallop feeding behavior was immediate when exposed
456 to toxic algae and this reaction was also observed in oyster (Pousse et al., 2018; Tran et al., 2010).
457 Therefore, rather than a toxin induced response, the avoidance of toxic particles is the most plausible
458 explanation, as suggested in previous studies (Coquereau et al., 2016; Lassus et al., 2004, 1999;
459 Pousse et al., 2018; Wildish et al., 1998).

460

461 4.1.3. Toxin accumulation dependence on filtration recovery duration and level

462 It is now well established that bivalve species, such as oysters, mussels or king scallops have different
463 feeding patterns. Indeed, our findings showing that toxin accumulation efficiency does not influence
464 toxin accumulation in king scallop differs from what has been previously shown in oysters (Pousse
465 et al., 2018). This suggests that there is no clear effects from pre-ingestion mechanisms or toxin ab-
466 sorption/depuration on the inter-individual variability in toxin accumulation profile in king scallop.
467 Our results also highlight that king scallops accumulating more toxins and displaying higher toxin
468 accumulation potential are more likely to start filtration earlier and to exhibit a lower CR_{II} (Fig. 5).
469 This suggests that the high inter-individual variability observed in toxin accumulation due to feeding
470 behavior was driven by two factors: i) the time taken by king scallops to resume filtration after its

471 inhibition and ii) the level of filtration recovery. Such results have recently been observed in *C. gigas*
472 (Pousse et al., 2018). Therefore, the variability in toxin accumulation due to differential behavior in
473 reducing filtration activity could be a general mechanism in bivalves when facing toxic microalgae,
474 although extra analyzes on other species should be performed to confirm this purpose. In addition,
475 long-term exposure experiments would be necessary to test whether feeding behaviors are still dis-
476 criminant in toxin accumulation variability. Furthermore, because environmental conditions were the
477 same for all tested individuals, we can reject the assumption of an environmental cause. Therefore,
478 further studies analyzing the mechanisms involved in such inter-individual variabilities, for instance
479 genomic and/or transcriptomic analyzes associated with ecophysiological data, could highlight po-
480 tential genetic profiles among bivalve species and could link genes to this specific behavior toward
481 toxic algae. In addition, epigenetic variations could also be investigated to determine the factors and
482 mechanisms influencing such differential behavior.

483 Our data show that respiration rates follow the same evolution as filtration rates until day 3, with a
484 rapid decrease in the presence of toxic algae. However, whereas the filtration profile remains low, the
485 overall respiration activity increases at day 4. Generally, respiration rates increase exponentially with
486 increasing rates of assimilation (Bayne et al., 1989). Comparing the present respiration rates with the
487 feeding rates over the 4-day experiment shows that a decoupling appears to occur at day 4. Respiration
488 rates rises have already been observed in bivalves exposed to environmental stress such as ocean
489 acidification (Pousse et al., 2020), low/high salinity (Peteiro et al., 2018) or toxic algae (Li et al.,
490 2002). In the present context, one hypothesis for this increase in respiration would be that the inges-
491 tion of *A. minutum* triggers a metabolic response corresponding to an immune reaction toward the
492 toxic algae and its toxins increasing king scallops respiration rate. It is for instance well known that
493 the consumption of toxic algae by bivalves induces an inflammatory response implying hemocytes
494 degranulation and diapedesis into the digestive tract to encapsulate toxic algae (Galimany et al., 2008;
495 Hégaret et al., 2009a).

496

497 **4.2. The effects of *A. minutum* on king scallops at the proteomic level**

498 **4.2.1. Cytoskeletal structure**

499 Over the 6 successfully identified proteins, 3 of them are cell structure proteins involved in
500 cytoskeleton composition. It is frequent to find cytoskeletal proteins differentially regulated in
501 proteomic studies because they are highly abundant or conserved proteins among species (Monsinjon
502 and Knigge, 2007). In our study, Filamin A and paramyosin were down regulated, whereas β -actin
503 was upregulated in toxin-exposed king scallops. These 3 proteins are main components of the
504 cytoskeleton orchestrating cell shape, adhesion and motility. More specifically, filamin A cross-links
505 to F-actin proteins, including β -actin, giving to the cells a dynamic three-dimensional structure
506 (Nakamura et al., 2011; Popowicz et al., 2006; Stossel et al., 2001). Therefore, modifications of
507 cytoskeleton composition in toxin-exposed mantle cells may depict a cytoskeleton disintegration or
508 restructuration. It is now well established that several phycotoxins display toxicity toward
509 cytoskeleton, especially actin. Indeed, the main molecular target of several microalgal toxins is
510 cytoskeleton. *In vitro* studies have demonstrated that pectenotoxin-2 was disrupting actin
511 organization in several cell types (Spector et al., 1999). Furthermore, DSP toxins such as okadaic acid
512 have also been reported to disturb cytoskeleton dynamic and integrity in several organisms including
513 bivalves (Huang et al., 2015; Vilariño et al., 2008; Yoon et al., 2008). Furthermore, Hégaret et al.
514 (2007) observed adductor-muscle paralysis in some oysters *Crassostrea virginica* exposed to another
515 PST producer, *Alexandrium fundyense*, which could be related to cytoskeleton mis-functioning.
516 Muscular contraction is an important pathway related to digestion that appeared affected by toxins.
517 Indeed, Mat et al. (2018) also observed over-expression of calmodulin, which regulates binding
518 between myosin and actin in smooth muscles, whereas the muscarinic acetylcholine receptor (M3R),
519 important in the contractile response in smooth muscle, particularly in gastrointestinal smooth
520 muscles, was down-regulated in *C. gigas* with high PST loads (Mat et al., 2018).

521

522 4.2.2. Detoxification

523 Even though no correspondence could be clearly established between toxin accumulation profiles and
524 proteomic analyzes, mass spectrometry results brought insights on the functioning of ecotoxicological
525 response. Firstly, not surprising in toxin-challenged experiments, a protein involved in detoxification
526 was identified. The Major Vault Protein (MVP) is the main component of ribonucleoprotein particles
527 called vault (Tanaka et al., 2009). Vaults were suggested to be involved in signaling, innate immunity
528 and detoxification (Berger et al., 2009). In particular, MVP is considered as a major multidrug re-
529 sistance protein since several studies have found high expression of MVP correlated with chemother-
530 apy resistance (Kickhoefer et al., 1998; Scheper et al., 1993). Additional studies have demonstrated
531 that MVP could have a role in drug molecule export from nucleus to cytoplasmic vesicles for seques-
532 tration (Herlevsen et al., 2007; Suprenant et al., 2007). Furthermore, other proteomic studies have
533 shown an increase of MVP in mussel, *Mytilus galloprovincialis*, when exposed to aquatic pollutants
534 such as Ag nanoparticles or a mixture of Cu and benzo(a)pyrene (Gomes et al., 2013; Maria et al.,
535 2013). Therefore, it appears that MVP would be involved in xenobiotic detoxification. Surprisingly
536 in our case, MVP is decreased when king scallops are exposed to PST producing *A. minutum*, which
537 suggests that there might be a mechanism blocking the MVP-dependent detoxification pathway. Pos-
538 sibly, *A. minutum* could be responsible for this blockage by secretion of molecules inhibiting detoxi-
539 fication pathways. Similar to our results, Tomanek and Zuzow have found a lower abundance of two
540 isoforms of VMP in *Mytilus galloprovincialis* subjected to temperature stress and have suggested that
541 it could be part of an antiapoptotic response (Tomanek and Zuzow, 2010), therefore corresponding
542 to a more general stress response rather than a specific detoxification process.

543

544 4.2.3. Glycolysis balance

545 The fructose-bisphosphate aldolase is one of the major proteins involved in glycolysis and
546 gluconeogenesis pathways. In our study we found a lower concentration of fructose-bisphosphate
547 aldolase in king scallops exposed to *A. minutum* than in the control group. This result suggests a

548 subsequent decrease of glycolysis in mantle tissue of PST challenged king scallops due to the
549 accumulation of toxins in this tissue. Lower levels of fructose-bisphosphate aldolase have been found
550 in the kuruma prawn, *Marsupenaeus japonicus*, under stressful, hypoxic conditions (Abe et al., 2007).
551 In the same way, it is possible that glycolysis in king scallop is down-regulated when exposed to
552 cytotoxic *A. minutum*. In another hand, transcriptomic studies have shown higher amounts of
553 fructose-bisphosphate aldolase transcripts in oyster exhibiting higher toxin accumulation (Mat et al.,
554 2018). Here again, there might be differences in metabolic responses against toxic algae exposure
555 within bivalves species. This opposite result could also come from differences in the experimental
556 design (as described above) suggesting that the age of animals or the BEC could have an impact on
557 the metabolic response.

558

559 4.2.4. Immune system regulation

560 Finally, we show that the killer cell lectin-like receptor subfamily B member 1B (KLRB1B also called
561 NKR-P1B) is more abundantly accumulated in *A. minutum* exposed king scallops. The NKR-P1B is
562 part of the receptor family regulating the cytotoxic activity of Natural Killer cells (NK). NK cells are
563 sentinels focused on the early detection of pathogens and their inhibitory receptor NKR-P1B plays a
564 key role in protecting healthy tissues from NK cell-mediated lysis (Balaji et al., 2018). NKR-P1B is
565 specifically expressed on the surface of NK cells in animals possessing adaptive immunity. Whereas
566 no adaptive immunity has been described in bivalves, several studies have demonstrated that blue
567 mussel previously exposed to toxic microalgae accumulated less PST than the ones exposed for the
568 first time (Shumway and Cucci, 1987). In the same way, recent studies have suggested the existence
569 of an immune memory in oysters (Lafont et al., 2019, 2017). Furthermore, Araya and collaborators
570 have shown the presence of NKR in hemocytes of soft-shell clams, *Mya arenaria*, suggesting a po-
571 tential cytotoxic activity from hemocytes (Araya et al., 2010). Other studies have also observed cy-
572 totoxic activity in blue mussel, *Mytilus edulis*, suggesting that hemocytes may act as NK cells (Han-
573 nam et al., 2009). Our findings that NKR-P1B is more abundant in toxic algae exposed king scallops

574 suggest a down-regulation of the hemocyte cytotoxic activity through specific receptors. Very little is
575 known about receptor mediated immune response and their corresponding intracellular signaling
576 pathways in bivalves. Further research at the molecular level would allow to better characterize re-
577 ceptors, signaling molecules and pathways orchestrating the immune response in order to better un-
578 derstand the mechanisms regulating the cytotoxic activity found in bivalves.

579

580 **5. Conclusion**

581 Our data show for the first time that juvenile king scallops *P. maximus* have an important inter-indi-
582 vidual variability in toxin accumulation driven by its feeding behavior. Our study further highlights
583 the effects of delay and level for filtration recovery on toxin accumulation in king scallops. Further-
584 more, proteomic analyzes suggest an effect of toxic algae *A. minutum* on immune response, cytoskel-
585 eton remodeling, detoxication and metabolism of king scallops.

586

587 *Conflict of Interest Disclosures*

588 The authors declare no competing financial interests.

589

590 *Acknowledgements*

591 This project was supported by the National Research Agency ANR CESA (ACCUTOX project ANR-
592 13-CESA-0019). Coraline Chapperon was supported by a postdoctoral fellowship from the Conseil
593 Départemental du Finistère. The authors gratefully acknowledge Isabelle Quéau and Adeline Bidault
594 who provided technical help for ecophysiological and proteomic experiments.

595

596 *Author contributions*

597 YE, EP, SA & CC: Collection and/or assembly of data, data analyzes and interpretation, manuscript
598 writing. HH: Conception and design, financial support, manuscript writing. VP: financial support,
599 manuscript writing. JFSM: Conception and design. FJ: financial support.

600

601 **References**

- 602 Abe, H., Hirai, S., Okada, S., 2007. Metabolic responses and arginine kinase expression under hy-
 603 poxic stress of the kuruma prawn *Marsupenaeus japonicus*. *Comp. Biochem. Physiol. A.*
 604 *Mol. Integr. Physiol.* 146, 40–46. <https://doi.org/10.1016/j.cbpa.2006.08.027>
- 605 Anderson, D.M., Alpermann, T.J., Cembella, A.D., Collos, Y., Masseret, E., Montresor, M., 2012.
 606 The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and
 607 impacts on human health. *Harmful Algae, Harmful Algae--The requirement for species-spe-*
 608 *cific information* 14, 10–35. <https://doi.org/10.1016/j.hal.2011.10.012>
- 609 Araya, M.T., Markham, F., Mateo, D.R., McKenna, P., Johnson, G.R., Berthe, F.C.J., Siah, A.,
 610 2010. Identification and expression of immune-related genes in hemocytes of soft-shell
 611 clams, *Mya arenaria*, challenged with *Vibrio splendidus*. *Fish Shellfish Immunol.* 29, 557–
 612 564. <https://doi.org/10.1016/j.fsi.2010.05.017>
- 613 Artigaud, S., Gauthier, O., Pichereau, V., 2013. Identifying differentially expressed proteins in two-
 614 dimensional electrophoresis experiments: inputs from transcriptomics statistical tools. *Bio-*
 615 *informatics* 29, 2729–2734. <https://doi.org/10.1093/bioinformatics/btt464>
- 616 Artigaud, S., Lacroix, C., Richard, J., Flye-Sainte-Marie, J., Bargelloni, L., Pichereau, V., 2015.
 617 Proteomic responses to hypoxia at different temperatures in the great scallop (*Pecten maxi-*
 618 *mus*). *PeerJ* 3, e871. <https://doi.org/10.7717/peerj.871>
- 619 Artigaud, S., Thorne, M.A.S., Richard, J., Lavaud, R., Jean, F., Flye-Sainte-Marie, J., Peck, L.S.,
 620 Pichereau, V., Clark, M.S., 2014. Deep sequencing of the mantle transcriptome of the great
 621 scallop *Pecten maximus*. *Mar. Genomics* 15, 3–4.
 622 <https://doi.org/10.1016/j.margen.2014.03.006>
- 623 Baird, R.H., 1958. Measurement of Condition in Mussels and Oysters. *ICES J. Mar. Sci.* 23, 249–
 624 257. <https://doi.org/10.1093/icesjms/23.2.249>
- 625 Balaji, G.R., Aguilar, O.A., Tanaka, M., Shingu-Vazquez, M.A., Fu, Z., Gully, B.S., Lanier, L.L.,
 626 Carlyle, J.R., Rossjohn, J., Berry, R., 2018. Recognition of host Clr-b by the inhibitory
 627 NKR-P1B receptor provides a basis for missing-self recognition. *Nat. Commun.* 9, 4623.
 628 <https://doi.org/10.1038/s41467-018-06989-2>
- 629 Bayne, B.L., Hawkins, A.J.S., Navarro, E., 1987. Feeding and digestion by the mussel *Mytilus edu-*
 630 *lis* L. (*Bivalvia*: *Mollusca*) in mixtures of silt and algal cells at low concentrations. *J. Exp.*
 631 *Mar. Biol. Ecol.* 111, 1–22. [https://doi.org/10.1016/0022-0981\(87\)90017-7](https://doi.org/10.1016/0022-0981(87)90017-7)
- 632 Bayne, B.L., Hawkins, A.J.S., Navarro, E., Iglesias, I.P., 1989. Effects of seston concentration on
 633 feeding, digestion and growth in the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* 55, 47–54.
- 634 Berger, W., Steiner, E., Grusch, M., Elbling, L., Micksche, M., 2009. Vaults and the major vault
 635 protein: Novel roles in signal pathway regulation and immunity. *Cell. Mol. Life Sci.* 66, 43–
 636 61. <https://doi.org/10.1007/s00018-008-8364-z>
- 637 Borcier, E., Morvezen, R., Boudry, P., Miner, P., Charrier, G., Laroche, J., Hegaret, H., 2017. Ef-
 638 fects of bioactive extracellular compounds and paralytic shellfish toxins produced by *Alex-*
 639 *andrium minutum* on growth and behaviour of juvenile great scallops *Pecten maximus*.
 640 *Aquat. Toxicol.* 184, 142–154. <https://doi.org/10.1016/j.aquatox.2017.01.009>
- 641 Bougrier, S., Lassus, P., Bardouil, M., Masselin, P., Truquet, P., 2003. Paralytic shellfish poison ac-
 642 cumulation yields and feeding time activity in the Pacific oyster (*Crassostrea gigas*) and
 643 king scallop (*Pecten maximus*). *Aquat. Living Resour.* 16, 347–352.
 644 [https://doi.org/10.1016/S0990-7440\(03\)00080-9](https://doi.org/10.1016/S0990-7440(03)00080-9)
- 645 Boullot, F., Castrec, J., Bidault, A., Dantas, N., Payton, L., Perrigault, M., Tran, D., Amzil, Z., Bou-
 646 dry, P., Soudant, P., Hégaret, H., Fabioux, C., 2017. Molecular Characterization of Voltage-

- 647 Gated Sodium Channels and Their Relations with Paralytic Shellfish Toxin Bioaccumula-
648 tion in the Pacific Oyster *Crassostrea gigas*. *Mar. Drugs* 15, 21.
649 <https://doi.org/10.3390/md15010021>
- 650 Bricelj, V.M., Shumway, S.E., 1998. Paralytic Shellfish Toxins in Bivalve Molluscs: Occurrence,
651 Transfer Kinetics, and Biotransformation. *Rev. Fish. Sci.* 6, 315–383.
652 <https://doi.org/10.1080/10641269891314294>
- 653 Castrec, J., Hégaret, H., Alunno-Bruscia, M., Picard, M., Soudant, P., Petton, B., Boulais, M., Su-
654 quet, M., Quéau, I., Ratiskol, D., Foulon, V., Le Goïc, N., Fabioux, C., 2019. The dinoflag-
655 ellate *Alexandrium minutum* affects development of the oyster *Crassostrea gigas*, through
656 parental or direct exposure. *Environ. Pollut.* 246, 827–836.
657 <https://doi.org/10.1016/j.envpol.2018.11.084>
- 658 Castrec, J., Soudant, P., Payton, L., Tran, D., Miner, P., Lambert, C., Le Goïc, N., Huvet, A., Quil-
659 lien, V., Bouillot, F., Amzil, Z., Hégaret, H., Fabioux, C., 2018. Bioactive extracellular com-
660 pounds produced by the dinoflagellate *Alexandrium minutum* are highly detrimental for
661 oysters. *Aquat. Toxicol.* 199, 188–198. <https://doi.org/10.1016/j.aquatox.2018.03.034>
- 662 Chauvaud, L., Donval, A., Thouzeau, G., Paulet, Y.-M., Nézan, E., 2001. Variations in food intake
663 of *Pecten maximus* (L.) from the Bay of Brest (France): Influence of environmental factors
664 and phytoplankton species composition. *Comptes Rendus Académie Sci. - Ser. III - Sci. Vie*
665 324, 743–755. [https://doi.org/10.1016/S0764-4469\(01\)01349-X](https://doi.org/10.1016/S0764-4469(01)01349-X)
- 666 Chauvaud, L., Thouzeau, G., Paulet, Y.-M., 1998. Effects of environmental factors on the daily
667 growth rate of *Pecten maximus* juveniles in the Bay of Brest (France). *J. Exp. Mar. Biol.*
668 *Ecol.* 227, 83–111. [https://doi.org/10.1016/S0022-0981\(97\)00263-3](https://doi.org/10.1016/S0022-0981(97)00263-3)
- 669 Contreras, A.M., Marsden, I.D., Munro, M.H.G., 2012a. Physiological Effects and Biotransfor-
670 mation of PSP Toxins in the New Zealand Scallop, *Pecten novaezelandiae*. *J. Shellfish Res.*
671 31, 1151–1159. <https://doi.org/10.2983/035.031.0426>
- 672 Contreras, A.M., Marsden, I.D., Munro, M.H.G., 2012b. Effects of short-term exposure to paralytic
673 shellfish toxins on clearance rates and toxin uptake in five species of New Zealand bivalve.
674 *Mar. Freshw. Res.* 63, 166–174. <https://doi.org/10.1071/MF11173>
- 675 Coquereau, L., Jolivet, A., Hégaret, H., Chauvaud, L., 2016. Short-Term Behavioural Responses of
676 the Great Scallop *Pecten maximus* Exposed to the Toxic Alga *Alexandrium minutum* Meas-
677 ured by Accelerometry and Passive Acoustics. *PLOS ONE* 11, e0160935.
678 <https://doi.org/10.1371/journal.pone.0160935>
- 679 Fabioux, C., Sulistiyani, Y., Haberkorn, H., Hégaret, H., Amzil, Z., Soudant, P., 2015. Exposure to
680 toxic *Alexandrium minutum* activates the detoxifying and antioxidant systems in gills of the
681 oyster *Crassostrea gigas*. *Harmful Algae* 48, 55–62.
682 <https://doi.org/10.1016/j.hal.2015.07.003>
- 683 FAO Fisheries and Aquaculture Department - Yearbook of Fishery and Aquaculture Statistics -
684 Capture production [WWW Document], n.d. URL [https://www.fao.org/fishery/static/Year-](https://www.fao.org/fishery/static/Yearbook/YB2019_USBcard/navigation/index_content_capture_e.htm)
685 [book/YB2019_USBcard/navigation/index_content_capture_e.htm](https://www.fao.org/fishery/static/Yearbook/YB2019_USBcard/navigation/index_content_capture_e.htm) (accessed 1.10.22).
- 686 Flye-Sainte-Marie, J., Pouvreau, S., Paillard, C., Jean, F., 2007. Impact of Brown Ring Disease on
687 the energy budget of the Manila clam *Ruditapes philippinarum*. *J. Exp. Mar. Biol. Ecol.* 349,
688 378–389. <https://doi.org/10.1016/j.jembe.2007.05.029>
- 689 Galimany, E., Sunila, I., Hégaret, H., Ramón, M., Wikfors, G.H., 2008. Experimental exposure of
690 the blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium fundyense*: His-
691 topathology, immune responses, and recovery. *Harmful Algae* 7, 702–711.
692 <https://doi.org/10.1016/j.hal.2008.02.006>
- 693 Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier,
694 L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F.,
695 Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang,
696 J.Y., Zhang, J., 2004. Bioconductor: open software development for computational biology
697 and bioinformatics. *Genome Biol.* 5, R80. <https://doi.org/10.1186/gb-2004-5-10-r80>

- 698 Geraci, J.R., Anderson, D.M., Timperi, R.J., St. Aubin, D.J., Early, G.A., Prescott, J.H., Mayo,
699 C.A., 1989. Humpback Whales (*Megaptera novaeangliae*) Fatally Poisoned by Dinoflagel-
700 late Toxin. *Can. J. Fish. Aquat. Sci.* 46, 1895–1898. <https://doi.org/10.1139/f89-238>
- 701 Gomes, T., Pereira, C.G., Cardoso, C., Bebianno, M.J., 2013. Differential protein expression in
702 mussels *Mytilus galloprovincialis* exposed to nano and ionic Ag. *Aquat. Toxicol.* 136–137,
703 79–90. <https://doi.org/10.1016/j.aquatox.2013.03.021>
- 704 Guillard, R.R.L., Hargraves, P.E., 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte.
705 *Phycologia* 32, 234–236. <https://doi.org/10.2216/i0031-8884-32-3-234.1>
- 706 Haberkorn, H., Lambert, C., Le Goïc, N., Guéguen, M., Moal, J., Palacios, E., Lassus, P., Soudant,
707 P., 2010a. Effects of *Alexandrium minutum* exposure upon physiological and hematological
708 variables of diploid and triploid oysters, *Crassostrea gigas*. *Aquat. Toxicol.* 97, 96–108.
709 <https://doi.org/10.1016/j.aquatox.2009.12.006>
- 710 Haberkorn, H., Lambert, C., Le Goïc, N., Moal, J., Suquet, M., Guéguen, M., Sunila, I., Soudant,
711 P., 2010b. Effects of *Alexandrium minutum* exposure on nutrition-related processes and re-
712 productive output in oysters *Crassostrea gigas*. *Harmful Algae* 9, 427–439.
713 <https://doi.org/10.1016/j.hal.2010.01.003>
- 714 Haberkorn, H., Tran, D., Massabuau, J.-C., Ciret, P., Savar, V., Soudant, P., 2011. Relationship be-
715 tween valve activity, microalgae concentration in the water and toxin accumulation in the
716 digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*.
717 *Mar. Pollut. Bull.* 62, 1191–1197. <https://doi.org/10.1016/j.marpolbul.2011.03.034>
- 718 Hannam, M.L., Bamber, S.D., Sundt, R.C., Galloway, T.S., 2009. Immune modulation in the blue
719 mussel *Mytilus edulis* exposed to North Sea produced water. *Environ. Pollut.* 157, 1939–
720 1944. <https://doi.org/10.1016/j.envpol.2008.12.031>
- 721 Hégaret, Hélène, da Silva, P.M., Sunila, I., Shumway, S.E., Dixon, M.S., Alix, J., Wikfors, G.H.,
722 Soudant, P., 2009a. Perkinsosis in the Manila clam *Ruditapes philippinarum* affects re-
723 sponses to the harmful-alga, *Prorocentrum minimum*. *J. Exp. Mar. Biol. Ecol.* 371, 112–120.
724 <https://doi.org/10.1016/j.jembe.2009.01.016>
- 725 Hégaret, Hélène, Wikfors, G., Shumway, S., 2009b. Diverse feeding responses of five species of
726 bivalve mollusc when exposed to three species of harmful algae. *J. Shellfish Res.* 26, 549–
727 559. [https://doi.org/10.2983/0730-8000\(2007\)26\[549:DFROFS\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2007)26[549:DFROFS]2.0.CO;2)
- 728 Hégaret, H., Wikfors, G.H., Shumway, S. E., 2009. 2 - Biotxin contamination and shellfish safety,
729 in: Shumway, Sandra E., Rodrick, G.E. (Eds.), *Shellfish Safety and Quality*, Woodhead
730 Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp.
731 43–80. <https://doi.org/10.1533/9781845695576.1.43>
- 732 Hégaret, H., Wikfors, G.H., Soudant, P., Lambert, C., Shumway, S.E., Bérard, J.B., Lassus, P.,
733 2007. Toxic dinoflagellates (*Alexandrium fundyense* and *A. catenella*) have minimal appar-
734 ent effects on oyster hemocytes. *Mar. Biol.* 152, 441–447. [https://doi.org/10.1007/s00227-](https://doi.org/10.1007/s00227-007-0703-3)
735 [007-0703-3](https://doi.org/10.1007/s00227-007-0703-3)
- 736 Herlevsen, M., Oxford, G., Owens, C.R., Conaway, M., Theodorescu, D., 2007. Depletion of major
737 vault protein increases doxorubicin sensitivity and nuclear accumulation and disrupts its se-
738 questration in lysosomes. *Mol. Cancer Ther.* 6, 1804–1813. [https://doi.org/10.1158/1535-](https://doi.org/10.1158/1535-7163.MCT-06-0372)
739 [7163.MCT-06-0372](https://doi.org/10.1158/1535-7163.MCT-06-0372)
- 740 Huang, L., Zou, Y., Weng, H., Li, H.-Y., Liu, J.-S., Yang, W.-D., 2015. Proteomic profile in *Perna*
741 *viridis* after exposed to *Prorocentrum lima*, a dinoflagellate producing DSP toxins. *Environ.*
742 *Pollut.* 196, 350–357. <https://doi.org/10.1016/j.envpol.2014.10.019>
- 743 Kickhoefer, V.A., Rajavel, K.S., Scheffer, G.L., Dalton, W.S., Scheper, R.J., Rome, L.H., 1998.
744 Vaults Are Up-regulated in Multidrug-resistant Cancer Cell Lines. *J. Biol. Chem.* 273,
745 8971–8974. <https://doi.org/10.1074/jbc.273.15.8971>
- 746 Lafont, M., Goncalves, P., Guo, X., Montagnani, C., Raftos, D., Green, T., 2019. Transgenerational
747 plasticity and antiviral immunity in the Pacific oyster (*Crassostrea gigas*) against Ostreid

- 748 herpesvirus 1 (OsHV-1). *Dev. Comp. Immunol.* 91, 17–25.
749 <https://doi.org/10.1016/j.dci.2018.09.022>
- 750 Lafont, M., Petton, B., Vergnes, A., Pauletto, M., Segarra, A., Gourbal, B., Montagnani, C., 2017.
751 Long-lasting antiviral innate immune priming in the Lophotrochozoan Pacific oyster,
752 *Crassostrea gigas*. *Sci. Rep.* 7, 13143. <https://doi.org/10.1038/s41598-017-13564-0>
- 753 Lassudrie, M., Soudant, P., Richard, G., Henry, N., Medhioub, W., da Silva, P.M., Donval, A., Bu-
754 nel, M., Le Goïc, N., Lambert, C., de Montaudouin, X., Fabioux, C., Hégaret, H., 2014.
755 Physiological responses of Manila clams *Venerupis* (=Ruditapes) philippinarum with vary-
756 ing parasite *Perkinsus olseni* burden to toxic algal *Alexandrium ostenfeldii* exposure. *Aquat.*
757 *Toxicol.* 154, 27–38. <https://doi.org/10.1016/j.aquatox.2014.05.002>
- 758 Lassus, P., Bardouil, M., Beliaeff, B., Masselin, P., Naviner, M., Truquet, P., 1999. Effect of a con-
759 tinuous supply of the toxic dinoflagellate *Alexandrium minutum* halim on the feeding be-
760 havior of the pacific oyster (*Crassostrea gigas* thunberg). *J. Shellfish Res.* 18, 211–216.
- 761 Lassus, P., Baron, R., Garen, P., Truquet, P., Masselin, P., Bardouil, M., Leguay, D., Amzil, Z.,
762 2004. Paralytic shellfish poison outbreaks in the Penzé estuary: Environmental factors af-
763 fecting toxin uptake in the oyster, *Crassostrea gigas*. *Aquat. Living Resour.* 17, 207–214.
764 <https://doi.org/10.1051/alr:2004012>
- 765 Legendre, P., 2014. *lmodel2: Model II Regression*. R Package Version 1.7-2 [WWW Document].
766 URL <http://CRAN.R-project.org/package=lmodel2> (accessed 11.15.19).
- 767 Leverone, J.R., Shumway, S.E., Blake, N.J., 2007. Comparative effects of the toxic dinoflagellate
768 *Karenia brevis* on clearance rates in juveniles of four bivalve molluscs from Florida, USA.
769 *Toxicon* 49, 634–645. <https://doi.org/10.1016/j.toxicon.2006.11.003>
- 770 Li, S.-C., Wang, W.-X., Hsieh, D.P.H., 2002. Effects of toxic dinoflagellate *Alexandrium tamarens*
771 on the energy budgets and growth of two marine bivalves. *Mar. Environ. Res.* 53, 145–160.
772 [https://doi.org/10.1016/S0141-1136\(01\)00117-9](https://doi.org/10.1016/S0141-1136(01)00117-9)
- 773 Long, M., Tallec, K., Soudant, P., Le Grand, F., Donval, A., Lambert, C., Sarthou, G., Jolley, D.F.,
774 Hégaret, H., 2018. Allelochemicals from *Alexandrium minutum* induce rapid inhibition of
775 metabolism and modify the membranes from *Chaetoceros muelleri*. *Algal Res.* 35, 508–518.
776 <https://doi.org/10.1016/j.algal.2018.09.023>
- 777 Mafra, L.L., Bricelj, V.M., Fennel, K., 2010. Domoic acid uptake and elimination kinetics in oys-
778 ters and mussels in relation to body size and anatomical distribution of toxin. *Aquat. Toxi-*
779 *col.* 100, 17–29. <https://doi.org/10.1016/j.aquatox.2010.07.002>
- 780 Maria, V.L., Gomes, T., Barreira, L., Bebianno, M.J., 2013. Impact of benzo(a)pyrene, Cu and their
781 mixture on the proteomic response of *Mytilus galloprovincialis*. *Aquat. Toxicol.* 144–145,
782 284–295. <https://doi.org/10.1016/j.aquatox.2013.10.009>
- 783 Mat, A.M., Haberkorn, H., Bourdineaud, J.-P., Massabuau, J.-C., Tran, D., 2013. Genetic and geno-
784 toxic impacts in the oyster *Crassostrea gigas* exposed to the harmful alga *Alexandrium*
785 *minutum*. *Aquat. Toxicol.* 140–141, 458–465. <https://doi.org/10.1016/j.aquatox.2013.07.008>
- 786 Mat, A.M., Klopp, C., Payton, L., Jeziorski, C., Chalopin, M., Amzil, Z., Tran, D., Wikfors, G.H.,
787 Hégaret, H., Soudant, P., Huvet, A., Fabioux, C., 2018. Oyster transcriptome response to Al-
788 exandrium exposure is related to saxitoxin load and characterized by disrupted digestion,
789 energy balance, and calcium and sodium signaling. *Aquat. Toxicol.* 199, 127–137.
790 <https://doi.org/10.1016/j.aquatox.2018.03.030>
- 791 Monsinjon, T., Knigge, T., 2007. Proteomic applications in ecotoxicology. *PROTEOMICS* 7,
792 2997–3009. <https://doi.org/10.1002/pmic.200700101>
- 793 Morono, A., Franco, J., Miranda, M., Reyero, M.I., Blanco, J., 2001. The effect of mussel size, tem-
794 perature, seston volume, food quality and volume-specific toxin concentration on the uptake
795 rate of PSP toxins by mussels (*Mytilus galloprovincialis* Lmk). *J. Exp. Mar. Biol. Ecol.* 257,
796 117–132. [https://doi.org/10.1016/S0022-0981\(00\)00336-1](https://doi.org/10.1016/S0022-0981(00)00336-1)
- 797 Nakamura, F., Stossel, T.P., Hartwig, J.H., 2011. The filamins. *Cell Adhes. Migr.* 5, 160–169.
798 <https://doi.org/10.4161/cam.5.2.14401>

- 799 Narahashi, T., Moore, J.W., 1968. Neuroactive agents and nerve membrane conductances. *J. Gen.*
800 *Physiol.* 51, 93.
- 801 Pauletto, M., Milan, M., Moreira, R., Novoa, B., Figueras, A., Babbucci, M., Patarnello, T.,
802 Bargelloni, L., 2014. Deep transcriptome sequencing of *Pecten maximus* hemocytes: A ge-
803 nomic resource for bivalve immunology. *Fish Shellfish Immunol.* 37, 154–165.
804 <https://doi.org/10.1016/j.fsi.2014.01.017>
- 805 Payton, L., Perrigault, M., Hoede, C., Massabuau, J.-C., Sow, M., Huvet, A., Boullot, F., Fabioux,
806 C., Hegaret, H., Tran, D., 2017. Remodeling of the cycling transcriptome of the oyster
807 *Crassostrea gigas* by the harmful algae *Alexandrium minutum*. *Sci. Rep.* 7.
808 <https://doi.org/10.1038/s41598-017-03797-4>
- 809 Peteiro, L.G., Woodin, S.A., Wethey, D.S., Costas-Costas, D., Martínez-Casal, A., Olabarria, C.,
810 Vázquez, E., 2018. Responses to salinity stress in bivalves: Evidence of ontogenetic changes
811 in energetic physiology on *Cerastoderma edule*. *Sci. Rep.* 8, 8329.
812 <https://doi.org/10.1038/s41598-018-26706-9>
- 813 Popowicz, G.M., Schleicher, M., Noegel, A.A., Holak, T.A., 2006. Filamins: promiscuous organiz-
814 ers of the cytoskeleton. *Trends Biochem. Sci.* 31, 411–419.
815 <https://doi.org/10.1016/j.tibs.2006.05.006>
- 816 Pousse, É., Flye-Sainte-Marie, J., Alunno-Bruscia, M., Hégarret, H., Jean, F., 2018. Sources of para-
817 lytic shellfish toxin accumulation variability in the Pacific oyster *Crassostrea gigas*. *Toxicon*
818 144, 14–22. <https://doi.org/10.1016/j.toxicon.2017.12.050>
- 819 Pousse, E., Poach, M.E., Redman, D.H., Sennefelder, G., White, L.E., Lindsay, J.M., Munroe, D.,
820 Hart, D., Hennen, D., Dixon, M.S., Li, Y., Wikfors, G.H., Meseck, S.L., 2020. Energetic re-
821 sponse of Atlantic surfclam *Spisula solidissima* to ocean acidification. *Mar. Pollut. Bull.*
822 161, 111740. <https://doi.org/10.1016/j.marpolbul.>
- 823 R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for
824 Statistical Computing, Vienna, Austria. <http://www.R-project.org>.
- 825 Reyero, M., Cacho, E., Martínez, A., Vázquez, J., Marina, A., Fraga, S., Franco, J.M., 1999. Evi-
826 dence of saxitoxin derivatives as causative agents in the 1997 mass mortality of monk seals
827 in the Cape Blanc Peninsula. *Nat. Toxins* 7, 311–315. [https://doi.org/10.1002/1522-7189\(199911/12\)7:6<311::AID-NT75>3.0.CO;2-I](https://doi.org/10.1002/1522-7189(199911/12)7:6<311::AID-NT75>3.0.CO;2-I)
- 829 Ritchie, J.M., Rogart, R.B., 1977. The binding of saxitoxin and tetrodotoxin to excitable tissue, in:
830 *Reviews of Physiology, Biochemistry and Pharmacology, Volume 79.* Springer, pp. 1–50.
- 831 Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K., 2015. limma powers
832 differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids*
833 *Res.* 43, e47–e47. <https://doi.org/10.1093/nar/gkv007>
- 834 Savina, M., Pouvreau, S., 2004. A comparative ecophysiological study of two infaunal filter-feed-
835 ing bivalves: *Paphia rhomboïdes* and *Glycymeris glycymeris*. *Aquaculture* 239, 289–306.
836 <https://doi.org/10.1016/j.aquaculture.2004.05.029>
- 837 Scheper, R.J., Broxterman, H.J., Scheffer, G.L., Kaaijk, P., Dalton, W.S., van Heijningen, T.H., van
838 Kalken, C.K., Slovak, M.L., de Vries, E.G., van der Valk, P., 1993. Overexpression of a
839 M(r) 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer*
840 *Res.* 53, 1475–1479.
- 841 Seger, A., Hallegraeff, G., Stone, D.A.J., Bansemer, M.S., Harwood, D.T., Turnbull, A., 2020. Up-
842 take of Paralytic Shellfish Toxins by Blacklip Abalone (*Haliotis rubra rubra* Leach) from
843 direct exposure to *Alexandrium catenella* microalgal cells and toxic aquaculture feed. *Harm-
844 ful Algae* 99, 101925. <https://doi.org/10.1016/j.hal.2020.101925>
- 845 Shumway, S.E., Cucci, T.L., 1987. The effects of the toxic dinoflagellate *Protogonyaulax tamaren-*
846 *sis* on the feeding and behaviour of bivalve molluscs. *Aquat. Toxicol.* 10, 9–27.
847 [https://doi.org/10.1016/0166-445X\(87\)90024-5](https://doi.org/10.1016/0166-445X(87)90024-5)

- 848 Spector, I., Braet, F., Shochet, N.R., Bubb, M.R., 1999. New anti-actin drugs in the study of the or-
 849 ganization and function of the actin cytoskeleton. *Microsc. Res. Tech.* 47, 18–37.
 850 [https://doi.org/10.1002/\(SICI\)1097-0029\(19991001\)47:1<18::AID-JEMT3>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0029(19991001)47:1<18::AID-JEMT3>3.0.CO;2-E)
- 851 Stossel, T.P., Condeelis, J., Cooley, L., Hartwig, J.H., Noegel, A., Schleicher, M., Shapiro, S.S.,
 852 2001. Filamins as integrators of cell mechanics and signalling. *Nat. Rev. Mol. Cell Biol.* 2,
 853 138–145. <https://doi.org/10.1038/35052082>
- 854 Suprenant, K.A., Bloom, N., Fang, J., Lushington, G., 2007. The major vault protein is related to
 855 the toxic anion resistance protein (TelA) family. *J. Exp. Biol.* 210, 946–955.
 856 <https://doi.org/10.1242/jeb.001800>
- 857 Tanaka, H., Kato, K., Yamashita, E., Sumizawa, T., Zhou, Y., Yao, M., Iwasaki, K., Yoshimura,
 858 M., Tsukihara, T., 2009. The Structure of Rat Liver Vault at 3.5 Angstrom Resolution 323,
 859 5.
- 860 Tomanek, L., Zuzow, M.J., 2010. The proteomic response of the mussel congeners *Mytilus gallo-*
 861 *provincialis* and *M. trossulus* to acute heat stress: implications for thermal tolerance limits
 862 and metabolic costs of thermal stress. *J. Exp. Biol.* 213, 3559–3574.
 863 <https://doi.org/10.1242/jeb.041228>
- 864 Tran, D., Haberkorn, H., Soudant, P., Ciret, P., Massabuau, J.-C., 2010. Behavioral responses of
 865 *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. *Aquaculture* 298,
 866 338–345. <https://doi.org/10.1016/j.aquaculture.2009.10.030>
- 867 Vale, C., Alfonso, A., Vieytes, M.R., Romarís, X.M., Arévalo, F., Botana, A.M., Botana, L.M.,
 868 2008. In Vitro and in Vivo Evaluation of Paralytic Shellfish Poisoning Toxin Potency and
 869 the Influence of the pH of Extraction. *Anal. Chem.* 80, 1770–1776.
 870 <https://doi.org/10.1021/ac7022266>
- 871 Vilariño, N., Ares, I.R., Cagide, E., Louzao, M.C., Vieytes, M.R., Yasumoto, T., Botana, L.M.,
 872 2008. Induction of actin cytoskeleton rearrangement by methyl okadaate – comparison with
 873 okadaic acid. *FEBS J.* 275, 926–934. <https://doi.org/10.1111/j.1742-4658.2008.06256.x>
- 874 Walne, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the
 875 genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. *Fish Invest Ser* 2 26.
- 876 Walne, P.R., 1966. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. H. M.
 877 Stationery Off.
- 878 Wildish, D., Lassus, P., Martin, J., Saulnier, A., Bardouil, M., 1998. Effect of the PSP-causing dino-
 879 flagellate, *Alexandrium* sp. on the initial feeding response of *Crassostrea gigas*. *Aquat. Liv-*
 880 *ing Resour.* 11, 35–43. [https://doi.org/10.1016/S0990-7440\(99\)80029-1](https://doi.org/10.1016/S0990-7440(99)80029-1)
- 881 Yoon, S.Y., Choi, J.E., Choi, J.M., Kim, D.H., 2008. Dynein cleavage and microtubule accumula-
 882 tion in okadaic acid-treated neurons. *Neurosci. Lett.* 437, 111–115.
 883 <https://doi.org/10.1016/j.neulet.2008.03.083>

884

885 **Figures**886 **Figure 1: Scheme depicting the experimental design.**

887 For each condition, 7 biological replicates were collected. T-iso: *Tisochrysis lutea*, C: *Chaetoceros*
 888 *muelleri*. Group TC-NA corresponds to scallops exposed to T-iso&C for days 1&2, and without algae
 889 for days 3&4 (n=7). Groups TC-A correspond to scallops exposed to T-iso&C for days 1&2 and to *A.*
 890 *minutum* for days 3&4 (n=18).

891

892 **Figure 2: Interindividual variability of toxin accumulation potential**

893 Accumulation potential corresponding to the ratio between final toxin content in DG and numbers of
 894 toxic algal present in the tank. Three profiles of toxin accumulation potential are separated in clusters.
 895 Low (blue empty squares), intermediate (yellow empty triangles) and high (red empty circles)
 896 accumulation clusters are composed of 5, 8 and 5 scallops, respectively. Three aberrant values were
 897 removed from the dataset. Full shapes are means \pm SD of all individuals of the corresponding cluster.
 898

899 **Figure 3: Toxin accumulation linked to feeding behavior.** a. Individual toxin concentration in
 900 scallop digestive gland (DG) at the end of the exposure (day 4, $\mu\text{g STX } 100 \text{ g}^{-1} \text{ DG}$) against the total
 901 numbers of *A. minutum* cells consumed for each scallop on days 3&4 per g of scallop (number of
 902 cells g^{-1}) for all assays (n=18). The line indicates the adjusted type II regression model. b. Graph
 903 shows clearance rates (L h^{-1}) from TC-A assays (days 1&2 exposition to *T. lutea* and *C. muelleri* and
 904 days 3&4 exposition to *A. minutum*) measured from day 1 to day 4 and standardized for a 7g scallop
 905 in total mass. Data have been highlighted according to the 3 clusters (low, medium and high
 906 accumulation potential), each empty shape representing an individual from the low \square , medium \triangle or
 907 high \circ accumulation cluster and filled shapes corresponding to the average values for 5h from the low
 908 \blacksquare , medium \blacktriangle and high \bullet accumulation clusters.

910 **Figure 4: Toxin accumulation and accumulation potential in great scallop depend on the time**
 911 **of recovery filtration and on clearance rate inhibition.** Graphs a&c show individual clearance rate
 912 inhibition index (CRII) as a function of respectively final toxin accumulation ($\mu\text{g STX } 100 \text{ g}^{-1} \text{ DG}$)
 913 (a) and accumulation potential (c). Graphs b&d show the time for filtration recovery as a function of
 914 respectively final toxin accumulation ($\mu\text{g STX } 100 \text{ g}^{-1} \text{ DG}$) (b) and accumulation potential (d). Circles
 915 refer to the different accumulation groups: blue for low, yellow for intermediate and red for high
 916 accumulation clusters.

917

918 **Figure 5: No correlation between accumulation potential and toxin accumulation efficiency at**
 919 **days 3&4.**

920 Toxin accumulation efficiency (TAE) corresponding to the ratio of final toxin content in *P. maximus*
 921 and the amount of toxin consumed compared to accumulation potential (ratio between final toxin
 922 content in DG and numbers of toxic algal present in the tank).

923

924 **Figure 6: Evolution of standardized respiration rates over the 4 experimental days.** a. Graph
 925 shows respiration rates (mg O₂ h⁻¹) for a standard 7g scallop in total mass from TC-A assays (days
 926 1&2 exposition to *T. lutea* and *C. muelleri* and days 3&4 exposition to *A. minutum*) measured from
 927 day 1 to day 4. Each empty symbol corresponds to all individual measurements performed on the low
 928 □, intermediate △ and high ○ accumulation clusters. Filled symbols correspond to the average values
 929 for 5h of each accumulation cluster: low ■, intermediate ▲ and high ●. b. Graph shows the daily mean
 930 respiration rates (mg O₂ h⁻¹) of all individuals (standard mass of 7g) for each day of experiment (D1-
 931 D4). Each bar indicates the mean percent (± SEM) of 18 individuals. Results were considered
 932 significantly different for P<0.01.

933

934 **Figure 7: Representative 2-DE gels (pH 4–7, SDS-PAGE 12%) of *Pecten maximus* mantle**
 935 **proteins in TC-NA and TC-A conditions.** Successfully identified protein spots are indicated on the
 936 2-DE gels by a line and associated spot number. a. No algae (TC-NA) and b. toxic algae (TC-A)
 937 conditions. Details on identified proteins are provided in Table 1. For each condition N=7.

938

939 **Table 1: MS/MS identified proteins differentially accumulated in *A. minutum* exposed scallops**
 940 **compared to control non-exposed group.** List of *Pecten maximus* mantle proteins differentially
 941 expressed between the 2 conditions (TC-A/TC-NA) and identified by MS/MS. Values correspond to
 942 the Log₂ Fold Change (FC) for the normalized volumes of spots between scallops in toxic algae (TC-

943 A) and in no algae (TC-NA) conditions. Protein abundance changed significantly between the 2
 944 conditions (moderate t-test paired-comparison, $fdr < 0.1$).

Name	MW	pHi	# peptides	Peptide sequences	FC	Spot #
Major vault protein	96 kDa	5.7	8	SFFLLPGER LLHADQEIR TFKDDFGVVR KEVVIDETIR AIPLDENEGIYVR TAGDEWLFEGPGTYIPR SVQLAIEITTSQEATAR IPPYYLHVLDQNLNVTR	-0.6	3
Fructose-bisphosphate aldolase	39 kDa	6.0	10	ATVLCLSR ATEQVLAFTYK KPWPLTFSFGR GILAADESTGSGVGR FAPINVENTEENR IWQGKDENVAAAGQK FAPINVENTEENRR ETPSYQAMLENANVLAR VDKGVVPLMGTDNECTTQGLDGLSER TVPPAVAGVTFLSGGQSEEDASINLNAINTDSCR	-0.7	7
Beta-actin	42 kDa	5.3	6	GYSFTTAAER IWHHTFYNELR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTFMFGIADR TTGIVLDSGDGVTHTVPIYEGYALPHAILR	1.2	1
Beta-actin	42 kDa		6	GYSFTTAAER IWHHTFYNELR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTFMFGIADR TTGIVLDSGDGVTHTVPIYEGYALPHAILR	0.9	12
Beta-actin	42 kDa		6	GYSFTTAAER IWHHTFYNELR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTFMFGIADR TTGIVLDSGDGVTHTVPIYEGYALPHAILR	0.5	8
Beta-actin	42 kDa		6	GYSFTTAAER IWHHTFYNELR SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK DLYANTVLSGGTTFMFGIADR TTGIVLDSGDGVTHTVPIYEGYALPHAILR	0.5	9
Beta-actin	42 kDa		3	GYSFTTAAER SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK	0.6	10
Paramyosin	99 kDa	5.6	7	LEEAFAFLR VSLQAEVEDLR SQLQVTLDDFKR IRDLEGELEAEQR LSDELREQEENYK LSEIQVNVLVNDKR DLELASAQYEAQESSTR	-1.1	2
Paramyosin	99 kDa		10	LEEAFAFLR VSLQAEVEDLR YEESEEAASILR SQLQVTLDDFKR IRDLEGELEAEQR LSDELREQEENYK LSEIQVNVLVNDKR DLELASAQYEAQESSTR QNLQVQLSALQSDYDNLNAR LTQENFDLQHQVQELDAANAGLAK	-0.9	11
Filamin A	282 kDa	5.5	5	VYVTPSIGDAR YAGSYVAGSPFK FNDEHIPQSPYR GEINQPCFENIYTR VTYKPTPEPGNYVINIK	-0.9	4
Killer cell lectin-like receptor subfamily B member 1B	31 kDa	4.5	6	TEWSTAINR TLSGFENEIR VSTSDIVYTGR LVFQTNEEAQFVMR TWGSGEPDGGTQTCGCTR NDAYVWVFLSNDEPVDTAVR	0.7	5
Killer cell lectin-like receptor subfamily B member 1B	31 kDa		7	TEWSTAINR TLSGFENEIR VSTSDIVYTGR ILKNEQAEIR FEESLTSEVVR LVFQTNEEAQFVMR TWGSGEPDGGTQTCGCTR	0.5	6

Figure 1

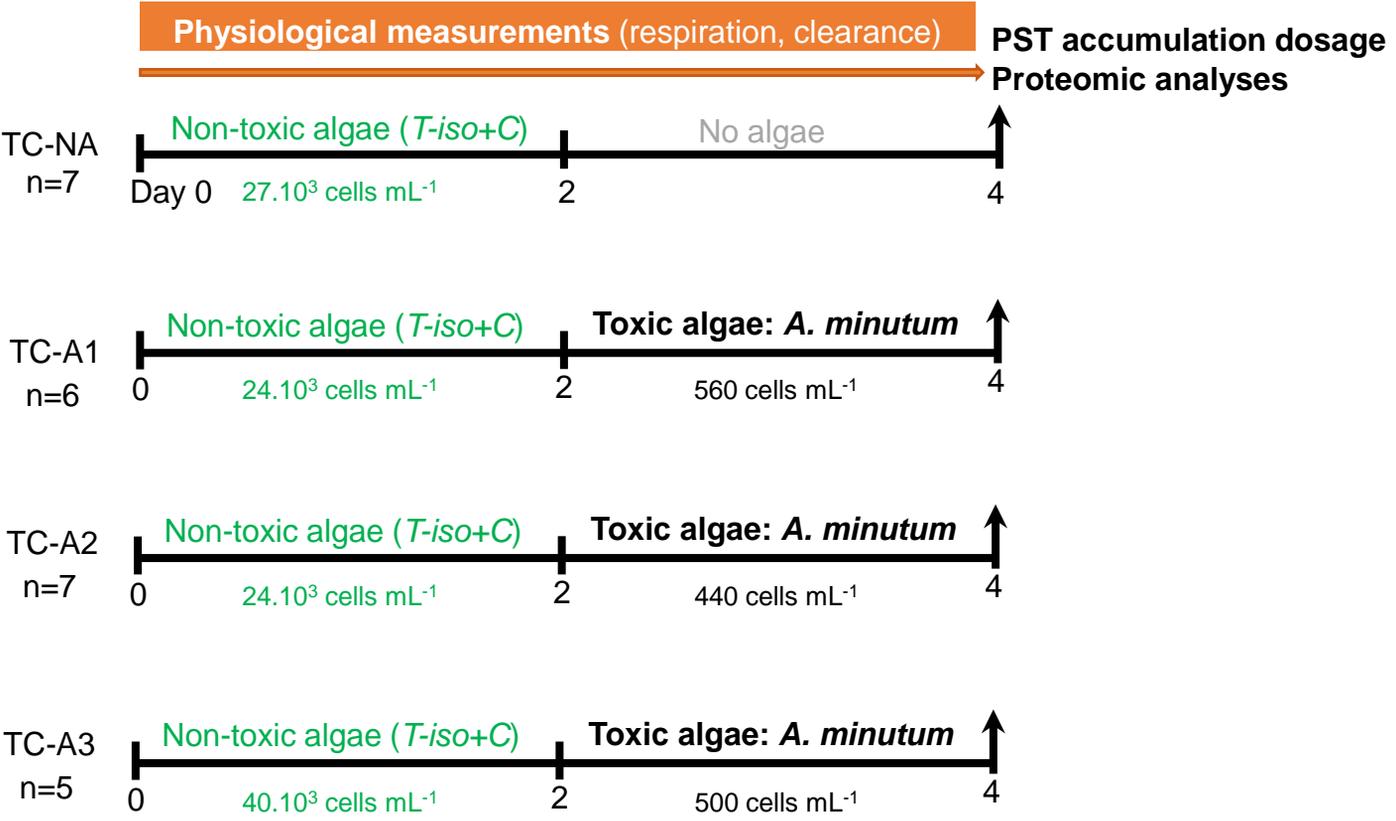


Figure 2

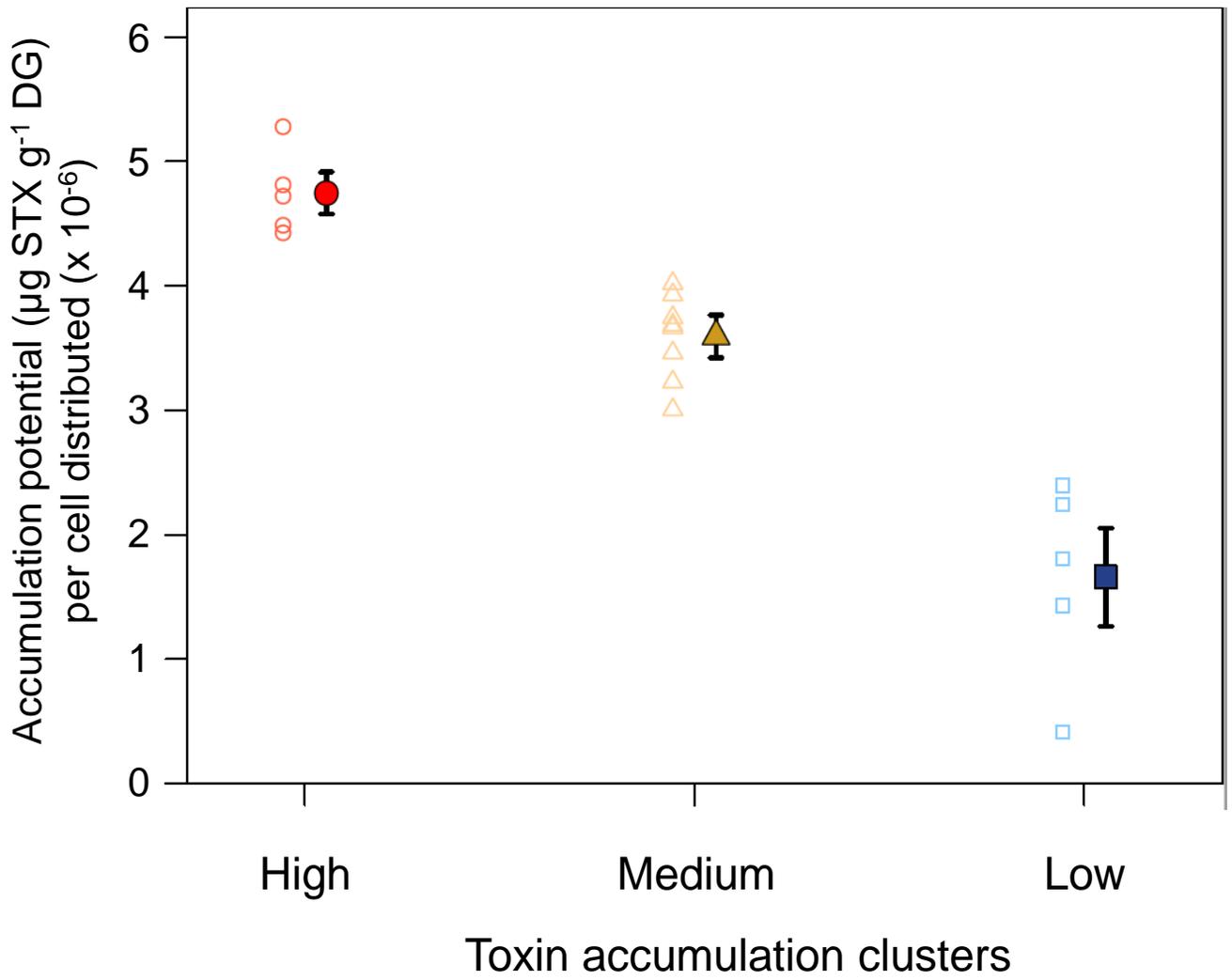
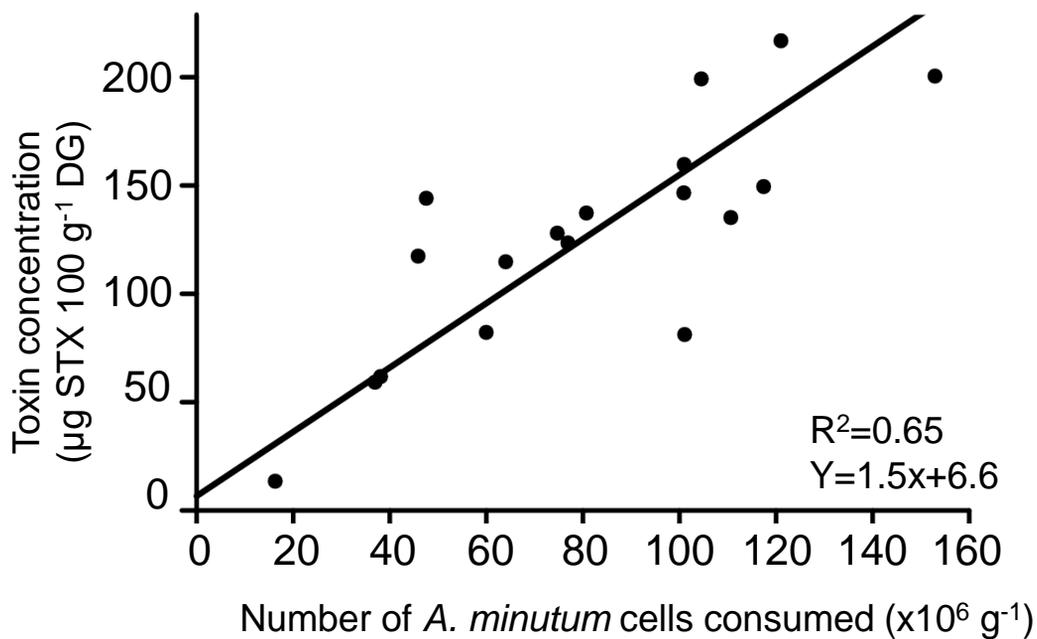


Figure 3

a



b

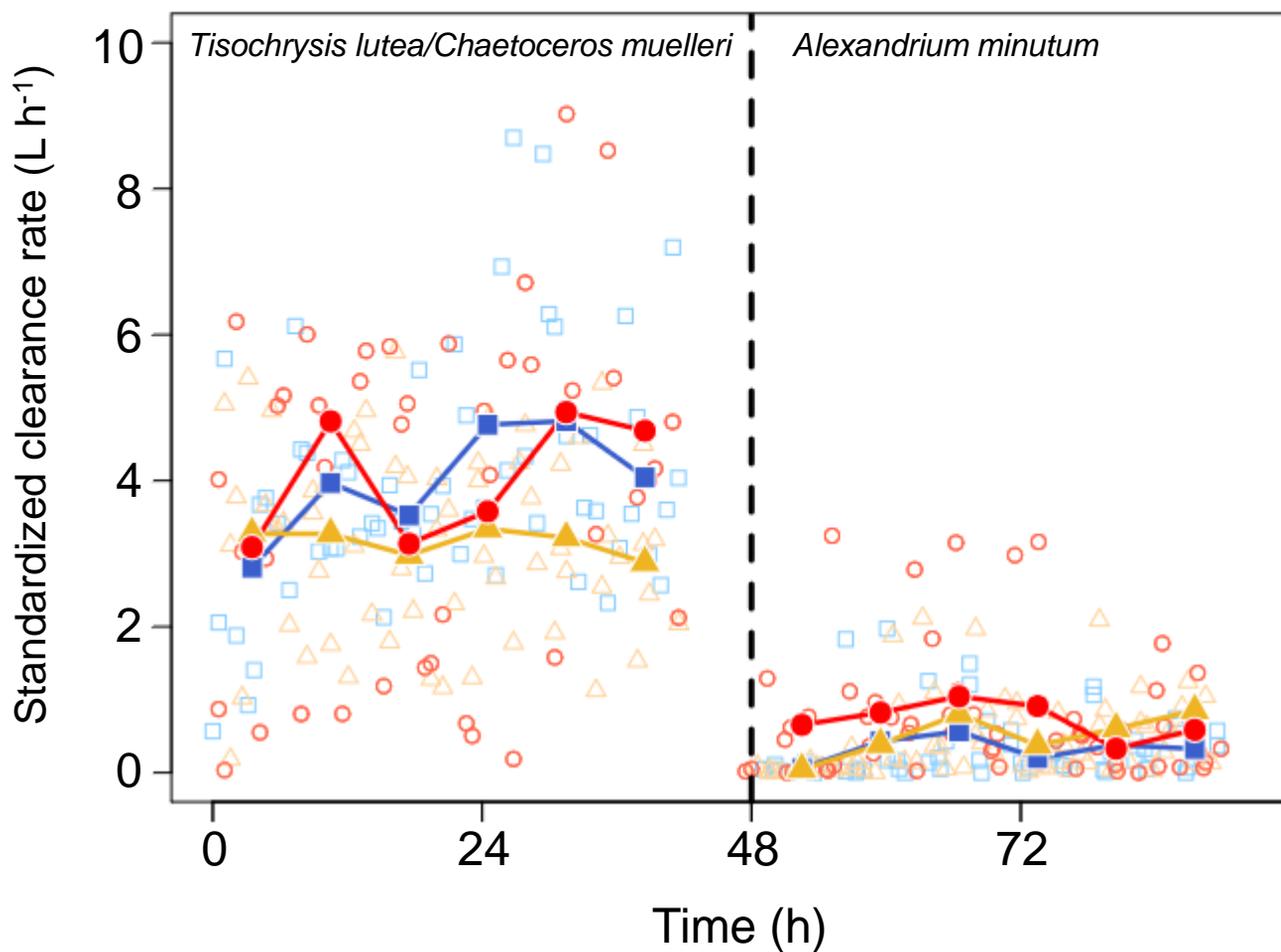
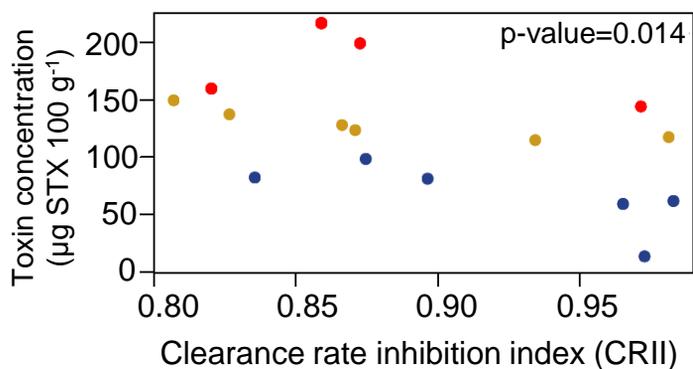
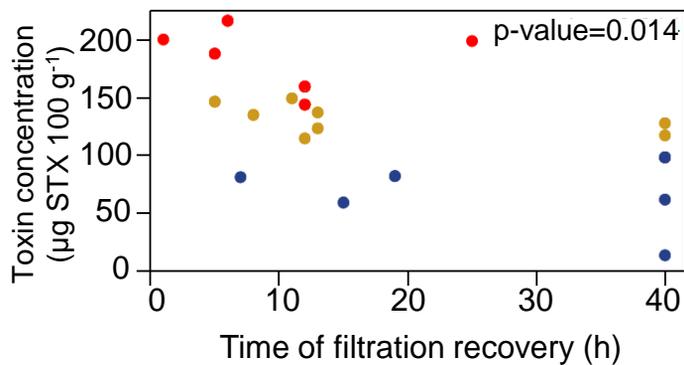


Figure 4

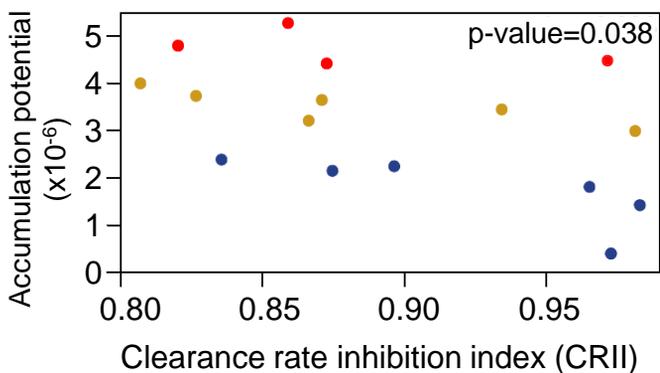
a



b



c



d

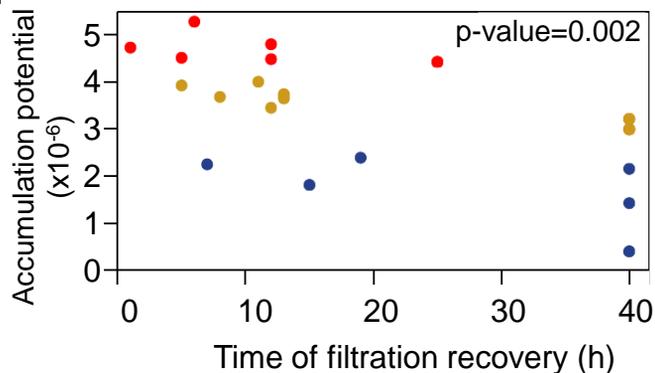


Figure 5

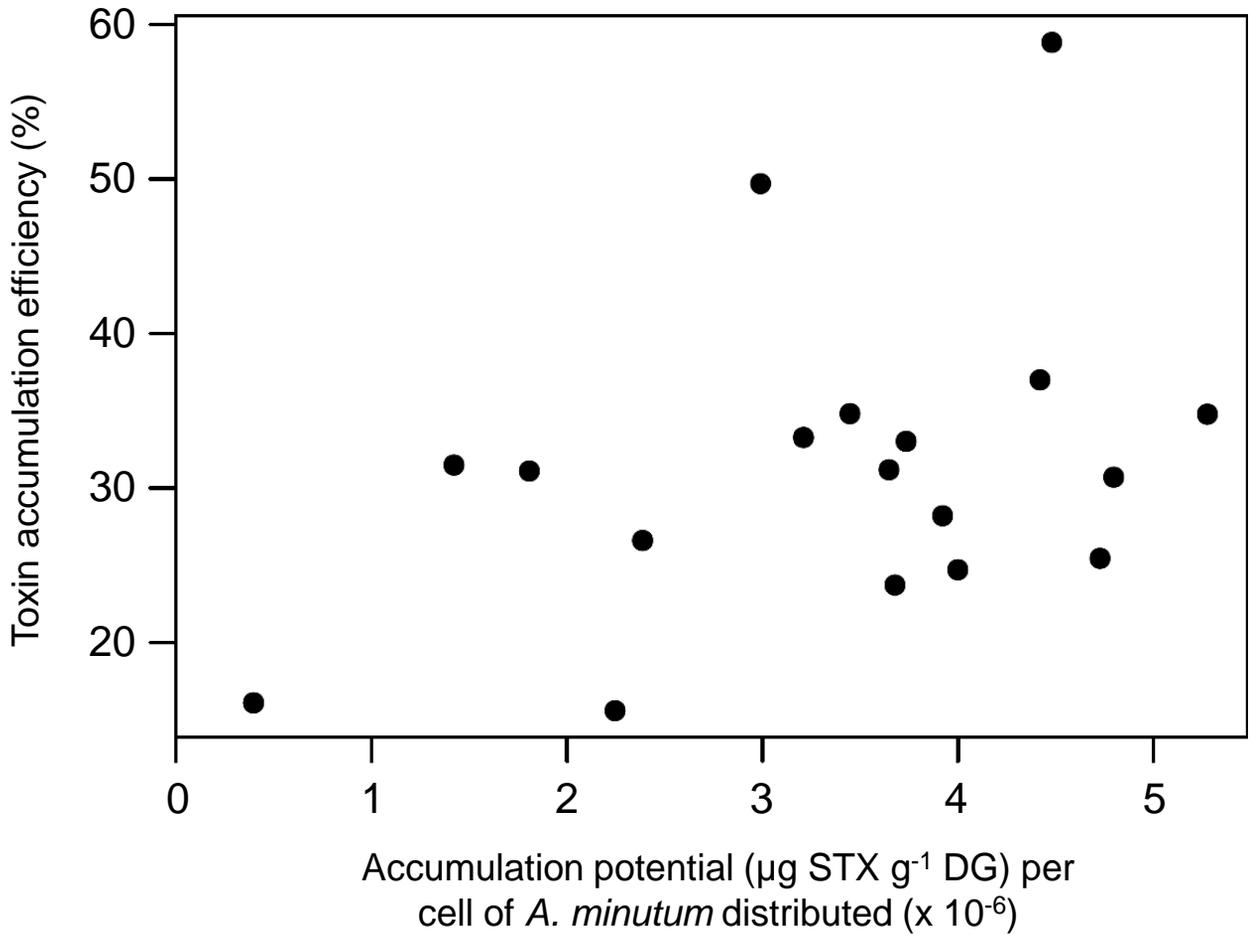


Figure 6

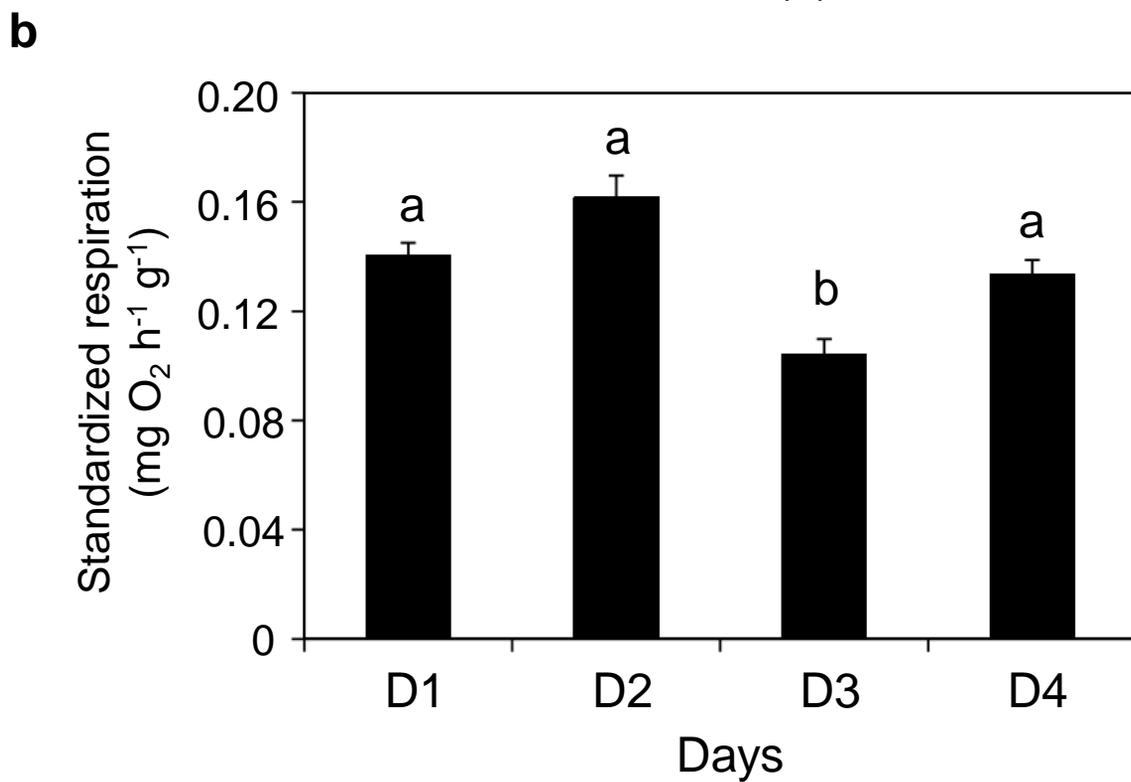
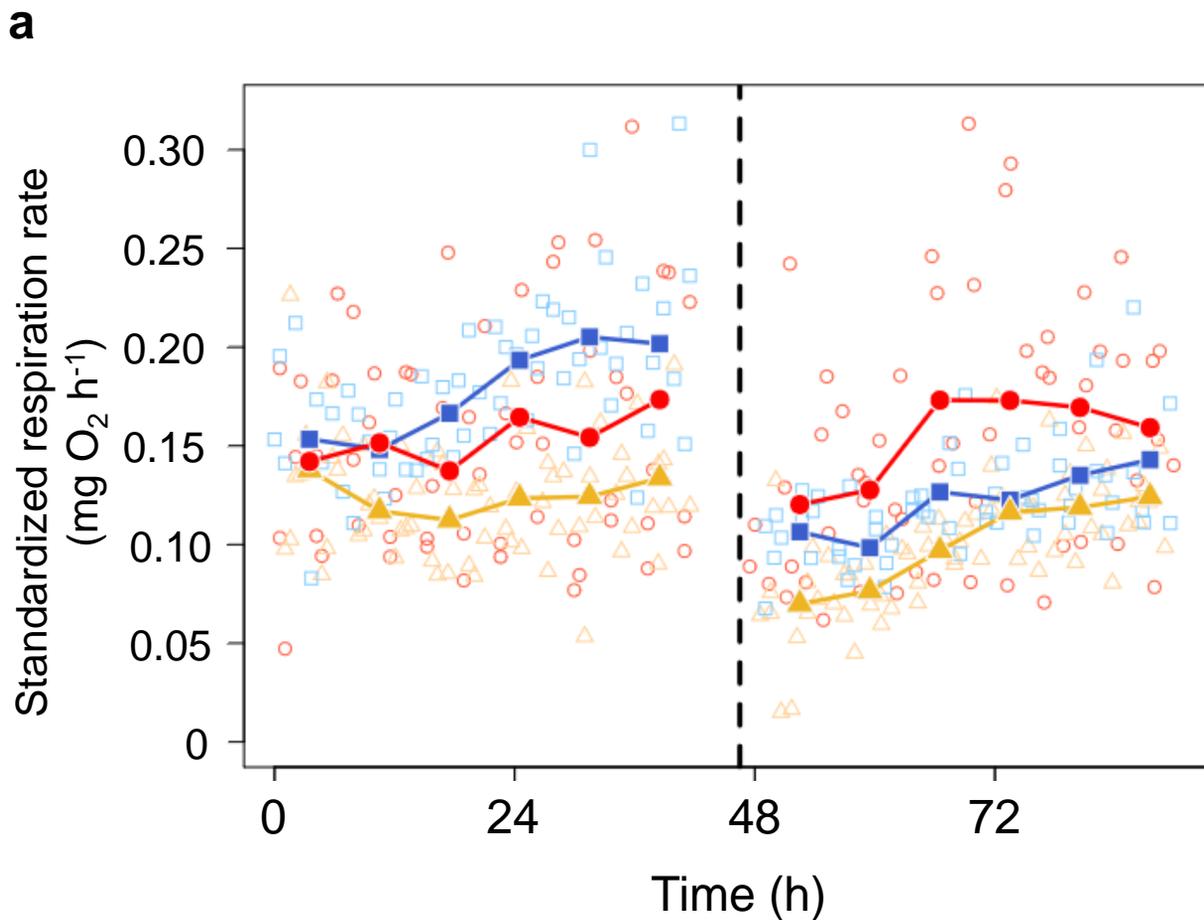
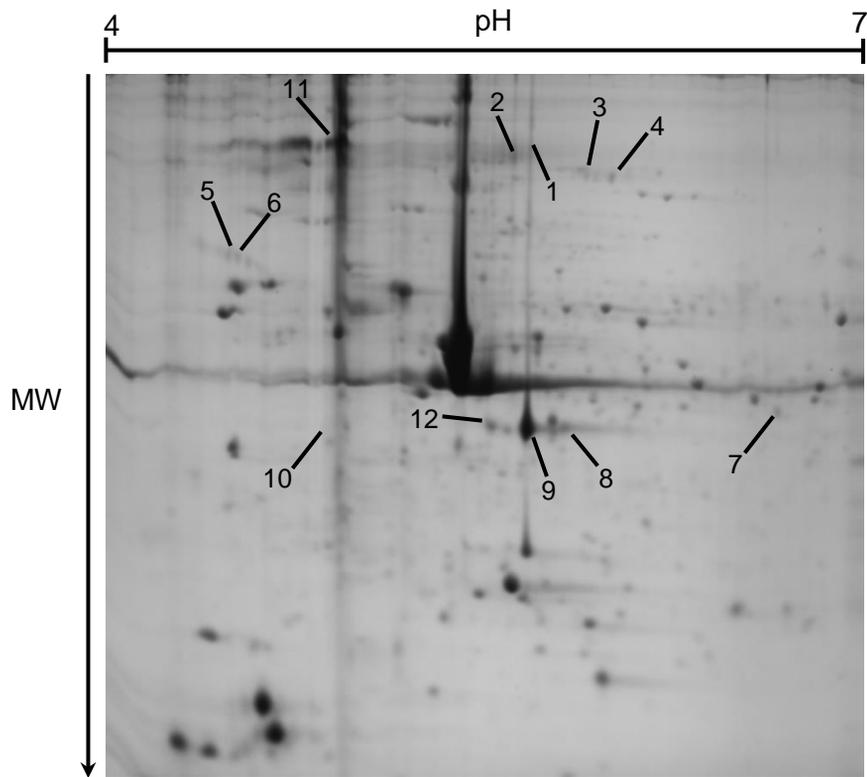


Figure 7

a



b

