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Allometric size-scaling of biometric growth parameters and metabolic and excretion rates. A comparative study of intertidal and subtidal populations of mussels (*Mytilus galloprovincialis*)

Kristina Arranz[a], Uxío Labarta[a]*, M. José Fernández-Reiriz[a] and Enrique Navarro[b]

[a]Consejo Superior de Investigaciones Científicas (CSIC), Instituto de Investigaciones Marinas (IIM), C/Eduardo Cabello 6, 36208 Vigo, Spain

[b]Departamento GAFFA (Animal Physiology), Facultad de Ciencia y Tecnología, Universidad del País Vasco/Euskal Herriko Unibertsitatea, Apartado 644, 48080 Bilbao, Spain

Abstract

Allometric relationships between biometric parameters (i.e., soft body and shell weights and shell organic content vs. shell length) as well as for routine and standard metabolic and ammonia excretion rates related to flesh weight and shell length were estimated and compared for subtidal and intertidal populations of *Mytilus galloprovincialis* in Galicia (NW Spain). This is the first report on allometric size–scaling of excretion and metabolic (both routine and standard) rates in this species. No evidences of differences in size–exponent were found between physiological rates or between both populations for any physiological rate. Intercepts of regression lines were significantly higher in subtidal than in intertidal mussels, indicating greater levels of energy expenditure in the former. However, metabolic scope for feeding and growth was about two–fold in intertidal mussels, pointing to a reduced growth efficiency compared with subtidal mussels. Evolution of biometric parameters of body components with size indicated that subtidal mussels allocated energy resources preferably into flesh growth, achieving higher condition indices, while intertidal mussels put more effort on shell calcification and thickening which resulted in heavier shells of reduced organic content. These

differentiated growth "strategies" of both populations could be related to their differences in growth efficiencies.

*Corresponding author. Tel.: +34 986231930, fax: +34 986292762. E-mail address: labarta@iim.csic.es (Uxío Labarta).

33 ## *Introduction*

34 The extensive culture of the mussel (*M. galloprovincialis*), with a production volume
35 that ranged between 200,000–300,000 tons, and a production value that exceeded 100
36 million euros in 2012 (www.pescadegalicia.com), is the main aquaculture industry in
37 Galicia (NW Spain). Mussels are cultured in floating systems (rafts) consisting of a
38 500m^2 wood structure anchored to the seafloor, from which culture ropes and/or seed
39 collectors are suspended. Nowadays, the number of ropes per raft is limited to 500 and
40 ca. 3300 rafts are located in the Galician Rias. Mussel culture is scheduled according to
41 the availability of natural resources for feeding and seed recruitment, the biological
42 cycle of mussels and the fluctuations of market demand (Labarta et al., 2004).

43 Producers collect mussel seeds for culture either form intertidal or subtidal habitats;
44 hence, the physiological and metabolic differences associated to the origin of the
45 individuals may constitute an important factor toward the optimization of mussel
46 production (Pérez-Camacho et al., 2013; 2014). Several factors have been invoked to
47 account for such differences in physiological behavior:

48 In the first place, intertidal populations are subjected to cycles of air exposure, which
49 implies intervals of hypoxic or anoxic conditions. Moreover, tidal cycles lead to
50 periodical shortages in feeding (Peterson & Black, 1988; Marsden & Weatherhead,
51 1999). Intertidal mussels cannot compensate for periods of starvation; even though
52 some advantages of the intertidal habitat have been identified, such as organic matter
53 resuspension and thermal fluctuations liable to improve energy gain at low tide by
54 increasing rates of digestion (Elvin & Gonor, 1979; Bayne et al., 1988). Storey &
55 Storey (1990) observed that organisms subjected to air exposure periods present a

56 reduced metabolic rate, considering this response as an energy saving mechanism acting
57 to compensate for the lesser feeding or energy acquisition time (Shick et al., 1988).

58 In the second place, physiological responses associated with the origin of individuals
59 have been considered as an indicator of the existence of genetic diversity (Rawson &
60 Hilbish, 1991; Widdows et al., 1984; Dickie et al., 1984). However, some other authors
61 suggest that these responses would reflect the persistence of original habitat influences
62 in the form of an "ecological memory" (Mallet et al., 1987; Okumuş & Stirling, 1994;
63 Pérez-Camacho et al., 1995; Labarta et al., 1997; Babarro et al., 2000a;b; 2003). In *M.*
64 *galloprovincialis*, such ecological memory is found to be responsible for the differences
65 in physiological condition between intertidal and subtidal populations. Hence, subtidal
66 individuals exhibited higher values of growth rate, condition index (Pérez-Camacho et
67 al., 1995; Babarro et al., 2003), energy reserves (i.e., triacylglycerol and phospholipid
68 levels) (Freites et al., 2002), and SFG ("Scope for growth", Labarta et al., 1997); since,
69 despite of their higher values of oxygen consumption (Babarro et al., 2000b) and
70 ammonia excretion rates, both clearance rate (Labarta et al., 1997; Babarro et al., 2000a)
71 and absorption efficiencies (Labarta et al., 1997) were also higher in subtidal mussels.

72 Differentiated growth trends encompass most of these physiological differences
73 between intertidal and subtidal mussel populations. Growth is frequently measured in
74 bivalves as changes in shell length or weight, but this approach tends to disregard
75 essential features of this phenomenon. For example, growth trajectories often differ for
76 shell and soft tissues according to environmental factors or variations in the
77 reproductive cycle (Hilbish, 1986; Borrero & Hilbish, 1988; Dame, 2012). Concerning
78 shell growth itself, shell architecture and organic content are important attributes often
79 subjected to variations between populations. Dynamics of shell formation includes
80 growth in both circumference and thickness (Gosling, 2003) as variables simultaneously

contributing to determine size and shape of bivalves. Habitat can be responsible for much of the variation in the relationships between biometric parameters accounting for different aspects of growth in mussels (Rao, 1953; Seed, 1973; Brown & Seed, 1977; Aldrich & Crowley, 1986). Since these relationships are known also to change along the life-span of individuals, the characterization of allometric scaling of these parameters to body size (usually shell length) in different populations constitutes a useful approach in the comparative analysis of habitat effects.

Among the various physiological components of growth, metabolic rate is a key parameter determining rates of growth in two related ways: In the context of the energy budget of an individual, metabolic rate constitutes, together with excretion rate, the main component of energy expenditure. At the same time, it summarizes the metabolic energy demands to sustain maintenance and growth processes. In the literature on metabolic rates in bivalves it is common use to distinguish between measurements performed on active fed organisms representative of routine rates and standard or resting rates characteristics of starved organisms (Bayne & Newell, 1983). In sessile continuous feeders, such as bivalves, the difference between both metabolic measurements represents the energy in excess of basal requirements used in the various activities of feeding, digestion and biosynthesis involved in tissue growth. This metabolic component has been recently designed as metabolic scope for feeding and growth (MSFG) (Tamayo et al., 2013).

As stated for biometric parameters, the analysis of allometric scaling of physiological rates to body size is meaningful in interpreting growth processes. Allometric relationships have been formalized as power functions of the form:

$$Y = a\,X^{b}$$

where Y is the biological variable, *a* the intercept, X the body mass, and *b* the allometric scaling exponent. Concerning metabolism, one of the most important points of controversy in scientific discussion about power functions is focused on the value of the exponent (for review see Glazier, 2005; White, 2011). Along many years different authors have reported that mass scaling exponents fluctuate within a range of values of 0.5–1 (Prosser, 1973; Withers, 1992; White et al., 2006). On account of observed variability, the assumption of a common weight exponent for metabolism (the proposed ¾ scaling law) is no longer tenable (Riisgård, 1998; Atanasov & Dimitrov, 2002; Bokma, 2004; Glazier, 2005; Muller–Landau et al., 2006; Reich et al., 2006; White et al., 2006; Glazier, 2008; 2009a; b; c; 2010; White, 2011).

Empirical knowledge of allometric exponents is of particular importance in the parameterization of bioenergetics growth models, where metabolic expenditure corresponding to the size groups has forcibly been estimated indirectly in different ways (Duarte et al., 2010). Particularly, in the case of *M. galloprovincialis* lack of specific information on allometric scaling values for any metabolic level has compelled size–standardization to be based on values reported for related species of *Mytilus*, mainly *M. edulis* (Navarro et al., 1991; Labarta et al., 1997; Babarro et al., 2000b; Tamayo, 2012; Anestis et al., 2010) and eventually *M. chilensis* (Sarà & Pusceddu, 2008).

In the context of the energy balance rates of nitrogen excretion (as ammonia-N) constitute a minor component of total energy losses (10% on average: Bayne & Newell, 1983); however, its determination is important as an indicator of changes in metabolizable substrates mainly occurring along the seasonal cycle.

Summarizing, habitat variation has been shown to promote differentiated growth trends in intertidal and subtidal populations of mussels that are relevant in regards to

129 suspended culture of this species. Such differentiation involves changing relationships
130 between biometric parameters representative of shell and soft tissue dynamics that can
131 be conveniently approached by means of allometric functions. Consequently, the aims
132 of this study were: (1) to compute allometric parameters for the scaling of flesh and
133 shell weight (both total and organic) to body size represented by shell length, (2) to
134 calculate allometric functions relating rates of energy loss (both metabolic and
135 ammonia–N excretion rates) to body size for subsequent comparison between intertidal
136 and subtidal populations, and (3) to analyze functional relationships of growth trends
137 associated to body size and habitat with the metabolic scope for feeding and growth
138 (MSFG).

Materials and methods

Collection and maintenance of mussels

Between September and October 2014 mussels *(Mytilus galloprovincialis)* were sampled from subtidal and intertidal habitats in Ria de Ares–Betanzos (Galicia, NW Spain), and brought to the laboratory where they were cleaned of epibionts and microbial biofilms with sterile scalpels and kept in open flow–through tanks of 20 L of capacity in seawater. The diet during the maintenance period consisted of a monoalgal suspension of *Rhodomonas lens* supplied in a continuous flow to each aquarium by a peristaltic pump (ISMATEC MPC Process). The concentration of food entering the tanks was established at 8000 cells ml^{-1}, at a flow rate of 10 L h^{-1}.

On the second day, shell lengths were measured to the nearest 0.5 mm with a caliper (Mitutoyo®) and individuals sorted in 8 size–classes in the range of 15–50 mm (Table 1). These groups were maintained in separate tanks at the above conditions for 15 days to let the mussels acclimate to laboratory conditions.

Physiological measurements

Metabolic rate

Metabolic rate was determined indirectly through the measure of oxygen consumption rate. Mussels were cleaned and placed in respirometers of about 780 ml of capacity, filled with filtered seawater (1μm), and maintained at a constant temperature (15ºC). Two respirometers were left without animals as a control in order to correct for bacterial respiration, electronic drift, etc. (Labarta et al., 1997). The number of mussels constituting each group is reported in Table 1. This distribution was chosen in order to promote a uniform decrement in oxygen concentration. Determinations started 15

minutes after placing the mussels in the respirometers in order to let the mussels open their valves and start the normal respiratory activity. Dissolved oxygen concentration (mg L^{-1}) was registered by a LDO probe connected to a HATCH HQ40d oxymeter. Determinations were concluded before oxygen concentration had dropped below 70% of the initial concentration. Routine metabolic rate (RMR) was estimated after a continuous feeding period, while standard metabolic rate (SMR) was determined after 72 h of starvation, when a stable level of respiration had been attained as based on previous studies (data not published). Respiration rates were calculated following the formula used by Babarro et al. (2000b), being modified in order to correct the oxygen consumed with the control chambers:

$$\frac{\qquad\qquad}{\qquad\qquad}$$

where is the difference between oxygen concentrationregistered in a respirometer with individuals from final to initial time, is the difference between oxygen concentration registered in control chambers from final to initial time, *Vol* represents the capacity (L) of the respirometer, *t* is the time (h) between final and initial oxygen registration and *n* means the number of individuals placed in the respirometer.

Ammonia excretion rate (VNH_4-N)

Ammonia (VNH_4-N) excretion rate was determined after placing the mussels cleaned of epibionts and biofilm in open Erlenmeyer flasks with 250 ml of filtered seawater (0.2 μm Millipore membranes). Temperature was maintained during the determinations by immersing the flasks in an isothermal bath. Two Erlenmeyer without animals were used as a control. After 120 min, water samples were collected from each Erlenmeyer flask

and frozen to –20ºC until analysis in the laboratory, according to the phenol–hypochlorite method described by Solórzano (1969). Excretion rates were calculated as:

$$\_\_\_\_\_ \quad –$$

where $VNH_4\text{-}N$ represents the ammonia excretion rate; μM and μM_c are the ammonia concentration estimated through the calibration curve in the sample and in the control chamber, respectively; *Vol* represents the capacity (ml) of the incubation chamber; and *t* is the incubation time (h).

Metabolic scope for feeding and growth

Metabolic scope for feeding and growth (MSFG) was computed as the difference between routine and standard metabolic rates and expressed as fraction of routine metabolic rate:

$$\_\_\_\_\_$$

Biometry and condition index

After concluding the physiological determinations, individuals were dissected to determine flesh and shell dry weight (100ºC for 24 h), as well as ash free dry weight (450ºC for 24 h). Condition Index (CI) was calculated according to Freeman (1974) using the following equation:

$$\_\_\_\_\_$$

Shell organic content (%) was estimated through the equation:

$$\_\_\_\_\_$$

205 *Data analysis*

206 Allometric relationships of oxygen consumption and ammonia excretion rates vs. size
207 were determined on log–log transformed data by linear regressions using the least
208 squares method. Allometric equations were compared through a covariance analysis
209 (ANCOVA test) (Zar, 1996). Assumptions of ANCOVA were verified using residual
210 plots (linearity), Kolmogorov–Smirnov tests (normality of residuals), Levene tests
211 (homoscedasticity) and Durbin Watson tests (independence of residuals). The level of
212 significance (α) for all analyses was set at $P = 0.05$. Statistical analyses were performed
213 with the statistical package R 2.15.2 (http://www.r-project.org/), using a custom made R
214 script based on Zar (1996).

Results:

Biometry and condition index

Condition index (CI) increased with size in both subtidal and intertidal mussels (Fig. 1A); however, in subtidal individuals, CI was significantly higher than in those from intertidal habitat. Shell organic content (OC %) (Fig. 1B) was characterized by a marked decline with size in both populations. In addition, subtidal mussels had a greater percentage of shell organic content than intertidal individuals at each size class.

Relationships of flesh weight (FW) to shell length (SL) for intertidal and subtidal populations were fitted by linear regression after log–log transformation (Fig. 2A). As there were not statistically significant differences between slopes (see Table 2), a common slope was calculated and *a* values recalculated for each population:

$$\text{Subtidal: } \log FW = 3.053 \log SL - 5.395$$

$$\text{Intertidal: } \log FW = 3.053 \log SL - 5.455$$

Relationships of shell weight (SW) to shell length (SL) were estimated as described above for flesh weight/shell length ratio (Fig. 2B). Again, lack of statistically significant differences between slopes (see Table 2) allowed a common slope to be calculated and *a* values were recalculated for each population:

$$\text{Subtidal: } \log SW = 2.637 \log SL - 4.052$$

$$\text{Intertidal: } \log SW = 2.637 \log SL - 3.863$$

Total shell organic content (g) vs. shell length (mm) did not show significant differences between slopes (see Table 2), while intercepts were statistically significant. Therefore, equations were recalculated according to their common slope:

237 Subtidal: Log OC = 2.415 Log SL – 4.985

238 Intertidal: Log OC = 2.415 Log SL – 4.926

239 *Allometries of respiration rates*

240 Allometric equations for routine and standard metabolic rates were fitted as a function
241 of dry weight (Fig. 3) and length (Fig. 4). Due to the lack of significant differences (see
242 Table 3) between slopes for routine metabolic rate (RMR) or standard metabolic rate
243 (SMR) in relation to dry mass (dry flesh weight, FW) corresponding to intertidal and
244 subtidal groups (Fig. 3A and 3B), common weight exponents were calculated and
245 intercepts (*a*) recalculated according to these common slopes:

246 Subtidal: Log RMR = 0.715 Log FW – 0.428

247 Intertidal: Log RMR = 0.715 Log FW – 0.485

248 Subtidal: Log SMR = 0.716 Log FW – 0.512

249 Intertidal: Log SMR = 0.716 Log FW – 0.661

250 Similarly, covariance analyses performed on regression lines for metabolic rates (both
251 RMR and SMR) in relation to shell length (SL) (Figure 4 A,B) resulted in lack of
252 significant differences in slope but significant differences in intercepts between
253 intertidal and subtidal groups (Table 3). Therefore, common slopes were computed, and
254 *a* values recalculated for each population:

255 Subtidal: Log RMR = 2.199 Log SL – 4.308

256 Intertidal: Log RMR = 2.199 Log SL – 4.410

257 Subtidal: Log SMR = 2.220 Log SL – 4.424

258 Intertidal: Log SMR = 2.220 Log SL – 4.618

259 *Metabolic scope for feeding and growth (MSFG)*

260 MSFG was expressed as a percentage of routine metabolic rate since mass scaling
261 exponents for both respiration rates were similar ($p > 0.05$) while intercepts were
262 significantly different ($p < 0.005$) (Table 3). Thus, recalculated *a* values were used for
263 this purpose. Therefore, MSFG represented 17.43% and 23.37% of routine rate in the
264 subtidal population, in terms of flesh weight and shell length, respectively; whereas
265 intertidal mussels showed a higher percentage of reduction, amounting to 33.33% and
266 38.05%, respectively.

267 *Allometries of ammonia excretion rates*

268 Relationships between ammonia excretion rate (VNH_4-N) and size were performed also
269 by regression analyses (Fig. 5). Equations relating ammonia excretion to dry flesh
270 weight (FW) (Fig. 5A) in intertidal and subtidal populations (Table 4) did not show
271 significant differences between slopes. Hence, equations were recalculated according to
272 their common slope:

273 $$\text{Subtidal: Log } VNH_4\text{-N} = 0.616 \text{ Log FW} + 1.217$$

274 $$\text{Intertidal: Log } VNH_4\text{-N} = 0.616 \text{ Log FW} + 1.138$$

275 Similarly, slopes of regressions relating ammonia excretion rate (VNH_4-N) to shell
276 length (SL) (Fig. 5B) did not differ statistically between origins (Table 4). Thus, a
277 common slope was calculated, recalculating then intercepts as the following form:

278 $$\text{Subtidal: Log } VNH_4\text{-N} = 1.910 \text{ Log SL} - 2.150$$

279 $$\text{Intertidal: Log } VNH_4\text{-N} = 1.910 \text{ Log SL} - 2.268$$

280 To assess a possible size-effect on the ratio of oxygen consumption to ammonia
281 excretion (the O:N ratio), regression lines for RMR and VNH_4vs. FW were compared

by ANCOVA (Table 5), resulting in absence of significant differences in slope for any population of mussels.

Discussion:

Biometry and condition index

The relevant amount of shell organics found in both populations suggests that the energy required for shell growth is not an insignificant portion of a bivalve's total energy budget, as stated previously by Jørgensen (1976); Rodhouse et al. (1984); Hawkins & Bayne (1985; 1992); Goulletquer & Wolowicz (1989); Wolowicz & Goulletquer (1999). Shell organic content (%) decreased with size in both subtidal and intertidal populations. Although subtidal mussels had higher levels of shell organic content (%), absolute shell organics (g) was only slightly lower in subtidal than in intertidal mussels due to the higher shell weight found in the latter group.

Subtidal mussels showed higher values of condition index (CI) than intertidal's. In some size classes (40–45 mm), subtidal values were about two–fold higher in relation to the intertidal ones. These results can be interpreted as indicative of a higher growth index (Smaal & Stralen, 1990; Pérez–Camacho et al., 1995). Pérez–Camacho et al. (1995) found similar results on *M. galloprovincialis*, which were attributed to the lesser feeding time in the intertidal population. In fact, use of energy reserves associated to reduced food availability have been reported in intertidal mussels (Freites et al., 2002), which could also explain the observed differences between populations concerning the CI in this experiment. On the other hand, broader fluctuations in flesh content of bigger individuals along the seasonal cycle are likely accounting for the increased variability recorded for CI in the largest size classes, since by the end of summer–early autumn some individuals are spawning while others are recovering from this event. The origin

306 of this variability would also account for greater CI fluctuation in subtidal mussels
307 endowed with a thinner shell.

308 Both FW/SL and SW/SL ratios, as well as CI, increased with size in each population.
309 However, subtidal mussels were characterized by a high flesh weight and low shell
310 weight per unit shell length relative to those in the intertidal habitat. Thippeswamy &
311 Joseph (1991; 1992) suggested that size of organisms is controlled by the ambient
312 coupled with the population selection strategies. Thus, shell dimensions are influenced
313 by the environmental conditions (Hemachandra & Thippeswamy, 2008). Our results
314 confirm previous studies on habitat differences regarding condition and biometry in
315 bivalves (Rao, 1953; Seed, 1973; Brown & Seed, 1977; Aldrich & Crowley, 1986). The
316 higher shell thickness found in intertidal bivalves in contrast to their subtidal
317 conspecifics could be explained as a protection strategy against the destructive effects of
318 wave action (Fox & Coe 1943; Raubenheimer & Cook 1990; Akester & Martel 2000;
319 Steffani & Branch, 2003). For instance, Akester & Martel (2000) found that mean shell
320 thickness at a typical wave-exposed site was about 60% greater than at a sheltered site.
321 It was also seen that some intertidal mussel species may increase shell thickness
322 –subsequently decreasing growth rates– in response to predation (Leonard et al., 1999;
323 Naddafi & Rudstam, 2014). The process of shell-thickening is thought to be mediated
324 by increasing calcification (Brookes, 2006, Brookes & Rochette, 2007, Freeman, 2007)
325 and would involve a decline in the percentage organic content of the shells (Brookes,
326 2006), as reported in the present work. Since 25 to 50% of the total body energy can be
327 allocated to shell production (Jørgensen, 1976; Griffiths & King, 1979; Gardner &
328 Thomas, 1987), thickening of shells can be considered to occur subjected to elevated
329 metabolic costs. Thus, higher costs of shell production in intertidal mussels would
330 account for slower growth whilst reduced condition (lower flesh weights) probably

331 reflects the poorer feeding conditions prevailing in this habitat (Aldrich & Crowley,
332 1986).

333 *Allometric scaling of respiration rates to body size*

334 Recorded values of size–scaling exponents for respiration in different species of the
335 genus *Mytilus* (for review see Winter, 1978; Bayne & Newell, 1983) fall in the range
336 0.65 to 0.87, that encloses the average value (0.78) reported for bivalves (Glazier,
337 2005). Most these fluctuations in weight exponents are attributable to the experimental
338 conditions under which determinations were performed, considering that some variables
339 such as temperature or season differ among studies. Activity level of endogenous origin
340 was an additional source of variation since these measurements combined routine as
341 well as standard rates (for review sees Bayne & Newell, 1983). This particular issue of a
342 relationship between metabolic size–exponents and activity levels has been recently
343 formalized by Glazier (2005) who put forward the metabolic level boundaries (MLB)
344 hypothesis.

345 According to Griffiths & Griffiths (1987), allometric scaling exponents (*b* values) for
346 metabolism in bivalves are subjected to minimal variations at the intraspecific level.
347 MLB hypothesis, by contrast, indicates that *b* values would increase with activity level
348 in ectothermic organisms (Glazier, 2009a). Results reported here revealed no
349 differences in scaling exponents between routine and standard metabolic rates. Hence, it
350 is possible that the increment in the activity level –from resting to active levels– is not
351 high enough to achieve a significant change in scaling exponents, since many other
352 studies (for review see Glazier, 2005; 2009a; Jensen et al., 2013) at the intraspecific
353 level have proved significant differences in scaling exponents based on activity level.

354 As previously stated, this is the first report on allometries of respiration rates for *M.*
355 *galloprovincialis* covering routine and standard levels and habitat differences. Scaling
356 exponents obtained for routine (0.715) and standard metabolic rate (0.716) vs. dry flesh
357 weight were similar to those estimated by Bayne et al. (1973) for *Mytilus edulis*. No
358 comparable data have been reported for the allometric relationship of respiration rate
359 and shell length (for review see Winter, 1978; Bayne & Newell, 1983; Glazier, 2005).
360 As for weight exponents, scaling exponents for length were found similar between
361 routine (2.199) and standard (2.220) metabolic rates. Regression analyses (Table 3)
362 provided models that accounted for 88 and 92% of the variation on oxygen consumption
363 based on shell length; which was exactly the same percentage of the variation than in
364 models based on dry flesh weight. These results allow using shell length as an
365 alternative to soft body weight in standardizing metabolic rates of mussels, which
366 represents some advantages. As measuring shell length is neither an invasive nor
367 destructive method, it makes possible to repeat measures throughout the time and allows
368 researchers to work with endangered species, enabling reintroduction of the specimens
369 in their habitat once measurements were concluded.

370 Regression intercepts (*a* values) have been reported to vary both among species and
371 depending on experimental conditions; particularly temperature and activity level
372 (Griffiths & Griffiths, 1987). Comparisons among different *a* values are frequently
373 made under the assumption that these coefficients are mass-independent, and
374 subsequently, considering that are independent of scaling exponents. As Carey et al.
375 (2013) adequately described, "any alteration of the value of the slope *b* necessarily
376 means that the intercept *a* will also change, and this confounds direct comparisons of
377 metabolic level using this metric"; so in this report comparisons between intercepts of

regression lines are made on the assumption of lack of statistical significance of differences among allometric scaling exponents.

Unlike allometric exponents, *a* values exhibited significant effects associated to both habitat and activity level. Compared with subtidal mussels, intertidal specimens experienced an 11.8% reduction in routine metabolic rate and 22.5% in standard metabolic rate. Specific restrictions found in the intertidal habitat might account for these metabolic reductions: metabolic expenditure can amount up to 84% of the absorbed energy in bivalves (56% on average in *M. edulis*) (Bayne & Newell, 1983), so that adjustments in respiration rate can operate as an efficient mechanism for saving energy, especially under limiting conditions of food availability. In this respect, lower rates of respiration recorded in intertidal *M. galloprovincialis* has been associated to limitations in feeding time imposed by tidal cycles (Babarro et al. 2000b). Moreover, reduced resting demands are known to diminish resource to anaerobiosis during air exposure periods (Shick et al., 1988), and this may help understanding why metabolic depression reported here for intertidal mussel concerns mainly to the standard rather than routine rates (see above). In addition to restrictions imposed to metabolic expenditure in the intertidal habitat, differences in metabolic rate between mussels from both populations could be enhanced by the specific demands of an increased gametogenic activity in subtidal mussels, as can be inferred from their greater CI and higher lipid content (Freites et al., 2002).

Metabolic scope for feeding and growth (MSFG)

Intercepts from regression lines were significantly different for routine and standard rates in both populations of mussels and this allowed using recalculated *a* values for computing MSFG. When expressed in relative terms (as a fraction of RMR) this

402 metabolic scope can be considered a nearly constant amount size–independent on
403 account of the lack of difference between the scaling exponents for both metabolic
404 levels (either in terms of soft body weight or shell length). However, the magnitude of
405 metabolic reduction experienced by starved mussels differed greatly between
406 populations, from 17 to 33% of total metabolic costs (represented by RMR) in subtidal
407 and intertidal mussels, respectively. These values are in the range reported for species of
408 *Mytilus*: 26 –45% in *M. edulis* (Bayne et al., 1989; Widdows & Hawkins, 1989) and 17
409 –53% in *M. galloprovincialis* (Tamayo et al., submitted). Greater metabolic investments
410 in growth processes exhibited by intertidal mussels contrasts with their reduced rates of
411 growth suggesting that growth efficiency would be considerably reduced, a condition
412 very likely associated to the elevated costs of shell production in the intertidal media
413 that were previously considered.

414 *Allometric scaling of ammonia excretion rates to body size*

415 Information on allometric scaling of rates of ammonia excretion to body weight in
416 bivalves is extremely scarce and no comparative data exist referred to shell length (for
417 review see Griffiths & Griffiths, 1987). There was no evidence in this study of any
418 effect of habitat on the scaling exponents of ammonia excretion rates *i.e.*, regression
419 models obtained for subtidal and intertidal populations showed no effect on the scaling
420 exponents. Bayne & Scullard (1977) reported variability both in habitat –rocky shore
421 and estuary–and season on nitrogen excretion rates. Results reported in the present
422 study concerning to habitat differences in a values are in agreement with Labarta et al.
423 (1997), who also found higher ammonia excretion rates in the subtidal population under
424 laboratory conditions.

425 Size exponents for metabolism and ammonia excretion were found no–significant for
426 any metabolic level or habitat, implying that O:N indices were size–independent. This
427 result agrees with reported data for *M. californianus* (Bayne et al., 1976a) but contrasts
428 with previous information on *M. edulis* (Bayne et al., 1976b) where O:N index was
429 found to increase or decrease with body size in resting or actively growing mussels,
430 respectively.

431 Summarizing, results report neither effect of habitat nor effect of activity level on the
432 allometric scaling exponent in both respiration and ammonia excretion rates. Allometric
433 scaling exponents based on weight were 0.715 and 0.716 for the routine and standard
434 respiration rates, respectively; while *b* values for the relationship between respiration
435 rates and shell length amounted to 2.199 and 2.220, respectively. Allometric scaling
436 exponents concerning ammonia excretion rates were 0.616 and 1.910 for weight and
437 shell length, respectively. Origin differences found in respiration rates could reflect
438 physiological compensations in the intertidal population for the lesser feeding time and
439 air exposure. The higher CI registered in subtidal mussels suggests a greater energy
440 budget than in intertidal mussels, despite the higher respiration and excretion rates of
441 subtidal individuals. This suggestion is confirmed by higher feeding rates (Babarro et
442 al., 2000a) and absorption efficiencies (Labarta et al., 1997) found for subtidal mussels
443 in previous studies. The differences in MSFG between populations could explain
444 differences in growth efficiencies; furthermore, the higher shell thickness found in the
445 intertidal individuals suggests that energy resources are allocated as a priority to shell
446 growth to the detriment of flesh growth.

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