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# Lipidome variations of deep-sea vent shrimps according to acclimation pressure : a homeoviscous response ?

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## ABSTRACT

The present study questions the ability of marine fauna to modulate the molecular composition of their membrane lipids, as a function of environmental hydrostatic pressure conditions. Specific variations in the cell lipidome composition are presented for the deep-sea hydrothermal vent shrimp *Mirocaris fortunata*, acclimated at the laboratory for several months at both natural (18 MPa) and atmospheric pressures, but fed with the same diet. These animals display physiological capabilities of acclimation to pressure variations, possibly driven by a particular composition of their lipid species. In particular, it is shown that shrimps exposed to atmospheric pressure exhibit a significantly lower level of the mono-unsaturated fatty acid, vaccenic acid (C18:1n-7), compared to those maintained at natural pressure. The observed fatty acid variations are consistent with a homeoviscous response, i.e. a modulation of the lipidome composition, in response to physical constraints, in order to balance the effects of pressure on membrane order. Additionally, supervised multivariate data analysis was performed for complex lipids and revealed an increase in levels of sterols, sphingomyelin, and ether-phosphatidylethanolamine lipids for animals exposed to atmospheric pressure, with respect to natural pressure. These observations strongly suggest that lipidic domains are involved in the shrimp's response to experimental conditions, and their consistency with a homeoviscous response to pressure is discussed.

30 **KEY WORDS** : Hydrothermal vent shrimps, Pressure tolerance, Viscosity, Homeostasis,  
31 Homeoviscous, Lipid signature, Lipidomes, AbyssBox

32

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37

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39 interests or personal relationships that could have appeared to influence the work reported in this  
40 paper.

41

42 **Contributions of authors** : BS designed the acclimation experiments, BS CD and AL wrote the  
43 manuscript, which was further reviewed by all authors. MZ JR DB supervised in situ sampling,  
44 transport, and maintenance of deep-sea shrimps, JR and MZ undertook conditioning of samples at  
45 the end of the acclimation period. DF GD AL undertook the determination of lipidomes by GC-MS,  
46 and CD AL carried out statistical analyses of data.

## 47 INTRODUCTION

48

49 Marine depths exceeding 1000 m occupy about 75% of the global ocean volume. The deep  
50 sea is therefore probably the largest biotope on earth (Fang et al., 2010), and discoveries in the last  
51 decades have brought the picture of a high biodiversity environment (Ramirez-Llodra et al., 2010).  
52 Along with these findings, another emerging view is that the deep sea has been the subject of  
53 successive colonisation events by shallow water species, spread over geological times (reviews in  
54 Priede & Froese, 2013, Brown & Thatje, 2014), and the opposite phenomenon (from deep to  
55 shallow waters) is also likely (Barnes & Kuklinsky, 2010, Brown & Thatje, 2014). The pace at  
56 which such migratory events occurred remains unknown : is it a "slow" phenomenon submitted to  
57 evolutionary speciation, or a "fast" one, driven by the migration of individual specimens capable of  
58 acclimatisation to new environmental conditions ?

59 The existence of a depth zonation in the distribution of extant species, possibly explained by  
60 a genetically constrained physiological tolerance regarding hydrostatic pressure variations (Carney  
61 2005, Brown & Thatje 2014) would support the first hypothesis. Hydrostatic pressure in the marine  
62 biosphere increases linearly with depth, ranging from 0.1 MPa at sea level (i.e. the so-called  
63 "atmospheric pressure"), to about 110 MPa in the deepest trenches, at a depth of about 11000m  
64 (Jamieson et al., 2010). Several studies have long documented severe impacts on behavioral and  
65 motor activity, and survival, either during pressurization experiments on shallow-water organisms  
66 (review in Pradillon 2012, Ammendolia et al., 2018), or by observations of decompressed deep-sea  
67 animals following their recovery at depth (reviews in MacDonald, 1997, Somero 1992).  
68 Furthermore, *in vitro* experiments have also established that pressure variations in the 10-50 MPa  
69 range impact on many aspects of biological systems, such as enzyme function, or trans-membrane  
70 signaling (reviews in Somero 1992, McDonald, 1997, Pradillon 2012). So, although other  
71 environmental parameters may vary between habitats at different depths (i.e. light intensity,  
72 temperature, food availability, oxygen levels, etc.. (Ramirez-Llodra et al., 2010)), and thereby  
73 possibly control the distribution of a species (Brown & Thatje, 2015), pressure alone would seem  
74 sufficient to restrict the occurrence of a species within a given depth range.

75 Some studies have opened the way to another (non-exclusive) hypothesis: direct  
76 colonisation by specimens capable of acclimatisation to new environmental conditions. Some *in situ*  
77 studies have reported spectacular bathymetric migrations, such as that of antarctic krill (*E. superba*,  
78 from the surface to a depth of beyond 3000 m, Clarke & Tyler, 2008), or Mediterranean red shrimp  
79 (*Aristeus antennatus*, depth from 80 m to 3300 m, Sarda et al., 2004). More recently, laboratory  
80 studies have shown that some shallow-water invertebrates could survive for several days at

81 pressures far beyond those encountered in the wild: the ditch shrimp *Palaemonetes varians*,  
82 exclusively reported in depths of less than 1m, presented survival of 70% after 28 days at a  
83 simulated depth of 1000 m (10 MPa, [Cottin et al., 2012](#)). Finally, the deep-sea hydrothermal vent  
84 shrimp *Mirocaris fortunata*, the subject of the present study, not only displays an important  
85 distribution of depth (from 850 to 4050 m, [Desbruyères et al., 2006](#), [Fouquet et al., 2008](#)), but has  
86 also been successfully acclimated for several months at atmospheric pressure ([Shillito et al., 2015](#)),  
87 far out of its reported natural bathymetric range. It has been argued that the potential to undertake  
88 bathymetric migration may be species-specific, suggesting there may be "winners and losers" in the  
89 face of near-future climate-driven migration patterns ([Ammendolia et al., 2018](#), [Brown & Thatje,](#)  
90 [2015](#)). In other words, some species would be able to display physiological plasticity in response to  
91 pressure variation (i.e. capable of acclimatisation), while others would not. Comparison between  
92 deep and shallow species point at a number of biological features which respond to pressure  
93 conditions, such as protein functioning ([Pradillon, 2012](#)) or osmolyte levels ([Yancey et al, 2013](#)),  
94 but most importantly, the molecular composition of biological membranes has long been identified  
95 as a highly pressure-sensitive feature (review in [Hazel & Williams, 1990](#)).

96 Biological membranes are a complex and dynamic assembly of lipids and proteins, and as  
97 for temperature, variations in hydrostatic pressure can modify the conformation and packing of the  
98 molecules composing these liquid-state lipidic bilayers, thereby impacting their molecular order.  
99 However, pressure and temperature effects appear to be in opposition: an increase in pressure tends  
100 to increase membrane order (i.e. "rigidifies" the membrane), while an increase in temperature  
101 lowers membrane order (i.e. "fluidizes" the membrane). Living organisms respond to such  
102 perturbations by modulating the molecular composition of their membranes, in order to oppose to  
103 the deleterious (ordering or disordering) effects of the environmental change, thereby allowing to  
104 maintain membrane order, and therefore cellular homeostasis ([Hazel, 1995](#), [Ernst et al., 2016](#)). This  
105 response is termed "homeoviscous", and was first observed regarding thermal variations in bacteria  
106 ([Sinensky, 1974](#)). Today it is widely accepted that all poikilothermic organisms (which do not  
107 control their temperature) may undertake such a response in the face of thermal variations,  
108 including within the lifespan of a given individual organism, i.e. as an acclimatory response ([Parent](#)  
109 [et al., 2008](#), for example). The variations in membrane compositions of deep-sea animals submitted  
110 to different pressures were first evidenced by comparing different fish species originating from  
111 various depths ([Cossins & MacDonald, 1986](#), [Behan et al., 1992](#)). An important study also showed  
112 that a given bacterial strain would modify its membrane composition within a few days, according  
113 to incubation pressure ([DeLong & Yayanos, 1985](#)). Since then, much of our knowledge on the  
114 homeoviscous response to pressure has come from studies of bacteria ([Wirsen et al., 1987](#), [Allen et](#)  
115 [al., 1999](#), [Grossi et al., 2010](#), [Fumiyoshi, 2013](#), [Wang et al., 2014](#), [Cario et al., 2015](#)), which are

116 more amenable to pressure incubation than metazoans, mainly because of a limitation in pressure  
117 equipments suitable for large organisms. Therefore, our knowledge of the homeoviscous response  
118 to pressure in animals remains limited. One of the major remaining questions is whether the above-  
119 mentioned "winners", i.e. species displaying acclimatisation capacities in response to pressure  
120 variation, are capable of a such a response.

121 Studies of the homeoviscous response to pressure have so far focused mainly on the study of  
122 the relative proportions of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and  
123 poly-unsaturated fatty acids (PUFA) which compose the hydrophobic fraction of membrane lipids.  
124 Schematically, high levels of SFA increase membrane order, while the presence of one or several  
125 double bonds in MUFA and PUFA lead to disordered assemblies ([Guschina & Harwood, 2006](#)).  
126 Therefore, organisms at high pressure are expected to display higher levels of unsaturated fatty  
127 acids and lower levels of SFA, thus counteracting the ordering effect of pressure, and a reverse  
128 situation is expected for exposure at low pressure. However, given the complexity of eucaryotic  
129 biomembranes, composed of several hundreds of different molecules, an emerging view is that the  
130 homeoviscous response is probably more complex than described up to now, and it is not only a  
131 matter of acyl chain composition. This view is supported by the growing outcome of contemporary  
132 quantitative lipidomics technology, which may allow to follow complex lipid changes during the  
133 homeoviscous response (review in [Ernst et al., 2016](#)). Finally, our understanding of the genetic and  
134 enzymatic regulation of the lipidomic response to pressure remains partial, and again owes much to  
135 studies of bacteria or yeast model organisms ([Fernandes et al., 2004](#), [Freitas et al., 2012](#)). More  
136 generally, the way by which membrane homeostasis is achieved remains unclear ([Ernst et al.,](#)  
137 [2018](#)).

138 We here present results regarding the lipidomic compositions of the deep-sea shrimp  
139 *Mirocaris fortunata*, successfully acclimated at the public aquarium of Océanopolis at different  
140 pressure regimes (as opposed to "acclimated" when occurring in the wild), in the context of a  
141 permanent public exhibition. Because of the importance in pressure variation between our  
142 experimental groups (18 MPa vs. atmospheric pressure), and because of the duration of the  
143 incubation in a controlled environment (several months, an exceptional situation regarding deep-sea  
144 fauna), our hypothesis was that potential lipidomic signature responses would be detectable, and  
145 that the additional use of high output lipidomics would help address the following questions :

- 146 1 - Are the observed successful acclimations accompanied by variations in lipidome composition,  
147 and if so, do these variations compare with homeoviscous responses documented in the literature?  
148 2 - Beyond revealing classic characteristics of a homeoviscous response, could the complex lipid  
149 analysis bring original insights regarding the response to hydrostatic pressure?

## 150 MATERIAL AND METHODS

### 151 Biological model

152 This study focuses on the hydrothermal vent shrimp *Mirocaris fortunata* (Martin &  
153 Christiansen, 1995). This species from the Alvinocarididae family is endemic to the Mid-Atlantic  
154 Ridge (MAR), where it can be encountered in proximity to vent openings, at temperatures around  
155 10 °C (Desbruyères et al., 2001, Desbruyères et al., 2006, Matabos et al., 2015). Believed to be an  
156 omnivorous shrimp (Colaço et al., 2007), *M. fortunata* is found in great abundance on  
157 *Bathymodiolus* mussel beds, making its collection relatively easy. This species has a wide  
158 bathymetric distribution (850 m to 4050 m depth, Desbruyères et al., 2006, Fouquet et al., 2008),  
159 thereby providing a good model to investigate pressure-driven homeoviscous adaptations of deep-  
160 sea organisms (Shillito et al., 2006). Moreover, several specimens originating from depths of 1700-  
161 1800 m have already been successfully acclimated in captivity for several years, both at *in situ* and  
162 atmospheric pressure at the Océanopolis public aquarium (Brest, France), (Shillito et al., 2015).  
163 These captive animals are the subjects of this study.

164

### 165 Specimen collection, transport and maintenance

166 Sampling of animals took place during two cruises, in July 2012 and 2013, respectively  
167 (Momarsat, 2012; Biobaz, 2013), on board the oceanographic vessels "Thalassa" (Momarsat, 2012)  
168 and "Pourquoi Pas?" (Biobaz, 2013) using the Remotely Operated Vehicle (ROV) Victor6000  
169 (IFREMER) at the Lucky Strike vent field (MAR, 37°17'6N, ~ 1750 m depth). Specimens were  
170 sampled with a suction device operated by the hydraulic arm of the ROV. Immediately after  
171 recovery onboard the ship, the shrimps were stored at atmospheric pressure in tanks of  
172 approximately 5 – 10 l of aerated seawater in the cold room (5 – 9 °C), in groups of few individuals  
173 (< 5). At the end of the cruises, the shrimps were shipped to the Océanopolis public aquarium (air  
174 freight by "Flying Sharks", Lisbon, Portugal).

175 On arrival at Océanopolis, husbandry of the shrimps was performed by staff members of the  
176 aquarium. Most of the shrimps were acclimated at atmospheric pressure in a dark room (10 °C) in  
177 groups of around 50 *M. fortunata* in ~ 80 l tanks each equipped with a 25W heating element  
178 wrapped in PVC foam. This heater provides a localised “hotspot” with respect to the surrounding 10  
179 °C environment, in the 13-28 °C range, in order to provide a thermal gradient mimicking their  
180 natural environment. Lights were switched on only a few minutes per day for cleaning, feeding and  
181 mortality counts. For the purposes of public exhibition, other individuals were maintained in a 16.5  
182 l pressurized aquarium named “AbyssBox” (described in Shillito et al., 2015), at a pressure of 18

183 MPa (thereby simulating the pressure prevailing in the natural environment of these shrimps). The  
184 background temperature was set to 10 °C by a temperature-regulation unit which cooled both the in-  
185 coming water (flow rate of 10 l/hour) and the experimental vessel. As for atmospheric-pressure  
186 maintenance, a localized hotspot was provided for the shrimps, consisting of a ring-shaped tube  
187 heated to 27 °C, wrapped in PVC foam. Because the pressurized shrimps were exposed to the  
188 public, they were submitted to about eleven hours of dim artificial light per day (see [Shillito et al.,](#)  
189 [2015](#) and [Matabos et al., 2015](#) for further details on shrimp maintenance and behaviour).

190

## 191 **Experimental design**

192 A first group (the BOX group) of five *M. fortunata* was maintained in the AbyssBox at a  
193 pressure of 18 MPa. The second group (the AP group) consisted of five *M. fortunata* maintained at  
194 atmospheric pressure. During the experiment which lasted five months, both groups were  
195 exclusively fed with Liptoqua food pellets (Liptosa, Madrid, Spain) every 4 – 5 days. These pellets  
196 were inert food (as opposed to live diet), apparently obtained from dried organic extracts, and their  
197 composition was not provided by the manufacturer. Shrimps in the AbyssBox were fed without  
198 decompression by an isobaric feeding line (see [Shillito et al., 2015](#)). At the end of the experiment,  
199 the shrimps (2 - 3 cm body length) were collected and dissected (immediately upon pressure release  
200 for the AbyssBox group). Abdominal muscle tissues and food pellets were subsequently frozen in  
201 liquid nitrogen.

202

## 203 **Lipid analysis**

### 204 *Fatty Acid Methyl Ester Analysis*

205 The frozen samples (muscle tissues and food pellets) were first homogenized using a tissue  
206 lyser apparatus (Quiagen, Les Ulis, France). A micro-method for total lipid extraction and  
207 methylation was applied from 20 µL serum. Fatty acid methyl esters (FAME) were prepared and  
208 assayed by GC-MS in the positive chemical ionization mode with ammonia as the reagent gas  
209 (GC6890-MS5975; Agilent Technologies, Les Ulis, France) as described previously ([Wolf &](#)  
210 [Quinn, 2008](#)). The response factors of fatty acids were calibrated with a weighed mixture (FAME  
211 Mix Supelco® 37, Sigma-Aldrich Chimie, L'Isle d'Abeau Chesnes, Saint Quentin-Fallavier,  
212 France). 25 fatty acids were detected and quantified.

213

214

215 ***Sterol Analysis***

216 Sterols in abdominal muscle tissue homogenates were extracted with a solvent mixture  
217 containing chloroform/methanol 2/1 (v/v) spiked with internal standards as described extensively  
218 elsewhere (Chevy et al., 2005). Briefly, lipids were partitioned in chloroform after the addition of  
219 saline and saponified by methanol potassium hydroxide (0.5 N, 60 °C, 15 min). The fatty acids  
220 released were then methylated with BF<sub>3</sub>-methanol (12%, 60 °C, 15 min) to prevent from interfering  
221 with the chromatography of sterols. Sterols were further re-extracted in hexane and silylated, and  
222 the resultant derivatives were separated by gas chromatography (GC) (Hewlett–Packard 6890  
223 series) in a medium polarity capillary column RTX-65, (Restek, Evry, France). The mass  
224 spectrometer (Agilent 5975 inert XL) in series with the GC was set up for the detection of positive  
225 ions, which were produced in the electron impact mode at 70 eV. Sterols were identified by the  
226 fragmentogram in the scanning mode, and quantified by selective monitoring of the specific ions  
227 after normalization with the internal standards and calibration with weighed standards.

228

229 ***Glycerophospholipid Analysis***

230 Total lipids were extracted from abdominal muscle tissues by the method of Folch et al.  
231 (1957). Individual phospholipid classes and their molecular species were quantified by a triple  
232 quadrupole Qtrap 6500, ABSciex (Les Ulis, France) in tandem with a Shimadzu Nexera XR liquid  
233 chromatography system (Shimadzu France, Marne la Vallée, France). A YMC-Pack PVA-Sil,  
234 bonded with a monomolecular polymer coating of vinyl alcohol (PVA) (Particle size : 5 µm Pore  
235 size : 120 Å, Usable pH range : 2.0-9.5 ; (YMC, Japan)) was used to separate the different lipid  
236 classes. Concentrations of the lipid compounds (including PE, PC, SM) were determined by  
237 comparing the peak area of each complex lipid with that of standards added with a known quantity.  
238 In total, 108 complex lipid compounds were quantified. The method is fully described in Wolf &  
239 Quinn (2008) and in Lamaziere et al. (2011).

240 **Statistical analysis**

241 In order to provide a broad view of the variations between the lipidomes of the shrimps in  
242 response to the two pressure conditions, Principal Component Analyses (PCA) were performed  
243 using RStudio software (1.1.4 version, packages ggplot2, factoextra, factoMineR, 2018 versions).  
244 The analysis was performed on both concentration and percentage data for the fatty acids and the  
245 complex lipids. This analysis also allowed to spot if any particular contamination occurred in some  
246 samples, by detecting outliers in the scores plot.

247           Following the PCAs, and in order to focus on the lipidome differences driven by pressure  
248 changes only, Orthogonal Projections to Latent Structures Discriminant Analyses (OPLS-DA) were  
249 then performed on the same data, using SIMCA 15.0 software (Umetrics, Umeå). The OPLS-DA,  
250 introduced by [Trygg & Wold \(2002\)](#), is a multivariate analysis for omics datasets, which helps to  
251 understand what distinguishes two groups of samples according to the factors that vary between  
252 them. It is an extension to the supervised PLS regression method ([Wold & Martens, 1983](#)), which  
253 removes variation from X (descriptor variables, here each lipid compound proportion or  
254 concentration) that is not correlated to Y (the variable to explain, here the pressure condition) in the  
255 first component of the model. Therefore, it is supposed to generate models easier to interpret  
256 compared to those generated by PLS-DA ([Checa et al., 2015](#), [Trygg & Wold, 2002](#)). The  
257 importance of variables on projection for the predictive component values (VIPpred) for the  
258 generated models were then calculated in order to detect which lipid compounds (fatty acids and  
259 complex lipids) were most responsible for the separation of the two groups of shrimps. They  
260 provide a score value for each independent variable X summarizing their importance in the  
261 projection used by the PLS model to explain Y ([Checa et al., 2015](#), [Galindo-Prieto et al., 2014](#)).  
262 Hence, the higher the VIPpred score of a variable, the more important for the explanation of Y. VIP  
263 values higher than one are generally admitted to consider a variable as significantly important  
264 ([Chong & Jun, 2005](#); [Sun et al., 2012](#)). Data were mean-centered and variance-scaled prior to PCA  
265 and PLS-DA multivariate analyses.

266           We subsequently tested the difference in mean proportion between the groups of pressure  
267 for the individual fatty acids (see Table 1). Data were tested with Shapiro–Wilks in order to  
268 evaluate their distribution. If the distribution was not normal, a non-parametric Wilcoxon-Mann  
269 Whitney test was applied. If the distribution was normal a t-test or a Welch test (if the variances of  
270 the two groups were different) was performed. All statistics were performed using an  $\alpha$ -level of  
271 0.05. These tests were performed using RStudio software.

272           In the case of groups of variables (sum of the saturated, mono-unsaturated, and poly-unsaturated  
273 fatty acids, shown in Figure 2), the two experimental groups were statistically compared, however  
274 without using Bonferroni corrections. Although debated among statisticians ([Nakagawa, 2004](#)), the  
275 use of such corrections accounts for the inflation of type I errors in multiple testing. The statistical  
276 power of the corresponding results has therefore not passed the rigor of Bonferroni corrections,  
277 however these data were not central in our approach, they were rather used as comparison points  
278 with other studies.

279

## 280 RESULTS

### 281 Overview of *M. fortunata* lipidomic response to acclimation pressure

282 The two groups of shrimp samples form two separated clusters in the first two PCA  
283 components performed on the concentration data for the fatty acids and the complex lipid contents  
284 (Figures S1-S2 in supplementary material). The two groups were segregated in two clusters  
285 opposed on a diagonal plan regarding their fatty acids concentrations, while they were opposed on  
286 the first axis regarding their complex lipid concentrations. No strong outlier was detected. The  
287 loading plots (not shown) and the raw data suggested that this clear segregation could be explained  
288 by generally higher lipid concentrations in BOX shrimps, with respect to AP shrimps. Indeed, mean  
289 concentration of fatty acids of BOX shrimps was 1317 +/- 283 ng/mg while that of AP shrimps was  
290 590 +/- 380 ng/mg. Similarly, the muscle of BOX shrimps contained higher levels of PE (431 +/-  
291 189 against 189 +/- 85 nanoM/mg) and PC (144 +/- 18 against 67 +/- 15 nanoM/mg). A similar  
292 trend was observed for sterol levels (9.56 +/- 2.11 nanoM/mg for BOX against 7.60 +/- 3.57  
293 nanoM/mg for AP shrimps), and also for SM levels (12.92 +/- 2.12 nanoM/mg for BOX, vs. 11.16  
294 +/- 1.86 nanoM/mg for AP shrimps).

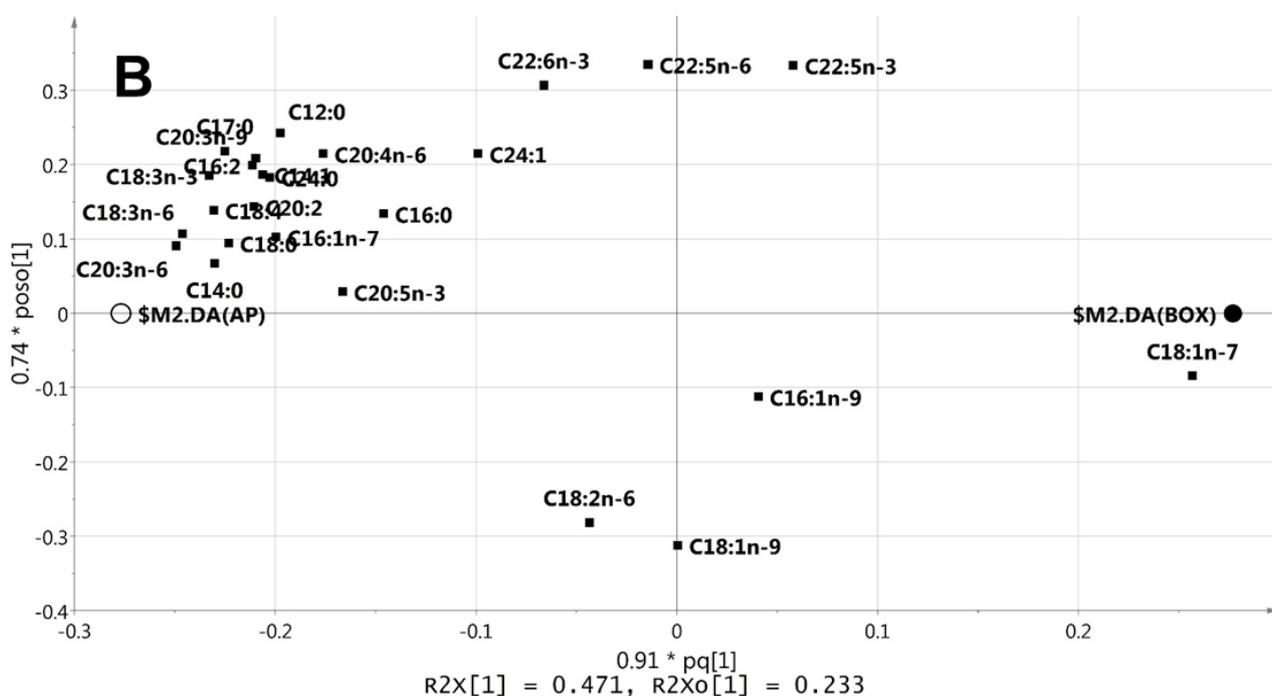
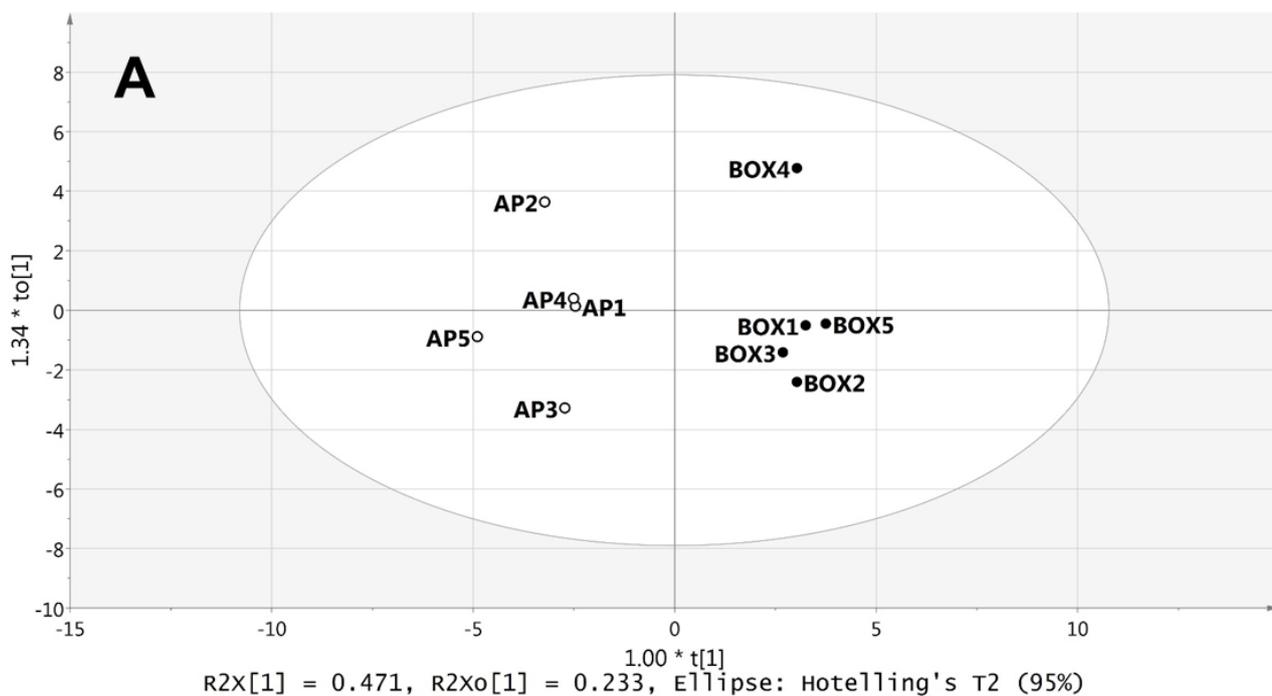
295

296 The two groups of shrimps also formed two distinct groups when the PCA analyses were  
297 performed on data expressed as percentage of total lipid fatty acids, in order to avoid size effects  
298 due to concentration differences (Figures S3-S4 in supplementary material). In the subsequent  
299 OPLS-DA regarding fatty acid percentages, the two groups of shrimps segregated through the first  
300 two components regarding their fatty acid compositions (Figure 1). In the score plot of the OPLS-  
301 DA performed on complex lipid compositions, the two groups were also well separated (Figure S5  
302 supplementary material). The two AP and BOX groups exhibited important phenotype  
303 dissimilarities leading to a clear segregation of the observations in the variability plane (first two  
304 components).

305

306

307



308

309

310 **Figure 1:** OPLS-DA score plot (A) and load plot (B) of the total fatty acids composition (%  
 311 weight) from *Mirocaris fortunata* muscle samples exposed to two pressure conditions,  
 312 atmospheric pressure (AP open circles, n=5) and 18 MPa (BOX full black circles, n=5).  
 313 Goodness of fit  $R^2 = 0.704$ , goodness of prediction by cross validation  $Q^2 = 0.846$ . In B, large  
 314 circles represent the epicentre of shrimp observation groups, noted \$M2.DA(AP) and  
 315 \$M2.DA(BOX) for AP and BOX shrimp groups, respectively.

316

317

318

% weight	BOX	SD	AP	SD	trend	p-value	Food
<b>C12:0</b>	<b>0,21</b>	0,13	<b>0,48</b>	0,28			0,06
<b>C14:0</b>	<b>0,46</b>	0,22	<b>1,29</b>	0,44	<b>UP</b>	<b>**</b>	1,60
<b>C14:1</b>	<b>0,02</b>	0,01	<b>0,06</b>	0,02	UP	*	0,03
<b>C16:0</b>	<b>15,04</b>	2,02	<b>16,93</b>	1,11			6,53
<b>C16:1n-9</b>	<b>5,04</b>	1,70	<b>4,60</b>	0,85			3,02
<b>C16:1n-7</b>	<b>0,08</b>	0,01	<b>0,12</b>	0,03	UP	*	0,17
<b>C16:2</b>	<b>0,03</b>	0,01	<b>0,06</b>	0,02	UP	*	0,28
<b>C17:0</b>	<b>0,12</b>	0,04	<b>0,23</b>	0,05	UP	*	0,08
<b>C18:0</b>	<b>1,69</b>	0,45	<b>2,72</b>	0,47	<b>UP</b>	<b>**</b>	0,85
<b>C18:1n-9</b>	<b>10,66</b>	3,44	<b>10,57</b>	1,89			5,78
<b>C18:1n-7</b>	<b>23,12</b>	1,54	<b>13,14</b>	2,03	<b>DOWN</b>	<b>**</b>	5,94
<b>C18:2n-6</b>	<b>1,73</b>	0,33	<b>1,96</b>	0,75			2,93
<b>C18:3n-6</b>	<b>0,13</b>	0,01	<b>0,23</b>	0,04	<b>UP</b>	<b>**</b>	0,55
<b>C18:3n-3</b>	<b>0,07</b>	0,04	<b>0,23</b>	0,09	<b>UP</b>	<b>**</b>	0,00
<b>C18:4</b>	<b>0,08</b>	0,01	<b>0,17</b>	0,05	<b>UP</b>	<b>**</b>	1,94
<b>C20:2</b>	<b>0,10</b>	0,02	<b>0,14</b>	0,01	UP	*	0,02
<b>C20:3n-9</b>	<b>0,07</b>	0,02	<b>0,12</b>	0,05			0,02
<b>C20:3n-6</b>	<b>0,04</b>	0,00	<b>0,08</b>	0,02			0,00
<b>C20:4n-6</b>	<b>0,68</b>	0,16	<b>1,01</b>	0,29	UP	*	0,20
<b>C20:5n-3</b>	<b>22,16</b>	3,65	<b>26,15</b>	2,24			46,38
<b>C22:5n-6</b>	<b>0,09</b>	0,05	<b>0,09</b>	-			0,04
<b>C22:5n-3</b>	<b>0,63</b>	0,15	<b>0,58</b>	0,12			0,59
<b>C22:6n-3</b>	<b>17,43</b>	2,83	<b>18,66</b>	2,10			22,81
<b>C24:0</b>	<b>0,11</b>	0,09	<b>0,30</b>	0,13			0,05
<b>C24:1</b>	<b>0,29</b>	0,08	<b>0,38</b>	0,14			0,13

319

320

321 **Table 1:** Average fatty acid composition (% weight) of the *Mirocaris fortunata* and food lipid data  
322 set. Trends between BOX (n = 5 ind.) and AP (n = 5 ind.) shrimps are indicated (\* symbol : p-value  
323 < 0.05, \*\* symbol : p-value < 0.01)

324

325

## 326 **Total fatty acid membrane composition**

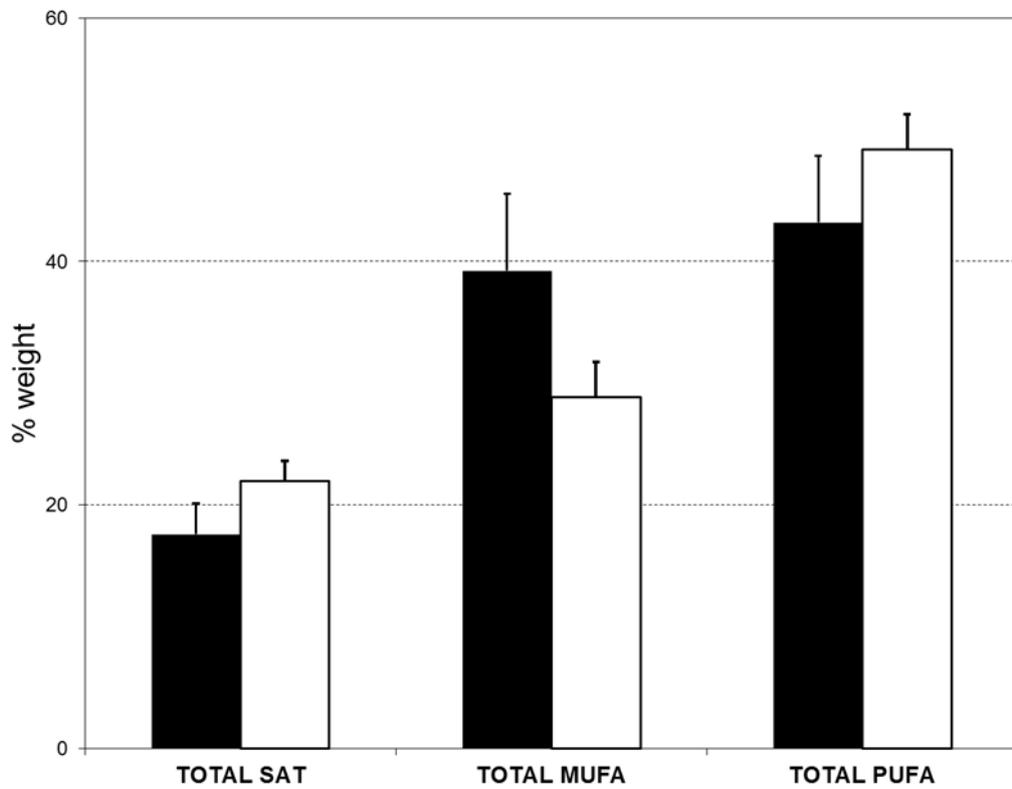
327 25 fatty acids were detected in the analysis (**Fig. 1A**, and **Table 1**). The AP shrimps contain  
328 a significantly higher proportion of saturated fatty acids than BOX shrimps (mean values : 21.95 %  
329 and 17.58 %, respectively, p-value = 0.013), and a significantly lower proportion of mono-  
330 unsaturated fatty acids (mean values : 28.86 % and 39.22 %, respectively, p-value = 0.011) (**Figure**  
331 **2**). No difference was detected between BOX and AP shrimps levels of poly-unsaturated fatty acids  
332 (p-value = 0.062).

333 Five predominant fatty acids each exceeded 10% of the composition, and accounted for 86.9  
334 % in terms of proportion : C16:0 (15.98 % on average in all the shrimps), C18:1 n-7 (18.13 % on  
335 average), C18:1 n-9 (10,61 % on average), C20:5 n-3 (known as “EPA”) (24.15 % on average) and  
336 C22:6 n-3 (known as “DHA”) (18.4 % on average) (**Table 1**). No significant difference was found  
337 between the levels of these predominant fatty acids within the two groups of shrimps, except for  
338 C18:1 n-7, a MUFA. AP shrimps’ proportions of C18:1 n-7 were significantly lower than for BOX  
339 shrimps (mean values : 13.14 % +/- 2.03, and 23.12 % +/- 1.54 respectively, p-value = 0.008). In  
340 the OPLS-DA load plot (**Fig. 1B**), this fatty acid appeared to strongly discriminate the two  
341 experimental groups. C18:1 n-7 also displayed the highest VIPpred score value (1.51) in the OPLS-  
342 DA model performed on the fatty acid compositions (not shown). C18:1 n-7 alone is likely to  
343 account for the higher proportion of MUFAs in BOX shrimps (**Table 1, Fig. 2**), as no other  
344 predominant fatty acid seemed to vary between the two groups. The other most discriminating fatty  
345 acids between the two groups of shrimps (according to their VIPpred values > 1, not shown), were  
346 C18:3 n-6, C18:0, C14:0, C18:3 n-3, C17:0, C16:2, C14:1, C20:2, C18:4, C16:1 n-7, C20:4 n-6.  
347 They were all present in proportions smaller than 3 % in the muscle of the shrimps, and these  
348 proportions were all significantly higher in AP shrimps (**Table 1**). Finally, the food was analyzed  
349 and two major components appeared to exceed 10% of the total composition : C20:5 n-3 (EPA,  
350 46.38 %) and C22:6 n-3 (DHA, 22.82 %).

## 351 352 **Fatty acid metrics conventionally used to assess membrane order**

353 The average chain length (ACL) index, introduced by [Poynter et al. \(1989\)](#) was calculated as  
354 follows for each shrimp sample:  $ACL = (\sum [ C_i ] \times i) / \sum [ C_i ]$ , where [ C<sub>i</sub> ] is the concentration of  
355 the fatty acids containing i carbon atoms. Average ACL index was 18.65 +/- 0.19 for BOX  
356 shrimps, and 18.68 +/- 0.13 for AP shrimps. The saturation ratio and unsaturation index were also  
357 calculated according to [Cossins & MacDonald \(1986\)](#), i.e. the ratio of the weight % of saturated to  
358 unsaturated fatty acids (for saturation ratio), and the sum of weight % multiplied by the number of  
359 double bonds for each component (for unsaturation index) . The saturation ratio was 0.214 +/- 0.038

360 for BOX shrimps, and 0.282 +/- 0.027 for AP shrimps. Finally, the unsaturation index was 265.7  
361 +/- 24.7 for BOX shrimps, and 284.7 +/- 15.9 for AP shrimps.  
362



363  
364  
365 **Figure 2:** Proportion (weight %) of saturated (SAT), mono-unsaturated (MUFAs) and poly-  
366 unsaturated (PUFAs) fatty acids in *Mirocaris fortunata* muscle after five months maintained at  
367 atmospheric pressure (AP, white bars, n = 5) or natural pressure (18MPa) in the Abyssbox (BOX ,  
368 black bars, n = 5). Mean values are shown and error bars correspond to the standard deviation.  
369

### 370 **Complex lipid membrane composition**

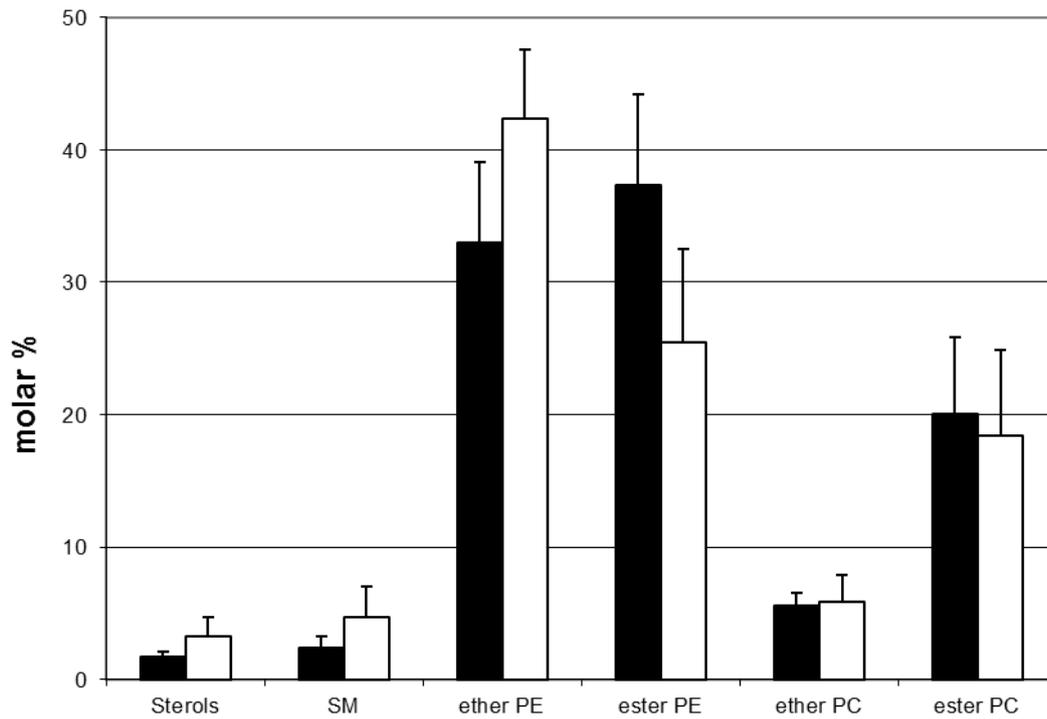
371 108 complex lipids were detected in the analysis, which targeted phosphatidylethanolamine  
372 (PE, 44 molecular species detected, including ether-PE (PE-O, 14 molecular species)),  
373 phosphatidylcholines (PC, 44 molecular species detected, including ether-PC (PC-O), 13 species),  
374 sphingomyelins (SM, 15 molecular species detected), and sterols (5 molecular species detected).  
375 For one sample (AP3), the sterol analysis could not be achieved for technical reasons, and therefore  
376 this individual observation was removed for the calculation of the following complex lipid  
377 proportions (molar %, **Figure 3**). Phosphatidylethanolamine (PE) was the dominant class in both  
378 groups of samples, accounting for 70.3 +/- 7.6 % of complex lipid data composition in BOX  
379 shrimps, and 67.8 +/- 11.7 % in AP shrimps (**Fig. 3**). Remarkably, these proportions were the  
380 addition of 33.0 % of ether-link PE (PE-O) and 37.3 % of ester-link PE for BOX shrimps, while  
381 they broke down in 42.3 % of ether-link PE (PE-O) and 25.5 % of ester-link PE, for AP shrimps.  
382 Phosphatidylcholines (PC) represented 25.6 +/- 6.5 % of complex lipid data composition of the  
383 BOX shrimps and 24.3 +/- 8.2 % of that of the AP shrimps. BOX shrimps contained 2.4 +/- 0.9 %  
384 of sphingomyelin (SM), while AP ones contained 4.7 +/- 2.3 %. Finally, sterols account for 1.7 +/-  
385 0.4 % in BOX shrimps and 3.2 +/- 1.5 % in AP shrimps.

386

387 The VIPpred scoring of the OPLS-DA was performed on the complex lipids proportion  
388 dataset (**Figure 4**), in order to understand which lipid compounds separated the two groups (**Fig. S5**  
389 **supplementary material**). **Figure 4** displays the 44 variables with VIPpred values higher than 1.  
390 Among the latter, 9 were sphingomyelins, all of which were dominant in the AP shrimps. All the  
391 detected sterols appeared in this figure (Cholesterol, 7-Lathosterol, Desmosterol, 7-DHC, 8-DHC),  
392 and were all present in higher proportions in the AP shrimps. Moreover, 23  
393 phosphatidylethanolamines were contained in this list. Among them, the 8 that include an ether link  
394 (PE O) were more abundant in proportion in AP shrimps and the great majority of the others  
395 (14/15), which include an ester link (PE), were more abundant in proportion in BOX shrimps. For  
396 seven phosphatidylethanolamines, the ether (PE-O) and corresponding ester (PE) species were both  
397 present in the list. This list also included seven PC (all ester species), 3 of which were found in  
398 larger proportion in AP shrimps samples, while the 4 other ones displayed higher levels in BOX  
399 shrimps.

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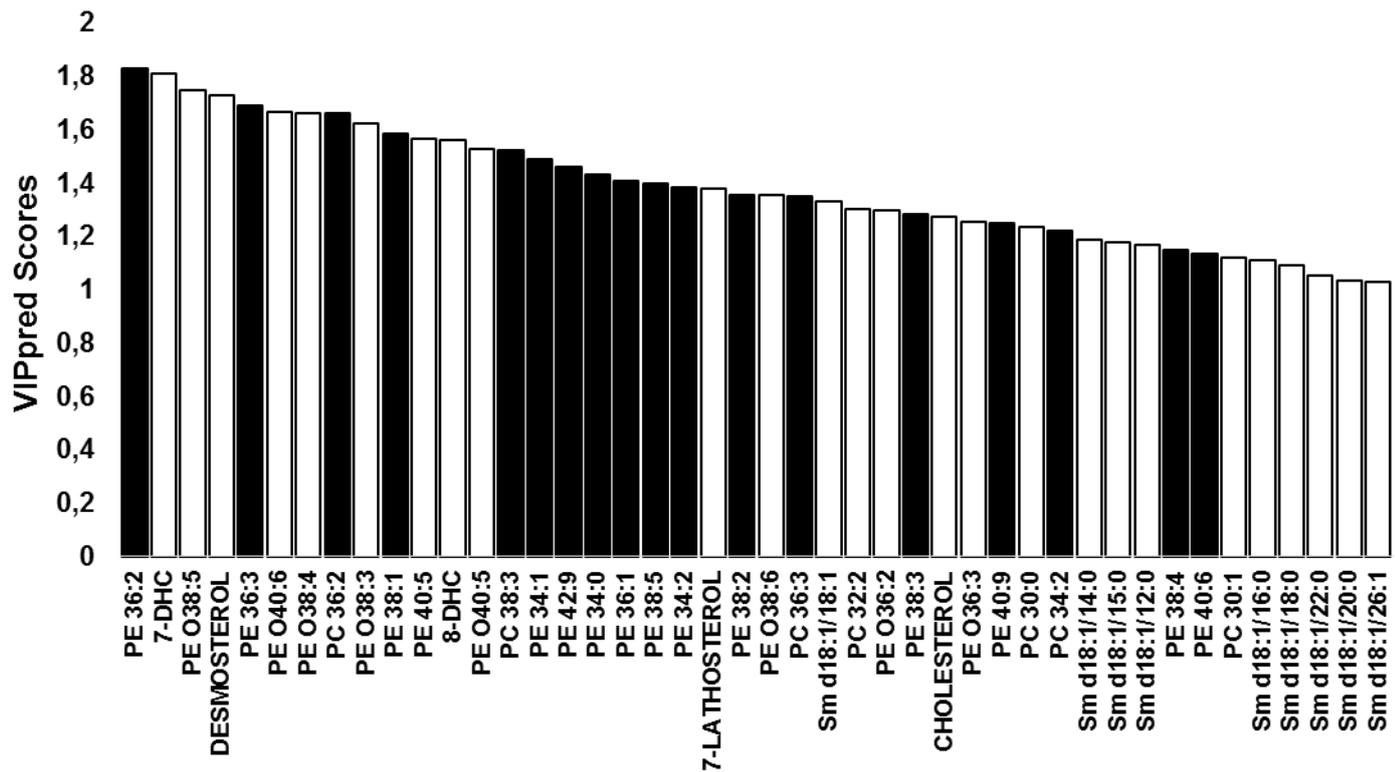
403

404

405 **Figure 3:** Complex lipids composition (molar %) of the data sets from *Mirocaris fortunata* muscle  
406 after five months maintained at atmospheric pressure (AP, white bars, n = 4) or natural pressure  
407 (18MPa) in the Abyssbox (BOX , black bars, n = 5). Mean values are shown and error bars  
408 correspond to the standard deviation.

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412

413 **Figure 4:** List of the variable importance for the projection (VIP) for the predictive components  
 414 with scores higher than 1 (arbitrary unit). These VIPs refer to the OPLS-DA performed on %  
 415 composition of complex lipids raw data. Each lipid molecular species is colored according to its  
 416 relative abundance within specific experimental conditions: white/black bars indicate higher levels  
 417 at atmospheric pressure (AP shrimps, n = 4)/18 MPa (BOX shrimps, n = 5) respectively.

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422 **DISCUSSION**

423 The aim of the present work was to test whether the successful acclimation of a deep-sea  
424 shrimp at two different pressures would allow to observe significant changes in membrane lipid  
425 composition, as expected according to the concept of homeoviscous adaptation (review in [Ernst et](#)  
426 [al., 2016](#)).

427 *The experimental context*

428 To our knowledge, it is the first time that lipidomic analyses have been conducted following  
429 long-term acclimation (i.e. several months) of deep-sea animals in captivity. The shrimps studied  
430 here had reached the Oceanopolis aquarium in July 2013 at the latest, and were sampled in  
431 December 2014, meaning a minimum acclimation period of 17 months, with identical food during  
432 the last five months.

433 While the AP group was maintained at atmospheric pressure, the BOX group was kept at its  
434 natural hydrostatic pressure, corresponding to the depth of the site of origin (1700-1800 m, [Shillito](#)  
435 [et al., 2015](#)). Except for hydrostatic pressure, our aim was to minimize environmental differences  
436 which could influence lipid compositions of the studied animals. Water chemistry, temperature and  
437 diet were the three main issues identified. Regarding nutrition, it is a well-known fact that lipid  
438 composition of an organism is directly influenced by the fatty acid content of its food, justifying the  
439 widespread use of fatty acids as tracers in trophic webs (review in [Kelly & Scheibling, 2012](#)).  
440 Therefore identical food was provided to both BOX and AP shrimps during the last five months  
441 prior to lipid analyses, in order to erase any differential diet effect on lipid composition. As to the  
442 thermal environment, it is also well established that habitat temperature has a direct effect on lipid  
443 composition of poikilothermic organisms ([Ernst et al., 2016](#)). In our experimental context, the  
444 global temperature was 10°C, however for both groups a "hotspot" was provided (24-27 °C), and  
445 AP and BOX shrimps were often seen aggregating on it, between periods of swimming or feeding  
446 activity within the 10°C environment ([Matabos et al., Shillito et al., 2015](#)). Therefore a possible  
447 effect of temperature on lipid composition cannot be ruled out, since we have no certainty on the  
448 actual thermal regime experienced by the experimental animals. It should be noted however that  
449 *Mirocaris fortunata* is a fairly eurythermal hydrothermal vent shrimp, with recorded habitat  
450 temperature ranging from 4 to 24 °C *in situ* ([Desbruyères et al., 2001](#)). Finally, regarding water  
451 chemistry, care was taken that both groups were supplied through the same seawater line.

452

453

454

455 ***Higher levels of FA and complex lipids in pressurised samples (BOX)***

456 The concentration data (in ng/mg or nanomol/mg, as opposed to relative data, in %) showed  
457 that the shrimps maintained at natural pressure (BOX shrimps, 18 MPa) were more concentrated in  
458 lipid compounds than those maintained at atmospheric pressure (AP shrimps, 0.1 MPa). Overall,  
459 mean concentration of fatty acids of BOX shrimps was two-fold of that of AP shrimps (1317 vs.  
460 590 ng/mg), as for PE (431 vs. 189 nanoM/mg) and PC (144 vs. 67 nanoM/mg) levels. Such a trend  
461 was already observed when comparing different animal species from various habitat depths  
462 (shrimps : [Yerlikaya et al., 2013](#), or other animal species, [Bockus & Seibel, 2016](#)). The reasons for  
463 such a phenomenon are unclear: although hydrostatic pressure was proposed as one among several  
464 possible drivers of this phenomenon, ecological factors such as food limitation yielding increased  
465 lipid storage were also proposed. In our case study, variations of the complex lipid species PE and  
466 PC show that this response involves membrane contents, rather than lipid reserves. The decrease in  
467 lipid levels in AP shrimps could then correspond to a limited microvilli or endomembrane system  
468 synthesis, or lower cell divisions rates, at atmospheric pressure. Further structural studies (using  
469 electron microscopy) would certainly help to test this hypothesis at the subcellular level.

470

471 ***The major FA components***

472 Four of the five major FA found in our samples showed no significant variation between the  
473 two experimental treatments. Among these, the two PUFA EPA (C20:5 n-3 / 24.15 %) and DHA  
474 (C22:6 n-3 / 18.4 %) display levels which may appear unusually high for adult hydrothermal vent  
475 fauna. The interpretation for lower levels in the wild (absence in several mid-Atlantic ridge animals,  
476 [Colaço et al., 2007](#), [Zhu et al., 2015](#), or less than 6% for adult *Mirocaris* shrimp, [Pond et al., 1997](#),  
477 and adult *Rimicaris* shrimp, [Streit et al., 2015](#)) is that EPA and DHA are considered as markers of a  
478 photosynthetically-derived diet, of planktonic origin ([Kelly & Scheibling 2012](#), [Parrish, 2013](#)).  
479 Therefore, they are rare in the hydrothermal vent food web, which is based on bacterial  
480 chemosynthesis ([Colaço et al., 2007](#)). The higher levels observed in our experimental specimens  
481 likely correspond to the EPA and DHA enrichment of the diet proposed in captivity (46 and 23%  
482 respectively, see **Table 1**). The two other major FA that did not show significant variation between  
483 our experiments are the SFA C16:0 (15.98 %), and the MUFA C18:1 n-9 (10.61 %). Such levels of  
484 these FA are commonly observed in marine organisms ([Kelly & Scheibling 2012](#), [Parrish, 2013](#)),  
485 including vent fauna ([Colaço et al., 2007](#)).

486

487 Finally, only one major FA showed a significant variation, the MUFA C18:1n-7 (vaccenic  
488 acid), reaching 23.12% +/- 1.54 for natural pressure acclimation, and 13.14% +/- 2.03 for  
489 atmospheric pressure acclimation (**Table 1**). In trophic studies, this FA is a common dietary tracer  
490 (Kelly & Scheibling 2012, Parrish, 2013) and for example the ratio C18:1n-9/C18:1n-7 may be  
491 used to discriminate carnivory from detritivory in deep-sea fauna (Drazen et al., 2008). At deep-sea  
492 vents, vaccenic acid appears as a major component in several species (Streit et al., 2015, Colaço et  
493 al., 2007), including Mirocaris (in the 6 to 21 % range, Pond et al., 1997). In the wild, these high  
494 levels reflect the importance of food sources of bacterial origin, in the chemosynthetically-based  
495 vent trophic web (Kelly & Scheibling 2012). In the present study, shrimps were in captivity and fed  
496 with industrial food pellets, which contain a significant amount of C18:1n-7 (about 6%, **Table 1**).  
497 Whether vaccenic acid in Mirocaris is here of endogenous or exogenous origin remains to be  
498 determined. However, given that identical diet was provided in both experiments (AP and BOX),  
499 the observed variation can not be explained by a diet effect.

500 In bacteria, C18:1n-7 is common and strongly involved in the homeoviscous response to  
501 temperature, with an increased biosynthesis facing cold temperatures, resulting in lower membrane  
502 order to counteract the ordering effect of cold temperatures (reviews in Zhang & Rock, 2008,  
503 Hulbert et al., 2014, Ernst et al., 2016). Recent studies demonstrated that for *E. coli*, C18:1n-7  
504 played a "disordering" role, and was the major MUFA involved in the regulation of membrane  
505 order of this bacteria (Budin et al., 2018). Regarding a homeoviscous response to pressure, an  
506 increased level of C18:1n-7 was also observed in response to increasing cultivation pressure, in the  
507 case of a deep-sea bacteria (Allen et al., 1999). Accordingly, variations in the level of this  
508 unsaturated FA impact membrane order, lower levels meaning higher order ("rigidifying effect"),  
509 and vice-versa. The important variation of vaccenic acid observed in our experiment is consistent  
510 with a homeoviscous response: exposed to atmospheric pressure, the shrimps displayed lower levels  
511 of C18:1n-7, in order to counteract the disordering ("fluidizing") effect of lower pressure. We  
512 therefore propose the existence of a homeoviscous response in the animals studied here, although  
513 further measurements of membrane order and composition in our experimental conditions are  
514 necessary for a complete demonstration.

515 Finally, it is interesting to note that C18:1n-7 (vaccenic acid) belongs to the same  
516 biosynthetic pathway as the FA C16:1n-7 (Kelly & Scheibling 2012) observed at greater levels in  
517 deep nematods (Van Campenhout et al., 2016) and fishes (Radnaeva et al., 2017), with respect to  
518 shallower species. Whether this pathway (desaturation of C16:0 towards C16:1n-7, followed by  
519 elongation towards C18:1n-7) was shut down following acclimation to atmospheric pressure (AP

520 samples), or whether C18:1n-7 was selectively accumulated through diet at natural pressure (BOX  
521 samples), remains to be investigated.

### 522 *The global response in FA composition*

523 The decrease in vaccenic acid levels observed upon acclimation to atmospheric pressure is  
524 likely responsible alone for the apparently lower levels of MUFA (**Fig. 2, Table 1**). This decrease  
525 occurred along with increases of several other fatty acids: an increase in SFA levels likely relates to  
526 the significant increases of C14:0, C18:0, and to a lesser extent C17:0 (**Table 1, Fig. 2**). These  
527 significant MUFA and SFA variations probably contribute to the apparently higher saturation ratio  
528 observed for atmospheric-pressure-acclimated shrimps (AP samples), and are also consistent with a  
529 homeoviscous response. Inversely, the situation seems unclear regarding PUFA variations: while  
530 several PUFA species clearly showed a marked up-regulation upon acclimation to atmospheric  
531 pressure (namely C18:3n-6, C18:3n-3, C18:4, and to a lesser extent C16:2, C20:2, and C20:4n-6,  
532 **Table 1**), these molecules nevertheless accounted for rather small proportions in the global  
533 compositions (less than 1%), and displayed even smaller variations, as opposed to the ca. 10%  
534 variation observed for vaccenic acid. At a global level furthermore (**Fig. 2**), there was no apparent  
535 variation regarding either PUFA global levels (figure 3), or unsaturation index (265.7 +/- 24.7 vs.  
536 284.7 +/- 15.9).

537 Most studies of lipid composition variations in response to increasing pressure and/or depth  
538 have emphasized a modulation of MUFA vs. SFA levels (increase vs. decrease), while the  
539 implication of PUFA variations is not clear. In the case of bacteria, some studies showed an  
540 involvement of PUFA (enhanced biosynthesis under increasing pressure, [DeLong & Yayanos, 1986](#),  
541 [Wang et al., 2014](#)), while others did not ([DeLong & Yayanos, 1985](#), [Allen et al., 1999](#), [Grossi et al.,](#)  
542 [2010](#)). The situation also remains to be clarified regarding animals. In a study on antarctic copepods  
543 sampled in the wild at increasing depths (from the surface down to approximately 1000m), [Pond et](#)  
544 [al. \(2014\)](#) observed a significant decrease of SFA (consistent with our results) in the form of C16:0  
545 and C18:0, but no specific variations of MUFA were mentioned (unlike our results). These studies  
546 also found a significant increase of the PUFA DHA (C22:6 n-3) with depth (not observed in our  
547 study), which they proposed to be consistent with homeoviscous adaptation theory. Another study  
548 on different fish species sampled from the surface down to 1600 m depth in lake Baïkal lead to  
549 decreased levels of SFA, and increased levels of MUFA (both consistent with our results, and with  
550 a homeoviscous response, but here mainly due to C16:1n-7 and C18:1n-9 regarding MUFAs), yet  
551 another trend for PUFA (decrease, including DHA), in deeper species ([Radnaeva et al., 2017](#)).  
552 Another study comparing shallow-water and deep-sea nematod species (1300 m depth) lead to a  
553 similar trend, regarding SFA (decrease), and MUFA (increase, C16:1n-7 in particular), but here

554 accompanied by an increase in PUFA levels (EPA and DHA in particular) for deeper species (Van  
555 Campenhout et al., 2016). PUFA may help regulate membrane order, as suggested from *in vitro*  
556 experiments (Guschina & Harwood 2006), however their role in that respect remains to be clarified  
557 when dealing with whole animals sampled from the wild, with reduced knowledge on the  
558 environmental and nutritional histories, as opposed to laboratory experiments. In our laboratory  
559 study however, samples of the same species, fed for several months on identical diet, did not appear  
560 to use PUFAs to adjust to experimental conditions.

561 Finally, the average chain length (ACL) calculated for each FA composition (BOX vs. AP  
562 samples) remained unchanged (18.65 +/- 0.19 for BOX shrimps, and 18.68 +/- 0.13). Shifts in ACL  
563 have been observed as a possible response to membrane order perturbation, following  
564 environmental stress in bacteria (review in Yoon et al., 2015). Such a response does not seem to  
565 occur in our study.

### 566 ***Variations in complex lipid composition***

567 As stated by Ernst et al. (2016), until now most studies have characterized the homeoviscous  
568 response with a specific focus on the acyl chain composition, involving their degree of saturation  
569 (relative compositions in terms of SFA MUFA PUFA). However, as a result of the development of  
570 quantitative lipidomics technology, an increasing number of studies emphasize the importance of  
571 complex lipidome variations of organisms facing changes in their thermal environment. To our  
572 knowledge, the complex lipid composition of organisms experiencing pressure variation has little  
573 been investigated so far, and hypotheses on lipid composition adjustments according to pressure are  
574 scarce. In this study, we examined the compositions in PC, PE, SM and sterols of our two shrimp  
575 pressure groups.

576 Cholesterol is known to regulate membrane order depending on its physical phase. It is  
577 supposed to favor the order in the liquid phase (Urbina et al., 1995, Ayee & Levitan, 2016). But few  
578 studies report a correlation between pressure and cholesterol content in metazoans. Vettier et al.  
579 (2006) compared the cholesterol levels in the yellow and the silver stages of the European eel. The  
580 yellow eel is the pre-migratory stage that lives in shallow fresh-waters. It never encounters high  
581 pressure, contrary to the silver stage, which can be encountered at close to 1000 m depth during its  
582 spawning migration (Righton et al., 2016). They suggested that the three-fold lower cholesterol  
583 levels in silver eels could contribute in maintaining membrane order during their migration at depth.  
584 Similarly, reduced proportions of cholesterol found in phocids were believed to help them cope  
585 with high hydrostatic pressure while deep-diving (Williams et al., 2001). In our results, sterols (and  
586 particularly cholesterol) were among the most discriminating complex lipids between the two

587 pressure groups (**Fig. 4**), representing on average 3.21 % of the complex lipids in the AP shrimps,  
588 and 1.69 % in the BOX shrimps (**Fig. 3**).

589 As for sterols, we observed that AP shrimps tend to have higher proportions of  
590 sphingomyelins than BOX shrimps on average (See **Figs 3-4**). Moreover, 9 out of the 15 detected  
591 sphingomyelins were important to discriminate the two groups, and all of them were dominant in  
592 AP shrimps (**Fig. 4**). This suggests that pressure also influences the SM levels. Finally, an  
593 intriguing discovery is that 8 discriminating ether PE were dominant in AP shrimps, while most of  
594 their corresponding ester versions (6) were dominant in BOX shrimps. It is interesting to note that  
595 in another study on yeast ether lipid deficient mutants ([Shi et al., 2016](#)), a reduction in C18:1n-7  
596 levels was observed, suggesting that production of these compounds were regulated by the same  
597 mechanisms, in order to regulate membrane order. Also, the increase of ether lipids levels in  
598 response to increasing temperature has long been reported, and their role in the homeoviscous  
599 response was suggested to contribute to thermal compensation of membrane function ([Hazel &  
600 Williams, 1990](#)).

601

602 Put together, cholesterol and sphingomyelins are known to be important components of  
603 ordered lipid domains originally named "rafts" ([Simons & Ikonen, 1997](#), [Foster et al., 2003](#)). More  
604 recently ether glycerophospholipids (PE species in particular), which also increase membrane order  
605 ([Demediuk et al., 1983](#)), were also shown to be components of these domains and were believed to  
606 stabilize these physical structures ([Wallner & Schmidz 2011](#), [Braverman & Moser, 2012](#);  
607 [Tulodzekia et al., 2016](#), [Koivuniemi, 2017](#), [Dean & Lodhi, 2018](#)). Our data would thus be  
608 consistent with the hypothesis of a modulation of the organization of these domains through specific  
609 changes in the lipid signature of AP shrimps. This particular phenotype would possibly increase  
610 membrane stability to maintain appropriate traffic and cell signaling, in response to the disordering  
611 effect of lower pressure. Indeed, these domains were also known to be involved in many other  
612 biological processes, such as signaling processes or vesicle traffic ([Foster et al., 2003](#)). More than  
613 just contributing to structural stability, modifications in lipid fingerprints in AP shrimps might be  
614 involved in regulating several cell functions impaired by the acclimation to atmospheric pressure. A  
615 combined lipid/protein study, to further investigate the proteins, the regulation pathways commonly  
616 associated with these domains and the variation of their relative gene expressions would allow to  
617 test this hypothesis.

618

619 **Conclusion**

620 This study provided new insights on the lipidomic response of organisms following  
621 acclimation to different pressures, and showed variations in lipid compositions, both at the fatty  
622 acids and the complex lipids levels. It is the very first time that this response has been studied in a  
623 deep-sea metazoan under long-term controlled conditions (pressure, temperature, food...), thanks to  
624 the use of rearing equipments at different pressures, a unique opportunity provided by the  
625 Océanopolis aquarium. It is also the first study reporting complex lipid variations in response to  
626 pressure acclimation. One limitation of our study is that the lipidomes are not exhaustive since  
627 several other complex lipids were not quantified (phosphatidylinositols, phosphatidylserines, ...),  
628 meaning a possible bias in the observed variations. However, the clear response in terms of  
629 vaccenic acid (C18:1n-7) variation, a molecule known to undoubtedly influence membrane order,  
630 strongly suggests the existence of a homeoviscous response (i.e. a regulation of the membrane's  
631 physical state) of *Mirocaris fortunata* following acclimation to atmospheric pressure. Whether the  
632 accompanying complex lipid variations participate to a homeoviscous adaptation remains to be  
633 determined. Further studies combining exhaustive lipidome determination and physical  
634 measurements of membrane fluidity under different pressure conditions are needed to make the link  
635 between these observations and membrane order. A complementary approach in order to better  
636 understand the mechanisms underlying this lipidomic response would be to target the enzymes  
637 involved in the production of the compounds which quantities differ, as well as follow the  
638 expression levels of the corresponding genes.

639  
640 Finally, the study presented here compares the lipidomes of shrimps that have been  
641 maintained in captivity at different pressures, for several months. The observed lipidomic response  
642 undoubtedly illustrates an acclimation process, thereby demonstrating the phenotypic plasticity of  
643 these organisms regarding environmental variations. Further investigations should aim at evaluating  
644 the kinetics of this response, by measuring lipidome variations throughout the acclimation period.  
645 Bearing in mind that *Mirocaris fortunata* is found from 850 m to 4050 m depth, this would surely  
646 provide new knowledge on the capacity of these shrimps to distribute over such a large bathymetric  
647 range in the wild, and on their potential to undertake successful migrations towards yet unoccupied  
648 depths, in response to near-future climate changes.

649  
650

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