

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

McCarthy, I.D.; Nicholls, R.; Malham, S.K.; Whiteley, N.M.

# Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology

DOI: 10.1016/j.cbpa.2015.10.019

Published: 23/10/2015

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):* McCarthy, I. D., Nicholls, R., Malham, S. K., & Whiteley, N. M. (2015). Validation of the flooding dose technique to determine fractional rates of protein synthesis in a model bivalve species, the blue mussel (Mytilus edulis L.). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 191, 166-173. https://doi.org/10.1016/j.cbpa.2015.10.019

#### Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.

- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

- **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

4	Ian D McCarthy <sup>a,b*</sup> , Ruth Nicholls <sup>a</sup> , Shelagh K Malham <sup>a</sup> and Nia M Whiteley <sup>c</sup>
5	<sup>a</sup> School of Ocean Sciences, College of Natural Sciences, Bangor University, Askew Street,
6	Menai Bridge, Anglesey, LL59 5AB, UK
7	<sup>b</sup> Laboratorio de Manejo, Ecologia e Conservação Marinha, Instituto Oceanográfico,
8	Universidade de São Paulo, Praça do Oceanográfico 191, Cidade Universitária, 05508-120
9	São Paulo SP, Brazil
10	<sup>c</sup> School of Biological Sciences, College of Natural Sciences, Bangor University, Deiniol
11	Road, Bangor, Gwynedd, LL57 2UW, UK
12	
13	* Corresponding author. Tel. 00 44 1248 382862;
14	E-mail address: i.mccarthy@bangor.ac.uk (I.D. McCarthy).
15	
16	Abstract
17	For the first time, use of the flooding dose technique using <sup>3</sup> H-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

- <sup>3</sup>H-phenylalanine into the posterior adductor muscle. Incorporation of <sup>3</sup>H-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 % d^{\text{-1}} for the gill, 2.5 $\pm$ 0.3 % d^{\text{-1}} for the mantle and 2.6 $\pm$ 0.3 % d^{\text{-1}} for the
29	whole-animal, respectively (mean values $\pm$ SEM). The whole-animal absolute rate of protein
30	synthesis was calculated as $18.9 \pm 0.6$ mg protein day <sup>-1</sup> . 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.

#### 36 Key words:

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

#### 39 **1. Introduction**

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

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

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

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, including aquatic ectotherms, because of the ease of tracer administration through a single 78 79 injection, the rapid flooding of the body free amino acid pools and the ability to selectively extract and measure free pool and protein-bound phenylalanine-specific radioactivities using 80 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 phenylalaninespecific radioactivity of the intracellular free amino acid pools is rapidly elevated following 83 84 injection; (2) the phenylalanine-specific radioactivity of the intracellular free amino acid pools either remain elevated and stable or show a slow linear decline over the incubation 85 period (Garlick et al., 1980, 1983); (3) the uptake of radiolabel into body protein is linear 86 87 over the incubation period; (4) the presence of a high dose of <sup>3</sup>H-L-phenylalanine does not 88 stimulate or depress rates of protein synthesis following injection. When applying the flooding dose technique to a new species for the first time, validation studies that examine the 89 90 changes in the free pool and protein-bound phenylalanine-specific radioactivities over time are essential (Fraser and Rodgers, 2007). 91

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

101 role, adaptive physiology for life in the intertidal and commercial importance of this taxa (e.g. Navarette and Menge, 1996; Ragnarsson and Raffaelli, 1999; Somero, 2002; Gosling, 102 2003; Gracey et al., 2008; Smith et al., 2014). In vivo rates of whole-animal protein synthesis 103 have been measured in bivalve molluscs of the genus *Mytilus* using <sup>15</sup>N-labelled algae and a 104 stochastic end-point model (e.g. Hawkins, 1985; Hawkins et al., 1989; Bayne and Hawkins, 105 1997). The results of these studies have provided some fundamental insights into the role that 106 107 inter-individual variation in protein turnover plays in individual growth performance, in terms of both growth rates and growth efficiency (Hawkins et al., 1989), have shown links between 108 109 genetic heterozygosity, protein turnover and growth performance (Hawkins et al., 1986; Bayne and Hawkins, 1997; Hawkins and Day, 1999) and examined the effects of salinity and 110 acute temperature change in the laboratory (Hawkins et al., 1987; Hawkins and Hilbish, 111 112 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-[<sup>3</sup>H]phenylalanine to measure whole-animal and tissue-specific rates of protein synthesis in 114 115 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 of protein synthesis in bivalves that are directly comparable with the range of other taxa that 117 have been studied using the flooding dose technique of Garlick et al. (1980). In addition, 118 whole-animal rates of protein synthesis can be compared with the data of Hawkins and co-119 workers (op cit) obtained using a stochastic endpoint model method. 120

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

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

133

#### 134 2.2 Radiolabel injection and incubation time course

Fractional rates of protein synthesis were measured using a modification of the flooding dose 135 136 technique (Garlick et al., 1980; Houlihan et al., 1995a). Immediately prior to injection, the mussels were removed from seawater, blotted dry and the shell valves were notched in the 137 vicinity of the posterior adductor muscle. Four groups of five mussels were injected, in turn, 138 into the sinus of the posterior adductor muscle with a solution containing 135 mM L-139 phenylalanine and L-[2, 6-<sup>3</sup>H]-phenylalanine (American Radiolabelled Chemicals (UK); 3.7 140 MBq ml<sup>-1</sup>). The injection volume used was 10  $\mu$ l g<sup>-1</sup> wet body mass (Houlihan et al., 1995a; 141 Fraser et al., 2002) with wet body mass (M<sub>W</sub>) estimated from shell length (S<sub>L</sub>) using the 142 following calibration equation for mussels at Tal y Foel:  $M_W = 0.00005 S_L^{2.74}$  (n = 50, r<sup>2</sup> = 143 0.927, p < 0.001; Busbridge, unpublished results). The phenylalanine-specific radioactivity of 144 the injection solution was  $838 \pm 33$  disintegrations per minute per nanomole phenylalanine 145 (dpm nmole<sup>-1</sup> phe). Following injection, each group of five injected mussels was placed in a 146 separate 5 l tank (20 x 15 x15cm) containing aerated seawater and left for either 1, 2, 4 or 6 h 147 to allow incorporation of the injected radiolabel. The fifth group was notched but not injected 148 and served as a control group (i.e. 0 h incorporation). After notching of the control group or 149 following incubation of the injected groups, each mussel was dissected by cutting through the 150

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

157

### 158 2.3 Laboratory analyses

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

176 Morgan et al., 1998; McCarthy and Fuiman, 2008). The concentration of phenylalanine in blue mussel tissue and whole-animal protein was measured in replicate samples (n=10) by 177 ion exchange analysis (PNAC, Department of Biochemistry, Cambridge University) for 178 179 mussels collected from the same collection site providing values of 112.4, 134,3 and 192.2 nmol phe mg<sup>-1</sup> protein for gill, mantle and whole-animal protein respectively. The 180 phenylalanine-specific radioactivity of the gill, mantle and whole-animal free pools (S<sub>a</sub>, dpm 181 nmol<sup>-1</sup> phe) was calculated by converting phenylalanine in the free pool to  $\beta$ -182 phenylethylamine (PEA) and extracting through heptane into 0.01M sulphuric acid and 183 184 measuring PEA content by fluorescence (Suzuki and Yagi, 1976) and PEA radioactivity by scintillation counting. 185 Fractional rates of protein synthesis (ks, expressed as a percentage of the protein mass 186 synthesised per day, %  $d^{-1}$ ) were calculated for gill, mantle and whole-animal samples as  $k_s =$ 187  $100.((S_b/S_a).(1440/t))$ , where  $S_b$  and  $S_a$  are the protein-bound and free pool phenylalanine 188 specific radioactivities (dpm nmole<sup>-1</sup> phe) and t is the incubation time (between injection and 189 190 freezing) in minutes and 1440 is the number of minutes in a day (Garlick et al., 1983). Whole-animal absolute rates of protein synthesis ( $A_s$ , mg protein synthesised per day, mg d<sup>-1</sup>) 191 were calculated as  $A_s = (k_s/100).(M_{prot})$  where  $M_{prot}$  is the whole-animal protein mass (mg). 192 RNA concentrations were expressed as the capacity for protein synthesis ( $C_s$ ,  $\mu g$  RNA mg<sup>-1</sup> 193 protein) and as RNA activity (k<sub>RNA</sub>, mg protein synthesised per mg RNA per day, mg mg<sup>-1</sup> d<sup>-</sup> 194 <sup>1</sup>) calculated as  $(10.k_s)/C_s$  (Sugden and Fuller, 1991). 195 196

197 2.4 Statistical analyses

All data are presented as mean values  $\pm$  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  $log_{10}$ -transformed if necessary to meet assumption for parametric statistical tests.

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

209

#### 210 **3. Results**

211 *3.1. Time course validation* 

212 The intracellular free pool specific radioactivities (S<sub>a</sub>) in the gill, mantle and whole-animal were elevated within one hour of injection (Fig. 1a-c) and remained elevated over the 6 h 213 time course with average  $S_a$  values of  $642 \pm 23$  dpm nmol<sup>-1</sup> phe for the gill,  $562 \pm 14$  dpm 214 nmol<sup>-1</sup> phe for the mantle and  $501 \pm 36$  dpm nmol<sup>-1</sup> phe for the whole-animal, respectively, 215 which were equivalent to  $77 \pm 3$  %,  $67 \pm 2$  % and  $60 \pm 4$  % of the specific radioactivity of 216 the injection solution. In the mantle and whole-animal, S<sub>a</sub> values were similar over the six 217 hour time course (mantle,  $F_{3,19}$ = 0.36, p = 0.78; whole-animal,  $F_{3,19}$ = 1.21, p = 0.34). In the 218 gill, S<sub>a</sub> was significantly higher after 2 h incubation compared with 4 h ( $F_{3,19}$ = 5.94, p = 219 0.006; Tukey *post-hoc* pairwise comparison, p = 0.003) but the other free pool S<sub>a</sub> values were 220 similar between 1 and 6 h incubation (Tukey *post-hoc* pairwise comparisons, p = 0.14 to p =221 0.99) (Fig. 1a). Thus, phenylalanine-specific radioactivities in the gill, mantle and whole-222 animal free pools remained elevated and stable over the 6 h time-course experiment. 223 The time course incorporation of <sup>3</sup>H phenylalanine into gill, mantle and whole-animal 224 protein pools are shown in Fig. 2a-c. The incorporation rates of radiolabel into all three 225

protein pools over the 6 h time course all exhibited significant linear relations and weredescribed 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 <sup>-1</sup> 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*

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

Data on the capacity for protein synthesis, RNA activity and fractional rates of protein synthesis for gill, mantle and whole-animal tissue are presented in Table 1. The capacity for protein synthesis (C<sub>s</sub>, Table 1) was significantly different between gill, mantle and whole animal tissue ( $F_{2,57}$ = 20.03, p < 0.001; Tukey's HSD pairwise comparisons, gill *vs* mantle or whole animal p < 0.01, mantle *vs* whole animal p < 0.05). However, RNA activity was significantly higher in gill tissue compared with the mantle and whole-animal ( $F_{2,57}$ = 37.34, p<0.001; Tukey's HSD pairwise comparisons, gill *vs.* mantle or whole animal both p < 0.01; mantle *vs* whole animal p > 0.05). This increased RNA activity resulted in significantly higher fractional rates of protein synthesis in gill tissue compared to the mantle and wholeanimal (ANOVA on log<sub>10</sub>-transformed data;  $F_{2,57}$ = 68.93, p < 0.001; Tukey's HSD pairwise comparisons, Gill vs. mantle or whole animal both p < 0.01, mantle *vs* whole animal p >0.05) (Table1).

258

#### 259 4. Discussion

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

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

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

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

The values obtained here for *M. edulis* at 15°C compare favourably with those 308 obtained by Hawkins and co-workers using <sup>15</sup>N-labelled algae measuring the tracer flux 309 through the animal by the appearance of <sup>15</sup>N in excreted ammonia and using a stochastic end-310 point model to determine absolute protein synthesis rates (Hawkins, 1985; Hawkins et al., 311 312 1986, 1989; Hawkins and Hilbish, 1992) (Fig. 4). Although the data set is limited, absolute rates of protein synthesis reported in the present study are of a magnitude expected for 313 mussels of this size at 15°C (rate predicted from regression, 20.9 mg d<sup>-1</sup>; observed rate 18.9 314 mg d<sup>-1</sup>) providing confidence in the measurements obtained. Synthesis rate data are compared 315 to shell length as body mass data are not comparable (wet mass data are presented in the 316 present study and dry mass data presented by Hawkins and co-workers) Similar concordance 317 between measurements using the flooding dose technique and the stochastic endpoint method 318 319 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. In most studies of protein metabolism in aquatic ectotherms, rates of protein synthesis 321 have been reported on a fractional basis, i.e. as a percentage of the protein mass synthesised 322 323 per day. Tables 2 and 3 summarise the whole-animal and tissue fractional rates of protein synthesis published for molluscs. The molluscan dataset set is limited to 10 studies in total 324

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 studies; Web of Science using Garlick et al. (1980) as a cited reference, search conducted 327 22/7/15]. The molluscan data focuses primarily on whole-animal rates in the Antarctic limpet 328 329 Nacella concinnus, on whole-animal and tissue rates in the dumpling squid Euprymna tasmanicus together with the whole-animal data for Mytilus edulis by Hawkins and co-330 workers (Tables 2 and 3). The whole-animal data presented in Table 2 can be described by 331 the following equation:  $Log_{10}k_s = 0.044T - 0.306 (r^2 = 0.821, n = 16, p < 0.0001)$ , where  $k_s$  is 332 the fractional rate of protein synthesis measured using the flooding dose technique and T is 333 water temperature (°C). This predicts a fractional rate of protein synthesis of 2.26 % d<sup>-1</sup> at 334  $15^{\circ}$ C which is close to the measured value (2.56 % d<sup>-1</sup>), indicating that the rate measured in 335 the present study is of the magnitude expected (although factors such as size and feeding 336 337 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 organs as well as in the whole-animal. Tissue rates of protein synthesis are important because 339 340 they can indicate specific responses to environmental change that may be masked in the whole-animal (Fraser and Rogers, 2007). The available data on tissue-specific fractional rates 341 of protein synthesis in molluscs are limited to 5 studies (Table 3) but in comparison to the 342 rates obtained in other aquatic ectotherms, the data show the same patterns in the ranking of 343 344 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 compared to muscle tissue [Table 3; crustaceans (*Carcinus maenas*, Houlihan et al., 1990a; 346 Nephrops norvegicus, Mente et al., 2011); fish (Oncorhynchus mykiss, Houlihan et al., 1986, 347 Foster et al., 1991; Salmo salar Martin et al., 1993)], usually associated with high tissue 348 capacities for protein synthesis and RNA activity (Table 1; Houlihan et al., 1990a, 1990b; 349 350 Foster et al., 1991; Carter et al., 2009). However, the exception appears to be mantle tissue in

young, fast-growing cephalopods (Table 3) where as part of their r-selected life histories
('living life in the fast lane', Carter et al., 2009), growth is rapid in order to reach maturity
and spawn within their short lifespans (usually < 1 year). Metabolically-active tissues such as</li>
the gills are likely to have higher rates of protein synthesis to allow for protein remodelling
and flexibility in enzyme production in keeping with the function of the gill as a specialised
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 technique to measure whole-animal and tissue-specific rates of protein synthesis in the blue 358 359 mussel Mytilus edulis. The use of this technique in measuring one of the major components of maintenance metabolism and growth will provide a valuable tool in furthering our 360 understanding of the intriguing relationship between protein synthesis rates, growth rates and 361 362 energy expenditure previously suggested by Hawkins and Day (1999) for this species. In addition, the comparative study of rates of protein turnover in a range of aquatic ectotherm 363 species within the 'fast-slow pace of life' continuum (Ricklefs and Wikelski, 2002) will 364 provide valuable insight into the physiological costs and energetic trade-offs associated with 365 fast growth/early maturation versus slow growth/delayed maturation (e.g. cephalopods versus 366 long-lived fish species). Indeed, validation of the flooding dose technique for bivalve 367 molluscs will allow protein turnover rates to be studied in a range of bivalve molluscs where 368 369 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 islandica, Butler et al., 2013) to determine the contribution of changing rates of protein 371 turnover to the ageing process and senescence. Finally, the study of tissue-specific and 372 373 whole-animal rates of protein synthesis will also provide understanding on the ability of this keystone marine invertebrate to adjust and respond to environmental fluctuations, such as that 374 375 expected as a result of climate change.

377 Acknowledgements

We thank Berwyn Roberts for collecting the mussels, Tom Busbridge for use of his unpublished data and Peter Sharratt (PNAC, Cambridge University) for conducting the amino acid analysis. This work was conducted as part of the project entitled: Shellfish Productivity in the Irish Sea, working towards a sustainable future (SUSFISH). SUSFISH is part-funded by the European Regional Development Fund (ERDF) through the Ireland Wales Territorial Co-operation (INTERREG 4A) Programme 2007–2013. IDM was funded by FAPESP (grant 14/21804-3 during the writing of this manuscript).

- 385
- 386

#### 387 **References**

- Bayne, B.L., Hawkins, A.J.S., 1997. Protein metabolism, the costs of growth, and genomic
  heterozygosity: experiments with the mussel *Mytilus galloprovincialis* Lmk. Physiol.
  Zool. 70, 391-402.
- Bowgen, A., Fraser, K.P.P., Peck, L.S., Clarke, A., 2007. Energetic cost of synthesizing
  proteins in Antarctic limpet, *Nacella concinna* (Strebel, 1908), is not temperature
  dependent. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R2266-R2274.
- Brocas, W.M., Reynolds, D.J., Butler, P.G., Richardson, C.A., Scourse, J.D., Ridgway, I.D.,
- Ramsay, K., 2013. The dog cockle, *Glycymeris glycymeris* (L.), a new annually-resolved
- sclerochronological archive for the Irish Sea. Palaeogeogr. Palaeoclimatol. Palaeoecol.
  373, 133-140.
- Butler, P.G., Wanamaker Jr, A.D., Scourse, J.D., Richardson, C.A., Reynolds, D.J., 2013.
  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., Moltschaniwskyj, 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.
- Carter, C.G., Mente, E., 2014. Protein synthesis in crustaceans: a review focused on feeding
  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,
- M.J., 1994. Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss*,
  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

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.
- Doherty, M.K., Whitfield, P.D., 2011. Proteomics moves from expression to turnover: update
  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 changes in protein synthesis in the Antarctic limpet Nacella concinna Strebel 1908. J. 425 Exp. Biol. 205, 3077-3086. 426
- 427 Fraser, K.P.P., Houlihan, D.F., Lutz, P.L., Leone-Kabler, S., Manuel, L., Brechin, J.G., 2001.

- Complete suppression of protein-synthesis during anoxia with no post-anoxia protein synthesis debt in red-eared slider turtle Trachemys scripta elegans. J. Exp. Biol. 204, 429 430 4353-4360.
- Fraser, K.P.P., Peck, L.S., Clarke, A., 2004. Protein synthesis, RNA concentrations, nitrogen 431 432 excretion, and metabolism vary seasonally in the Antarctic holothurian Heterocucumis steineni (Ludwig 1898). Physiol. Biochem. Zool. 77, 556-569. 433
- Fraser, K.P.P., Rodgers, A.D., 2007. Protein metabolism in marine animals: the underlying 434 435 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 cost and contribution to oxygen consumption. Comp. Biochem. Physiol. A 119, 459-467. 437
- Fuery, C.J., Withers, P.C., Hobbs, A.A., Guppy, M., 1998a. The role of protein synthesis 438
- during metabolic depression in the Australian desert frog Neobatruchus centralis. Comp. 439 440 Biochem. Physiol. A 119, 469-476.
- Garlick, P.J., Fern, M., Preedy, V.R., 1983. The effect of insulin infusion and food intake on 441 muscle protein synthesis in postabsorptive rats. Biochem. J. 210, 669-676. 442
- Garlick, P.J., McNurlan, M.A., Essen, P., Wernerman, J., 1994. Measurement of tissue 443 protein-synthesis rates in-vivo - a critical analysis of contrasting methods. Am. J. 444 Physiol. Endoc. Metab. 282, E287-E297. 445
- Garlick, P.J., McNurlan, M.A., Preedy, V.R., 1980. A rapid and convenient technique for 446 measuring the rate of protein synthesis in tissues by injection of 3H phenylalanine. 447 Biochem. J. 217, 507-516. 448

- Gosling, E., 2003. *Bivalve Molluscs: Biology, Ecology and Culture*. Wiley-Blackwell, 454
  pp.
- 451 Gracey, A.Y., Chaney, M.L., Boomhower, J.P., Tyburczy, W.R., Connor, K., Somero, G.N.,
- 2008. Rhythms of gene expression in a fluctuating intertidal environment. Curr. Biol. 18,1501-1507.
- Hawkins, A.J.S., 1985. Relationships between the synthesis and breakdown of protein,
  dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L. Oecologia (Berlin) 66, 42-49.
- Hawkins, A.J.S., Bayne, B.L., Day, A.J., 1986. Protein turnover, physiological energetics and
  heterozygosity in the blue mussel, *Mytilus edulis*: the basis of variable age-specific
- 459 growth. Proc. R. Soc. Lond. B 229, 161-176.
- Hawkins, A.J.S. and Day A.J., 1999. Metabolic interrelations underlying the physiological
  and evolutionary advantages of genetic diversity. Am. Zool. 39, 401-411.
- Hawkins, A.J.S. Hilbish, T.J., 1992. The costs of cell volume regulation: protein metabolism
  during hyperosmotic adjustment. J. Mar. Biol. Ass. U.K. 72, 569-578.
- Hawkins, A.J.S., Widdows, J., Bayne, B.L., 1989. The relevance of whole-body protein
  metabolism to measured costs of maintenance and growth in *Mytilus edulis*. Physiol.
  Zool. 62, 745-763
- Hawkins, A.J.S., Wilson, I.A., Bayne, B.L., 1987. Thermal responses reflect protein turnover
  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.

- Houlihan, D.F., Carter, C.G., McCarthy, I.D., 1995b. Protein synthesis in fish. In: P.
  Hochachka and P. A. Walsh (Eds.), Biochemistry and Molecular Biology of Fishes, 4,
  Elsevier, Amsterdam, pp. 191-219.
- Houlihan, D.F., McMillan, D.N., Agnisola, C., Trara Genoino, I., Foti, L., 1990b. Protein
  synthesis and growth in *Octopus vulgaris*. Mar. Biol. 106, 251-259.
- Houlihan, D.F., McMillan, D.N., Laurent, P., 1986. Growth rates, protein synthesis, and
  protein degradation rates in rainbow trout: effects of body size. Physiol. Zool. 59. 482479 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.
- Houlihan, D.F., Waring, C.P., Mathers, E., Gray, C., 1990a. Protein synthesis and oxygen
  consumtion of the shore crab *Carcinus maenas* after a meal. Physiol. Zool. 63, 735-756.
- Houlihan, D.F., Wieser, W., Foster, A., Brechin, J., 1992. In vivo protein synthesis rates in
  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.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with
  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.
- Malham, S.K., Hutchinson, T.H., Longshaw, M. 2012. A review of the biology of European
  cockles (*Cerastoderma* spp.). J. Mar. Biol. Assoc. UK 92, 1563–1577.

- Martin, N.B., Houlihan, D.F., Talbot, C., Palmer, R.M., 1993. Protein metabolism during
  sexual maturation in female Atlantic salmon (*Salmo salar* L). Fish Physiol. Bochem. 12,
  131-141.
- McCarthy, I.D., Fuiman, L.A., 2008. Growth and protein metabolism in red drum (*Sciaenops ocellatus*) larvae exposed to environmental levels of atrazine and malathion. Aquat.
  Toxicol. 88, 220-229.
- McCarthy, I.D., Houlihan, D.F., Carter, C.G., (1994). Individual variation in protein turnover
  and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Proc. R. Soc.
  Lond. B 257, 141-147.
- McCarthy, I.D., Moksness, E., Pavlov, D.P., Houlhan, D.F., 1999. Effects of water
  temperature on protein synthesis and protein growth in juvenile Atlantic wolfish
  (*Anarhichas lupus*). Can. J. Fish. Aquat. Sci. 56, 231-241
- McNurlan, M.A., Essen, P., Heys, S.D., Buchan, V., Garlick, P.J., Wernerman, J., 1991.
  Measurement of protein synthesis in human skeletal-muscle further investigation of the
  flooding technique. Clin. Sci. 81, 557-564.
- McNurlan, M.A., Tomkins, A.M., Garlick, P.J., 1979. Effect of starvation on the rate of
  protein synthesis in rat liver and small intestine. Biochem. J. 178, 373-379.
- Mejbaum, W., 1939. Über die bestimmung kleiner pentosemengen insbesondere in derivaten
  der adenylsaüre. Hoppe-Seyler's Z. Physiol. Chem. 258, 117-120.
- Mente, E., Carter, C.G., Barnes (Katersky), R.S., Karapanagiotidis, I.T., 2011. Protein
  synthesis in wild-caught Norway lobster (*Nephrops norvegicus* L.). J. Exp. Mar. Biol.
  Ecol. 409, 208-214.
- Moltschaniwskyj, N.A., Carter, C.G., 2010. Protein synthesis, degradation and retention:
  Mechanisms of indeterminate growth in cephalopods. Physiol. Biochem. Zool. 83, 9971008.

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
- ammonia) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 19, 377-389.
- Navarette, S.A., Menge, B.A., 1996. Keystone predation and interaction strength: interactive
  effects of predators on their main prey. Ecol. Monog. 66, 409-429.
- Pakay, J.L., Withers, P.C., Hobbs, A.A., Guppy, M., 2002. In vivo downregulation of protein
  synthesis in the snail *Helix apersa* during estivation. Am. J. Physiol. Regul. Integr.
  Comp. Physiol. 283, R197-R204.
- Ragnarsson, S.A., Raffaelli, D., 1999. Effects of the mussel *Mytilus edulis* L. on the
  invertebrate fauna of sediments. J. Exp. Mar. Biol. Ecol. 241, 31-43.
- Rastrick, S.P.S. Whiteley, N.M., 2013. Influence of natural thermal gradients on whole
  animal rates of protein synthesis in marine gammarid amphipods. PLoS ONE 8, e60050.
- 537 doi:10.1371/journal.pone.0060050.
- Rennie, M.J., Smith, K., Watt, P.W., 1994. Measurement of human tissue protein-synthesis 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
- Schacterle, G.R, Pollack, R.I., 1973. Simplified method for quantitative assay of small
  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.
- 549Availableonlineat<a href="http://www.devotes-project.eu/wp-">http://www.devotes-project.eu/wp-</a>550content/uploads/2014/07/DEVOTES-D6-1-Keystones.pdf
- 551 Somero, G.N., 2002. Thermal physiology and vertical zonation of intertidal animals: Optima,
- limits, and costs of living. Int. Comp. Physiol. 42, 780-789.
- Sugden, P.H., Fuller, S,J., 1991. Regulation of protein turnover in skeletal and cardiac
  muscle. Biochem.J. 273, 21-37.
- Suzuki, O., Yagi, K., 1976. A fluorometric assay for phenylethylamine in rat brain. Anal.
  Biochem. 75, 192-200.
- Wagenmakers, A.J., 1999. Tracers to investigate protein and amino acid metabolism in
  human subjects. Proc. Nutr. Soc. 58, 987-1000.
- Waterlow, J.C., 1995. Whole-body protein turnover in humans past, present, and future.
  Ann. Rev. Nutr. 15, 57-92.
- Wilson, R.W., Wood, C.M., Houlihan, D.F., 1996. Growth and protein turnover during
  acclimation to acid and aluminium in juvenile rainbow trout (*Oncorhynchus mykiss*).
- 563 Can. J. Aquat. Sci. 53, 802-811.
- 564
- 565
- 566

#### 567 **Figure Legends**

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

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

581

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

587

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



Fig. 1



Fig. 2



Fig. 3



## 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
ks	$9.54\pm0.78$	$2.51\pm0.30^{\rm a}$	$2.58\pm0.29^{\rm a}$
$(\% d^{-1})$			
Cs	$50.81 \pm 1.88$	$40.44 \pm 2.62$	$32.69 \pm 1.39$
(µg RNA mg <sup>-1</sup> protein)			
k <sub>RNA</sub>	$1.98\pm0.15$	$0.62\pm0.07^{\rm a}$	$0.83\pm0.11^{\rm a}$
(mg protein mg <sup>-1</sup> RNA d <sup>-1</sup> )			

617

618 Mean  $\pm$  SEM (n=20).

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

Same letter, along a row, indicate no significant difference following one-way ANOVA andTukey HSD pairwise comparisons.

622

624	<b>Table 2.</b> Summary of whole-animal fractional rates of protein synthesis ( $k_s$ , % d <sup>-1</sup> ) for	r
625	Phylum Mollusca.	

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

 $626 T^{\circ}C - Temperature; S_L - shell length M_W - wet mass; M_D - dry mass$ 

**Table 3.** Summary of fractional rates of protein synthesis ( $k_s$ , % d<sup>-1</sup>) in various body tissues 627

for Phylum Mollusca. 628

Species	Tracer	Size	Tissue	$k_s$ (% d <sup>-1</sup> )	T°C & Reference
Bivalvia					
Mytilus edulis	<sup>3</sup> H	$\begin{array}{l} 61 \text{ mm } S_L \\ 4.00 \text{ g } M_W \end{array}$	Gill Mantle	9.54 2.51	15°C This study
Gastropoda	311	A	Hamatal	2.2	22 - 250C
Helix aspersa	Ή	Awake 4.2 g M <sub>w</sub>	Foot <sup>2</sup>	3.3 2.6	22 - 25°C Pakay et al. (2002)
		Aestivate 3.8 g M <sub>W</sub>	Hepato <sup>1</sup> Foot <sup>2</sup>	0.8 1.4	
Octopus vulgaris	<sup>3</sup> H	14.8 g Mw	Arm	2.92	22°C
1 0		0	Arm Tip	3.10	Houlihan et al.
			Brain	3.64	(1990b)
			Branchial	3.19	
			Gill	2.96	
			Mantle	2.33	
			Renal	2.36	
			Appendage		
			Stomach	2.94	
Euprymna	<sup>3</sup> H	2.8 g M <sub>W</sub>	Arms	5.37	18°C
tasmanica		-	Digestive	11.31	Carter et al. (2009)
			Gland		
			Anterior	6.13	
			Mantle		
			Posterior	6.86	
			Mantle		
		14.8 a Mw	Arms	1 /3	20°C
		14.0 g WIW	Digestive	9.74	Carter et al. (2009)
			Gland	9.21	
			Anterior Mantle	0.56	
			Posterior	0.26	
			Mantle	0.36	
	<sup>3</sup> H	Immature	Gonad*	9.67	18°C
		3.33 g Mw	Mantle	4.56	Moltschaniwskyj and Carter (2013)
		Mature	Gonad	12.23	18°C
		$5.58 \ g \ M_W$	Mantle	1.67	Moltschaniwskyj and Carter (2013)

 $S_L$  – shell length;  $M_W$  – wet mass;  $M_D$  – dry mass. <sup>1</sup> = Hepatopancreas; <sup>2</sup> = Foot muscle; \* estimated from Figure 2a in Moltschaniwskyj and 630

Carter (2013) 631