MALACOLOGIA, 2022, 64(2): 257–267

ALLOMETRY IN THE FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA* L.): MUSSELS TEND TO GROW FLATTER AT HIGHER WATER SPEED

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ABSTRACT

The freshwater pearl mussel (*Margaritifera margaritifera*) is one of the longest-lived invertebrate species in the world and one of the most threatened freshwater animals in Europe. Its southernmost populations, located in northwestern Spain, are in a critical conservation situation and are still understudied. Here we calibrate a non-invasive method for calculating the volume of the shell and use it to study the ontogenetic scaling of shell volume on shell length. We characterized ontogenetic growth and determined allometric relationships in 16 *M. margaritifera* northwestern Spain populations by using ordinary least squares regression, major axis and reduced major axis methods. We estimated topographic slopes of the sampling points using a GIS system, as a proxy of water speed. We measured 803 shells and found that the volume of the shell can precisely be estimated using three linear measurements. We found evidence for negative allometry of shell volume in the global sample and in 11 populations. We hypothesized that water speed would affect allometric patterns of local populations. Results suggest a negative relationship between the allometric slope and the topographic slope of the river section inhabited by *M. margaritifera*. We propose that when water speed is higher, larger mussels become proportionally flatter than in locations where water current is slower, allowing them to burrow more easily in the sediment. Our method will allow estimation of *M. margaritifera* biomass and ontogenetic growth without killing any specimens, which will contribute to conservation programs for this species.

Key words: Unionidae, freshwater mussel, shell volume, allometry, water speed.

INTRODUCTION

Bivalves of the order Unionida are globally imperilled (Lydeard et al., 2004; Strayer, 2006). The freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) is among the most critically threatened freshwater bivalves worldwide (Lopes-Lima et al., 2017; Geist et al., 2018). This species satisfies criteria of an indicator, flagship, keystone and umbrella taxon and can thus be considered a useful target species to be included in conservation programs and management measures to promote healthy aquatic ecosystem functioning (Geist, 2010). Recently, a European initiative has been developed to provide guidelines on standardized sampling methods to design national monitoring programmes (Boon et al., 2019), indicating the need to study the size and viability of *M. margaritifera* populations.

It is well known that *M. margaritifera* is one of the longest-lived animal species in the world and one of the most threatened freshwater bivalves in Europe (Ziuganov et al., 2000). It inhabits cool, clean streams and rivers used by salmonids, but numerous studies in Europe demonstrated a catastrophic decline over most of its range since the last decades of the twentieth century (Bauer, 1988; Young et al., 2001) which led to its listing as an endangered species (Moorkens et al., 2017). Changes in land use, especially intensification of agriculture and forestry, have lead many pearl mussel populations to extinction, even in places with low human population density (Popov, 2015). Galician populations (northwestern Spain) of *M.*

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margaritifera have been studied from several perspectives, such as growth rates, genetic variability, population density and spatial distribution, principally in relation to their conservation (San Miguel et al., 2004; Bouza et al., 2007; Outeiro et al., 2008; Lois et al., 2015).

The populations of *M. margaritifera* in the Iberian Peninsula are mostly located in Galicia, that is, at the southern limit of the area of the geographic distribution of the species (Lois et al., 2014). It has been suggested that these peripheral populations, with smaller maximum shell length, faster growth rates, shorter-lived and very low genetic variability in comparison with northern populations, appear to be close to their limits of existence (San Miguel et al., 2004; Bouza et al., 2007). In the context of global warming, these southern populations are at high risk of extinction. Therefore, there is a critical need for increased knowledge on many aspects of their biology, particularly traits related to phenotypic variability and integration.

Studying freshwater bivalve ontogenetic growth and determining allometric relationships is basic for generating useful information for managing resources and understanding how variable environmental conditions affect shell morphology (Palmer, 1990). Allometric growth (either positive or negative) is the general rule in nature. Very few organisms grow isometrically, with juveniles appearing as miniature replicas of adults (Gotelli & Ellison, 2013). Allometry is a useful tool when the goal is to obtain estimates of variables that are linked to body size (most commonly represented as body mass) such as carrying capacity and the intrinsic rate of increase (Peters, 1990). The allometry of life-history traits (such as body size) is relevant to efforts for conservation of threatened and endangered species, which should be based in a good knowledge of their reproductive biology (Calder, 1996).

Our research goal was to calibrate a non-invasive method for calculating the volume of the shell of *M. margaritifera*. To this end, we studied how shell dimensions (length, height, convexity) and shell volume (as a surrogate of body size) are related, and therefore if linear dimensions could be used to evaluate variation in other life history traits such as ontogenetic growth in 16 *M. margaritifera* northwestern Spain populations. Furthermore, it is equally relevant to find out if some ecological variables such as mussel density or water variables (e.g., velocity, temperature) can influence allometric growth. To this end, we compiled data from our unpublished previous work, the literature, and estimated water speed using the topographic slope of the sampling location as a proxy.

MATERIAL AND METHODS

Estimation of Shell Volume

There are over 2,000 dry shells of *M. margaritifera* in the collection of the Genetics Department of the University of Santiago de Compostela in the Campus of Lugo. Many shells (specially the oldest) are often cracked or prone to cracking, and consequently 53 specimens were selected, being chosen to show a maximum range in volume (1.5 to 114 ml) for comparison to calculated volumes (Vc).

For every shell we used the following procedure: we introduced each specimen (an empty shell with two valves perfectly closed by a tight laboratory film band) into a test tube with volumetric calibration marks. Prior to sealing the shell, we put some pellets inside the shell to eliminate buoyancy. Then we poured tap water into the test tube to immerse exactly and completely the shell. According to the shell size, different test tubes and pipettes were employed to estimate the volume to the nearest 0.1 ml. If for a specific shell, for example, we poured 3 ml of tap water and the test tube mark indicated 7 ml, the difference was taken as an estimation of the observed shell volume (Vo). We used an approximation described by Eagar (1977) to compute theoretical volumes considering the closed valve like an ellipsoid. Thus, the volume (in litres) is given by

$$(4/3)(\pi LCH)/8,$$

and this expression was simplified to

$$Vc = (LCH)/2 \qquad [1]$$

where the radii or axes are ((Ziuganov et al., 1994) one-half of the product of maximum Length (L, measured to the nearest 0.01 mm along the major axis of the shell, using a Mitutoyo calliper), Convexity (C, the greatest distance between the valves of the closed shell), and Height (H, the greatest distance between dorsal and ventral margins). Measurements were done following Ziuganov et al. (1994), as indicated in Figure 1. Measurements for each shell are provided in the data repository Zenodo, with the DOI 10.5281/zenodo.5593148.

Analysis of Natural Populations

We analysed 16 populations of *M. margaritifera* occurring in Galicia and Asturias

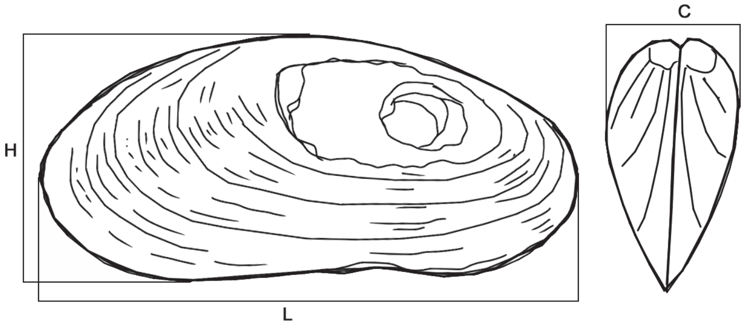

FIG. 1. Schematic representation of a shell of *Margaritifera margaritifera*, with the measurements taken to estimate shell volume. Based on Ziuganov et al., 1994.

(northwestern Spain; Table 1). In previous reports (Álvarez-Claudio et al., 2000; San Miguel et al., 2004; Outeiro et al., 2008; Lois et al., 2014, 2015) these populations and many others were examined for growth rates, densities and other characteristics. Since *M. margaritifera* is protected by European law, collecting was carried out with a permit from the Galician government (Xunta de Galicia). Live specimens were left undisturbed, and therefore only empty shells were taken to be measured, and we selected those in the best condition and showing the maximum possible ranges in size.

TABLE 1. Populations of *M. margaritifera* included in this study. Altitude (in m) was obtained from GoogleEarth. The slope (%) was calculated from a buffer 1000 m upstream (see Methods). Summer temperature is the average of temperatures from 20 June to 20 September 2014–2016. River length, flow and basin surface were obtained from the literature (Río Barja & Rodríguez Lestegás, 1992). Mussel density is expressed in individuals/m^2. Exact coordinates of sampling points are not given because the species is endangered, to protect the populations from illegal collecting (*obtained from Wikipedia, **obtained from other sources).

River	Altitude	Slope	Summer Temp.	River Length (km)	Flow (m^3/s)	Basin Surface (km^2)	Basin	Mussel Density
Arnego	296	1.2	16.3	57	9.4	378.7**	Ulla	0.62
Camba	680	3.0	14.1	56	6.3	166	Miño	2.43
Eo	158	0.7	18.3	92	24.4	700	Eo	1.34
Landro	13	3.4	15.9	31	7.1	269.6	Landro	0.57
Limia	556	0.4	19.1	108	28.0	1,328.8	Limia	0.35
Mandeo	18	2.8	19.4	56	14.1	456.9	Mandeo	0.40
Masma	26	1.5	17.7	46	6.3	291.3	Masma	0.89
Mera	416	2.8	16.5	29	4.2	126.9	Mera	0.08
Narcea	91	0.8	-	91*	43.4*	1,135*	Nalón	-
Narla	402	1.2	17.6	24	3.8	205	Miño	0.50
Ouro	42	0.7	16.9	30	3.4	188.9	Ouro	0.60
Salas	844	0.2	16.2	35	-	145**	Limia	0.66
Tambre	145	0.4	20.6	125	54.1	1,530	Tambre	0.15
Tea	26	0.5	18.2	50	17.6	411	Miño	0.37
Trimaz	414	0.6	-	50	21.2	889	Miño	0.01
Ulla	326	0.8	17.5	132	79.3	2,803.6	Ulla	0.27

To characterize the environment of each population, the coordinates of the sampling point were visualized in a GIS system, over a digital terrain model obtained from the National Geographic Institute of Spain, with a resolution of 25 m. We then estimated the altitude of the sampling point and, calculated the altitude of a point in the river situated in a buffer 1 km upstream. The topographical slope was then calculated as the difference between altitudes divided by the distance and multiplied by 100, to obtain the slope in percentage. Values oscillated between 0.2 and 2.9% (Table 1). We also compiled river length, surface basin and average water flow of the river from several bibliographic sources (mainly from Río Barja & Rodríguez Lestegás, 1992), and the estimated density of *M. margaritifera* population from our samplings (details in Table 1). Water temperature was estimated using dataloggers that took a measurement every 15 min or every hour. We calculated the average water temperature of summers 2014–2016, from 20 June to 20 September, because this is the period where mussel growth should be maximum. No data are available for two of the rivers (Trimaz and Narcea).

Statistics

Measurement error was estimated from a set of 22 shells from 8 populations that were measured three times by the same researcher. An ANOVA was used to partition the variance into within and among individuals (Yezerinac et al., 1992). The mean squared deviations within individuals (MS_{within}) estimated the within-individual component of variance, and the among-individual component was calculated by dividing (MS_{among} - MS_{within}) by the number of repeated measurements (3 in this case) (Sokal & Rohlf, 2012). This analysis indicates that Length and Height have very low measurement error (0.13% and 1.51%, respectively). However, the Convexity was more prone to error (13.74%).

We computed a simple linear regression to test the accuracy of calculated shell volume (Vc) to predict the observed volume (Vo), measured with water displacement.

To study allometric patterns, we computed the regression of calculated shell volume on shell length. Under isometry, the expected slope of a log-log regression is 3, given that volume is proportional to the cube of length. We calculated the slopes using ordinary least-squares regression (OLS), a method that assumes that the *x* variable is measured without error (a good approximation given that error was 0.13%), whereas the *y* variable is affected by measurement error (Warton et al., 2006). Given that both length and estimated volume have measurement error, we also calculated the regression lines using Major Axis (MA), and Reduced Major Axis (RMA), which assume the same magnitude of measurement error in both variables (Warton et al., 2006), and that have been recommended for allometric studies (Labarbera, 1989), although some authors consider this claim unjustified (Smith, 2009). Using procedure RL FUNCTIONAL in Genstat (2020) we estimated the intercept, slope and mean value of the relationship between $\log_{10}$(length) and $\log_{10}$(Vc), and estimated the confidence interval of the slope by means of a bootstrap procedure with 1,000 simulations. The relationship between allometric slope and environmental variables was explored using regression and correlation analyses.

Over the text, mean values are presented with their standard errors and sample size. Statistical analyses were done with Genstat 20th edition (GenStat, 2020) and xlStat 2020 (www.xlstat.com).

RESULTS

Estimation of Shell Volume

The observed volumes (Vo) were plotted against calculated volumes (Vc) showing a good linearity, with an $r^2 = 0.99$ (Fig. 2). Thus, the expression [1] is a reliable approximation to calculate the volume of a shell, and calculated shell volume (Vc) was used to study the allometry of shells from the different populations.

Analysis of Natural Populations

The global sample included 803 shells, with a mean length of 84.7 ± 0.5 mm and a volume of 42.0 ± 0.6 mL (Table 2). The relationship between $\log_{10}$(shell length) and $\log_{10}$(calculated volume) is presented in Figure 3, with the regression lines from all three methods (OLS $r^2 = 0.97$). The overall estimate of the slope (including all populations) is 2.75 ± 0.02 from OLS, 2.83 ± 0.02 from MA and 2.80 ± 0.02 from RMA, in all cases significantly smaller than the expected slope ($b = 3$), indicating negative allometry.

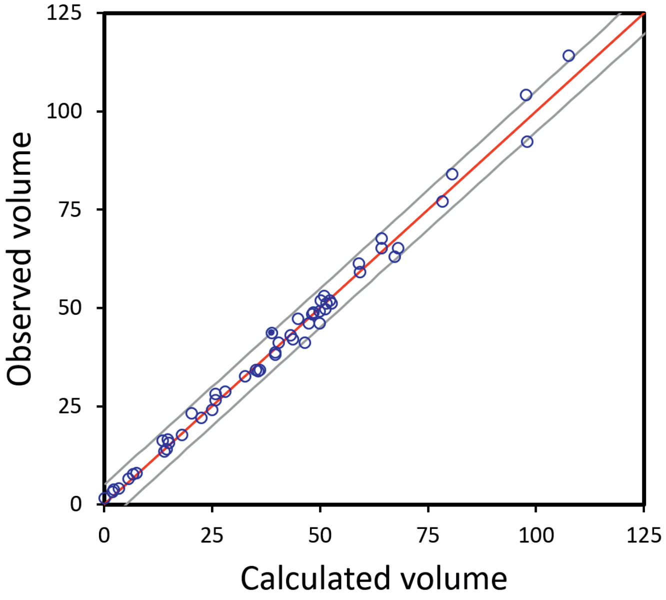

FIG. 2. Observed shell volumes (Vo, in mL) of 53 specimens of *M. margaritifera* plotted against their calculated volumes (Vc) by the expression [1]. Least-squares regression line: Vo = -1.211+1.017*Vc, $r^2 = 0.99$, $p < 0.001$. Grey lines indicate 95% confidence limits.

TABLE 2. Basic statistics for shell dimensions by population.

		Shell length (mm)					Shell volume (mL)				
Population	N	Min	Max	Range	Mean	SE	Min	Max	Range	Mean	SE
Arnego	22	71.35	90.50	19.15	82.90	1.18	23.53	45.80	22.27	34.64	1.44
Camba	87	42.69	108.36	65.67	85.89	1.60	5.41	76.87	71.46	42.02	1.85
Eo	140	35.84	111.24	75.40	85.94	1.20	3.24	80.58	77.35	41.28	1.44
Landro	55	82.14	117.32	35.18	101.58	0.85	42.87	89.54	46.68	65.16	1.45
Limia	50	22.33	105.58	83.25	77.55	3.02	0.92	94.36	93.44	42.60	3.80
Mandeo	38	59.50	111.24	51.74	84.26	1.32	15.08	81.75	66.68	40.64	1.66
Masma	76	30.30	98.37	68.07	80.82	1.59	2.07	57.43	55.36	34.99	1.44
Mera	50	72.47	96.77	24.30	87.79	0.81	24.77	61.25	36.48	42.55	1.06
Narcea	12	38.72	99.76	61.04	71.62	4.91	9.63	66.82	57.18	28.49	4.99
Narla	31	51.36	101.65	50.29	87.69	2.59	10.11	66.75	56.64	48.02	2.50
Ouro	62	57.43	103.13	45.70	84.23	1.35	15.94	68.86	52.92	42.40	1.56
Salas	24	34.68	83.02	48.34	65.24	2.16	3.43	32.76	29.33	19.62	1.38
Tambre	30	68.74	106.67	37.93	93.56	1.35	23.40	71.53	48.13	50.51	1.98
Tea	46	28.22	122.00	93.78	77.33	2.98	1.65	111.22	109.57	39.44	3.69
Trimaz	22	67.76	90.56	22.80	82.10	1.20	22.38	46.13	23.75	35.71	1.36
Ulla	62	15.34	103.61	88.27	83.24	2.13	0.29	71.63	71.34	41.35	2.12
Total	803	15.34	122.00	106.66	84.66	0.53	0.29	111.22	110.93	41.95	0.62

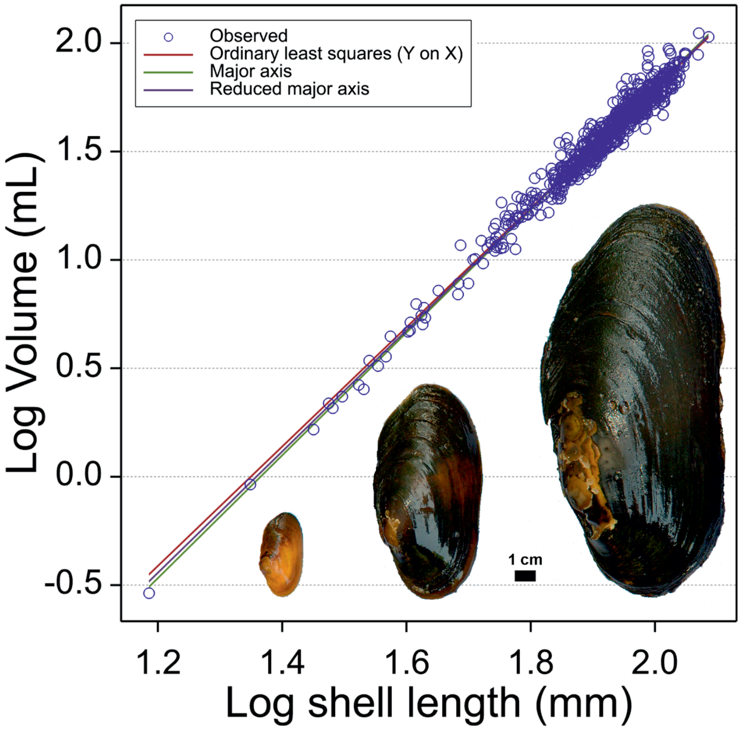

FIG. 3. The relationship between shell length and calculated volume in 803 specimens of *M. margaritifera* from rivers from NW Spain. The three regression lines are also plotted. Three individuals are presented at the same scale, to show variations in shell proportions with size (Scale bar = 1 cm).

Table 3 presents the estimates of slopes and their associated confidence intervals by population. In 11 out of the 16 populations the estimated slopes (under OLS) were also significantly smaller than the expected. Estimates of slopes using RMA or RA indicate also negative allometry in the same populations, except for rivers Landro, Mera and Trimaz. In other words, larger shells had a lower volume than the expected under isometry. In no case there was evidence for positive allometry. However, we found evidence for a positive association between the estimate of slope using OLS and the range in shell length among populations ($r = 0.57$, $p = 0.020$), but not using MA ($r = 0.02$, $p = 0.930$) or RMA ($r = 0.32$, $p = 0.225$). Sample size did not correlate with the estimated slope using any of the methods (range of r from -0.13 to -0.25, $p > 0.05$), suggesting that sample sizes were enough for slope estimation.

We hypothesized that water speed would affect allometric patterns of local populations. To test this idea, we calculated the relationship between the allometric slope (OLS) and the topographic slope. A simple linear regression suggests a negative relationship ($F_{1,14} = 4.04$, $p = 0.064$) (Fig. 4), with a Pearson correlation coefficient of -0.48, $p = 0.079$. The Spearman rank correlation coefficient was also negative, but not significant ($r_s = -0.35$, $p = 0.215$). Given that multiple variables can affect the allometric slopes, we tested the possible effects of mussel density, topographic slope, and summer temperature using partial correlations (controlling for the effect of the rest of variables). Correlation values were 0.05 for density, -0.37 for the topographic slope and 0.09 for summer temperature, again suggesting an effect of the topographic slope.

TABLE 3. Allometric slopes of the relationship between $\log_{10}$ of calculated shell volume and $\log_{10}$ of shell length for 16 populations of *M. margaritifera* from NW Spain, with Standard error (SE) and 95% confidence intervals from a bootstrap procedure. Values significantly different from the expected slope under isometry (b = 3) are presented in **bold**. The values estimated from all three methods are very similar and give the same conclusions, with the only exception of population Trimaz when using Major Axis regression.

	Ordinary Least Squares (OLS)				Major Axis (MA)				Reduced Major Axis (RMA)			
Population	slope	SE	lower	upper	slope	SE	lower	upper	slope	SE	lower	upper
Arnego	2.806	0.270	2.416	3.486	3.093	0.296	2.616	3.769	2.962	0.278	2.533	3.632
Camba	**2.569**	0.079	2.399	2.707	**2.670**	0.073	2.518	2.793	**2.626**	0.074	2.468	2.751
Eo	**2.743**	0.056	2.627	2.839	**2.805**	0.050	2.704	2.899	**2.778**	0.052	2.671	2.870
Landro	**2.397**	0.217	2.117	2.741	2.798	0.217	2.450	3.319	2.617	0.175	2.318	3.030
Limia	2.980	0.048	2.903	3.093	3.020	0.058	2.936	3.165	3.002	0.053	2.922	3.134
Mandeo	**2.604**	0.132	2.254	2.783	**2.711**	0.095	2.528	2.887	**2.664**	0.104	2.420	2.828
Masma	**2.752**	0.050	2.631	2.832	**2.799**	0.053	2.714	2.928	**2.778**	0.049	2.679	2.883
Mera	**2.504**	0.204	2.082	2.876	3.064	0.194	2.726	3.498	2.803	0.180	2.466	3.174
Narcea	3.055	0.151	2.792	3.407	3.114	0.163	2.841	3.485	3.088	0.155	2.821	3.437
Narla	**2.607**	0.100	2.322	2.703	**2.644**	0.083	2.418	2.736	**2.628**	0.089	2.381	2.717
Ouro	**2.368**	0.091	2.172	2.539	**2.516**	0.081	2.368	2.687	**2.453**	0.079	2.288	2.614
Salas	**2.656**	0.080	2.394	2.745	**2.672**	0.072	2.435	2.759	**2.665**	0.075	2.416	2.753
Tambre	2.564	0.214	2.246	3.152	2.984	0.425	2.573	4.225	2.791	0.289	2.485	3.648
Tea	2.929	0.077	2.722	3.030	2.983	0.063	2.833	3.079	2.959	0.068	2.788	3.055
Trimaz	**2.621**	0.125	2.436	2.917	2.688	0.135	2.494	3.012	**2.659**	0.129	2.471	2.970
Ulla	**2.808**	0.056	2.677	2.863	**2.827**	0.046	2.720	2.879	**2.819**	0.050	2.702	2.871

DISCUSSION

Our results indicate that the volume of the shell of *M. margaritifera* can precisely be estimated using equation [1], based on three linear measurements. These variables have low measurement error, but care should be taken when measuring shell convexity. The required values can be obtained without damaging the animal, which is appropriate when working with rare and endangered freshwater mussels. Furthermore our technique also enables the estimation of biomass, which is a key parameter for evaluation of the relevance of freshwater mussels as ecosystem services providers (Atkinson et al., 2020). This method allowed the calculation of shell volume in a sample of 803 shells from 16 rivers in northwestern Spain. We found evidence for negative allometry of shell volume in the global sample and in 11 populations. Finally, we also found evidence for inter-population variation in allometric slopes, which seems related to water speed.

The use of three different methods to estimate slopes gave almost the same results (Table 3), providing more support to the conclusions. However, in 6–8 of the 16 populations (depending on the method), the slope did not significantly deviate from isometry, suggesting that some environmental and/or genetic factors affect the volume of the shell in local populations. Galician populations of *M. margaritifera* are apparently hermaphroditic, which is likely to be due to their low mussel densities (Bauer, 1987). This fact excludes sex-ratio as a relevant factor on shell morphology, which has been shown to affect mussel width in populations where individuals are not hermaphroditic (Wilson et al., 2012). It is interesting to note that using OLS there was a positive correlation between the range in shell length included in the sample and the estimated slope. This find-

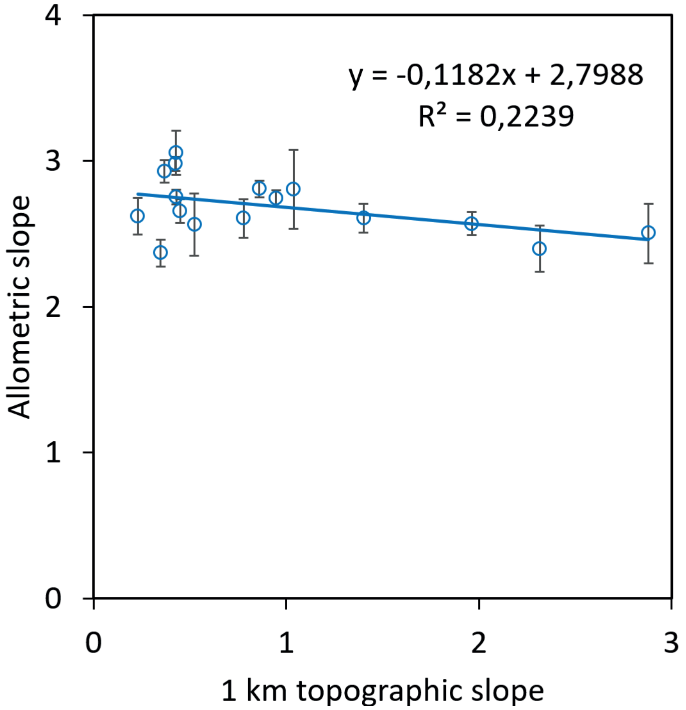

FIG. 4. The relationship between allometric slope (± SE) and topographic slope (1 km upstream) in 16 *M. margaritifera* populations from NW Spain. Mussels tended to be less tumid at sites with higher topographic slopes, where we expect water speed to be higher.

ing is suggestive that the true slope might be close to 3 when the range in shell length covers all the variation of shell size. Extreme values, either very small or very large, have a strong effect on the estimation of the slope of the linear relationship, and precisely these extreme values are absent when range is small. Even if our study included a large sample, it might be necessary to obtain more data for very small and very large mussels, to increase the precision of the estimates of the slope.

A recent study has evaluated the relationship between shell length and mass in 43 species of unionid mussels from North America. Atkinson et al. (2020) found an overall slope of 3.08 ± 0.36 at the species level for soft tissue dry mass, and 3.09 ± 0.45 for shell dry mass, indicating isometry. However, they found large variation among species and tribes. At the tribe level, the slope averaged 2.80 ± 0.15 and 2.67 ± 0.40, for soft tissue and shell respectively, indicating negative allometry. Note that the first value is identical to our estimate for *M. margaritifera* using RMA. However, the estimate of the slope was greater for the tribe Lampsilini than for any other tribe, with 6 (out of 10) species showing *b* values significantly larger than 3 (Atkinson et al., 2020). These species become broader with size. Therefore, in certain groups of unionid mussels negative allometry is the rule (e.g., *Amblema plicata, Elliptio complanata, M. margaritifera*), but in others positive allometry is more common. In the first case, larger mussels become narrower (and perhaps heavier), likely enabling them to protect themselves into the substrate when flow is intense (Atkinson et al., 2020). Maximum water velocity is a critical factor in the distribution of *M. margaritifera*, favouring those individuals that are dorsally arched and heavier (Eagar, 1977).

We found variability in length-volume relationships among populations of *M. margaritifera* (Table 3). Our results suggest that when water speed is higher, larger mussels are less tumid than in locations where water current is slower (Fig. 4). This may indicate that under these conditions, mussels need to be able to burrow themselves to avoid being pulled away by the water and being less tumid would be favoured. However, detailed analyses of water current indicate that flow speed is quite homogeneous near the bed where mussels are living (Moorkens & Killeen, 2014). Therefore, our results are to be taken as suggestive rather than as evidence of a causal relationship. Another possible confounding factor is the fact that brooding mussels are typically wider than non-brooding specimens (Wilson et al., 2012). However, our populations have very low densities and in these conditions the predominant strategy is hermaphroditism (Bauer, 1987), and therefore the proportion of non-brooding individuals is expected to be small.

In benthic-type aquatic organisms it has been argued that the surface ($\propto L^2$) and body volume ($\propto L^3$) are conditioned by hydrodynamic forces that influence the acceleration, drag and lift reaction (Koehl, 1996; García-March et al., 2007): thus, these organisms have a physical constraint on body form not experienced by creatures in steady waters. One century ago, Ortmann (1920) working in North America, found that unionid mussels were rather compressed or flat in the headwaters, and more convex and swollen in large rivers. The same observation is reported for other species in Europe, noting that there is often a negative correlation between the relative height (H/L ratio), the presence of a straight ventral border (which favours burrowing) and the water velocity of the habitat (Eagar, 1948). This observation is suggestive of a negative allometry of volume against size in the headwaters, which is what we found in general, and in some of the populations, apparently due to faster flow in these stations. However, in the marine mussel *Mytilus edulis* (which is wave-swept and not under unidirectional flow), individuals tended to be narrower (flatter) at high density and at low food level, and the slope of volume against length increased with decreasing food level (Alunno-Bruscia et al., 2001). Therefore, food availability is another factor that may have an influence in the variability of form in river mussels. In our dataset, mussel density did not correlate with the allometric slope, suggesting that food availability might not be different in rivers with contrasting densities. However, except for rivers Camba and Eo, mussel density was low and similar among populations, with an average of 0.61 ± 0.16 (N = 15) individuals/m^2 (Table 1), a fact that might explain why we could not detect any effect of mussel density on allometric patterns among populations. Densities of *M. margaritifera* in northwestern Spain are very low compared to the extreme cases of Russia, with a record of 1,000 individuals/m^2 (Popov, 2015), or the densities of Irish populations, in the range of 10–50 individuals/m^2 (Moorkens & Killeen, 2014).

Allometric models built at the species level provide the most accurate estimates of soft tissue and shell biomass for unionid mussels (Atkinson et al., 2020). Our results indicate that in *M. margaritifera* the general pattern of growth is negative allometry. The methods described in this paper would be useful to estimate mussel biomass and the ecosystem services they provide, such as water filtration and nutrient cycling (Lummer et al., 2016; Atkinson et al., 2020).

ACKNOWLEDGEMENTS

We are very grateful to Martiño Cabana, for his help calculating topographic slopes using GIS. ACR was supported by a grant from the Spanish Ministry of Science, Innovation and Universities, including ERD funds (PGC2018-096656-B-I00). Additional funding was obtained from Xunta de Galicia within “Programa de Consolidación e Estructuración de Unidades de Investigación Competitivas” (ED431D 2017/22 and ED431B 2020/16).

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Revised ms. accepted November 23, 2021