LENGTH–WEIGHT RELATIONSHIP FOR THE FRESHWATER MUSSEL *UNIO RAVOISIERI* (MOLLUSCA: BIVALVIA: UNIONIDAE) FROM DIFFERENT TUNISIAN AQUATIC ECOSYSTEMS
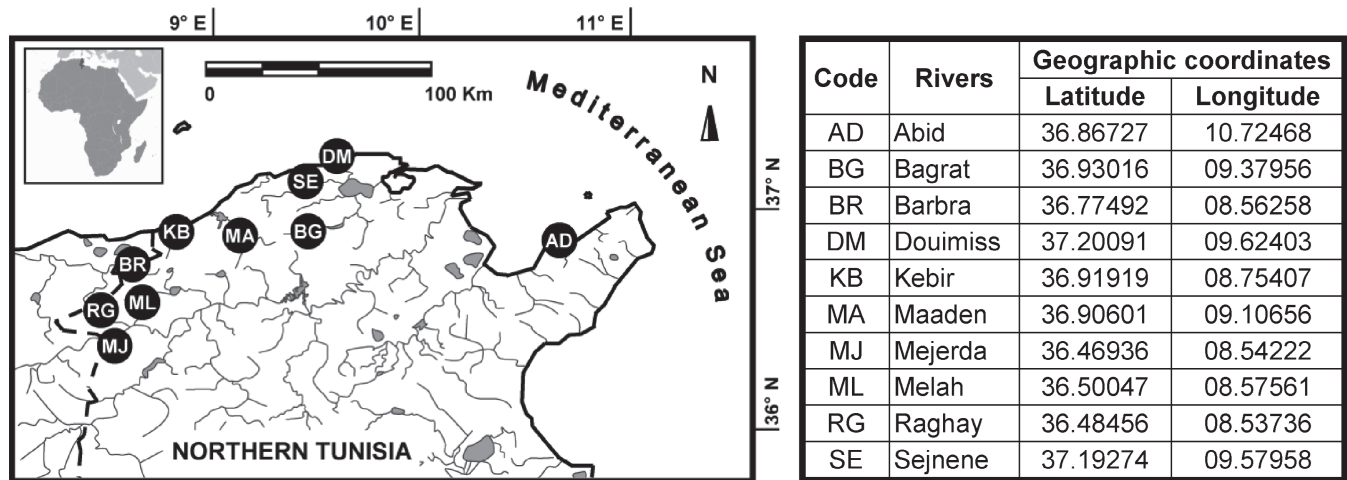
The freshwater mussels of the Unionidae family are bivalve molluscs widely distributed across the continents of the world, with the exception of South America[1] and Antarctica. This family forms the most diversified group of freshwater mussels at the specific level, with currently 753 identified species represented by 153 genera[1]. In Tunisia, the diversity of unionid mussels is low, with only five species currently recognised[2]. Among them, there is *Unio ravoisieri* (Deshayes, 1847) which presents the most common freshwater mussel species in North Africa. *U. ravoisieri*'s geographic distribution in Tunisia is limited to the humid and sub-humid bioclimatic regions and it lives in lotic and lentic ecosystems in the north.

Unio ravoisieri plays an important ecological role in these aquatic ecosystems since it is considered to be a powerful filter feeder that cleans the water by removing particles from both the water column and interstitial sediments[3]. It also provides many other ecosystem services such as supporting nutrient recycling and storage, contributes to the structural integrity of the habitat, helps to modify the substrate and food web, and serves as an environmental monitor[3]. As an endemic and vulnerable species in its geographic area[2, 4, 5], studies on its genetic diversity and its population dynamics are necessary to enhance its conservation.

Length and weight are two biological data measurements that are directly linked to growth and reflect the fitness and wellbeing of the species within their spatio-temporal context. Studies of length–weight relationships are of great importance in stock assessment research[6] since they provide information about conversion of growth in length equations to growth in weight for use in stock assessment models, allowing the estimation of biomass from length observations these are useful for inter-regional comparisons of life histories of species[6, 7, 8].

In this study, length–weight relationship parameters are reported for the freshwater mussel *Unio ravoisieri* collected from different aquatic ecosystem rivers in northern Tunisia. A total of 293 *Unio ravoisieri* specimens were collected by visual inspection and by hand from 10 rivers monthly during January 2012 to July 2012 (Fig. 1). The sampled rivers cover four major watersheds: Mejerda watershed with 3 sampled localities (Mejerda, Melah and Raghay rivers), North-West watershed with 3 sampled localities (Barbra, Kebir and Maaden rivers), Ichkeul watershed

Code	Rivers	Geographic coordinates	
		Latitude	Longitude
AD	Abid	36.86727	10.72468
BG	Bagrat	36.93016	09.37956
BR	Barbra	36.77492	08.56258
DM	Douimiss	37.20091	09.62403
KB	Kebir	36.91919	08.75407
MA	Maaden	36.90601	09.10656
MJ	Mejerda	36.46936	08.54222
ML	Melah	36.50047	08.57561
RG	Raghay	36.48456	08.53736
SE	Sejnene	37.19274	09.57958

Figure 1 Map of northern Tunisian rivers showing locations of the populations and the geographic coordinates for *Unio ravoisieri* samples.

with 3 sampled localities (Bagrat, Douimiss and Sejnene rivers) and Cap Bon watershed with a single sampled locality (Abid river).

Identification of freshwater mussels to species level was carried out based firstly on external shell morphology and colour as described by Khalloufi et al.[2] and then were verified using allozyme diagnostic loci as indicated in Fassatoui et al.[9]. Damaged individuals were discarded and not considered in the analyses.

Bivalve shell length (SL) was determined to the nearest 0.01mm with a digital calliper (RUPAC µ) using the maximum distance between anterior and posterior shell margins. Total fresh body weight (W_b), including shell, was measured using a digital balance with a precision of 0.001g. The association degree between SL and W_b variables was calculated by the determination coefficient (r^2).

The length-weight relationship was examined applying the classical allometric model of Le Cren[10]: $W_b=aSL^b$, where a is the y-intercept parameter and b is the slope or allometric coefficient. The linearized equation was done with ordinary least-squares regression[11] after Log-transformation: $Ln (W_b)=Ln (a)+b Ln (SL)$. The relation between length and weight is used to provide information on the condition of freshwater mussel, and help to determine whether somatic growth is isometric ($b=3$) or negative allometric ($b < 3$) or positive allometric ($b > 3$). The appropriate model for fitting allometric

data and to estimate b parameter is the reduced major axis regression, known as standardized major axis method[11], so we used this method. Confidence limits at 95% of the slopes b were estimated and Student's t-test was applied to test the departures from isometry.

As sample size is limited in our study, the length-weight relationship was investigated for each locality for all combined specimens. This was applied to all samples with more than 10 specimens, as it was recommended by Froese et al.[8]. Moreover, the factors such as habitat, seasonal effect, age, sex and maturity stage which can affect the length-weight relationship were not taken into consideration. For these reasons, the estimated a and b parameters should be considered as mean annual values.

The comparison of slopes b between localities was carried out using an analysis of variance (ANOVA). All of the statistical analyses were performed with the free R environment version 3.5.1[12] using smatr package[13]. The significance level for all statistical tests was taken as $p < 0.05$.

There were significant positive associations between shell length and total weight variables within all populations, with high values of the coefficients of determination ($r^2 > 0.865$, $p < 0.001$) (Table 1). The growth coefficients b of the length-weight relationships ranged between 2.545 and 3.285 among populations and were mostly lower than 3, except for the b values of the samples of Mejerda and Raghay Rivers. Overall,

Table 1 Descriptive statistics and estimated parameters of length-weight relationship for samples of the freshwater mussel *Unio ravoisieri* (combined sexes) collected during 2012 from northern Tunisian Rivers. *N*, sample size; *SL*, total shell length; W_b, total body weight; SE, standard error; *a*, intercept; *b*, slope; CI, confidence intervals; r^2, coefficient of determination; TG, types of growth (I, isometry; A+, positive allometry; A-, negative allometry). Significant *b* values ($p < 0.05$) are shown in **bold** after evaluation by Student's *t*-test using smatr package[13] under R project environment. Samples coded as in Fig. 1.

Sample code	N	SL±SE (cm)	Wb±SE (g)	a	b	CI at 95% of b	p-value	TG	r^2
AD	40	6.280±0.11	30.04±1.50	0.1414	2.951	2.773–3.141	0.600	I	0.960
BG	40	5.370±0.13	18.67±1.46	0.1264	2.979	2.809–3.158	0.807	I	0.899
BR	23	7.562±0.22	47.21±4.10	0.1250	2.973	2.701–3.272	0.845	I	0.947
DM	10	5.296±0.19	17.56±1.82	0.1462	2.922	2.455–3.479	0.739	I	0.949
KB	34	4.804±0.08	12.28±0.60	0.1460	2.866	2.670–3.076	0.198	I	0.937
MA	19	5.651±0.26	21.87±2.47	0.1748	**2.756**	2.604–2.918	0.006	A-	0.962
MJ	27	7.725±0.21	51.13±4.45	0.0809	3.285	2.888–3.737	0.159	I	0.888
ML	33	5.578±0.12	21.96±1.28	0.2283	**2.721**	2.486–2.977	0.034	A-	0.946
RG	32	5.045±0.10	15.63±1.05	0.1035	3.107	2.942–3.282	0.200	I	0.962
SE	35	5.401±0.06	18.79±0.56	0.3620	**2.545**	2.212–2.927	0.022	A-	0.865

Unio ravoisieri in the northern Tunisia rivers exhibits isometric to negative allometry. Three populations have slopes significantly less than 3 (Maaden, Melah and Sejnene populations), while the remaining populations display slopes statistically equal to 3. The intercept a parameters varied relatively little among populations from 0.081 to 0.362 with a mean of 0.163±0.025. The comparison of slopes *b* between populations by the ANOVA test showed a slight significant difference (*df*=9, *F*-test=2.337, *p*=0.014).

This study provided the first data on length-weight relationships for the freshwater mussel *Unio ravoisieri* collected over a large area of different aquatic ecosystem rivers of northern Tunisia. Almost all length-weight relationships showed an isometric growth of freshwater mussels suggesting balanced growth between shell length and weight. However, only three samples from Maaden, Melah and Sejnene rivers demonstrated negative allometric growth suggesting that the weight gain is slower than the growth in length, as inferred from low significant *b*-values. This might be attributed to environmental conditions, food availability or linked to intrinsic factors specific to each population such as reproduction and demographic structure. The *b*-values of the length-weight relationship for the *Unio ravoisieri* in the present study were in the same range as those that were recently obtained on a reduced geographic scale of Ichkeul watershed[9]. Similarly, our results of *b*-values were much closer to those observed in other unionid species such as, for example, *Unio pictorum mancus* from Lake Maggiore and Lake Candia of Italy[14], *Sinanodonta woodiana* from Po River Basin of Italy[15] and *Unio durieui* from Ichkeul watershed of Tunisia[9].

Our analyses revealed slight significant difference of slopes *b* between populations, which indicates that there are heterogeneities between the regions. The geographical heterogeneities in allometric growth have serious implications for the management of the species. Accordingly, any future conservation should take into account this geographic pattern. This study fulfilled the aims set for it, and the data presented might constitute a valuable guideline for establishing future biometric studies for this freshwater mussel collected through the northern Tunisia.

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