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1 **Validation of the flooding dose technique to determine fractional rates of protein**
2 **synthesis in a model bivalve species, the blue mussel (*Mytilus edulis* L.)**

3

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15

16 **Abstract**

17 For the first time, use of the flooding dose technique using ³H-Phenylalanine is validated for
18 measuring whole-animal and tissue-specific rates of protein synthesis in the blue mussel
19 *Mytilus edulis* (61 mm shell length; 4.0 g fresh body mass). Following injection, the
20 phenylalanine-specific radioactivities in the gill, mantle and whole-animal free pools were
21 elevated within one hour and remained elevated and stable for up to 6 h following injection of
22 ³H-phenylalanine into the posterior adductor muscle. Incorporation of ³H-phenylalanine into
23 body protein was linear over time following injection and the non-significant intercepts for
24 the regressions suggested incorporation into body protein occurred rapidly after injection.
25 These results validate the technique for measuring rates of protein synthesis in mussels. There

26 were no differences in the calculated rates following 1-6 h incubation in gill, mantle or
27 whole-animal and fractional rates of protein synthesis from the combined time course data
28 were $9.5 \pm 0.8 \text{ \% d}^{-1}$ for the gill, $2.5 \pm 0.3 \text{ \% d}^{-1}$ for the mantle and $2.6 \pm 0.3 \text{ \% d}^{-1}$ for the
29 whole-animal, respectively (mean values \pm SEM). The whole-animal absolute rate of protein
30 synthesis was calculated as $18.9 \pm 0.6 \text{ mg protein day}^{-1}$. The use of this technique in
31 measuring one of the major components of maintenance metabolism and growth will provide
32 a valuable and convenient tool in furthering our understanding of the protein metabolism and
33 energetics of this keystone marine invertebrate and its ability to adjust and respond to
34 fluctuations, such as that expected as a result of climate change.

35

36 **Key words:**

37 Bivalve, Flooding dose technique, Mussel, *Mytilus edulis*, Protein synthesis

38

39 **1. Introduction**

40 Proteins are central to life playing a key role in maintenance metabolism through the
41 synthesis and breakdown of structural, catalytic and other metabolically-active proteins, with
42 growth occurring when rates of protein synthesis exceed breakdown (Doherty and Whitfield,
43 2011). It has been estimated that protein synthesis accounts for 11 to 42% of the basal
44 metabolism in ectothermic and endothermic animals with protein breakdown accounting for
45 an additional energetic cost which, although currently unquantified, may be equivalent to the
46 cost of synthesis (Houlihan et al., 1995a; Fraser and Rogers, 2007). Thus, the cycle of protein
47 turnover (i.e. patterns of synthesis and breakdown) is a fundamental physiological process
48 underlying not only changing patterns of tissue replacement and growth during ontogeny and
49 ageing, but also providing the mechanisms allowing animals to adapt to changing
50 environmental conditions and planes of nutrition. A number of techniques have been

51 developed to measure *in vivo* rates of protein synthesis (Waterlow, 1995; Houlihan et al.,
52 1995a, 1995b; Wagenmakers, 1999). Essentially these techniques all adopt a similar approach
53 in that a stable or radioactive isotope-labelled tracer is introduced to the animal and the rate
54 of incorporation of the isotope into body protein, or its appearance in metabolic end-products
55 over time, is measured in order to determine rates of protein synthesis. These methodologies,
56 however, vary in their underlying methodological assumptions, choice of tracer (stable
57 isotope or radioisotope tracer; use of labelled amino acids such as phenylalanine, leucine etc
58 or uniformly-labelled protein), method of tracer administration (*e.g.* constant infusion, single
59 high dose or multiple bolus injection), or choice of immediate precursor pool (*e.g.* free amino
60 acids or amino-acyl-tRNAs) (Waterlow, 1995; Davis and Reeds, 2001).

61 The flooding dose technique of Garlick et al. (1980) has been one of the major tracer
62 methods used to measure rates of protein synthesis in endotherms and ectotherms. It was
63 originally developed to measure fractional rates of protein synthesis in rats but the
64 methodology has been applied to other vertebrate and invertebrate taxa (see reviews by
65 Houlihan et al., 1995a; Fraser and Rodgers, 2007; Carter and Mente, 2014). Using this
66 technique, a labelled amino acid (usually L-[³H]-phenylalanine in non-human studies) is
67 injected as a single large dose to rapidly swamp all the body free amino acid pools with the
68 label (hence the term ‘flooding dose’) and to ensure that the specific radioactivities of all the
69 various body compartments are closely related. Validation work has shown that labelling in
70 the free amino acid and the aminoacyl-transfer RNA are equilibrated following a flooding
71 dose (Davis et al., 1999; Davis and Reeds, 2001; Caso et al., 2002). After a known
72 incorporation period, samples can be obtained through biopsy (in humans, usually to measure
73 rates of protein synthesis in muscle tissue) or through killing the animal and taking tissue
74 samples for subsequent analysis of rates of protein synthesis in different tissues (*e.g.* liver,
75 muscle), or homogenising the whole animal to obtain a whole-animal rate of protein

76 synthesis. The flooding dose technique of Garlick et al. (1980) has become one of the
77 standard techniques for measuring *in vivo* rates of protein synthesis in most animals,
78 including aquatic ectotherms, because of the ease of tracer administration through a single
79 injection, the rapid flooding of the body free amino acid pools and the ability to selectively
80 extract and measure free pool and protein-bound phenylalanine-specific radioactivities using
81 standard biochemical assays. Validation of the flooding dose technique, however, is essential
82 to ensure success and the following key criteria need to be fulfilled: (1) the phenylalanine-
83 specific radioactivity of the intracellular free amino acid pools is rapidly elevated following
84 injection; (2) the phenylalanine-specific radioactivity of the intracellular free amino acid
85 pools either remain elevated and stable or show a slow linear decline over the incubation
86 period (Garlick et al., 1980, 1983); (3) the uptake of radiolabel into body protein is linear
87 over the incubation period; (4) the presence of a high dose of ^3H -L-phenylalanine does not
88 stimulate or depress rates of protein synthesis following injection. When applying the
89 flooding dose technique to a new species for the first time, validation studies that examine the
90 changes in the free pool and protein-bound phenylalanine-specific radioactivities over time
91 are essential (Fraser and Rodgers, 2007).

92 In the aquatic environment, the majority of studies on protein synthesis using the
93 flooding dose have focused on fish and crustaceans (Houlihan et al., 1995b; Fraser and
94 Rodgers, 2007; Carter and Mente, 2014), although the technique has also been applied to
95 other vertebrate and invertebrate taxa, albeit less often: amphibians (Fuery et al., 1998a,
96 1998b), reptiles (Fraser et al., 2001), holothurian echinoderms (Fraser et al., 2004) and
97 gastropod (Fraser et al., 2002; Bowgen et al., 2007) and cephalopod (Houlihan et al., 1990b;
98 Carter et al., 2009; Moltschaniwskyj and Carter, 2010; Moltschaniwskyj et al., 2013)
99 molluscs. However, the flooding dose technique has not previously been used to determine
100 rates of protein synthesis in bivalve molluscs, which is surprising given the key ecological

101 role, adaptive physiology for life in the intertidal and commercial importance of this taxa
102 (e.g. Navarette and Menge, 1996; Ragnarsson and Raffaelli, 1999; Somero, 2002; Gosling,
103 2003; Gracey et al., 2008; Smith et al., 2014). *In vivo* rates of whole-animal protein synthesis
104 have been measured in bivalve molluscs of the genus *Mytilus* using ¹⁵N-labelled algae and a
105 stochastic end-point model (e.g. Hawkins, 1985; Hawkins et al., 1989; Bayne and Hawkins,
106 1997). The results of these studies have provided some fundamental insights into the role that
107 inter-individual variation in protein turnover plays in individual growth performance, in terms
108 of both growth rates and growth efficiency (Hawkins et al., 1989), have shown links between
109 genetic heterozygosity, protein turnover and growth performance (Hawkins et al., 1986;
110 Bayne and Hawkins, 1997; Hawkins and Day, 1999) and examined the effects of salinity and
111 acute temperature change in the laboratory (Hawkins et al., 1987; Hawkins and Hilbish,
112 1992) and seasonal changes in the field on protein metabolism in *M. edulis* (Hawkins, 1985).

113 The aim of the current study is to validate the flooding dose technique using L-[³H]-
114 phenylalanine to measure whole-animal and tissue-specific rates of protein synthesis in
115 bivalve molluscs using the commercially and ecologically important species the blue mussel
116 *Mytilus edulis* as a model. Validation will allow whole-animal and tissue-specific measures
117 of protein synthesis in bivalves that are directly comparable with the range of other taxa that
118 have been studied using the flooding dose technique of Garlick et al. (1980). In addition,
119 whole-animal rates of protein synthesis can be compared with the data of Hawkins and co-
120 workers (*op cit*) obtained using a stochastic endpoint model method.

121

122 **2. Materials and Methods**

123 *2.1 Animal collection*

124 Twenty five blue mussels (*Mytilus edulis* L.) were collected from the high intertidal zone at
125 Tal y Foel, Menai Strait, North Wales in June 2010 and returned to the flow-through seawater

126 aquarium at the School of Ocean Sciences. On the shore, individual mussels were selected to
127 be greater than 50mm shell length and free from any visible signs of damage such as chips or
128 holes in the shell. The mussels [shell length, 61.0 mm \pm 0.8 mm (mean \pm SEM), range 54.2 to
129 68.8 mm] were left overnight in ambient environmental conditions for Menai Bridge (15.1
130 °C, 33.1 PSU) and divided into five groups of five mussels the following morning for use in
131 the validation experiment. Shell length was not different in the five time-course groups
132 (ANOVA; $F_{3,24} = 0.97$, $p = 0.45$).

133

134 2.2 Radiolabel injection and incubation time course

135 Fractional rates of protein synthesis were measured using a modification of the flooding dose
136 technique (Garlick et al., 1980; Houlihan et al., 1995a). Immediately prior to injection, the
137 mussels were removed from seawater, blotted dry and the shell valves were notched in the
138 vicinity of the posterior adductor muscle. Four groups of five mussels were injected, in turn,
139 into the sinus of the posterior adductor muscle with a solution containing 135 mM L-
140 phenylalanine and L-[2, 6-³H]-phenylalanine (American Radiolabelled Chemicals (UK); 3.7
141 MBq ml⁻¹). The injection volume used was 10 μ l g⁻¹ wet body mass (Houlihan et al., 1995a;
142 Fraser et al., 2002) with wet body mass (M_w) estimated from shell length (S_L) using the
143 following calibration equation for mussels at Tal y Foel: $M_w = 0.00005S_L^{2.74}$ ($n = 50$, $r^2 =$
144 0.927 , $p < 0.001$; Busbridge, unpublished results). The phenylalanine-specific radioactivity of
145 the injection solution was 838 ± 33 disintegrations per minute per nanomole phenylalanine
146 (dpm nmole⁻¹ phe). Following injection, each group of five injected mussels was placed in a
147 separate 5 l tank (20 x 15 x 15cm) containing aerated seawater and left for either 1, 2, 4 or 6 h
148 to allow incorporation of the injected radiolabel. The fifth group was notched but not injected
149 and served as a control group (*i.e.* 0 h incorporation). After notching of the control group or
150 following incubation of the injected groups, each mussel was dissected by cutting through the

151 posterior adductor muscle and the total fresh body tissue rinsed with distilled water, briefly
152 blotted and weighed (fresh body mass 4.00 ± 0.22 g; range 2.22 to 6.58 g). Samples (*ca.* 200
153 mg) of tissue were removed from the gill and mantle and placed in microcentrifuge tubes and
154 the remaining mussel tissue (henceforth referred to as the whole-animal) placed in a foil bag.
155 All dissections were completed within 5 min and the samples were frozen in liquid nitrogen
156 and stored at -80°C until further laboratory analysis.

157

158 *2.3 Laboratory analyses*

159 Duplicate gill and mantle tissue samples (*ca.* 100 mg) from each mussel were homogenised in
160 2 ml 0.2M perchloric acid (PCA) and centrifuged (6000 g, 4°C 15 min) to separate the
161 intracellular free pool from the precipitated protein pellet and RNA. Whole-animal samples
162 were homogenised in 40 ml 0.2M PCA, vortex mixed and duplicate 2 ml subsamples
163 removed (Fraser et al., 2002) and treated in the same way as gill and mantle tissue samples.
164 The treatment of gill, mantle and whole-animal samples to measure the free pool and protein-
165 bound phenylalanine specific radioactivity followed the protocol outlined in Houlihan et al.
166 (1995a) and McCarthy and Fuiman (2008). In brief, the protein pellet was solubilised in 0.3M
167 NaOH and the protein content was measured using the Folin-phenol method of Lowry et al.
168 (1951) as modified by Schacterle and Pollack (1973) using bovine serum albumin as a
169 standard and RNA content measured using the Orcinol assay (Mejbaum, 1939) using Type IV
170 calf liver RNA (SIGMA) as a standard. The protein-bound phenylalanine specific
171 radioactivity (S_b , dpm nmol^{-1} phe) was calculated from the radioactivity of the solubilised
172 protein (dpm mg^{-1} protein), determined by scintillation counting (Perkin Elmer
173 WinSpectralTM 1414 Liquid Scintillation counter using Optiphase 'HiSafe' scintillant at a
174 counting efficiency of 37%), divided by the concentration of phenylalanine (nmol phe mg^{-1}
175 protein) in that tissue or for the whole-animal (Houlihan et al., 1992; Wilson et al., 1996;

176 Morgan et al., 1998; McCarthy and Fuiman, 2008). The concentration of phenylalanine in
177 blue mussel tissue and whole-animal protein was measured in replicate samples (n=10) by
178 ion exchange analysis (PNAC, Department of Biochemistry, Cambridge University) for
179 mussels collected from the same collection site providing values of 112.4, 134.3 and 192.2
180 nmol phe mg⁻¹ protein for gill, mantle and whole-animal protein respectively. The
181 phenylalanine-specific radioactivity of the gill, mantle and whole-animal free pools (S_a, dpm
182 nmol⁻¹ phe) was calculated by converting phenylalanine in the free pool to β-
183 phenylethylamine (PEA) and extracting through heptane into 0.01M sulphuric acid and
184 measuring PEA content by fluorescence (Suzuki and Yagi, 1976) and PEA radioactivity by
185 scintillation counting.

186 Fractional rates of protein synthesis (k_s, expressed as a percentage of the protein mass
187 synthesised per day, % d⁻¹) were calculated for gill, mantle and whole-animal samples as k_s =
188 100.((S_b/S_a). (1440/t)), where S_b and S_a are the protein-bound and free pool phenylalanine
189 specific radioactivities (dpm nmole⁻¹ phe) and t is the incubation time (between injection and
190 freezing) in minutes and 1440 is the number of minutes in a day (Garlick et al., 1983).

191 Whole-animal absolute rates of protein synthesis (A_s, mg protein synthesised per day, mg d⁻¹)
192 were calculated as A_s = (k_s/100).(M_{prot}) where M_{prot} is the whole-animal protein mass (mg).

193 RNA concentrations were expressed as the capacity for protein synthesis (C_s, μg RNA mg⁻¹
194 protein) and as RNA activity (k_{RNA}, mg protein synthesised per mg RNA per day, mg mg⁻¹ d⁻¹)
195 calculated as (10.k_s)/C_s (Sugden and Fuller, 1991).

196

197 *2.4 Statistical analyses*

198 All data are presented as mean values ± SEM. All data were tested for normality
199 (Kolmogorov-Smirnov test) and equal variance (Levene's test) prior to statistical analysis and
200 data were log₁₀-transformed if necessary to meet assumption for parametric statistical tests.

201 Least-squares linear regression analysis was used to describe the incorporation of radiolabel
202 into gill, mantle and whole-animal protein over time. Free pool S_a values and fractional rates
203 of protein synthesis after 1, 2, 4 and 6 h incubation were compared using a one-way
204 ANOVA. Similarly, fractional rates of protein synthesis, C_s and k_{RNA} values in gill, mantle
205 and the whole mussel were compared using a one-way ANOVA. Where the ANOVA was
206 significant, post-hoc pairwise comparisons were conducted using Tukey's HSD test. All
207 statistical analyses were conducted using SPSS for Windows v20 using a significance value
208 of $\alpha = 0.05$.

209

210 **3. Results**

211 *3.1. Time course validation*

212 The intracellular free pool specific radioactivities (S_a) in the gill, mantle and whole-animal
213 were elevated within one hour of injection (Fig. 1a-c) and remained elevated over the 6 h
214 time course with average S_a values of 642 ± 23 dpm nmol⁻¹ phe for the gill, 562 ± 14 dpm
215 nmol⁻¹ phe for the mantle and 501 ± 36 dpm nmol⁻¹ phe for the whole-animal, respectively,
216 which were equivalent to 77 ± 3 %, 67 ± 2 % and 60 ± 4 % of the specific radioactivity of
217 the injection solution. In the mantle and whole-animal, S_a values were similar over the six
218 hour time course (mantle, $F_{3,19} = 0.36$, $p = 0.78$; whole-animal, $F_{3,19} = 1.21$, $p = 0.34$). In the
219 gill, S_a was significantly higher after 2 h incubation compared with 4 h ($F_{3,19} = 5.94$, $p =$
220 0.006 ; Tukey *post-hoc* pairwise comparison, $p = 0.003$) but the other free pool S_a values were
221 similar between 1 and 6 h incubation (Tukey *post-hoc* pairwise comparisons, $p = 0.14$ to $p =$
222 0.99) (Fig. 1a). Thus, phenylalanine-specific radioactivities in the gill, mantle and whole-
223 animal free pools remained elevated and stable over the 6 h time-course experiment.

224 The time course incorporation of ³H phenylalanine into gill, mantle and whole-animal
225 protein pools are shown in Fig. 2a-c. The incorporation rates of radiolabel into all three

226 protein pools over the 6 h time course all exhibited significant linear relations and were
227 described by the following equations:

228 Gill $S_b = 0.408 + 2.292t$ ($r^2 = 0.989$, $n = 5$, $p < 0.001$)

229 Mantle $S_b = 0.255 + 0.427t$ ($r^2 = 0.968$, $n = 5$, $p = 0.002$)

230 Whole-animal $S_b = 0.119 + 0.445t$ ($r^2 = 0.967$, $n = 5$, $p = 0.002$)

231 where S_b is the protein-bound phenylalanine specific radioactivity (dpm nmol⁻¹ phe) and t is
232 incubation time (h). The intercept values in each regression line were not significantly
233 different from zero ($p = 0.15$ to 0.45).

234

235 3.2 Rates of protein synthesis

236 Fractional rates of protein synthesis for gill and mantle tissue and the whole-animal
237 calculated from the time-course data are presented in Fig. 3. Fractional rates of protein
238 synthesis were highest in gill tissue at *ca.* 8-10 % d⁻¹ compared to mantle tissue and whole-
239 animal rates where rates of protein synthesis were *ca.* 2-3.5 % d⁻¹. There were no differences
240 in the calculated rates following 1-6 h incubation in the gill ($F_{3,19} = 0.64$, $p = 0.60$), mantle
241 ($F_{3,19} = 1.27$, $p = 0.32$) and whole-animal ($F_{3,19} = 0.93$, $p = 0.45$) and the average fractional
242 rates of protein synthesis from the combined time course data were 9.5 ± 0.8 % d⁻¹ for the
243 gill, 2.5 ± 0.3 % d⁻¹ for the mantle and 2.6 ± 0.3 % d⁻¹ for the whole-animal, respectively
244 (Table 1). The whole body absolute rate of protein synthesis was calculated as 18.9 ± 0.6 mg
245 protein day⁻¹.

246 Data on the capacity for protein synthesis, RNA activity and fractional rates of protein
247 synthesis for gill, mantle and whole-animal tissue are presented in Table 1. The capacity for
248 protein synthesis (C_s , Table 1) was significantly different between gill, mantle and whole
249 animal tissue ($F_{2,57} = 20.03$, $p < 0.001$; Tukey's HSD pairwise comparisons, gill *vs* mantle or
250 whole animal $p < 0.01$, mantle *vs* whole animal $p < 0.05$). However, RNA activity was

251 significantly higher in gill tissue compared with the mantle and whole-animal ($F_{2,57} = 37.34$, p
252 < 0.001 ; Tukey's HSD pairwise comparisons, gill *vs.* mantle or whole animal both $p < 0.01$;
253 mantle *vs.* whole animal $p > 0.05$). This increased RNA activity resulted in significantly
254 higher fractional rates of protein synthesis in gill tissue compared to the mantle and whole-
255 animal (ANOVA on \log_{10} -transformed data; $F_{2,57} = 68.93$, $p < 0.001$; Tukey's HSD pairwise
256 comparisons, Gill *vs.* mantle or whole animal both $p < 0.01$, mantle *vs.* whole animal $p >$
257 0.05) (Table1).

258

259 **4. Discussion**

260 The results of the present study validate the use of the 'flooding dose' method of Garlick et
261 al. (1980) using ^3H -phenylalanine to determine, for the first time, whole animal and tissue-
262 specific fractional rates of protein synthesis in bivalve molluscs. The blue mussel, *Mytilus*
263 *edulis*, was chosen as a direct comparison for previous determinations of protein synthesis
264 carried out using the stochastic end-point model and the stable isotope ^{15}N (*e.g.* Hawkins,
265 1985; Hawkins et al., 1986; Hawkins et al., 1989). The time-course data presented in Figs 1
266 and 2 fulfil the first three validation criteria for the technique (see Introduction) as following
267 injection into the posterior adductor muscle, free pool phenylalanine-specific radioactivities
268 in the gill, mantle and whole-animal were elevated within one hour of injection (criteria 1)
269 and remained elevated and stable (criteria 2) for a further 5 h (Fig. 1). Subsequent
270 incorporation of radiolabel into body protein was linear (Fig. 2; criteria 3) and the non-
271 significant intercepts for the regression lines describing the time-course incorporation rates of
272 radiolabel into gill, mantle and whole-animal protein suggest rapid equilibration within the
273 body free amino acid pools and incorporation into body protein occurred rapidly after
274 injection. The fourth validation criteria, that the swamping of the body free amino acid pools
275 with phenylalanine does not affect rates of protein synthesis, has been examined in humans

276 and shown to be the case (e.g. McNurlan et al., 1979; Garlick et al., 1980; McNurlan et al.,
277 1991; Garlick et al., 1994; but see Rennie et al., 1994). However, this validation criteria has
278 been little studied in non-mammalian animals (Loughna and Goldspink, 1985) and it has been
279 assumed that there is no stimulatory effect of the flooding dose on rates of protein synthesis
280 (Houlihan et al., 1995a; Fraser and Rodgers, 2007). Clearly, further validation of this criteria
281 is required for aquatic ectotherms but may be problematic due to differing time scales of
282 tracer administration and incorporation using different methodologies.

283 The free pool phenylalanine-specific radioactivities in the gill, mantle and whole-
284 animal were lower than the specific radioactivity of the injection solution (with mean values
285 of 77%, 67% and 60%, respectively). However, these values are within the range of
286 phenylalanine-specific radioactivities attained within tissue and whole-animal free pools for a
287 range of taxa with the percentage similarity to the specific radioactivity of the injection
288 dependent on method of administration. Injection directly into the circulating body fluid via a
289 blood vessel or sinus has resulted in free pool phenylalanine-specific radioactivities that are
290 usually > 80 % the value of the injection solution (e.g. Houlihan et al., 1990a, 1990b; Foster
291 et al., 1991; McCarthy et al., 1994) compared to injection into a body cavity such as the
292 coelom (73%, Fraser et al., 2004) or peritoneum (60%, McCarthy et al., 1999) or the uptake
293 from bathing in radiolabel (74%, Houlihan et al., 1995c; 42%, McCarthy and Fuiman, 2008).
294 It is unlikely that the lower specific radioactivities observed in the gill and mantle are the
295 result of the injection volume remaining as a bolus in the adductor muscle and not
296 distributing throughout the body within the 6 h incubation period because the whole-animal
297 free pool phenylalanine-specific radioactivity also differs from that of the injection solution
298 and therefore does not include any residual undistributed injection solution within the
299 posterior adductor muscle. In addition, the non-significant intercepts for the time-course
300 incorporation of radiolabel into body protein suggest rapid distribution throughout the body.

301 The lower tissue/whole-animal free pools reported here are most likely due to the combined
302 effect of dilution of the injection solution by baseline phenylalanine in the free amino acid
303 pools in the mussel and by leakage of radiolabel from the injection site following withdrawal
304 of the needle. Fraser et al. (2004) report that these two factors combined resulted in a 27%
305 reduction in the body wall free-pool phenylalanine-specific radioactivity in the Antarctic
306 holothurian *Heterocucumis steini* following a flooding dose injection with dilution
307 accounting for a 12.5% reduction and the remainder assumed lost by leakage.

308 The values obtained here for *M. edulis* at 15°C compare favourably with those
309 obtained by Hawkins and co-workers using ¹⁵N-labelled algae measuring the tracer flux
310 through the animal by the appearance of ¹⁵N in excreted ammonia and using a stochastic end-
311 point model to determine absolute protein synthesis rates (Hawkins, 1985; Hawkins et al.,
312 1986, 1989; Hawkins and Hilbish, 1992) (Fig. 4). Although the data set is limited, absolute
313 rates of protein synthesis reported in the present study are of a magnitude expected for
314 mussels of this size at 15°C (rate predicted from regression, 20.9 mg d⁻¹; observed rate 18.9
315 mg d⁻¹) providing confidence in the measurements obtained. Synthesis rate data are compared
316 to shell length as body mass data are not comparable (wet mass data are presented in the
317 present study and dry mass data presented by Hawkins and co-workers) Similar concordance
318 between measurements using the flooding dose technique and the stochastic endpoint method
319 has also been reported for fish (Carter et al., 1994; Houlihan et al., 1995b). However, further
320 cross-calibration studies between methodologies in aquatic ectotherms are recommended.

321 In most studies of protein metabolism in aquatic ectotherms, rates of protein synthesis
322 have been reported on a fractional basis, i.e. as a percentage of the protein mass synthesised
323 per day. Tables 2 and 3 summarise the whole-animal and tissue fractional rates of protein
324 synthesis published for molluscs. The molluscan dataset set is limited to 10 studies in total
325 compared to the larger data sets available for crustaceans [15 studies cited in review by

326 Carter and Mente (2014) plus Rastrick and Whiteley (2013)] and for fishes [*ca.* 70 published
327 studies; Web of Science using Garlick et al. (1980) as a cited reference, search conducted
328 22/7/15]. The molluscan data focuses primarily on whole-animal rates in the Antarctic limpet
329 *Nacella concinnus*, on whole-animal and tissue rates in the dumpling squid *Euprymna*
330 *tasmanicus* together with the whole-animal data for *Mytilus edulis* by Hawkins and co-
331 workers (Tables 2 and 3). The whole-animal data presented in Table 2 can be described by
332 the following equation: $\text{Log}_{10}k_s = 0.044T - 0.306$ ($r^2 = 0.821$, $n = 16$, $p < 0.0001$), where k_s is
333 the fractional rate of protein synthesis measured using the flooding dose technique and T is
334 water temperature ($^{\circ}\text{C}$). This predicts a fractional rate of protein synthesis of 2.26 % d^{-1} at
335 15°C which is close to the measured value (2.56 % d^{-1}), indicating that the rate measured in
336 the present study is of the magnitude expected (although factors such as size and feeding
337 history have not been accounted for in this estimate). One of the advantages of the flooding
338 dose technique is that it enables rates of protein synthesis to be measured in tissues and
339 organs as well as in the whole-animal. Tissue rates of protein synthesis are important because
340 they can indicate specific responses to environmental change that may be masked in the
341 whole-animal (Fraser and Rogers, 2007). The available data on tissue-specific fractional rates
342 of protein synthesis in molluscs are limited to 5 studies (Table 3) but in comparison to the
343 rates obtained in other aquatic ectotherms, the data show the same patterns in the ranking of
344 the relative rates obtained for different tissues. Fractional rates of protein synthesis are higher
345 in metabolically-active tissues such as the liver/hepatopancreas, gonads, gill and gut organs
346 compared to muscle tissue [Table 3; crustaceans (*Carcinus maenas*, Houlihan et al., 1990a;
347 *Nephrops norvegicus*, Mente et al., 2011); fish (*Oncorhynchus mykiss*, Houlihan et al., 1986,
348 Foster et al., 1991; *Salmo salar* Martin et al., 1993)], usually associated with high tissue
349 capacities for protein synthesis and RNA activity (Table 1; Houlihan et al., 1990a, 1990b;
350 Foster et al., 1991; Carter et al., 2009). However, the exception appears to be mantle tissue in

351 young, fast-growing cephalopods (Table 3) where as part of their r-selected life histories
352 ('living life in the fast lane', Carter et al., 2009), growth is rapid in order to reach maturity
353 and spawn within their short lifespans (usually < 1 year). Metabolically-active tissues such as
354 the gills are likely to have higher rates of protein synthesis to allow for protein remodelling
355 and flexibility in enzyme production in keeping with the function of the gill as a specialised
356 site for both gas and ion exchange (Lyndon and Houlihan, 1998).

357 In summary, the results of the present study validate the use of the flooding dose
358 technique to measure whole-animal and tissue-specific rates of protein synthesis in the blue
359 mussel *Mytilus edulis*. The use of this technique in measuring one of the major components
360 of maintenance metabolism and growth will provide a valuable tool in furthering our
361 understanding of the intriguing relationship between protein synthesis rates, growth rates and
362 energy expenditure previously suggested by Hawkins and Day (1999) for this species. In
363 addition, the comparative study of rates of protein turnover in a range of aquatic ectotherm
364 species within the 'fast-slow pace of life' continuum (Ricklefs and Wikelski, 2002) will
365 provide valuable insight into the physiological costs and energetic trade-offs associated with
366 fast growth/early maturation versus slow growth/delayed maturation (e.g. cephalopods versus
367 long-lived fish species). Indeed, validation of the flooding dose technique for bivalve
368 molluscs will allow protein turnover rates to be studied in a range of bivalve molluscs where
369 lifespan can vary from less than 5 years (e.g. *Cerastoderma edule*, Malham et al., 2012) to
370 decades (e.g. *Glycymeris glycymeris*, Brocas et al., 2013) or hundreds of years (e.g. *Arctica*
371 *islandica*, Butler et al., 2013) to determine the contribution of changing rates of protein
372 turnover to the ageing process and senescence. Finally, the study of tissue-specific and
373 whole-animal rates of protein synthesis will also provide understanding on the ability of this
374 keystone marine invertebrate to adjust and respond to environmental fluctuations, such as that
375 expected as a result of climate change.

376

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385

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387 **References**

388 Bayne, B.L., Hawkins, A.J.S., 1997. Protein metabolism, the costs of growth, and genomic
389 heterozygosity: experiments with the mussel *Mytilus galloprovincialis* Lmk. *Physiol.*
390 *Zool.* 70, 391-402.

391 Bowgen, A., Fraser, K.P.P., Peck, L.S., Clarke, A., 2007. Energetic cost of synthesizing
392 proteins in Antarctic limpet, *Nacella concinna* (Strebel, 1908), is not temperature
393 dependent. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R2266-R2274.

394 Brocas, W.M., Reynolds, D.J., Butler, P.G., Richardson, C.A., Scourse, J.D., Ridgway, I.D.,
395 Ramsay, K., 2013. The dog cockle, *Glycymeris glycymeris* (L.), a new annually-resolved
396 sclerochronological archive for the Irish Sea. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*
397 373, 133-140.

398 Butler, P.G., Wanamaker Jr, A.D., Scourse, J.D., Richardson, C.A., Reynolds, D.J., 2013.
399 Variability of marine climate on the North Icelandic Shelf in a 1357 year proxy archive

400 based on growth increments in the bivalve *Arctica islandica*. *Palaeogeogr.*
401 *Palaeoclimatol. Paleoecol.* 373, 141–151.

402 Carter, C.G., Lynch, K.A., Moltshaniwskyj, N.A., 2009. Protein synthesis in a solitary
403 benthic cephalopod, the Southern dumpling squid (*Euprymna tasmanica*). *Comp.*
404 *Biochem. Physiol. A* 153, 185-190.

405 Carter, C.G., Mente, E., 2014. Protein synthesis in crustaceans: a review focused on feeding
406 and nutrition. *Cent. Eur. J. Biol.* 9, 1-10.

407 Carter, C.G., Owen, S.F., He, Z.-Y., Watt, P.W., Scrimgeour, C., Houlihan, D.F., Rennie,
408 M.J., 1994. Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss*,
409 using a stable isotope. *J. Exp. Biol.* 189, 279-284.

410 Caso, G., Ford, G.C., Nair, K.S., Garlick, P.J., McNurlan, M.A., 2002. Aminoacyl-tRNA
411 enrichment after a flood of labeled phenylalanine: insulin effect on muscle protein
412 synthesis. *Am. J. Physiol. Endoc. Metab.* 282, E1029-E1038.

413 Davis, T.A., Fiorotto, M.L., Nguyen, H.V., Burrin, D.G., 1999. Aminoacyl-tRNA and tissue
414 free amino acid pools are equilibrated after a flooding dose of phenylalanine. *Am. J.*
415 *Physiol. Endoc. Metab.* 277, E103-E109.

416 Davis, T.A., Reeds, P.J., 2001. Of flux and flooding: the advantages and problems of
417 different isotopic methods for quantifying protein turnover in vivo : II. Methods based on
418 the incorporation of a tracer. *Curr. Opin. Clin. Nutr. Metab. Care* 4, 51-56.

419 Doherty, M.K., Whitfield, P.D., 2011. Proteomics moves from expression to turnover: update
420 and future perspectives. *Expert Rev. Proteomics* 8, 325-334.

421 Foster, A.R., Houlihan, D.F., Gray, C., Medale, F., Fauconneau, B., Kaushik, S.J., Le Bail,
422 P.Y., 1991. The effects of ovine growth hormone on protein turnover in rainbow trout.
423 *Gen. Comp. Endocrinol.* 82, 111-120.

424 Fraser, K.P.P., Clarke, A., Peck, L.S., 2002. Low-temperature protein metabolism: seasonal
425 changes in protein synthesis in the Antarctic limpet *Nacella concinna* Strebel 1908. J.
426 Exp. Biol. 205, 3077-3086.

427 Fraser, K.P.P., Houlihan, D.F., Lutz, P.L., Leone-Kabler, S., Manuel, L., Brechin, J.G., 2001.
428 Complete suppression of protein-synthesis during anoxia with no post-anoxia protein
429 synthesis debt in red-eared slider turtle *Trachemys scripta elegans*. J. Exp. Biol. 204,
430 4353-4360.

431 Fraser, K.P.P., Peck, L.S., Clarke, A., 2004. Protein synthesis, RNA concentrations, nitrogen
432 excretion, and metabolism vary seasonally in the Antarctic holothurian *Heterocucumis*
433 *steineni* (Ludwig 1898). Physiol. Biochem. Zool. 77, 556-569.

434 Fraser, K.P.P., Rodgers, A.D., 2007. Protein metabolism in marine animals: the underlying
435 mechanisms of growth. Adv. Mar. Biol. 52, 267-362.

436 Fuery, C.J., Withers, P.C., Guppy, M., 1998a. Protein synthesis in the liver of *Bufo marinus* –
437 cost and contribution to oxygen consumption. Comp. Biochem. Physiol. A 119, 459-467.

438 Fuery, C.J., Withers, P.C., Hobbs, A.A., Guppy, M., 1998a. The role of protein synthesis
439 during metabolic depression in the Australian desert frog *Neobatrachus centralis*. Comp.
440 Biochem. Physiol. A 119, 469-476.

441 Garlick, P.J., Fern, M., Preedy, V.R., 1983. The effect of insulin infusion and food intake on
442 muscle protein synthesis in postabsorptive rats. Biochem. J. 210, 669-676.

443 Garlick, P.J., McNurlan, M.A., Essen, P., Wernerman, J., 1994. Measurement of tissue
444 protein-synthesis rates in-vivo - a critical analysis of contrasting methods. Am. J.
445 Physiol. Endoc. Metab. 282, E287-E297.

446 Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for
447 measuring the rate of protein synthesis in tissues by injection of ³H phenylalanine.
448 Biochem. J. 217, 507-516.

449 Gosling, E., 2003. *Bivalve Molluscs: Biology, Ecology and Culture*. Wiley-Blackwell, 454
450 pp.

451 Gracey, A.Y., Chaney, M.L., Boomhower, J.P., Tyburczy, W.R., Connor, K., Somero, G.N.,
452 2008. Rhythms of gene expression in a fluctuating intertidal environment. *Curr. Biol.* 18,
453 1501-1507.

454 Hawkins, A.J.S., 1985. Relationships between the synthesis and breakdown of protein,
455 dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus*
456 *edulis* L. *Oecologia* (Berlin) 66, 42-49.

457 Hawkins, A.J.S., Bayne, B.L., Day, A.J., 1986. Protein turnover, physiological energetics and
458 heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific
459 growth. *Proc. R. Soc. Lond. B* 229, 161-176.

460 Hawkins, A.J.S. and Day A.J., 1999. Metabolic interrelations underlying the physiological
461 and evolutionary advantages of genetic diversity. *Am. Zool.* **39**, 401-411.

462 Hawkins, A.J.S. Hilbish, T.J., 1992. The costs of cell volume regulation: protein metabolism
463 during hyperosmotic adjustment. *J. Mar. Biol. Ass. U.K.* 72, 569-578.

464 Hawkins, A.J.S., Widdows, J., Bayne, B.L., 1989. The relevance of whole-body protein
465 metabolism to measured costs of maintenance and growth in *Mytilus edulis*. *Physiol.*
466 *Zool.* 62, 745-763

467 Hawkins, A.J.S., Wilson, I.A., Bayne, B.L., 1987. Thermal responses reflect protein turnover
468 in *Mytilus edulis* L. *Funct. Ecol.* 1, 339-351.

469 Houlihan, D.F., Carter, C.G., McCarthy, I.D., 1995a. Protein turnover in animals. In: Wright,
470 P.J., Walsh, P.A. (Eds.), *Nitrogen Metabolism and Excretion*, CRC Press, Boca Raton,
471 FL, pp. 1-32.

472 Houlihan, D.F., Carter, C.G., McCarthy, I.D., 1995b. Protein synthesis in fish. In: P.
473 Hochachka and P. A. Walsh (Eds.), *Biochemistry and Molecular Biology of Fishes*, 4,
474 Elsevier, Amsterdam, pp. 191-219.

475 Houlihan, D.F., McMillan, D.N., Agnisola, C., Trara Genoino, I., Foti, L., 1990b. Protein
476 synthesis and growth in *Octopus vulgaris*. *Mar. Biol.* 106, 251-259.

477 Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and
478 protein degradation rates in rainbow trout: effects of body size. *Physiol. Zool.* 59. 482-
479 493.

480 Houlihan, D.F., Pedersen, B.H., Steffensen, J.F., Brechin, J., 1995c. Protein synthesis, growth
481 and energetics in larval herring (*Clupea harengus*) at different feeding regimes. *Fish*
482 *Physiol. Biochem.* 14, 195-208.

483 Houlihan, D.F., Waring, C.P., Mathers, E., Gray, C., 1990a. Protein synthesis and oxygen
484 consumption of the shore crab *Carcinus maenas* after a meal. *Physiol. Zool.* 63, 735-756.

485 Houlihan, D.F., Wieser, W., Foster, A., Brechin, J., 1992. In vivo protein synthesis rates in
486 larval nase (*Chondrostoma nasus* L.). *Can.J. Zool.* 70, 2436-2440.

487 Loughna, P.T., Goldspink, G., 1985. Muscle protein synthesis rates during temperature
488 acclimation in a eurythermal (*Cyprinus carpio*) and a stenothermal (*Salmo gairdneri*)
489 species of teleost. *J. Exp. Biol.* 118, 267-276.

490 Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with
491 the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.

492 Lyndon, A.R., Houlihan, D.F., 1998. Gill protein turnover: costs of adaptation. *Comp.*
493 *Biochem. Physiol. A* 119, 27-34.

494 Malham, S.K., Hutchinson, T.H., Longshaw, M. 2012. A review of the biology of European
495 cockles (*Cerastoderma* spp.). *J. Mar. Biol. Assoc. UK* 92, 1563–1577.

496 Martin, N.B., Houlihan, D.F., Talbot, C., Palmer, R.M., 1993. Protein metabolism during
497 sexual maturation in female Atlantic salmon (*Salmo salar* L). Fish Physiol. Biochem. 12,
498 131-141.

499 McCarthy, I.D., Fuiman, L.A., 2008. Growth and protein metabolism in red drum (*Sciaenops*
500 *ocellatus*) larvae exposed to environmental levels of atrazine and malathion. Aquat.
501 Toxicol. 88, 220-229.

502 McCarthy, I.D., Houlihan, D.F., Carter, C.G., (1994). Individual variation in protein turnover
503 and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Proc. R. Soc.
504 Lond. B 257, 141-147. .

505 McCarthy, I.D., Moksness, E., Pavlov, D.P., Houlihan, D.F., 1999. Effects of water
506 temperature on protein synthesis and protein growth in juvenile Atlantic wolfish
507 (*Anarhichas lupus*). Can. J. Fish. Aquat. Sci. 56, 231-241

508 McNurlan, M.A., Essen, P., Heys, S.D., Buchan, V., Garlick, P.J., Wernerman, J., 1991.
509 Measurement of protein synthesis in human skeletal-muscle - further investigation of the
510 flooding technique. Clin. Sci. 81, 557-564.

511 McNurlan, M.A., Tomkins, A.M., Garlick, P.J., 1979. Effect of starvation on the rate of
512 protein synthesis in rat liver and small intestine. Biochem. J. 178, 373-379.

513 Mejbbaum, W., 1939. Über die bestimmung kleiner pentosemengen insbesondere in derivaten
514 der adenylsäure. Hoppe-Seyler's Z. Physiol. Chem. 258, 117-120.

515 Mente, E., Carter, C.G., Barnes (Katersky), R.S., Karapanagiotidis, I.T., 2011. Protein
516 synthesis in wild-caught Norway lobster (*Nephrops norvegicus* L.). J. Exp. Mar. Biol.
517 Ecol. 409, 208-214.

518 Moltschaniwskyj, N.A., Carter, C.G., 2010. Protein synthesis, degradation and retention:
519 Mechanisms of indeterminate growth in cephalopods. Physiol. Biochem. Zool. 83, 997-
520 1008.

521 Moltschaniwskyj, N.A., Carter, C.G., 2013 The adaptive response of protein turnover to the
522 energetic demands of reproduction in a cephalopod. *Physiol. Biochem. Zool.* 86, 119-
523 126.

524 Morgan, I.J., D’Cruz, L.M., Dockray, J.J., Linton, T.K., McDonald, D.G., Wood, C.M., 1998.
525 The effects of elevated winter temperatures and sub-lethal pollutants (low pH, elevated
526 ammonia) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus*
527 *mykiss*). *Fish Physiol. Biochem.* 19, 377-389.

528 Navarette, S.A., Menge, B.A., 1996. Keystone predation and interaction strength: interactive
529 effects of predators on their main prey. *Ecol. Monog.* 66, 409-429.

530 Pakay, J.L., Withers, P.C., Hobbs, A.A., Guppy, M., 2002. In vivo downregulation of protein
531 synthesis in the snail *Helix apersa* during estivation. *Am. J. Physiol. Regul. Integr.*
532 *Comp. Physiol.* 283, R197-R204.

533 Ragnarsson, S.A., Raffaelli, D., 1999. Effects of the mussel *Mytilus edulis* L. on the
534 invertebrate fauna of sediments. *J. Exp. Mar. Biol. Ecol.* 241, 31-43.

535 Rastrick, S.P.S. Whiteley, N.M., 2013. Influence of natural thermal gradients on whole
536 animal rates of protein synthesis in marine gammarid amphipods. *PLoS ONE* 8, e60050.
537 doi:10.1371/journal.pone.0060050.

538 Rennie, M.J., Smith, K., Watt, P.W., 1994. Measurement of human tissue protein-synthesis -
539 an optimal approach. *Am. J. Physiol. Endoc. Metab.* 282, E298-E307.

540 Ricklefs, R.E., Wikelski, M., 2002. The physiology/life-history nexus. *Trends Ecol. Evol.* 17,
541 462–468

542 Schacterle, G.R, Pollack, R.I., 1973. Simplified method for quantitative assay of small
543 amounts of protein in biologic material. *Anal. Biochem.* 51, 654-655.

544 Smith, C., Papadopoulou, N., Sevastou, K., Franco, A., Teixeira, H., Piroddi, C.,
545 Katsanevakis, S., Fürhaupter, K., Beauchard, O., Cochrane, S., Ramsvatn, S., Feral, J.-P.,

546 Chenuil, A., David, R., Kiriakopoulou, N., Zaiko, A., Moncheva, S., Stefanova, K.,
547 Churilova, T., Kryvenko O., 2014. Report on identification of keystone species and
548 processes across regional seas. Deliverable 6.1, DEVOTES Project. 105 pp + 1 Annex.
549 Available online at [http://www.devotes-project.eu/wp-](http://www.devotes-project.eu/wp-content/uploads/2014/07/DEVOTES-D6-1-Keystones.pdf)
550 [content/uploads/2014/07/DEVOTES-D6-1-Keystones.pdf](http://www.devotes-project.eu/wp-content/uploads/2014/07/DEVOTES-D6-1-Keystones.pdf)
551 Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: Optima,
552 limits, and costs of living. *Int. Comp. Physiol.* 42, 780-789.
553 Sugden, P.H., Fuller, S.J., 1991. Regulation of protein turnover in skeletal and cardiac
554 muscle. *Biochem.J.* 273, 21-37.
555 Suzuki, O., Yagi, K., 1976. A fluorometric assay for phenylethylamine in rat brain. *Anal.*
556 *Biochem.* 75, 192-200.
557 Wagenmakers, A.J., 1999. Tracers to investigate protein and amino acid metabolism in
558 human subjects. *Proc. Nutr. Soc.* 58, 987-1000.
559 Waterlow, J.C., 1995. Whole-body protein turnover in humans - past, present, and future.
560 *Ann. Rev. Nutr.* 15, 57-92.
561 Wilson, R.W., Wood, C.M., Houlihan, D.F., 1996. Growth and protein turnover during
562 acclimation to acid and aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*).
563 *Can. J. Aquat. Sci.* 53, 802-811.
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566

567 **Figure Legends**

568 **Fig. 1.** Intracellular free pool phenylalanine-specific radioactivity (S_a , dpm nmol⁻¹ phe) in (a)
569 gill, (b) mantle and (c) whole-animal tissue of blue mussel *Mytilus edulis* (shell length 61.0
570 mm \pm 0.8 mm; fresh body mass 4.00 \pm 0.22 g) after 1–6 h incubation following a single
571 flooding dose injection of a solution containing 135 mM phenylalanine/L-[2,6-³H]-
572 phenylalanine (3.6 MBq ml⁻¹) into the posterior adductor muscle. S_a values for uninjected
573 control mussels are plotted at time zero. Data are presented as mean values \pm SEM (n = 5).

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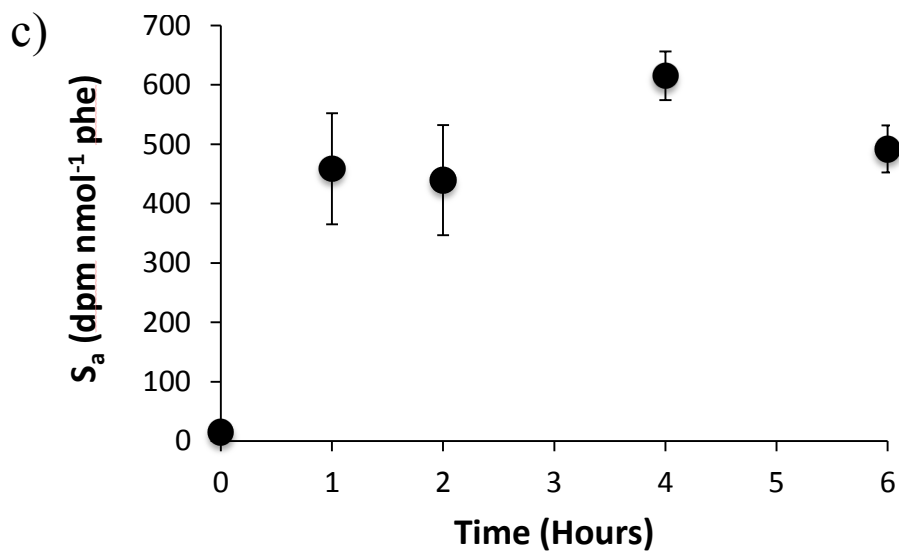
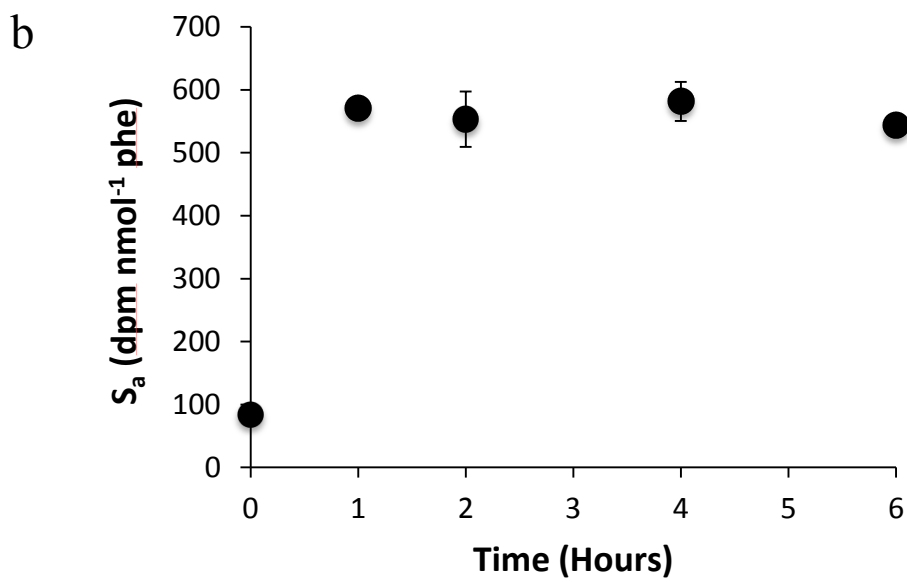
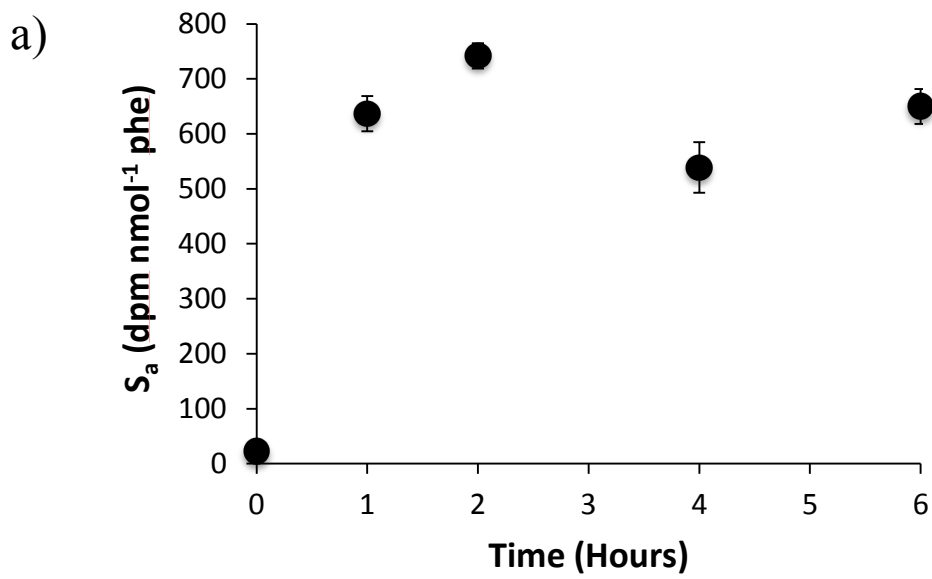
575 **Fig. 2.** Protein-bound phenylalanine-specific radioactivity (S_b , dpm nmol⁻¹ phe) in (a) gill, (b)
576 mantle and (c) whole-animal tissue of blue mussel *Mytilus edulis* (shell length, 61.0 mm \pm 0.8
577 mm; fresh body mass 4.00 \pm 0.22 g) after 1–6 h incubation following a single flooding dose
578 injection of a solution containing 135 mM phenylalanine/L-[2,6-³H]-phenylalanine (3.6 MBq
579 ml⁻¹) into the posterior adductor muscle. S_b values for uninjected control mussels are plotted
580 at time zero. Data are presented as mean values \pm SEM (n = 5).

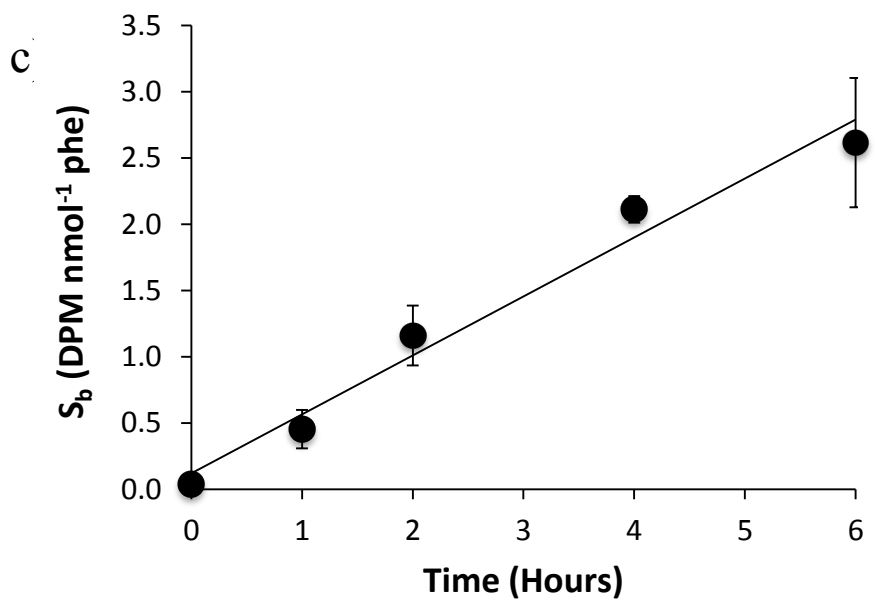
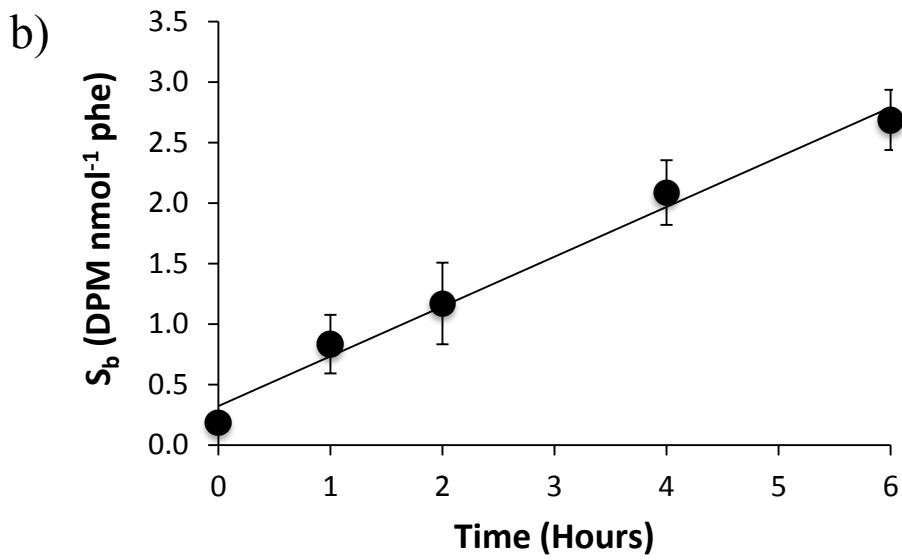
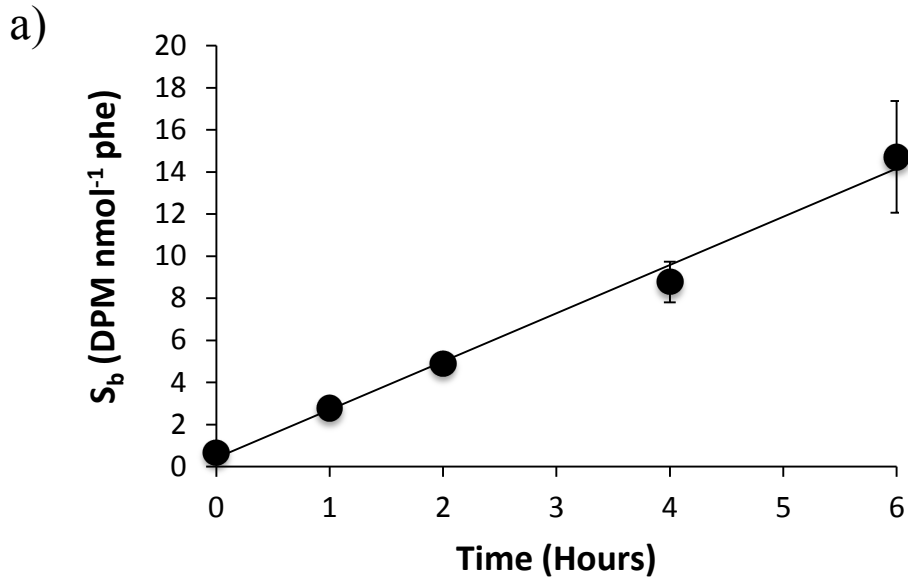
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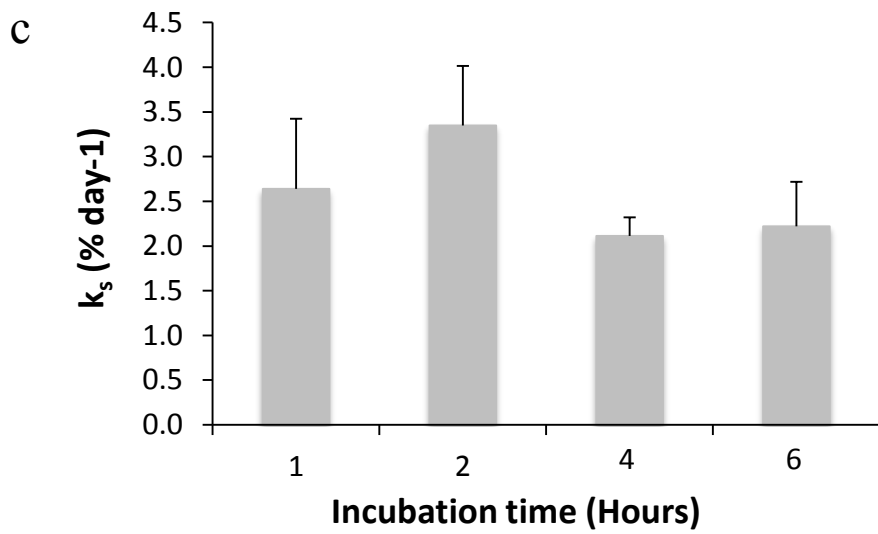
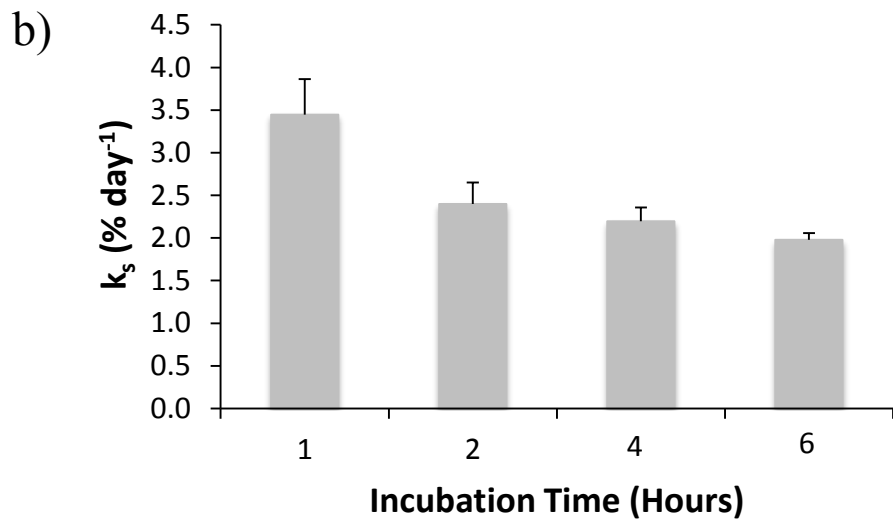
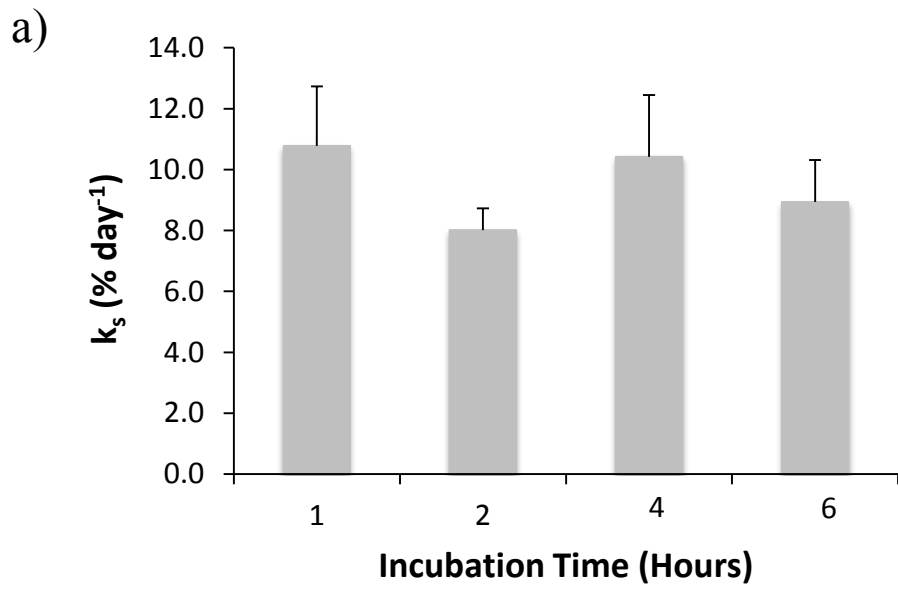
582 **Fig. 3.** Fractional rates of protein synthesis (k_s , % d⁻¹) in the blue mussel *Mytilus edulis* (shell
583 length, 61.0 mm \pm 0.8 mm; fresh body mass 4.00 \pm 0.22 g) after 1–6 h incubation following a
584 single flooding dose injection of a solution containing 135 mM phenylalanine/L-[2,6-³H]-
585 phenylalanine (3.6 MBq ml⁻¹). Data (mean \pm SEM, n = 5) are presented for (a) gill, (b)
586 mantle and (c) whole-animal.

587

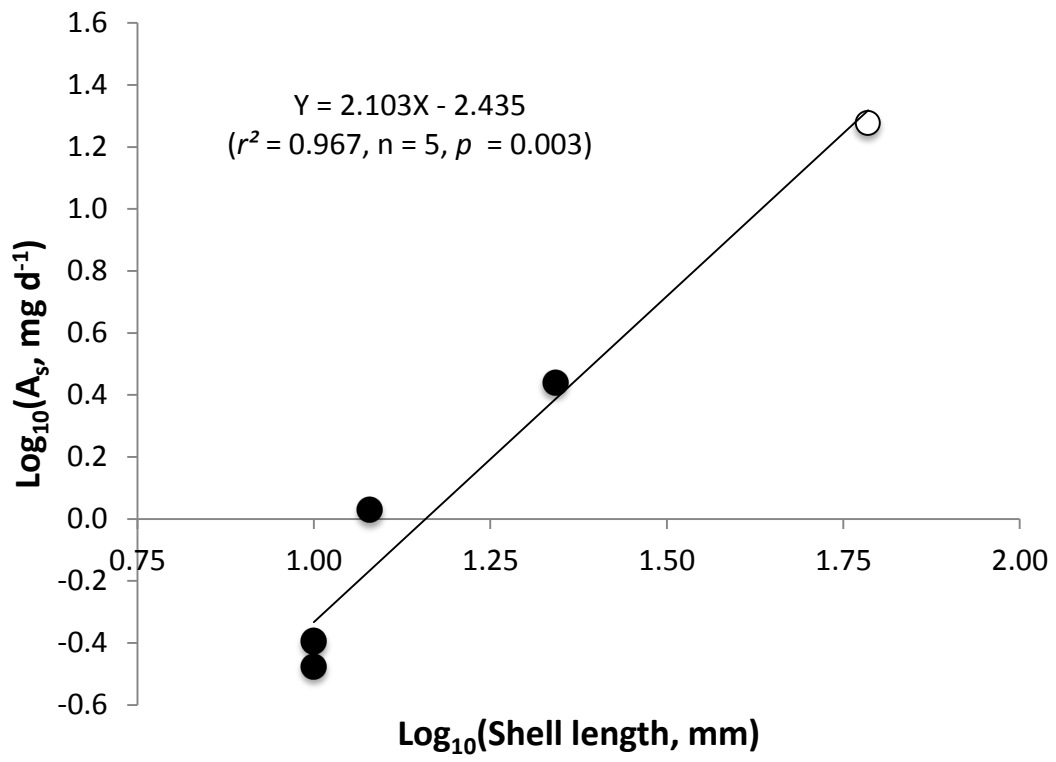
588 **Fig. 4.** Absolute rates of protein synthesis (A_s , mg Protein day⁻¹) for *Mytilus edulis* at 15°C.
589 Data are taken from the present study (open circle) and from Hawkins et al. (1986), Hawkins
590 et al. (1989) and Hawkins and Hilbish (1992). Data from Hawkins and co-workers have been
591 corrected back to their original rates from the mass-corrected data reported based on dry mass
592 data presented in these papers.







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Fig. 4

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613 **Table 1.**

614 Protein metabolism in the gill, mantle and whole-animal tissue of the blue mussel *Mytilus*
615 *edulis* (shell length, 61.0 mm \pm 0.8 mm; fresh mass 4.00 \pm 0.22 g, 15°C).

616

	Gill	Mantle	Whole-animal
k_s (% d ⁻¹)	9.54 \pm 0.78	2.51 \pm 0.30 ^a	2.58 \pm 0.29 ^a
C_s (μ g RNA mg ⁻¹ protein)	50.81 \pm 1.88	40.44 \pm 2.62	32.69 \pm 1.39
k_{RNA} (mg protein mg ⁻¹ RNA d ⁻¹)	1.98 \pm 0.15	0.62 \pm 0.07 ^a	0.83 \pm 0.11 ^a

617

618 Mean \pm SEM (n=20).

619 k_s fractional rate of protein synthesis, C_s capacity for protein synthesis; k_{RNA} RNA activity.

620 Same letter, along a row, indicate no significant difference following one-way ANOVA and
621 Tukey HSD pairwise comparisons.

622

623

624 **Table 2.** Summary of whole-animal fractional rates of protein synthesis (k_s , % d⁻¹) for
 625 Phylum Mollusca.

Species	Tracer	Size	T°C	k_s (% d ⁻¹)	Reference	
Bivalvia						
<i>Mytilus edulis</i>	³ H	61 mm S _L 4.00 g M _w	15°C	2.58	This study	
	¹⁵ N	10 mm S _L 10 mg M _D	15°C	5.4	Hawkins et al. (1989)	
	¹⁵ N	45-57 mm S _L 523 mg M _D	9°C 13°C 13°C	0.29 0.58 0.53	Hawkins (1985)	
Gastropoda						
<i>Nacella concinna</i>	³ H	25.2 mm S _L 2.05 g M _w	0.58°C	0.40	Fraser et al. (2002)	
			-0.36°C	0.56		
			-1.05°C	0.35		
			-1.35°C	0.27		
	³ H	25.7 mm S _L 1.98 g M _w	-0.47°C	0.80	Fraser et al. (2007)	
			27.7 mm S _L 2.24 g M _w	-1.62°C		0.55
	³ H	23.9 mm S _L 1.50 g M _w	-1.5°C	0.50	Fraser et al. (2007)	
			24.6 mm S _L 1.59 g M _w	1.0°C		0.85
			24.3 mm S _L 1.55 g M _w	3.5°C		0.50
			23.5 mm S _L 1.21 g M _w	6.0°C		0.55
Cephalopoda						
<i>Octopus vulgaris</i>	³ H	199 g M _w	22.0°C	3.8	Houlihan et al. (1990b)	
<i>Euprymna tasmanica</i>	³ H	2.8 g M _w	18.0°C	9.45	Carter et al. (2009)	
		14.8 g M _w		1.49		
	³ H	60 d old 0.92 g M _w	20.0°C	4.78	Moltschaniwskyj and Carter (2010)	
		100 d old 3.72 g M _w		3.77		
		140 d old 6.54 g M _w		3.10		

626 T°C - Temperature; S_L – shell length M_w – wet mass; M_D – dry mass

627 **Table 3.** Summary of fractional rates of protein synthesis (k_s , % d⁻¹) in various body tissues
 628 for Phylum Mollusca.

Species	Tracer	Size	Tissue	k_s (% d ⁻¹)	T°C & Reference		
Bivalvia							
<i>Mytilus edulis</i>	³ H	61 mm S _L	Gill	9.54	15°C		
		4.00 g M _w	Mantle	2.51	This study		
Gastropoda							
<i>Helix aspersa</i>	³ H	Awake	Hepato ¹	3.3	22 - 25°C		
		4.2 g M _w	Foot ²	2.6	Pakay et al. (2002)		
		Aestivate	Hepato ¹	0.8			
		3.8 g M _w	Foot ²	1.4			
<i>Octopus vulgaris</i>	³ H	14.8 g M _w	Arm	2.92	22°C		
			Arm Tip	3.10	Houlihan et al.		
			Brain	3.64	(1990b)		
			Branchial heart	3.19			
			Gill	2.96			
			Mantle	2.33			
			Renal	2.36			
			Appendage				
			Stomach	2.94			
			<i>Euprymna tasmanica</i>	³ H	2.8 g M _w	Arms	5.37
Digestive Gland	11.31	Carter et al. (2009)					
Anterior Mantle	6.13						
Posterior Mantle	6.86						
14.8 g M _w	Arms	1.43				20°C	
	Digestive Gland	9.24				Carter et al. (2009)	
	Anterior Mantle	0.56					
	Posterior Mantle	0.36					
	³ H	Immature 3.33 g M _w			Gonad*	9.67	18°C
					Mantle	4.56	Moltschaniwskyj and Carter (2013)
Mature 5.58 g M _w		Gonad			12.23	18°C	
		Mantle			1.67	Moltschaniwskyj and Carter (2013)	

629 S_L – shell length; M_w – wet mass; M_D – dry mass.

630 ¹ = Hepatopancreas; ² = Foot muscle; * estimated from Figure 2a in Moltschaniwskyj and
 631 Carter (2013)