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Research Article

Effect of body size on the energetic physiology of the West Indian top shell *Cittarium pica* (Linnaeus, 1758)

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ABSTRACT. *Cittarium pica* is an endangered Caribbean gastropod of ecological and economic importance. In order to provide the basis for the aquaculture development and/or population management of this species, the relationships among its body size and physiological variables associated with the energetic balance were assessed. Individual measurements of rates of egestion (ER), ingestion (IR), absorption (AR), oxygen consumption (OCR), ammonia excretion (UR), mucus production (MR), absorption efficiency (AE) and scope for growth (SFG), were carried out on specimens between 0.01 and 12.07 g of dry weight of the soft parts (DW) under controlled laboratory conditions. Most of the variables showed positive potential relationships with the DW of the animals (ER mg h^{-1} = 1.51 $DW^{0.37}$, IR mg h^{-1} = 4.13 $DW^{0.32}$, AR = 2.56 $DW^{0.30}$, OCR mL O_2 h^{-1} = 0.38 $DW^{0.74}$, UR µg NH_4 -N h^{-1} = 19.17 $DW^{0.78}$, MR mg h^{-1} = 1.28 $DW^{0.14}$ and SFG = 36.92 $DW^{0.22}$). AE did not show any relationship with this variable. The large snails had faster input and output of energy compared to the small specimens, also having proportionally greater energetic expenditures, mucus production, and energetic balance.

Keywords: *Cittarium pica*; Tegulidae; gastropods; ecophysiology; growth; Caribbean Sea

INTRODUCTION

The West Indian top shell *Cittarium pica* (Linnaeus, 1758) is an herbivorous and detritivorous gastropod that lives in the rocky intertidal of the Caribbean Sea, which is considered among those of higher ecological and commercial importance of the region (Randall, 1964; Robertson, 2003). Its populations have been overexploited due to the interest in their meat and shell (Robertson, 2003; Osorno & Díaz, 2006; Rosique *et al*., 2008), as well as their easily accessible habitat (Lozano & Estrada, 2008). It is currently considered an endangered species (Ardila *et al*., 2002). Therefore, different conservation strategies have been proposed including the development of technologies for aquaculture and restocking, as well as the population monitoring and management programs (Bell, 1992; Osorno *et al*., 2009).

In mollusks, the measurement of the energetic balance or scope for growth (SFG) is one of the quickest approaches, with fewer requirements of specimens and most accurate for the fitness determinations of an organism or population (Riisgård & Randlov, 1981; Bayne & Newell, 1983; Widdows, 1985; Grant & Cranford, 1991; Navarro *et al*., 2006; Filgueira *et al*., 2011; Larsen *et al*., 2014). This index measures the inputs and outputs of the energy of an organism, estimating the quantity of energy that it is available for growth and/or reproduction. It has been useful for different purposes such as the aquaculture developing (Farías *et al*., 2003; Velasco, 2006, 2007), the health status diagnosing of populations (Campillo *et al*., 2013), the and population ecology modeling (Velasco & Navarro, 2002, 2005; Toro *et al*., 2003; Albentosa *et al*., 2012b; Zhang *et al*., 2015).

The energetic physiology or scope for growth (SFG), has been studied in gastropods such as *Ancylus fluviatilis* (Calow, 1975), *Bullia digitalis* (Stenton-Dozey & Brown, 1988), *Buccinum undatum* (Kideys, 1998), *Chorus giganteus* (Navarro *et al*., 2002, 2006), *Concholepas concholepas* (Navarro & Torrijos, 1995), *Haliotis tuberculata* (Peck *et al*., 1987), *Haliotis midae*

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(Barkai & Griffiths, 1987, 1988), *Haliotis fulgens* (Farías *et al*., 2003), *Nassarius conoidalis* (Liu *et al*., 2011; Zhang *et al*., 2015), *Nassarius siquijorensis* (Liu *et al*., 2011), *Patella vulgata* (Davies *et al*., 1990), *Planorbis contortus* (Calow, 1975), *Polinices duplicatus* (Huebner & Edwards, 1981) and *Thais lapillus* (Stickle & Bayne, 1982). These studies have shown that body size is one of the intrinsic factors that most affect the energetic physiological variables, being necessary to determine the level of dependence of each physiological variable about the animal size and standardize them as a previous step for the study of other endogenous or exogenous factors.

The goal of this study was to assess the relationships between the body size and the physiological variables related to the energetic balance of *C. pica,* as the basis for the determination of the optimal environmental conditions for aquaculture and/or restocking purposes, as well as for the health status diagnosing of their populations.

MATERIALS AND METHODS

Collection and acclimatization of specimens

Smaller size specimens (<20 mm in diameter of the shell) of *Cittarium pica* were collected manually under low tide conditions on the rocky coast of Monohuaca (11°16'15.61"N, 74°12'15.45"W), Taganga Bay (Santa Marta, Colombia), while the larger animals were obtained from local fishermen. The coastal waters of the region of Santa Marta have a temperature of 23-34°C, salinity of 28.1-37.2 (Garcés-Ordóñez *et al*., 2016), pH of 7.28-8.54 (Vivas-Aguas *et al*., 2015) and oxygen concentration of 3.5-7.6 mg O_2 L^{-1} (Vivas-Aguas *et al*. 2012).

Small and large animals were transported to the Laboratory of Mollusks and Microalgae at Universidad del Magdalena, in plastic boxes under dry conditions. They were cleaned of epibionts, marked, and acclimatized to laboratory conditions for three weeks. For this purpose, pairs of animals of similar size were put together within a 10 L plastic aquarium. These aquariums were maintained with continuous flow (100% h^{-1}) of microfiltered seawater (1 µm), at a temperature of 27 ± 1°C, salinity 37 ± 1, pH 7.7 ± 1.0 and oxygen concentrations 5.06 ± 0.21 mg L^{-1} (80 ± 4%). The feces were removed daily by siphoning. A polyvinylchloride (PVC) plate (15x22 cm) coated with an artificial biofilm was placed daily in each aquarium as food. The artificial biofilm was made impregnating the plates with a mixture of dried and ground (<1 mm) microalgae *Spirulina* sp. (15 mg mL^{-1}), macroalgae *Padina gymnospora* (15 mg mL^{-1}) and eggshell (5 mg mL^{-1}), which was agglutinated with agar (10 mg mL^{-1}) and gelatin (15 mg mL^{-1}).

Experimental design and physiological determinations

Twenty-seven specimens of different sizes, which presented a more active feeding behavior during the period of acclimatization, were used to examine the responses of the variables of energetic balance. Animals had shell lengths (SL) between 10.7 and 92.6 mm, wet live weights (LW) between 0.5 and 319.1 g and dry weight of soft parts (DW) between 0.01 and 12.07 g. Each animal was placed individually in the same aquarium and water conditions described for acclimatization, including three control aquariums without animals and with three biofilm plates.

Feed characterization and physiological feeding determinations were done for 6 h (between 03:00 and 09:00 h), when the maximal feces production rate was observed during acclimatization. Three samples of fed (plates with artificial biofilm) were taken before fed animals and each two hours during animal feeding, from each control aquarium (12 samples in total). The total dry weight and the organic content of feed (>200 mg of dry weight) were analyzed following the gravimetric methodology of Strickland & Parsons (1972). Each sample was scraped and filtered on glass-fiber filters (diameter = 0.45 µm; Millipore) which had been previously washed with distilled water, ashed at 450°C for 4 h, and weighed. The filtrate was washed with 3% ammonium formate, dried at 70°C for 48 h, and weighed; the net dry weight represented the total dry matter in the diet. The organic material content (*f*) of the samples was determined from the ash-free dry weight, obtained by ashing the filters at 450°C for 4 h and re-weighing. Additionally, the energetic content of the other three feed samples (1 g) was measured using a microcalorimeter (IKA® C 200 and precision of 1 J mg^{-1}). The feces produced by each specimen were quantitatively collected, with Pasteur pipettes, and frozen every 2 h throughout 6 h (three samples per animal).

The total dry weight and the organic content of the feces collected were analyzed as was mentioned above for feed. The feeding rates were measured as described below, following the biodeposition method (Iglesias *et al*., 1998). The central assumption of the biodeposition method is that ingested particulate inorganic matter can be used as an inert tracer of feeding and digestive processes. This indirect method was selected in order to avoid the overestimation risk of direct food intake quantification attributed to the detachment of the artificial biofilm from the plates caused by the animal activity over the biofilm.

Ingestion rate (IR)

$$IR\ (mg\ h^{-1}) = IIR + OIR$$

$$IIR\ (mg\ h^{-1}) = IER$$

$$OIR\ (mg\ h^{-1}) = IER \times (f / (1 - f))$$

where, IIR: the inorganic ingestion rate; OIR: the organic ingestion rate (mg h^{-1}); IER: the inorganic egestion rate or rate of feces production (mg h^{-1}) and *f*: the organic material content in the feed (77.6 ± 2.2%).

Absorption rate (AR)

$$AR\ (mg\ h^{-1}) = OIR - OER$$

where OIR: organic ingestion rate (mg h^{-1}), and OER: organic egestion rate (mg h^{-1}).

Absorption efficiency (AE)

$$AE\ (\%) = (AR / OIR) \times 100$$

where AR: absorption rate (mg h^{-1}), and OIR: organic ingestion rate (mg h^{-1}).

Individual measurements of oxygen consumption, excretion, and mucus production rates were made with snails recently fed. The animals were individually incubated three times for 2 h (from 09:00 to 15:00 h) in sealed respirometer chambers of 0.28 L for snails between 9 and 25 mm, 0.82 mL for those between 26 and 60 mm, and 3.1 L for snails among 60 and 69 mm. There were two control chambers without animals for each chamber size. Chambers were changed and filled with filtered and aerated seawater of the same temperature and salinity described above. The oxygen concentration in the water was estimated using the Winkler method modified by Carritt & Carpenter (Strickland & Parsons, 1972). The oxygen consumption rate (OCR) was determined by following the equation (Widdows, 1985).

$$OCR\ (mL\ O_2\ h^{-1}) = [(Oc \times vc) - (Oi \times vi)] / t$$

where O: final oxygen concentration (mL O_2 L^{-1}), c: chamber control, i: experimental chamber, v: volume of the chamber (L) and t time of incubation (h). Initial and final values of oxygen concentration in the control chambers were in average 106% of O_2 saturation, while the final values of oxygen concentration in the experimental chambers were in average 86% of O_2 saturation.

The ammonia concentration was determined by using the Solorzano (1969) colorimetric method. The ammonia excretion rate (UR) was calculated by following the equation of Widdows (1985).

$$UR\ (\mu g\ NH_4\text{-}N\ h^{-1}) = 28 \times [(Xi / vi) - (Xc / vc)] / t$$

where X: final NH_4-N (µM L^{-1}) concentration, I: experimental chamber, c: control chamber, v: volume of chamber (L), t: time of incubation (h), and 28: conversion factor of µM to µg NH_4-N.

The feces produced in the respirometer chambers were eliminated and the mucus adhered to the inside was refrigerated after being collected quantitatively every two hours using a silicone spatula. Mucus material collected from each animal was joined in order to have representative samples. The total dry weight of mucus and its energetic content were determined by following the methodologies previously described to characterize the feed.

Mucus production rate (MR)

$$MR\ (mg\ h^{-1}) = (Wf_{70°C} - Wf_0) / t$$

where: $Wf_{70°C}$: weight of the filter with mucus after being dried at 70ºC (mg), $Wf_{0:}$ weight of the empty filter after being dried at 70ºC (mg), and t: time of incubation.

The scope for growth (SFG) was calculated according to the energy balance equation of Winberg (1960) as the difference between absorbed energy (AR) and the energy used for metabolic purposes (OCR), the losses of energy due to excretion of nitrogen waste (UR) and the production of mucus (M).

$$SFG\ (J\ h^{-1}) = A - (OC + U + M)$$

where, A = AR (J h^{-1}) = AR × 23.98 J mg^{-1}; OC = OCR (J h^{-1}) = OCR × 20.08 J mL^{-1} O_2 (Gnaiger, 1983), U = UR (J h^{-1}) = UR × 0.0248 J µg NH_4-N h^{-1} (Elliot & Davinson, 1975) and M = MR (J h^{-1}) = MR × 3.8 J mg^{-1}.

At the end of the experiment, the shell length (SL), live wet weight (LW) and dry weight of soft parts (DW) were individually determined in each experimental animal, as well as their sex based on gonad coloration, which is green in females, cream for males and brown for those undetermined.

Statistical analysis

In order to examine the existence of relationships between the morphometric measurements (SL, LW and DW), as well as among DW and physiological variables (ER, IR, AE, AR, OCR, UR, MR and SFG), linear regression analysis were done, after verifying the homogeneity of variances of the variables (Cochran C test) and checking the normality of the models residuals (Kolmogorov-Smirnov test). The statistical analyses were achieved using the Statgraphics Centurion XV program. For all the significance decisions, α value of 0.05 was used.

RESULTS

Positive potential relationships were found among SL and LW (Table 1, Fig. 1a) and among SL and DW (Table 1, Fig. 1b), with exponents of 2.95 and 2.76, respectively. On the other hand, LW and DW showed a

Table 1. Regression analysis between morphological measurements and energetic physiological rates of *Cittarium pica* and its body dry weight (DW). LW: live weight, SL: shell length, ER: egestion rate, IR: ingestion rate, AR: absorption rate, OCR: oxygen consumption rate, UR: ammonia excretion rate, MR: mucous production rate, and SFG: scope for growth.

Dependent variable	Equation	df	F	*P*	R^2
LW	$0.0004\ SL^{2.95}$	26	7058.35	0.0001	0.99
SL	$4x10^{-5}\ DW^{2.75}$	26	180.11	0.0001	0.88
DW	27.68 LW - 3.26	26	1039.36	0.0001	0.97
ER	$1.51\ DW^{0.73}$	26	75.07	0.0001	0.75
IR	$4.13\ DW^{0.32}$	26	78.90	0.0001	0.76
AR	$2.56\ DW^{0.30}$	26	66.05	0.0001	0.73
OCR	$0.38\ DW^{0.74}$	26	79.66	0.0001	0.76
UR	$19.17\ DW^{0.78}$	26	54.87	0.0001	0.78
MR	$1.28\ DW^{0.14}$	26	33.46	0.0001	0.57
SFG	$36.92\ DW^{0.22}$	26	15.38	0.0006	0.38

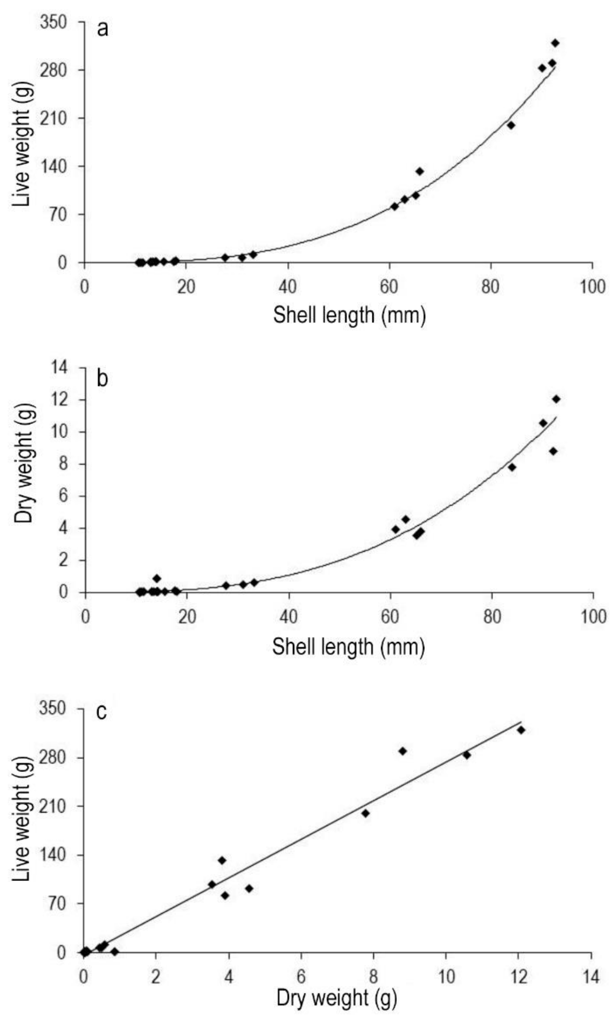

Figure 1. Relationships of *Cittarium pica* between a) live wet weight (LW) and shell length (SL), b) dry weight of soft tissues (DW) and SL, and c) LW and DW.

direct relationship (Table 1, Fig. 1c) with a slope of 27.68. Eighty percent of the animals studied showed undetermined sex, while 10% were male and the remaining 10% were female.

The organisms with SL <20 mm ingested a dry weight of around 4% of its LW per day, while the larger organisms, SL >50 mm, ingested approximately 0.14% of its LW per day. The absorption efficiency (AE) fluctuated between 59 and 95%, without any significantly equation with respect to the DW (degree of freedom df = 26, $P > 0.05$) (Fig. 3a). The absorption rate (AR) fluctuated between 0.3 and 6.8 mg h^{-1}, and its variation was associated with the DW of the animals with a direct potential relationship, with an exponent of 0.30 (Table 1, Fig. 3b).

The egestion rate (ER) oscillated between 0.2 and 5.3 mg h^{-1}, showing an increase together with the DW of the organisms in a positive potential relationship with an exponent of 0.37 (Table 1, Fig. 2a). The values of the ingestion rate (IR) varied between 0.6 and 11.7 mg h^{-1}, verifying a direct potential relationship with the DW, with an exponent of 0.32 (Table 1, Fig. 2b).

The OCR varied between 0.01 and 3.81 mL O_2 h^{-1}, demonstrating a positive potential relationship between this variable and the DW of the animals, with an exponent of 0.74 (Table 1, Fig. 4a). On the other hand, the UR varied between 0.4 and 241.8 µg NH_4-N h^{-1}, showing a potential positive response with DW and b value of 0.78 (Table 1, Fig. 4b). The MR varied between 0.6 and 2.5 mg h^{-1}, with a positive potential increase of its values together with the DW of the animals and showing an exponent of 0.14 (Table 1, Fig. 4c).

The SFG fluctuated between 1.3 and 107.3 J h^{-1}, with a positive potential relationship with the DW (Table 1, Fig. 5a). From an average of energy ingested

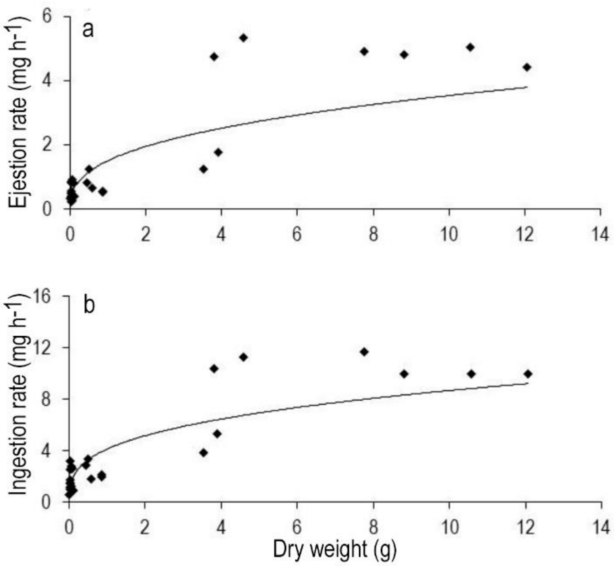

Figure 2. Relationships between the dry weight of *Cittarium pica* soft tissues (DW) and a) feces production rate (FR), and b) ingestion rate (IR).

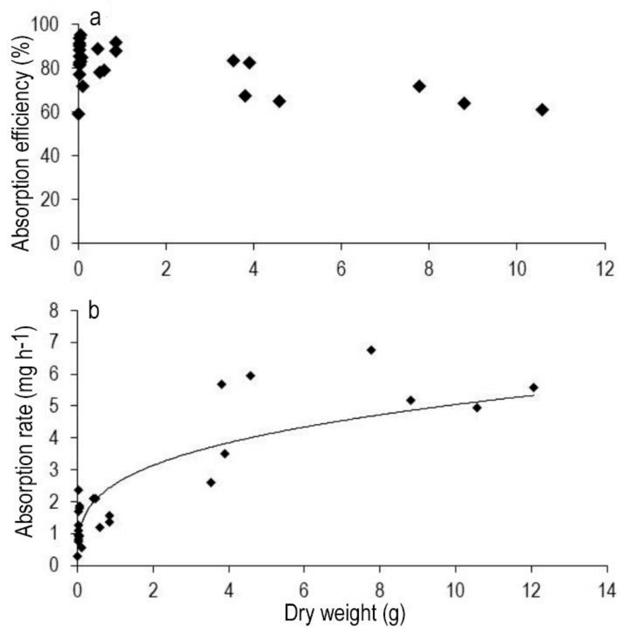

Figure 3. Relationships between the dry weight of *Cittarium pica* soft tissues (DW) and a) absorption efficiency (AE), and b) absorption rate (AR).

in the food of 77.8 ± 9 J h^{-1}, an average of 20 ± 2% was lost in the feces, 12 ± 3% was used in functional processes, 1 ± 0.2% was lost in nitrogenous wastes and 9 ± 1% in organic compounds of mucus, leaving 58 ± 4% available for growth and reproduction (Fig. 5b).

DISCUSSION

The potential relationships encountered between shell length (SL), live weight (LW) and dry weight of soft parts (DW) of *Cittarium pica* show that the growth in weight is around three times the growth in length. Therefore, the growth measurement of this species is more accurate using weight than length units. Similar relationships and exponents have been reported previously for the same species (Randall, 1964) and in other gastropods such as *Buccinum undatum* (Kideys, 1998), *Haliotis fulgens* (Farías *et al.*, 2003), *Lymnaea stagnalis* (Zonneveld & Kooijman, 1989), *Nerita peloronta, N. tessellate, N. versicolor* (Hughes, 1971), *Pomacea patula* (García-Ulloa *et al*., 2006), *Strombus gigas* (Aldana-Aranda *et al*., 1996). The values of the coefficient of determination and the level of probability found for the equation that relates the DW and LW of *C. pica* (R^2 = 0.97 and P < 0.01) suggest that the intraspecific variations that can exist in the content of water of the tissues or the density of the shell among animals do not introduce large biases in determining DW from LW. Therefore, in future physiological or bioecological studies, this equation can be confidently used to estimate the physiological rates in terms of DW of the animals found indirectly from their LW. This practice avoids killing the experimental animals, which can be returned to their natural habitat, making this type of study harmless and viable, considering the status of the vulnerability of the natural populations.

The pattern of increase in the rates of feces production (ER), ingestion (IR) and absorption (AR) of *C. pica*, together with its body size, coincides with that found in other gastropods such as *Ancylus fluviatilis* (Calow, 1975), *Buccinum undatum* (Kideys, 1998), *B. digitalis* (Stenton-Dozey & Brown, 1988), *Concholepas concholepas* (Navarro & Torrijos, 1995), *Haliotis fulgens* (Farías *et al.*, 2003), *H. midae* (Barkai & Griffiths, 1987, 1988), *H. tuberculata* (Peck *et al.*, 1987), *Polinices duplicatus* (Huebner & Edwards, 1981) and *Planorbis contortus* (Calow, 1975). The higher feeding rates of the mollusks have been related to greater capacities of the digestive tract and the digestive gland (Bayne & Newell, 1983; Ibarrola *et al.*, 2008; Albentosa *et al*., 2012a), which are greater in bigger organisms (Bayne & Newell, 1983). Then, the increase in the feeding rates of *C. pica*, together with the size, can be due to the higher capacity of the digestive system of the large specimens.

The lack of relationship among the absorption efficiency (AE) and the body size of *C. pica* agrees with results reported in *A. fluviatilis, P. contortus* (Calow, 1975), *C. concholepas* (Navarro & Torrijos, 1995), *H. fulgens* (Farías *et al.*, 2003) and *H. midae* (Barkai & Griffiths, 1987, 1988). Then, although the IR values of large specimens, were higher than in the small ones,

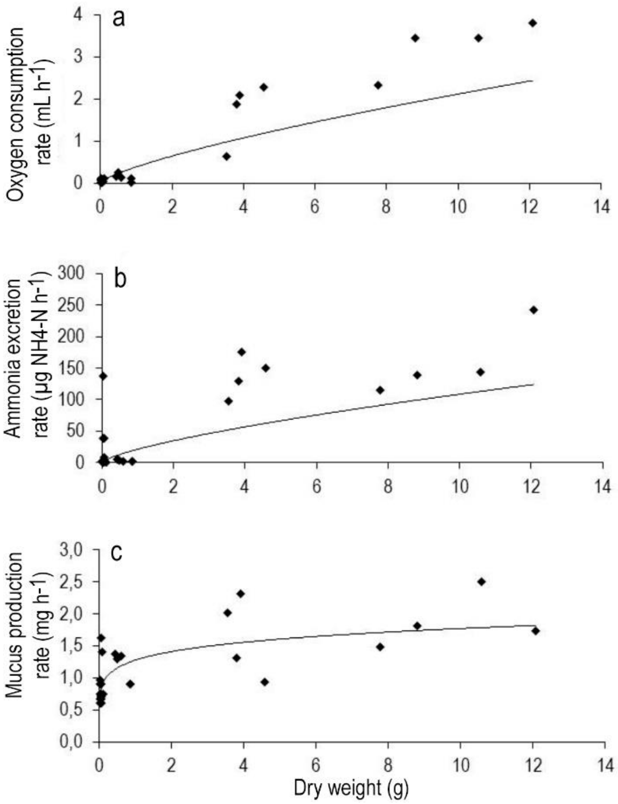

Figure 4. Relationships between the dry weight of *Cittarium pica* soft tissues (DW) and: a) oxygen consumption rate (OCR), b) ammonia excretion rate (UR), and c) mucus production rate (MR).

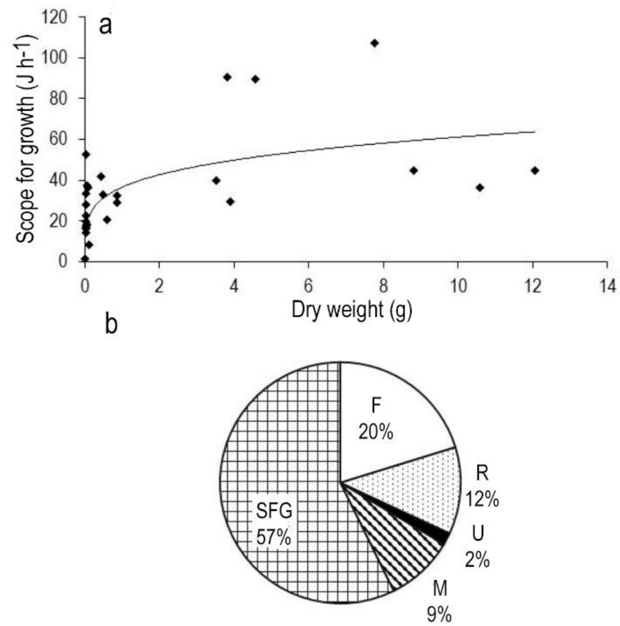

Figure 5. a) The relationship between the scope for growth (SFG) of *Cittarium pica* and the dry weight of its soft tissues (DW), b) percentage of ingested energy lost in the feces (F), used in the animal functioning (R), lost in urine (U) and mucus (M), and available for growth and reproduction (SFG).

their residence time, in the digestive tract, and/or enzymatic action values was higher too, producing similar AE values.

The positive potential relationship found between the body size and the oxygen consumption rate (OCR) of *C. pica,* with an exponent around 0.7, is concordant with that found in *B. undatum* (Kideys, 1998), *B. digitalis* (Stenton-Dozey & Brown, 1988), *C. concholepas* (Navarro & Torrijos, 1995), *H. fulgens* (Farías *et al.,* 2003), *H. midae* (Barkai & Griffiths, 1987, 1988), *H. tuberculata* (Peck *et al*., 1987), *P. duplicatus* (Huebner & Edwards, 1981), *T. lapillus* (Stickle & Bayne, 1982) and another 32 species of gastropods studied by Marsden *et al*. (2012). Such results show that the large organisms of *C. pica* have greater energy demand than the small ones, which has been explained in other mollusks by the higher energy requirement for maintaining and functioning of a greater tissue mass (Hawkins & Bayne, 1991).

The ammonia excretion rate (UR) of *C. pica* was positively related to the body size, according with other gastropods like *B. digitalis* (Stenton-Dozey & Brown, 1988), *C. concholepas* (Navarro & Torrijos, 1995), *H. midae* (Barkai & Griffiths, 1987, 1988), *H. fulgens* (Farías *et al*., 2003), *H. tuberculata* (Peck *et al*., 1987) and *T. lapillus* (Stickle & Bayne, 1982). Those results have been attributed to the higher protein catabolism at higher AR values.

The increase of the mucus production rate (MR) of *C. pica* with the body size is concordant with that reported for *B. undatum* (Kideys, 1998), *C. concholepas* (Navarro & Torrijos, 1995), *H. tuberculata* (Peck *et al.*, 1987) and *P. vulgata* (Davies *et al*., 1990). The MR of gastropods is related to the adhesion activity, locomotion and/or the foot size. Those who are more active and/or have larger foot produce higher amounts of mucus per unit of time (Peck *et al*. 1987). In *C. pica,* higher activity was observed in smaller specimens than in the larger ones under the experimental conditions, so it is probable that the greater MR of the larger animals is associated to its greater foot size.

The positive relationship between the SFG of *C. pica* and the dry weight shows that the availability of energy in order to grow and/or reproduce is higher in larger animals,s can be explained by the greater quantity of energy absorbed by large animals. In other gastropods, positive relationships have been reported between the SFG and body size such as *C. concholepas* for the spring and summer months (Navarro & Torrijos, 1995), *H. fulgens* (Farías *et al*., 2003) and *H. midae* (Barkai & Griffiths, 1988); as well as negative relationships in *C. concholepas* for the fall and winter months (Navarro & Torrijos, 1995) and in *H. tuberculata* (Peck *et al*., 1987).

The average percentage of ingested energy available for growth and reproduction in *C. pica* (58%), is higher or similar compared to that reported for other species under optimal conditions such as *H. fulgens* (39%; Farías *et al*., 2003), *H. midae* (5%; Barkai & Griffiths, 1988), *H. tuberculata* (25-37%; Peck *et al*., 1987), *C. concholepas* (40-50%; Navarro & Torrijos, 1995) and in *Chorus giganteus* (55-60%) (Navarro *et al*., 2002). These results are related to the efficiency of the organisms to absorb and use the energy ingested in order to carry out their functions, organisms that have a low relationship among oxygen consumption and the amount of energy absorbed are more efficient. Then, it is possible to consider that the diet and conditions under which *C. pica* was maintained in this study allowed for efficient absorption and use of the food energy in order to achieve their basic functions and leave a considerable quantity of energy for growth and reproduction.

In conclusion, although the larger animals of *C. pica* showed higher energetic expenditure and losses of energy than smaller organisms, they also ingest and absorb a greater amount of food and also have higher energy for growth and reproduction. The equations and b values obtained in this study are the basis for the expedite and innocuous identification of the optimal environmental conditions of this species for aquaculture and/or population restoration purposes.

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