



HAL
open science

Exposure to low pH induces molecular level changes in the marine worm, *Platynereis dumerilii*

Janine Wäge, Adelaide Lerebours, Jörg D Hardege, Jeanette Rotchell

► To cite this version:

Janine Wäge, Adelaide Lerebours, Jörg D Hardege, Jeanette Rotchell. Exposure to low pH induces molecular level changes in the marine worm, *Platynereis dumerilii*. *Ecotoxicology and Environmental Safety*, 2016, 124, pp.105-110. 10.1016/j.ecoenv.2015.10.008 . hal-02155105

HAL Id: hal-02155105

<https://hal.science/hal-02155105v1>

Submitted on 13 Jun 2019

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
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

**Exposure to low pH induces molecular level changes in the marine
worm, *Platynereis dumerilii***

Janine Wäge¹, Adelaide Lerebours^{1,2}, Jörg D. Hardege¹, Jeanette M. Rotchell^{1§}

¹School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham
Road, Hull, HU6 7RX, United Kingdom

²Current address: School of Biological Sciences, Institute of Marine Sciences University of
Portsmouth, Langstone Harbour, Ferry Road, Eastney, Portsmouth, P04 9LY, United Kingdom

§Corresponding author:

j.rotchell@hull.ac.uk

Tel: +44 (0)1482 465333

Fax: +44 (0)1482 465458

20 ABSTRACT

21 Fossil fuel emissions and changes in net land use lead to an increase in atmospheric CO₂
22 concentration and a subsequent decrease of ocean pH. Noticeable effects on organisms'
23 calcification rate, shell structure and energy metabolism have been reported in the literature. To
24 date, little is known about the molecular mechanisms altered under low pH exposure, especially in
25 non-calcifying organisms. We used a suppression subtractive hybridisation (SSH) approach to
26 characterise differentially expressed genes isolated from *Platynereis dumerilii*, a non-calcifying
27 marine polychaeta species, kept at normal and low pH conditions. Several gene sequences have
28 been identified as differentially regulated. These are involved in processes previously considered as
29 indicators of environment change, such as energy metabolism (NADH dehydrogenase, 2-
30 oxoglutarate dehydrogenase, cytochrome c oxidase and ATP synthase subunit F), while others are
31 involved in cytoskeleton function (paramyosin and calponin) and immune defence (fucoselectin-1 and
32 paneth cell-specific alpha-defensin) processes. This is the first study of differential gene expression
33 in a non-calcifying, marine polychaete exposed to low pH seawater conditions and suggests that
34 mechanisms of impact may include additional pathways not previously identified as impacted by
35 low pH in other species.

36

37 *Keywords:*

38 pH, acidification, polychaete, gene expression

39

40 **Highlights**

41

42 • Differentially expressed genes were isolated from *P. dumerilii* kept at low pH conditions.

43

44 • Transcripts indicating change in energy metabolism, cytoskeleton and immunity were
45 observed.

46

47 • The results indicate that non-calcifying organisms are impacted at low pH exposures.

48

49

10 Introduction

51 The ocean and atmosphere exchange large amounts of carbon dioxide (CO₂) with the atmos-
52 pheric concentration of CO₂ increasing by 40% since pre-industrial times (IPCC, Climate Change
53 2013). For the past 200 years the oceans have absorbed approximately one-third of the CO₂ pro-
54 duced by human activity (Sabine et al., 2004) resulting in a measurable decrease of ocean pH. The
55 ocean's surface pH has dropped by 0.1 pH-units which is equivalent to a 26% increase in hydrogen
56 (H⁺) ion concentration (IPCC, Climate Change 2013) leading to a current average pH of ocean sur-
57 face water at around pH 8.1 (IGBP, Ocean Acidification Summary for Policymakers, 2013). Fur-
58 thermore, global ocean surface pH is predicted to decrease to ~7.8 by 2100 (IPCC, Climate Change
59 2013). For the last 800 000 years the CO₂ concentration in the atmosphere has thus remained rela-
60 tively stable (172-300 ppmv) (Lüthi et al., 2008) as compared to the predicted values expected for
61 the end of the century (1071 ppmv) (Plattner et al., 2001).

62 Rapid changes in water chemistry, termed ocean acidification, are predicted to lower the
63 stability and productivity of ecosystems by loss of biodiversity and keystone sensitive species
64 (Barry et al., 2011). The precise mechanisms are predicted to include many different physiological
65 processes such as photosynthesis, calcification, acid-base homeostasis, respiration / gas exchange
66 and metabolic rate (Melzner et al., 2009; Gattuso et al., 1999; Seibel and Walsh, 2003). To date, the
67 main focus of research has resided on calcifying organisms and molecular-level gene expression
68 impacts following short term low pH exposure (O'Donnell et al., 2010; Todgham and Hoffmann
69 2009; Stumpp et al., 2011; Martin et al., 2011; Parker et al., 2011; Tomanek et al., 2011; Wong et
70 al., 2011; Moya et al., 2012; Dineshram et al., 2012, 2013; Pespeni et al., 2013; Vidal-Dupiol et al.,
71 2013). Recent work also suggests that non-calcifying organisms, such as fish, may also be impacted
72 by acidification (Fabry et al., 2008; Wittmann and Pörtner, 2013). Even where taxa are able to tol-
73 erate high CO₂ water exposure, there are still apparent impacts in terms of altered energy require-
74 ments for basic biological functions, enzyme activities and metabolic shifts (Pörtner et al., 2000;
75 Seibel and Welsh, 2003; O'Donnell et al., 2010; Wong et al., 2011; Pespeni et al., 2013; Vidal-

76 Dupiol et al., 2013). Specifically, genes involved in energy metabolism and biomineralisation in
77 Japanese rice fish (*Oryzias latipes*), larval sea urchins (*Strongylocentrotus purpuratus*) and oyster
78 (*Crassostrea hongkongensis*) have been shown to be down regulated (Tseng et al., 2013; Todgham
79 and Hofmann, 2009; Dineshram et al., 2013), while genes for ion regulation and acid-base balance
80 pathways have been reported as up-regulated (O'Donnell et al., 2010) in organisms kept at low pH
81 relative to control pH exposure conditions. The data is inconsistent however in that up regulation of
82 energy metabolism genes have also been reported in sea urchin (*S. purpuratus*) and coral
83 (*Pocillopora damicornis*) studies under similar exposure conditions (Stumpp et al., 2011; Vidal-
84 Dupiol et al., 2013). Further biological processes that are potentially impacted by acidification in-
85 clude cell signalling and cytoskeleton assembly (Dineshram et al., 2012). An experimental exposure
86 using oysters (*C. virginica* and *C. hongkongensis*) exposed to high pCO₂ conditions for two weeks,
87 revealed up-regulation of several proteins involved in cytoskeleton and oxidative stress (Tomanek
88 et al., 2011; Dineshram et al., 2013). It is therefore clear that changes in seawater pH have an im-
89 pact on aquatic organisms, both calcifying and non-calcifying, and that multiple cellular processes
90 may be affected.

91 In order to understand the molecular-level impacts of acidic pH exposure on the polychaeta
92 species, *P. dumerilii*, differentially - regulated pH-specific mRNA transcripts from worms main-
93 tained at control (8.2) and acidified (7.8) pH levels were isolated and identified. *P. dumerilii* is a
94 model organism used for the study of molecular development, evolution, neurobiology, ecology and
95 toxicology (Hardege, 1999; Hutchinson et al., 1995; Tessmar-Raible and Arendt, 2003) and,
96 relevantly, can be found in, and perhaps adapted to, naturally occurring acidified habitats (Cigliano
97 et al., 2010; Calosi et al., 2013). The experimental conditions used in this study represent a pH
98 stress scenario predicted to occur in the surface ocean water column within the next century (Gat-
99 tuso and Lavigne, 2009).

100

101 **2. Methods**

102 **2.1. Animals and experimental exposure**

103 *P. dumerilii* (mean mass \pm SEM: pH 8.2 worms 1 week: 13 ± 1.55 mg, n=8; pH 7.8 worms 1 week:
104 10.94 ± 1.76 mg, n=8) from the laboratory culture supplied by EMBL Heidelberg (Germany) were
105 used for the experiment. Atokus worms, a sexually immature stage of the worm, were used to
106 reduce natural variation linked to maturation, reproduction and sex. Specimens were raised in
107 filtered natural seawater (~pH 8.2). For the low pH exposure regime, 8 worms were kept in closed
108 plastic containers (2000 cm³) with approximately 800 ml filtered natural seawater of pH 7.8
109 (salinity 35 ppt) at a light regime of 16 hrs light/ 8 hrs dark at 18 °C simulating summer conditions.
110 A further 8 worms were kept at pH 8.2 and in the same experimental conditions as for controls. A
111 complete water change was conducted each day. Hydrochloric acid (1M) and sodium hydroxide
112 (1M) were used to maintain pH levels. Although not identical to the seawater carbonate chemistry
113 changes expected for 2100, the procedure is based on the Le Chatelier's principle and the carbon
114 equilibrium system. Adjusting pH with CO₂ or mineral acid (in a closed system) leads to the same
115 partial pressure of CO₂ and the same CO₂ concentration (Gattuso et al., 2010). After 7 days, chosen
116 to reflect an acclimation time point used in a number of ocean acidification studies (Tomenek et al.,
117 2011; Parker et al., 2011; Moya et al., 2012), each single worm was immediately immersed in
118 800 μ l of RNALater solution (Sigma-Aldrich Company Ltd., Gillingham, U.K.) and stored at -80°C
119 until molecular analysis.

120

121 **2.2. Total RNA isolation**

122 Total RNA was extracted from individual worms kept at normal and low pH using High Pure RNA
123 Tissue (Roche, Burgess Hill, U.K.) reagents using the protocol described in the manufacturer's
124 procedures. The RNA concentration was measured using a Qubit[®] Fluorometer (Life Technologies,
125 Paisley, U.K.) and the integrity of the RNA checked using a standard ethidium bromide stained 1%
126 formaldehyde agarose gel.

128 **2.3. Suppressive Subtractive Hybridization (SSH), sub-cloning and sequencing**

129 The SSH technique was used to isolate and enrich differentially expressed genes between *P.*
130 *dumerilii* kept at normal and low pH conditions. Equal amounts of RNA were pooled from each
131 worm (n=8 worms/group), with a total of 2.5 µg RNA for each pool. cDNA was synthesised using
132 the SMARTer™ PCR cDNA Synthesis Kit reagents and manufacturer's protocol (Clontech, Saint-
133 Germain-en-Laye, France). The forward and reverse subtracted libraries were produced using PCR-
134 Select cDNA Subtraction reagents (Clontech, Saint-Germain-en-Laye, France) according to the
135 manufacturer's protocol. The differential PCR products generated by SSH were inserted in a
136 pCR^R2.1 linearized vector (Life Technologies, Paisley, U.K.) and the constructs were transformed
137 into competent DH10B-T *E.coli* (Life Technologies, Paisley, U.K.). Two hundred randomly
138 selected colonies from each subtracted library were inoculated in LB broth and screened by PCR for
139 inserts using vector-based primers. Fifty two clones were sent for commercial sequencing
140 (Macrogen Europe, Amsterdam, Netherlands). Sequence identities were obtained by BLAST
141 searches against the NCBI nucleic acid and protein databases as well as the PLATYpopsys database
142 (<http://hydra.cos.uni-heidelberg.de/pops/>). Using the BLAST search results, sequence reads with an
143 *E*-value <10⁻⁵ were discarded.

144 **2.4. Quantitative RT-PCR (qRT-PCR) validation**

145 *Target* mRNAs, identified using SSH, were selected for validation using qRT-PCR according to
146 MIQE guidelines (Bustin et al., 2009). Total RNA was isolated from 8 individual worm tissues per
147 exposure group using High Pure RNA Tissue Kit reagents (Roche, Burgess Hill, U.K.) and treated
148 with RNA-free DNase I (Qiagen, Manchester, U.K.). Template RNA was then removed using
149 RNase H enzyme and 10 × buffer (Thermo Fisher Scientific, Loughborough, U.K.) with a 45 min
150 incubation at 37°C. The RNA concentrations were measured with the Quant-iT RNA assay kit and
151 Qubit[®] fluorometer (Life Technologies, Paisley, U.K.). Reverse transcription of 140 ng of total

152 RNA from each individual worm sample (based on the typical yield from each RNA extraction)
153 was carried out using SuperScript VILO cDNA Synthesis reagents (Life Technologies, Paisley,
154 U.K.) and following the manufacturer's instructions. qRT-PCR reactions were performed in
155 duplicate, in a final volume of 20 μ l containing 10 μ l of qPCR Fast Start SYBR Green Master Rox
156 (Roche, Burgess Hill, U.K.). 1 μ l of cDNA and the corresponding primers (see concentrations used
157 in Table 1) were used. Amplification were performed in a CFX96 Real Time PCR Detection
158 System (Bio-Rad, Hemel Hempstead, U.K.) and using the following conditions: after 2 min at 95°C,
159 45 cycles at 95°C for 10 sec, 60°C for 1 min and 72°C for 1 min. To generate the melt curve, a
160 heating step gradient from 5 sec at 60°C to 5 sec at 95°C, was added to the end of the PCR run. For
161 each of the *target* mRNAs, the melting curve, gel picture and sequences were analysed in order to
162 verify the specificity of the amplified products. The amplification efficiency of each primer pair
163 was calculated using a ten times dilution series of cDNA. Additionally, the crossing point (Cp) was
164 detected for each *target* mRNA and normalised to the reference genes *α -tubulin (Tub)*, and *18S*
165 *rRNA (18S)* (Zheng et al., 2011; Won et al., 2011).

166

167 **2.5 Statistical analyses**

168 Statistical analyses were carried out using GraphPad InStat v3 (GraphPad Software Inc., La Jolla,
169 U.S.A.). All data were tested for normality of residuals and the homogeneity of variance.
170 Significance for relative gene expression was tested using an unpaired *t*-test. Outliers were
171 identified, and removed, if they differed by more than twice the standard deviation of the mean. For
172 *paramyosin* (pH 8.2) one value was identified as an outlier (as defined by the MIQE guidelines,
173 Bustin et al., 2009) and excluded from the statistical analysis. Statistical significance was accepted
174 at $p < 0.05$.

175

176

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size (bp)	Primer concentration (nM)
<i>18S rRNA</i>	GCGCATTTATCAGCACAAAGA	CTTGGATGTGGTAGCCGTTT	239	50
<i>α-Tub</i>	CTTCAAGGTCGGCATCAACT	TGGCAGTGGTATTGCTCAAC	101	100
<i>Calponin</i>	GGAGCCAGTGTGCTTGGT	AGCCTGTCCAGACTTGTCCA	126	100
<i>Paramysin</i>	AGAACGCTGAGGGTGAATTG	CGAGCTGGAGCCTGTCCGCA	183	80
<i>Cytochrome c oxidase</i>	GCGCAGATGTTTCGTATGCTA	GAGCCTACTCGGCATCTGTC	197	100
<i>NADH</i>	CGAACCGGATTATGGCTTTG	GGGAATTTGTCCCGTCTGCA	147	100

177

178 **Table 1. Target and reference gene primer sequences, amplicon sizes and primer**
179 **concentrations used for qPCR.**

180

181 **3. Results**

182 **3.1. SSH analysis**

183 A total of fifty-two differentially expressed mRNA sequences were isolated and then compared
184 with sequences in the NCBI GenBank database and PLATYpopsys database ([http://hydra.cos.uni-](http://hydra.cos.uni-heidelberg.de/pps/styled-2/)
185 [heidelberg.de/pps/styled-2/](http://hydra.cos.uni-heidelberg.de/pps/styled-2/)) using the blastn and blastx algorithms. 32% of the sequences libraries
186 matched to genes from different organisms, predominantly invertebrates (Table 2). The remaining
187 sequences showed either similarity to unidentified hypothetical proteins or showed no similarity
188 with the sequences available on the database.

189

190

191

192

193

194

195

196

Clone accession no.	Gene identity	Length (bp)	Homolog species	E-value	GenBank accession no.	Functional category
Up-regulated in pH 7.8:						
JZ820677	Paramyosin*	380	<i>Mytilus galloprovincialis</i>	0.0	O96064.1	Cytoskeleton
	Calponin*	50	<i>Echinococcus granulosus</i>	$4E^{-143}$	CDJ18009.1	Cytoskeleton
KP640621	ribosomal protein L34 (Rpl34)	230	<i>Cerebratulus lacteus</i>	$1E^{-15}$	KJ526218.1	Protein synthesis
JZ820678	DNA replication complex GINS protein PSF3	765	<i>Saccoglossus kowalevskii</i>	$8E^{-13}$	NM_001184840.1	DNA replication
Down-regulated in pH 7.8:						
JZ820679	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7-like	292	<i>Ceratitidis capitata</i>	$3E^{-7}$	XM_004520945.1	Energy metabolism
JZ820680	Cytochrome c oxidase subunit 6A	489	<i>Poecilia formosa</i>	$4E^{-14}$	XM_007557010.1	Energy metabolism
JZ820681	2-Oxoglutarate dehydrogenase*	316	<i>Pseudopodoces humilis</i>	0.0	XP_005532951.1	Energy metabolism
JZ820682	ATP synthase F chain	350	<i>Ixodes scapularis</i>	$4E^{-6}$	XM_002399310.1	Energy metabolism
KP640622	16S ribosomal RNA	500	<i>Hediste diadroma</i>	$8E^{-150}$	AB703100.1	Protein synthesis
JZ820683	Serine protease 55*	168	<i>Pteropus alecto</i>	$2E^{-22}$	XP_006906396.1	Hydrolysis of peptide bonds
JZ820684	Ferritin	571	<i>Perinereis aibuhitensis</i>	$1E^{-173}$	KJ784305.1	Iron storage
JZ820685	WNK lysine deficient protein kinase 1 (WNK1)	234	<i>H. sapiens</i>	$3E^{-05}$	NG_007984.2	Ion transport
JZ820686	Calcium-binding and coiled-coil domain-containing protein 2*	363	<i>Peromyscus maniculatus bairdii</i>	$1E^{-6}$	XP_006971971.1	
JZ820687	IQ and ubiquitin-like domain-containing protein	711	<i>S. purpuratus</i>	$7E^{-64}$	XM_789342.3	Predicted: cilia formation
JZ820688	fucosyltransferase-1	323	<i>Anguilla japonica</i>	$1E^{-05}$	AB037867.1	Immune defense
JZ820689	Paneth cell-specific alpha-defensin	366	<i>Equus caballus</i>	$5E^{-5}$	NM_001166074.1	Host defence

197

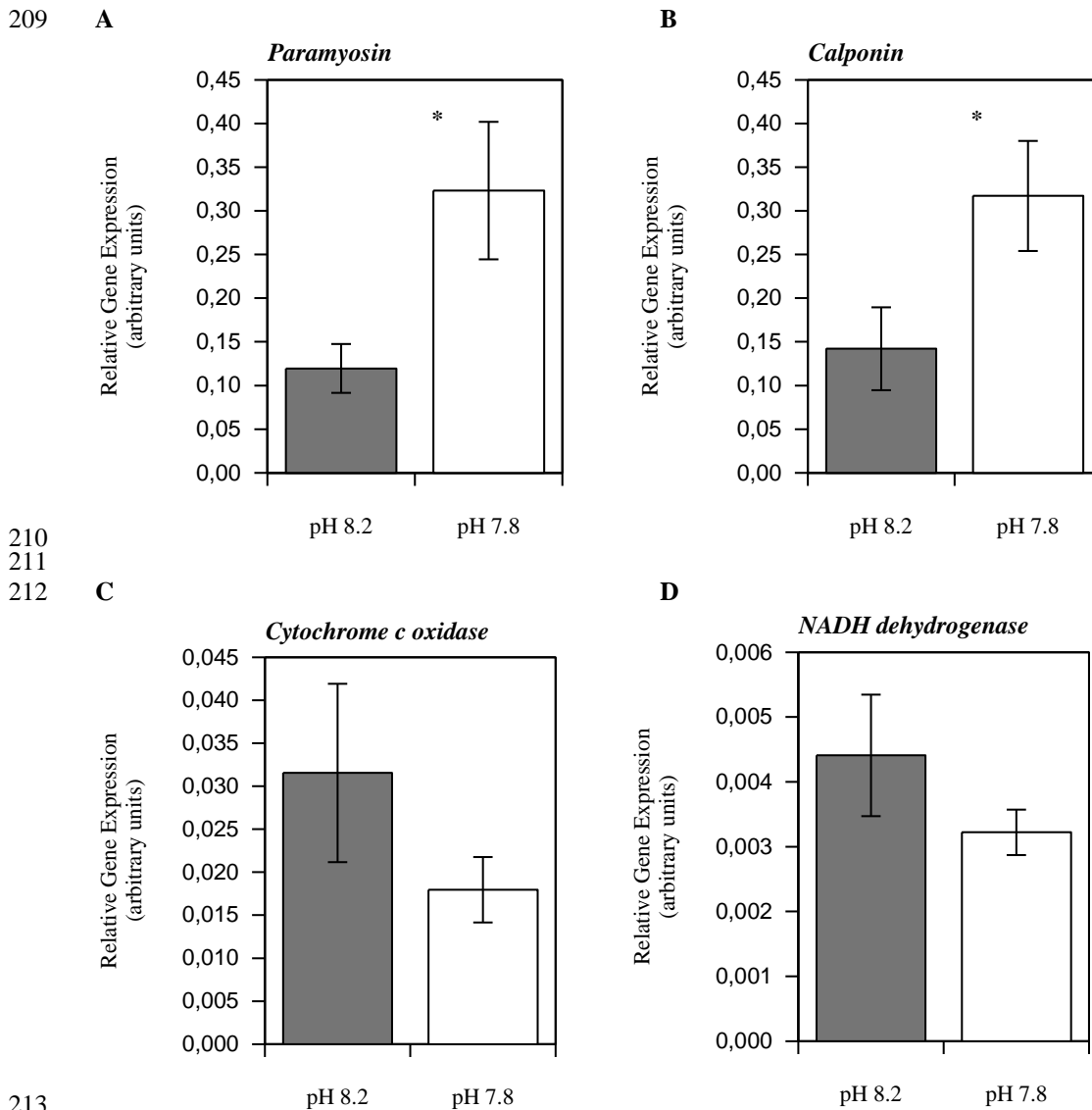
198 **Table 2 - Differentially expressed (subtracted) mRNAs in *P. dumerilii* maintained at normal**
199 **or low pH conditions.** * indicates hits with PLATYpopsys only and corresponding E-value
200 represents the re-blast result of the matched sequence.

201

202 3.2. Validation of differentially expressed mRNA transcripts

203 Four *target* mRNAs were selected to validate the SSH differential transcription results by qRT-PCR
204 (Figure 1). *Calponin* and *paramyosin*, were significantly ($p=0.0440$; $p=0.0386$) up-regulated in
205 worms kept at low pH compared with worms kept at normal pH (Figure 1A-1B). *Cytochrome c*

206 *oxidase* and *NADH dehydrogenase* were down-regulated in worms kept at pH 7.8 compared with
207 worms kept at pH 8.2 (Figure 1C-1D), though the results were not significant ($p=0.2615$;
208 $p=0.2757$).



213 **Figure 1.** qPCR analysis of selected transcripts identified by SSH as differentially expressed in
214 worms maintained at low pH seawater conditions relative to normal pH conditions. $n=8$, (with the
215 exception of $n=7$ for paramyosin at pH 8.2), $*p<0.05$.

217

218 4. Discussion

219 Using the SSH approach we generated libraries enriched for genes that vary between normal pH
220 (pH 8.2) and simulated acidification (of pH 7.8) conditions from the marine polychaete worm *P.*

221 *dumerilii*. In the subtractions reported here two separate libraries were constructed using: a) cDNA
222 from worms exposed to normal pH for one week as driver (reverse library), and b) cDNA from
223 worms exposed to acidified pH conditions for one week as tester (forward library). From these li-
224 braries, 33% of the sequences obtained were identified, which is comparable to similar studies using
225 invertebrates (6–44%, Boutet et al., 2008; Craft et al., 2010; Ciocan et al., 2011; Ciocan et al.,
226 2012).

227 Several mRNA transcripts were identified and validated as up regulated in *P. dumerilii* kept
228 under low pH seawater conditions (Table 2; Figure 1). Amongst these transcripts were proteins
229 involved in metabolism, cytoskeleton function (paramyosin and calponin), protein synthesis
230 (Ribosomal protein L34 and 16S ribosomal RNA), and DNA replication (GINS protein Psf3)
231 functions. For calponin, the sequence isolated is short (at 50 bp) and, as such, only a tentative
232 identity can be assumed. In every case, further work is merited that would ideally seek full sequence
233 lengths and additional functional protein studies.

234 Changes to metabolic rates have often been observed in organisms under stress conditions.
235 Down regulation of *2-oxoglutarate dehydrogenase*, *NADH-dehydrogenase*, *cytochrome c oxidase*,
236 and *ATP synthase*, collectively representing genes involved in the Krebs cycle and the
237 mitochondrial electron transport chain, indicate a reduction in oxidative metabolism and capacity to
238 generate ATP and NADPH. Similar down regulation of such metabolism-related genes has been
239 reported in coral (*A. millepora*) and sea urchin (*S. purpuratus*) larvae after 28 days high pCO₂
240 exposure and 40 hours post hatching with medium level CO₂ exposure treatment respectively
241 (Kaniewksa et al., 2012; Todgham and Hoffmann, 2009). In a study using the scleractinian coral, *P.*
242 *damicornis*, *2-oxoglutarate dehydrogenase* was observed to be slightly up regulated at pH 7.8 and
243 subsequently down-regulated at pH 7.4 (Vidal-Dupiol et al., 2013). Also, in contrast to our findings,
244 Vidal-Dupiol et al. (2013) reported an enrichment of genes involved in oxidative phosphorylation
245 (among them NADH dehydrogenase) at pH 7.8 and 7.2 in coral. Oyster (*C. hongkongensis*) and sea
246 urchin (*Lytechinus pictus*) larvae behaved similarly to the worms however, with an apparent down

247 regulation of *cytochrome c oxidase* and *NADH dehydrogenase* at low pH (Dineshram et al., 2013;
248 O'Donnell et al., 2010).

249 Of the cytoskeleton function transcripts identified, *paramyosin* is primarily a muscle thick
250 filament protein that is common in invertebrate species (Hooper et al., 2008). Unusually, paramy-
251 osin has roles in both muscle contraction as well as immunoregulation (Gobert et al., 2005). Nema-
252 tode muscles consist of a contractile part made up of fibres with myosin, actin, tropomyosin and
253 paramyosin, and a non-contractile part supplying energy requirements (Hooper et al., 2008). Re-
254 lated to this is another of the isolated transcripts, *calponin*. Calponin (calcium- and calmodulin-
255 binding troponin T-like protein) is a calcium-binding protein that inhibits ATPase activity of myo-
256 sin in smooth muscle (Castagnone-Sereno et al., 2001). An up regulation of *paramyosin* and
257 *calponin* transcripts following acidic pH seawater exposure is consistent with other investigations
258 reported in the literature. Adult oysters (*C. virginica*) and oyster larvae (*C. hongkongensis*) exposed
259 to pH 7.9 – pH 7.5 displayed increased calponin-2 and myosin (light chain) expression respectively
260 (Tomanek et al., 2011; Dineshram et al., 2013).

261 In addition to muscle microfilament upregulation, there was also an apparent increase in
262 protein synthesis related (ribosomal protein L34) and DNA replication (GINS protein Psf3)
263 processes (Table 2). Such changes are common following stress conditions and consistent with
264 findings for other invertebrate species, including oysters (*C. virginica*) exposed to low pH
265 (Tomanek et al., 2011). Specifically, Psf3, is a protein from the Psf family, involved in cell cycle,
266 and has been observed herein as up regulated in worms kept at low pH (7.8) similarly to the *Psf2*
267 transcript identified in sea urchin (*S. purpuratus*) larvae kept under a moderate level of acidification
268 conditions (Todgham and Hofmann, 2009). In contrast, the marine coccolithophore, *Emiliana*
269 *huxleyi*, responded to low pH, with a down-regulation in the chloroplastic 30S ribosomal protein S7
270 (Jones et al., 2013).

271 Down regulation of the immune-related transcripts *fuclectin* and *paneth cell-specific alpha*
272 *defensin* was observed in worms kept at low pH conditions (Table 1). Fuclectins are fucose bin-

273 ding proteins that have a pathogen recognition role in fish (Honda et al., 2000), while alpha
274 defensins are microbiocidal and cytotoxic peptides involved in host defence (Szyk et al., 2006).
275 Defensins have previously been isolated from invertebrate species such as the horseshoe crab
276 (*Tachypleus tridentatus*, Kawabata, 2010) and oyster (*C.gigas*, Rosa et al., 2011), but, to our
277 knowledge, this represents the first such isolation from a polychaete species (Smith et al., 2010).
278 Immunosuppression upon contaminant exposure is well documented for marine vertebrates (de
279 Swart et al., 1996). To what extent pH changes will similarly impact the immune system of
280 invertebrates is less extensively studied. Bibby et al. (2008) reported that several components of
281 mussel (*Mytilus edulis*) immune response, including suppressed phagocytosis, were modulated
282 following (32 day) exposure to acidified seawater. A similar trend in immune suppression has also
283 been reported in the star fish *Asterius rubens* (Henroth et al., 2011). Calcium ion concentrations
284 have been suggested as the mechanism by which acidification causes suppression of the immune
285 system (Bibby et al., 2008).

286 The other genes down regulated in worms exposed to low pH include (IQ ubiquitin-like
287 domain-containing protein (IQUB), ferritin and WNK1 (Table 1). IQUB is a putative cilia protein
288 (Lai et al., 2011) previously observed as down regulated in larvae hatched from gastropod egg mas-
289 ses that had been co-exposed to temperature and UVB stresses (Fischer and Phillips, 2014). Ferritin
290 stores iron and protects cells from iron-induced redox damage while also controlling its' release for
291 different enzymatic reactions (Theil, 1987). In an investigation of the interactive effects of
292 acidification (using elevated CO₂ levels for a duration of 4 to 5 weeks) and metal exposure in
293 oysters (*C. virginica*), Goetze et al. (2014) reported pCO₂ potentiation of metal-induced ferritin ex-
294 pression. WNK1 down regulation in worms exposed to low pH is also interesting in that WNK1 in
295 vertebrates is a serine-threonine kinase expressed in kidneys that phosphorylates synaptotagmin 2,
296 leading to activation of the epithelial sodium channel and also stimulates endocytosis of ROMK1,
297 an ATP dependent potassium channel (Kahle et al., 2006). The observed differential expression of
298 the WNK1 gene is therefore interesting because no common sodium, calcium nor carbonate trans-

299 porter protein transcripts are among those differentially expressed in the worms kept at low pH in
300 this study, and as such WNK1 may represent an alternative mechanism of ion transport and balance
301 in this worm relative to other species.

302

303 **5. Conclusion**

304 Using an SSH transcriptomic approach, differentially expressed genes were identified in
305 worms kept a low pH seawater conditions. Such worms represent a non-calcifying marine species.
306 Particularly novel findings are differentially regulated transcripts involved in cytoskeleton processes
307 and the immune system. These findings demonstrate that non-calcifying organisms, as well as
308 calcifying organisms, are also at risk of potential future ocean acidification impacts.

309

310 **Conflict of Interest Statement**

311 None declared.

312

313 **Acknowledgements**

314 We would like to thank the Arendt Group (EMBL Heidelberg) for access to the
315 PLATYpopsys database. We would also like to thank Tomas A. Larsson (Department of Chemistry
316 & Molecular Biology, University of Gothenburg) for conducting the laboratory exposure
317 experiment.

318

319 **References**

- 320 Barry, J.P., Widdicombe, S., Hall-Spencer, J.M., 2011. Effects of ocean acidification on marine
321 biodiversity and ecosystem function, in: Gattuso, J.-P., Hansson, L. (Eds.), Ocean acidifica-
322 tion. Oxford University Press, New York, pp.192-209.
- 323 Bibby, R., Widdicombe, S., Parry, H., Spicer, J., Pipe, R., 2008. Effects of ocean acidification on
324 the immune response of the blue mussel *Mytilus edulis*. *Aquat. Biol.* 2, 67-74.

325 Boutet, I., Moraga, D., Marinovic, L., Obreque, J., Chavez-Crooker, P., 2008. Characterization of
326 reproduction-specific genes in a marine bivalve mollusc: influence of maturation stage and
327 sex on mRNA expression. *Gene* 407, 130-138.

328 Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan,
329 T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T. (2009). The MIQE guide-
330 lines: minimum information for publication of quantitative real-time PCR experiments. *Clin.*
331 *Chem.* 55, 611-622.

332 Calosi, P., Rastrick, S.P.S., Lombardi, C., de Guzman, H.J., Davidson, L., Jahnke, M., Giangrande,
333 A., Hardege, J.D., Schulze, A., Spicer, J.I., Gambi, M.C. 2013. Adaptation and
334 acclimatisation to ocean acidification in marine ectotherms: an *in situ* transplant experiment at
335 a shallow CO₂ vent system. *Phil. Trans. R. Soc. B.* 368, 20120444.

336 Castagnone-Sereno, P., Leroy, F., Abad, P. (2001). cDNA cloning and expression analysis of a cal-
337 ponin gene from the plant-parasitic nematode *Meloidogyne incognita*. *Mol. Biochem. Parsit.*
338 112, 149-152.

339 Cigliano, M., Gambi, M.C., Rodolfo-Metalpa, R., Patti, F.P., Hall-Spencer, J.M., 2010. Effects of
340 ocean acidification on invertebrate settlement at volcanic CO₂ vents. *Mar. Biol.* 157, 2489-
341 2502.

342 Ciocan, C.M., Cubero-Leon, E., Minier, C., Rotchell, J.M., 2011. Identification of reproduction-
343 specific genes associated with maturation and estrogen exposure in a marine bivalve *Mytilus*
344 *edulis*. *PloS One* 6, e22326.

345 Ciocan, C.M., Cubero-Leon, E., Peck, M.R., Langston, W.J., Pope, N., Minier, C., Rotchell, J.M.,
346 2012. Intersex in scrobicularia plana: Transcriptomic analysis reveals novel genes involved in
347 endocrine disruption. *Environ. Sci. Technol.* 46, 12936-12942.

348 Craft, J.A., Gilbert, J.A., Temperton, B., Dempsey, K.E., Ashelford, K., Tiwari, B., Hutchinson,
349 T.H., Chipman, J.K., 2010. Pyrosequencing of *Mytilus galloprovincialis* cDNAs: tissue
350 specific expression patterns. *PLoS One* 5, e8875.

351 De Swart, R.L., Ross, P.S., Vos, J.G., Osterhaus, A.D., 2006. Impaired immunity in harbour seals
352 (*Phoca vitulina*) exposed to bioaccumulated environmental contaminants: review of a long-
353 term feeding study. *Environ. Health Perspect.* 104, 823-828.

354 Dineshram, R., Wong, K.K.W., Xiao, S., Yu, Z., Qian, P.Y., Thiyagarajan, V., 2012. Analysis of
355 Pacific oyster larval proteome and its response to high-CO₂. *Mar. Pollut. Bull.* 64, 2160-2167.

356 Dineshram, R., Thiyagarajan, V., Lane, A., Ziniu, Y., Xiao, S., Leung, P.T.Y., 2013. Elevated CO₂
357 alters larval proteome and its phosphorylation status in the commercial oyster, *Crassostrea*
358 *hongkongensis*. *Mar. Biol.* 160, 2189-2205.

359 Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine
360 fauna and ecosystem processes. *ICES J. Mar. Sci.* 65, 414–432.

361 Fischer, J., Phillips, N.E., 2014. Carry-over effects of multiple stressors on benthic embryos are
362 mediated by larval exposure to elevated UVB and temperature. *Glob. Change. Biol.* 20, 2108-
363 2116.

364 Gattuso, J.-P., Allemand, D., Frankignoulle, M., 1999. Photosynthesis and calcification at cellular,
365 organismal and community levels in coral reefs: a review on interactions and control by car-
366 bonate chemistry. *Am. Zool.* 39, 160-183.

367 Gattuso, J.-P., Lavigne, H., 2009. Technical note: Approaches and software tools to investigate the
368 impact of ocean acidification. *Biogeosciences* 6, 2121-2133.

369 Gobert, G.N., McManus, D.P., 2005. Update on paramyosin in parasitic worms. *Parasitol. Int.* 54,
370 101-107.

371 Goetze, S., Matoo, O.B., Beniash, E., Saborowski, R., Sokolova, I.M., 2014. Interactive effects of
372 CO₂ and trace metals on the proteasome activity and cellular stress response of marine
373 bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. *Aquat. Toxicol.* 149, 65-82.

374 Hardege, J.D., 1999. Nereidid polychaetes as model organisms for marine chemical ecology.
375 *Hydrobiologia* 402, 145–161.

376 Henroth, B., Baden, S., Thorndyke, M., Dupont, S., 2011. Immune suppression of the echinoderm
377 *Asterias rubens* (L.) following long-term ocean acidification. *Aquat. Toxicol.* 103, 222-224.

378 Honda, S., Kashiwagi, M., Miyamoto, K., Takei, Y., Hirose, S., 2000. Multiplicity, structures, and
379 endocrine and exocrine natures of eel fucose-binding lectins. *J. Biol. Chem.* 275, 33151-
380 33157.

381 Hooper, S.L., Hobbs, K.H., Thuma, J.B., 2008. Invertebrate muscles: thin and thick filament struc-
382 ture; molecular basis of contraction and its regulation, catch and asynchronous muscle. *Prog.*
383 *Neurobiol.* 86, 72-127.

384 Hutchinson, T.H., Jha, A.N., Dixon, D.R., 1995. The polychaete *Platynereis dumerilii* (Audouin
385 and Milne-Edwards): a new species for assessing the hazardous potential of chemicals in the
386 marine environment. *Ecotox. Environ. Safe.* 31, 271–281.

387 IPCC, 2013. Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis.*
388 *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental*
389 *Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J.
390 Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (Eds.)]. Cambridge University Press,
391 Cambridge, United Kingdom and New York, NY, USA.

392 IGBP, IOC, SCOR, 2013. *Ocean Acidification Summary for Policymakers – Third Symposium on*
393 *the Ocean in a High-CO₂ World.* International Geosphere-Biosphere Programme, Stockholm,
394 Sweden.

395 Jones, B.M., Iglesias-Rodriguez, M.D., Skipp, P.J., Edwards, R.J., Greaves, M.J., Young, J.R.,
396 Elderfield, H., O'Connor, C.D., 2013. Responses of the *Emiliana huxleyi* proteome to ocean
397 acidification. *PloS One* 8, no. 4, e61868.

398 Kahle, K.T., Rinehart, J., Ring, A., Gimenez, I., Gamba, G., Hebert, S.C., Lifton, R.P., 2006. WNK
399 protein kinases modulate cellular Cl⁻ flux by altering the phosphorylation state of the Na-K-
400 Cl and K-Cl cotransporters. *Physiology* 21, 326-335.

401 Kaniewksa, P., Campbell, P.R., Kline, D.I., Rodriguez-Lanetty, M., Miller, D.J., Dove, S., Hoegh-
402 Guldborg, O., 2012. Major cellular and physiological impacts of ocean acidification on a reef
403 building coral. *PloS One* 7, no. 4, e34659.

404 Kawabata, S.I., 2010. Immunocompetent molecules and their response network in horseshoe crabs,
405 in: Söderhäll, K. (Eds.), *Invertebrate Immunity*. Springer, New York, pp. 122-136.

406 Lai, C.K., Gupta, N., Wen, X., Rangell, L., Chih, B., Peterson, A.S., Bazan, J.F., Li, L., Scales, S.J.,
407 2011. Functional characterization of putative cilia genes by high-content analysis. *Mol. Biol.*
408 *Cell*. 22, 1104-1119.

409 Lüthi, D., Le Floch, M., Bereiter, B., Blunier, T., Barnola, J.-M., Siegenthaler, U., Raynaud, D.,
410 Jouzel, J., Fischer, H., Kawamura, K., Stocker, T.F., 2008. High-resolution carbon dioxide
411 concentration record 650,000–800,000 years before present. *Nature* 453, 379-382.

412 Martin, S., Richier, S., Pedrotti, M.-L., Dupont, S., Castejon, C., Gerakis, Y., Kerros, M.-E.,
413 Oberhänsli, F., Teyssié, J.-L., Jeffree, R., Gattuso, J.-P., 2011. Early development and
414 molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO₂-
415 driven acidification. *J. Exp. Biol.* 214, 1357-1368.

416 Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C.,
417 Bleich, M., Pörtner, H.-O., 2009. Physiological basis for high CO₂ tolerance in marine
418 ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences Discus-*
419 *sions* 6, 2313-2331.

420 Moya, A., Huisman, L., Ball, E.E., Hayward, D.C., Grasso, L.C., Chua, C.M., Woo, H.N., Gattuso,
421 J.-P., Forêt, S., Miller, D.J., 2012. Whole transcriptome analysis of the coral *Acropora*
422 *millepora* reveals complex responses to CO₂-driven acidification during the initiation of
423 calcification. *Mol. Ecol.* 21, 2440-2454.

424 O'Donnell, M.J., Todgham, A.E., Sewell, M.A., Hammond, L.M., Ruggiero, K., Fangué, N.A.,
425 Zippay, M.L., Hofmann, G.E., 2010. Ocean acidification alters skeletogenesis and gene ex-
426 pression in larval sea urchins. *Mar. Ecol. Prog. Ser.* 398, 157-171.

427 Parker, L.M., Ross, P.M., Raftos, D., Thompson, E., O'Connor, W.A., 2011. The proteomic re-
428 sponse of larvae of the Sydney rock oyster, *Saccostrea glomerata* to elevated pCO₂. Aust.
429 Zool. 35, 1011-1023.

430 Pespeni, M.H., Sanford, E., Gaylord, B., Hill, T.M., Hosfelt, J.D., Jaris, H.K., LaVigne, M., Lenz,
431 E.A., Russell, A.D., Young, M.K., Palumbi, S.R., 2013. Evolutionary change during experi-
432 mental ocean acidification. Proc. Natl. Acad. Sci. U.S.A. 110, 6937–6942.

433 Plattner, G.-K., Joos, F., Stocker, T.F., Marchal, O., 2001. Feedback mechanisms and sensitivities
434 of ocean carbon uptake under global warming. Tellus B 53, 564-592.

435 Pörtner, H.O., Bock, C., Reipschlag, A., 2000. Modulation of the cost of pHi regulation during
436 metabolic depression: a (31) P-NMR study in invertebrate (*Sipunculus nudus*) isolated
437 muscle. J. Exp. Biol. 203, 2417-2428.

438 Rosa, R. D., Santini, A., Fievet, J., Bulet, P., Destoumieux-Garzón, D., & Bachère, E. (2011). Big
439 defensins, a diverse family of antimicrobial peptides that follows different patterns of expres-
440 sion in hemocytes of the oyster *Crassostrea gigas*. PLoS One, 6, e25594.

441 Sabine, C.L., Feely, R.A., Gruber, N., Key, R.M., Lee, K., Bullister, J.L., Wanninkhof, R., Wong,
442 C.S., Wallace, D.W.R., Tilbrook, B., Millero, F.J., Peng, T.-H., Kozyr, A., Ono, T., Rios,
443 A.F., 2004. The oceanic sink for anthropogenic CO₂. Science 305, 367-371.

444 Seibel, B.A., Walsh, P.J., 2003. Biological impacts of deep-sea carbon dioxide injection inferred
445 from indices of physiological performance. J. Exp. Biol. 206, 641-650.

446 Smith, V.J., Desbois, A.P., Dyrinda, E.A., 2010. Conventional and unconventional antimicrobials
447 from fish, marine invertebrates and micro-algae. Marine Drugs 8,1213-1262.

448 Stumpp, M., Dupont, S., Thorndyke, M.C., Melzner, F., 2011. CO₂ induced seawater acidification
449 impacts sea urchin larval development II: Gene expression patterns in pluteus larvae. Comp.
450 Biochem. Physiol. 160, 320-330.

451 Szyk, A., Wu, Z., Tucker, K., Yang, D., Lu, W., Lubkowski, J., 2006. Crystal structures of human
452 alpha-defensins HNP4, HD5, HD6. Protein Sci. 15, 2749-2760.

453 Tessmar-Raible, K., Arendt, D., 2003. Emerging systems: between vertebrates and arthropods, the
454 Lophotrochozoa. *Curr. Opin. Genet. Dev.* 13, 331-340.

455 Theil, E.C., 1987. Ferritin: structure, gene regulation, and cellular function in animals, plants and
456 microorganisms. *Ann. Rev. Biochem.* 56, 289-315.

457 Todgham, A.E., Hofmann, G.E., 2009. Transcriptomic response of sea urchin larvae
458 *Strongylocentrotus purpuratus* to CO₂-driven seawater acidification. *J. Exp. Biol.* 212, 2579-
459 2594.

460 Tomanek, L., Zuzow, M.J., Ivanina, A.V., Beniash, E., Sokolova, I.M., 2011. Proteomic response to
461 elevated PCO₂ level in eastern oysters, *Crassostrea virginica*: evidence for oxidative stress. *J.*
462 *Exp. Biol.* 214, 1836-1844.

463 Tseng, Y.C., Hu, M.Y., Stumpp, M., Lin, L.Y., Melzner, F., Hwang, P.P., 2013. CO₂-driven sea-
464 water acidification differentially affects development and molecular plasticity along life his-
465 tory of fish (*Oryzias latipes*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 165, 119-130.

466 Vidal-Dupiol, J., Zoccola, D., Tambutté, E., Grunau, C., Cosseau, C., Smith, K.M., Freitag, M.,
467 Dheilly, N.M., Allemand, D., Tambutté, S., 2013. Genes related to ion-transport and energy
468 production are upregulated in response to CO₂-driven pH decrease in corals: New insights
469 from transcriptome analysis. *PLoS ONE* 8, e58652; DOI:10.1371/journal.pone.0058652

470 Wittmann, C., Pörtner, H.-O., 2013. Sensitivities of extant animal taxa to ocean acidification. *Na-*
471 *ture Climate Change* 3, 995–1001.

472 Won, E.-J., Kim, R.-O., Rhee, J.-S., Park, G.S., Lee, J., Shin, K.-H., Lee, Y.-M., Lee, J.-S., 2011.
473 Response of glutathione *S*-transferase (*GST*) genes to cadmium exposure in the marine pollu-
474 tion indicator worm, *Perinereis nuntia*. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 154,
475 82-92.

476 Wong, K.K.W., Lane, A.C., Leung, P.T.Y., Thiyagarajan, V., 2011. Response of larval barnacle
477 proteome to CO₂-driven seawater acidification. *Comp. Biochem. Physiol.* 6, 310-321.

478 Zheng, S., Qiu, X., Chen, B., Yu, X., Lin, K., Bian, M., Liu, Z., Huang, H., Yu, W., 2011. Toxicity
479 evaluation of benzo[*a*]pyrene on the polychaete *Perinereis nuntia* using subtractive cDNA
480 libraries. *Aquat. Toxicol.* 105, 279-291.

481

482

483

484

485

486

487

488

489

490

491