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

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20 ABSTRACT

21 Fossil fuel emissions and changes in net land use lead to an increase in atmospheric CO<sub>2</sub>  
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.

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

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49

## 10 Introduction

51 The ocean and atmosphere exchange large amounts of carbon dioxide (CO<sub>2</sub>) with the atmos-  
52 pheric concentration of CO<sub>2</sub> 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 CO<sub>2</sub> 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<sup>+</sup>) 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 pCO<sub>2</sub> 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:  $13 \pm 1.55$  mg, n=8; pH 7.8 worms 1 week:  
104  $10.94 \pm 1.76$  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 ( $\sim$ pH 8.2). For the low pH exposure regime, 8 worms were kept in closed  
108 plastic containers ( $2000\text{ cm}^3$ ) 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 CO<sub>2</sub> or mineral acid (in a closed system) leads to the same  
115 partial pressure of CO<sub>2</sub> and the same CO<sub>2</sub> 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  $\mu$ 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<sup>®</sup> 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<sup>R</sup>2.1 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<sup>-5</sup> 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  $\mu$ l containing 10  $\mu$ l of qPCR Fast Start SYBR Green Master Rox  
156 (Roche, Burgess Hill, U.K.). 1  $\mu$ 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  *$\alpha$ -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  $p < 0.05$ .

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Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size (bp)	Primer concentration (nM)
<i>18S rRNA</i>	GCGCATTATCAGCACAAGA	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.

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

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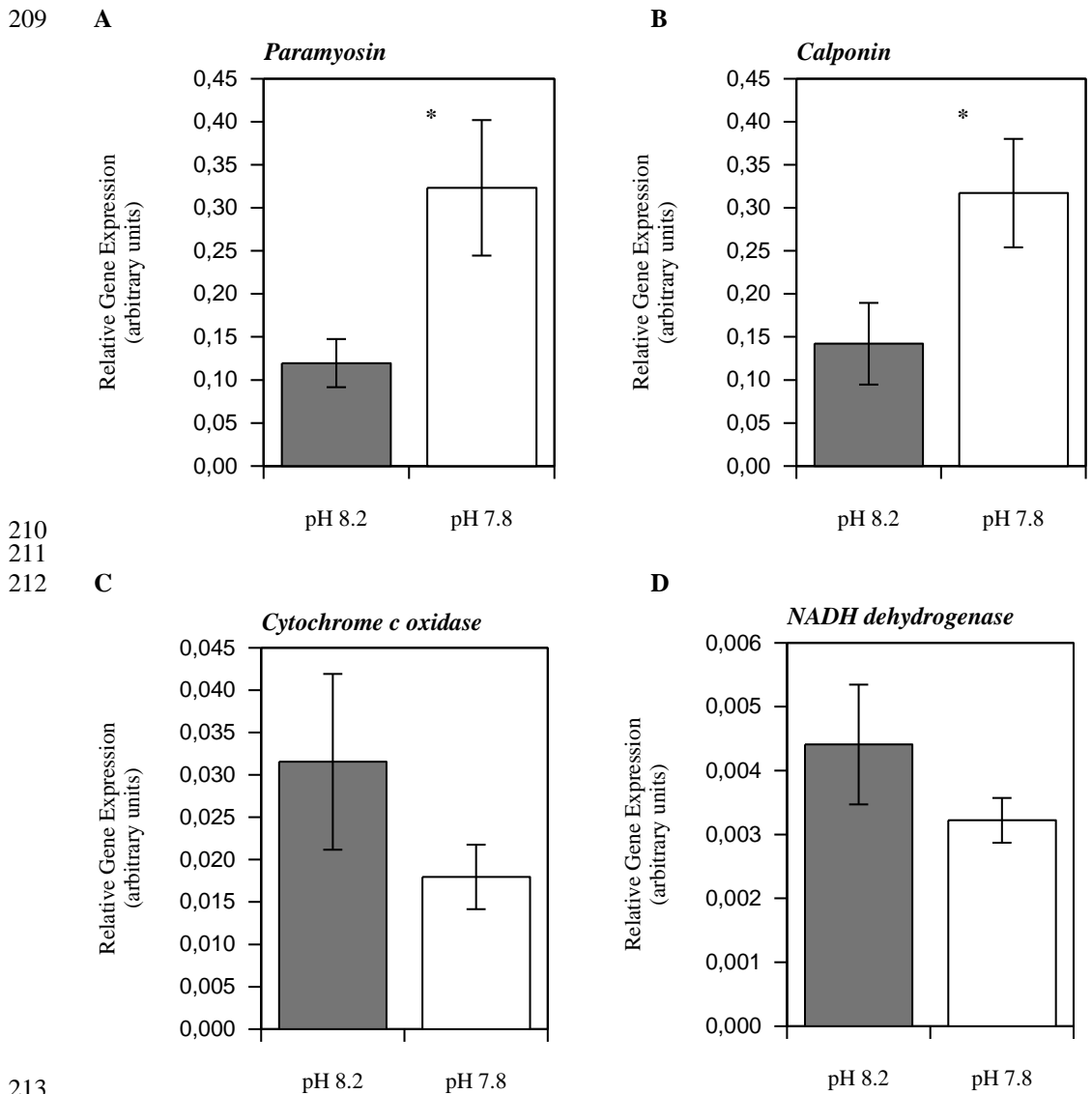
198 **Table 2 - Differentially expressed (subtracted) mRNAs in *P. dumerilii* maintained at normal**  
199 **or low pH conditions.** \* indicates hits with PLATYpopsys only and corresponding E-value  
200 represents the re-blast result of the matched sequence.

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 pCO<sub>2</sub>  
240 exposure and 40 hours post hatching with medium level CO<sub>2</sub> 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 CO<sub>2</sub> levels for a duration of 4 to 5 weeks) and metal exposure in  
293 oysters (*C. virginica*), Goetze et al. (2014) reported pCO<sub>2</sub> 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

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