

Growth models and longevity of freshwater pearl mussels (*Margaritifera margaritifera*) in Spain

E. San Miguel, S. Monserrat, C. Fernández, R. Amaro, M. Hermida, P. Ondina, and C.R. Altaba

Abstract: Growth rates of populations of the freshwater pearl mussel, *Margaritifera margaritifera* (L., 1758), in north-western Spain were analysed based on measurements of annual annuli and using two nonlinear functions for length-at-age data sets: von Bertalanffy's growth model and a hyperbolic function. These populations reach the smallest maximum shell length (90.5 mm) and have the shortest life-span (35 years) and the highest growth rate (k in von Bertalanffy's model $>0.1\text{-year}^{-1}$, on average) known for this species. The two models were similar in performance and were well fitted (around 99%) to shell-length-at-age data, although the hyperbolic function appears to be applicable only from 6 years of age. The growth rate (either k or k' from the hyperbolic function) showed a large and significant variation across populations, both among and within drainages.

Résumé : Nous avons analysé les taux de croissance de populations de la moule perlière d'eau douce, *Margaritifera margaritifera* (L., 1758), dans le nord-ouest de l'Espagne d'après les mesures des annulus annuels à l'aide de deux fonctions non linéaires pour les données de longueur en fonction de l'âge, soit le modèle de croissance de von Bertalanffy et une fonction hyperbolique. Ces populations atteignent la plus petite taille maximale (90,5 mm), la durée de vie la plus courte (35 ans) et le taux de croissance le plus élevé (k du modèle de von Bertalanffy en moyenne $>0,1\text{-an}^{-1}$) connus chez cette espèce. Les deux modèles affichent des performances semblables et sont également bien ajustés (autour de 99 %) aux données de longueur de la coquille en fonction de l'âge, bien que la fonction hyperbolique semble pouvoir s'appliquer à partir de l'âge de 6 ans. Le taux de croissance (k ou k' de la fonction hyperbolique) montre des variations importantes et significatives d'une population à l'autre, tant dans un même bassin versant que d'un bassin versant à l'autre.

[Traduit par la Rédaction]

Introduction

The key to protecting and managing rare or endangered species lies in understanding their biological relationships to their environment; this involves acquiring information on the characteristics of the species, such as size and growth (Primack 1995, 1998). Thus, comparative analyses of growth traits can shed light on the trade-offs that drive the evolution of life histories (Roff 1984), as well as on conservation strategies that are efficient. In this paper we report one such analysis involving detailed individual growth records from isolated conspecific populations of an endangered species exhibiting extreme longevity. Here we show that growth can be fitted by a simple equation from which several relevant life-history parameters can be accurately derived.

The striking phenotypic variability of unionoids (naiads, or freshwater mussels) has long been a nightmare for taxon-

omists, resulting in enormous lists of synonyms for many species (Haas 1969; Altaba 1996). Yet it also provides an opportunity for understanding how habitat differences translate to individual variability (Tevesz and Carter 1980; Watters 1994). Indeed, such variation is largely a response to environmental conditions, and thus can be interpreted as an adaptation to the unpredictable and changing nature of freshwater habitats. This plasticity can be traced in individual shells, because size and shape at all ages remain recorded as growth rings, or annuli, essentially in the same way as in tree trunks. Through analysis of annual size increments, variations in growth rate and changes in shape throughout an individual naiad's life can thus be reconstructed (Mutvei et al. 1994; Altaba et al. 2001; Bauer and Wächtler 2001).

The freshwater pearl mussel, *Margaritifera margaritifera* (L., 1758), is one of the longest lived animal species in the

Received 17 March 2004. Accepted 27 July 2004. Published on the NRC Research Press Web site at <http://cjz.nrc.ca> on 10 November 2004.

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world. Its life-span can reach 100–190 years in northern populations, but it can achieve a maximum age of up to 210 years (Ziuganov et al. 2000). Furthermore, in this species different characteristics such as maximum age, maximum size, and growth pattern exhibit remarkable variation (Comfort 1957; Hendelberg 1961; Bauer 1983, 1992; Chesney et al. 1993; Mutvei et al. 1994; Beasley and Roberts 1999; Ziuganov et al. 1994, 2000; Mutvei and Westermarck 2001). A comparative study on a wide geographic scale showed that in this species, reproductive output is correlated positively with both maximum age (A_{\max}) and maximum shell length (L_{\max}), but negatively with growth rate (Bauer 1992). Sometimes differences in growth characteristics of *M. margaritifera* occur even within the same drainage (Hastie et al. 2000). All these traits render *M. margaritifera* an excellent candidate for studies of growth variations and their impact on life history.

Margaritifera margaritifera is one of the most threatened naiads in Europe. Its range originally extended throughout the Atlantic and Arctic drainages of Europe, as well as throughout northeastern North America. It is almost always found in acidic waters, and only exceptionally in limestone areas (Chesney et al. 1993). However, through the 20th century it experienced catastrophic declines or even local extinction throughout most of its range (Clarke 1981; Bauer 1983, 1986, 1988; Young and Williams 1983; International Union for the Conservation of Nature and Natural Resources 1990; Beasley and Roberts 1996; Ziuganov et al. 1998; Altaba 2000; Young et al. 2001; Velasco et al. 2002; Reis 2003).

Data on growth of Iberian populations of *M. margaritifera* are very scarce (Ziuganov et al. 2000), but are badly needed in order to design effective conservation strategies. The purposes of this study, involving most Iberian populations of *M. margaritifera*, were (i) to estimate A_{\max} and L_{\max} ; (ii) to measure yearly growth increments in the permanent record provided by shells; (iii) to compare two nonlinear growth models for age-at-length data: the classical von Bertalanffy's growth function (VBGF; Ricker 1979) and a three-parameter hyperbolic growth function (HGF; Altaba et al. 2001); though HGF is not usually employed for studying animal growth, its application is very simple; and (iv) to test if there is any association between growth parameters provided by these models. When compared against logistic and power models, VBGF was found to be the most suitable function (Hastie et al. 2000). Here we explore a new function based on a hyperbolic curve (Altaba et al. 2001), which also provides asymptotic length and a growth rate.

Material and methods

Populations

All populations studied occur in northwestern Spain (15 from Galicia and 1 from Asturias). They were collected in nine different drainages, and were identified by the name of the sampled river. In two of the drainages more than one river was sampled: the Mera, Narla, and Trimaz rivers in the Miño drainage and the Arnego and Ulla rivers in the Ulla drainage. The seven remaining rivers (Eo, Landro, Mandeo, Masma, Narcea, Ouro, Tambre) belong to separate drainages. When several samples were taken in the same river (at

Table 1. Observed maximum shell lengths (L_{\max}) and ages (A_{\max}) for Spanish *Margaritifera margaritifera* populations.

Population	N	L_{\max} (mm)	A_{\max} (years)
Arnego	22	90.5	48
Eo ₁	44	111.24	50
Eo ₂	52	107.01	43
Eo ₃	39	105.66	44
Landro	44	117.32	44
Mandeo	38	111.24	48
Masma ₁	25	98.37	42
Masma ₂	39	95.05	45
Mera	49	96.77	49
Narcea	12	99.76	35
Narla	30	101.65	53
Ouro ₁	26	100.2	40
Ouro ₂	33	103.13	64
Tambre	27	106.67	65
Trimaz	22	90.56	52
Ulla	26	98.45	45

sites separated by at least 2 km), they were designated by a subscripted number (Eo₁, Eo₂, Eo₃; Masma₁, Masma₂; Ouro₁, Ouro₂; see Table 1). This scheme allowed nested comparisons within and among rivers and drainages. In a preliminary report (Ziuganov et al. 2000) some of these populations (Eo, Landro, Masma, Mera, Ouro, Ulla) were examined for L_{\max} and A_{\max} ; the present work is based on new, independent, and more extensive sampling.

Since *M. margaritifera* is protected by European law (listed in Annex V of the Habitats Directive), collecting was always carried out under the supervision of forest rangers from the autonomous government (Xunta de Galicia). Dead (often fresh) or live (no more than 10 individuals) mussels were taken from each locality. The Narcea sample (consisting entirely of empty shells) was supplied by an individual collector.

Age calibrations

The yearly periodicity of deep annuli on the shells of *M. margaritifera* has been validated by several authors (Hendelberg 1961; Bauer 1992; Ziuganov et al. 1994, 2000). To render these annuli clearly visible, shells were placed in a 5% solution of KOH at 50 °C to carefully remove the periostracum (external organic layer of the valves). A problem arose with old specimens because fairly large areas around the umbos are often badly abraded, thus hampering observation of several of the earliest annuli. To avoid the problem caused by this absence of data, VBGF was used (in the way suggested by Bauer 1992) to determine the age of the first discernible annulus. In addition, as in the Eo river, very young shells were sometimes used to establish a standard pattern for age calibration. Depending on the particular locality, this earliest scorable ring represents the age of 2 (Narcea), 3 (Eo₂, Eo₃, Mandeo, Masma₂, Ouro₁, and Ouro₂), 4 (Narla, Landro, and Tambre), or even 5 (Arnego, Eo₁, Masma₁, Mera, Trimaz, and Ulla) years.

Growth parameters

Counting yearly annuli, age was estimated for each individual. Total length was measured to the nearest 0.01 mm along the major (anteroposterior) axis of the shell, using a digital calliper (Mitutoyo 500). Emphasis was placed on finding the largest shell. Readings were taken from the oldest and the longest specimens from each locality to estimate A_{\max} and L_{\max} for every population. L_{\max} gives an indication of the size of the soft parts and is closely related to reproductive traits (Bauer 1992).

Distances between consecutive annuli (each annulus was measured along its longest axis) were measured to calculate annual growth increments. These values were averaged for each consecutive age among all specimens in a population. Plots of annual growth versus age showed the timing of growth-rate variations.

Length-at-age data were also employed to fit two nonlinear growth models, VBGF and HGF. Both functions provide a generalized description of growth patterns and facilitate comparisons of growth among and within species at various times and places.

VBGF has been applied to fishes, crustaceans, molluscs, reptiles, birds, and plants (Roff 1992). It is often written in the form

$$L_t = L_{\infty}[1 - \exp(-k(t - t_0))]$$

where L_t is length (in mm) at age t (in years), L_{∞} is the asymptotic length (theoretical final length), k is the growth rate (year^{-1}), and t_0 is the hypothetical starting time at which an individual would have been zero-sized if it had always grown according to this function. The origin of the growth curve is set by t_0 , although it is often omitted, as it is generally very small and has little influence on the growth trajectory. The parameter k measures the growth rate; for example (assuming $t_0 = 0$), when $k = 1.0$, an individual attains 90% of its asymptotic length in 2.3 years, while if $k = 0.1$, it takes 23 years to reach this percentage.

Model parameters and their approximate standard errors were estimated using nonlinear-regression analysis in lieu of the classical Ford-Walford method (Bagenal 1978). Nonlinear regression, recommended by several authors (Gallucci and Quinn 1979; Cerrato 1990; Motulsky 1996; Goong and Chew 2001), facilitates quantitative comparisons among the parameters of the equation. Parameters were estimated by means of JMP package software (SAS Institute Inc. 1995).

HGF is an expression similar to that commonly used in enzymology, and belongs to a family of functions known as hyperbolae, given by

$$L_t = L_0 + \frac{L_{\infty}t}{t_1 + t}$$

where L_t is length (in mm) at age t (in years) above the initial length at birth (L_0). The parameter L_0 may be assumed to be very small. For this reason, a two-parameter hyperbolic model (i.e., assuming $L_0 = 0$) may be used without significant loss of generality. This model also exhibits asymptotic behaviour. The parameter L_{∞} can be interpreted as the maximum size that an average specimen would reach if it lived indefinitely. Parameter t_1 (the age at which length is half the

asymptotic value) may be interpreted as the growth rate. In fact, the growth rate dL_t/dt for HGF is

$$\frac{dL_t}{dt} = \frac{L_{\infty}t_1}{(t_1 + t)^2}$$

Then L_{∞}/t_1 ($\text{mm}\cdot\text{year}^{-1}$) represents the growth rate at birth ($t = 0$). This rate is clearly nonconstant in time, but if it was maintained in the following years, an average individual would reach maximum size in t_1 years.

The growth of *M. margaritifera* is well fitted by HGF. However, residuals are always significantly larger than the mean during the early years. This suggests that this species could follow different growth behaviour at an early age and then gradually adjust to a hyperbolic-like growth model. Indeed, adjustments improve markedly when only data after the first 6 years are considered. Thus, we introduced a slight modification of the hyperbolic expression to take into account the fact that it may be applied after a given age, t_0 ($t_0 = 6$ years in our case). This seems to complicate the mathematical expression, yet the essential features of the model remain the same

$$L_t = L_0 + \frac{L_1(t - t_0)}{t_1 + (t - t_0)}$$

L_0 is now the individual size at age t_0 , or when the hyperbolic growth model starts to be valid. The maximum size, above the initial size (L_0), is now L_1 ; therefore, L_{∞} is given by $(L_0 + L_1)$. Now t_1 is the age at which length is half the asymptotic value (L_1), so L_1/t_1 is the growth rate at the onset (t_0). The instantaneous growth rate (L_1/t_1 ; $\text{mm}\cdot\text{year}^{-1}$) in the sixth year was called k' . We also employed nonlinear regression to compute the parameters (as well as their approximate standard errors) of the hyperbolic function.

The performance of both VBGF and HGF models was assessed by visual inspection of the lines fitted, combined with analyses of residuals quantified by the coefficient of determination (r^2), which is the proportion of the total variance explained by the model for each population ($SS_{\text{model}}/SS_{\text{total}}$).

Results

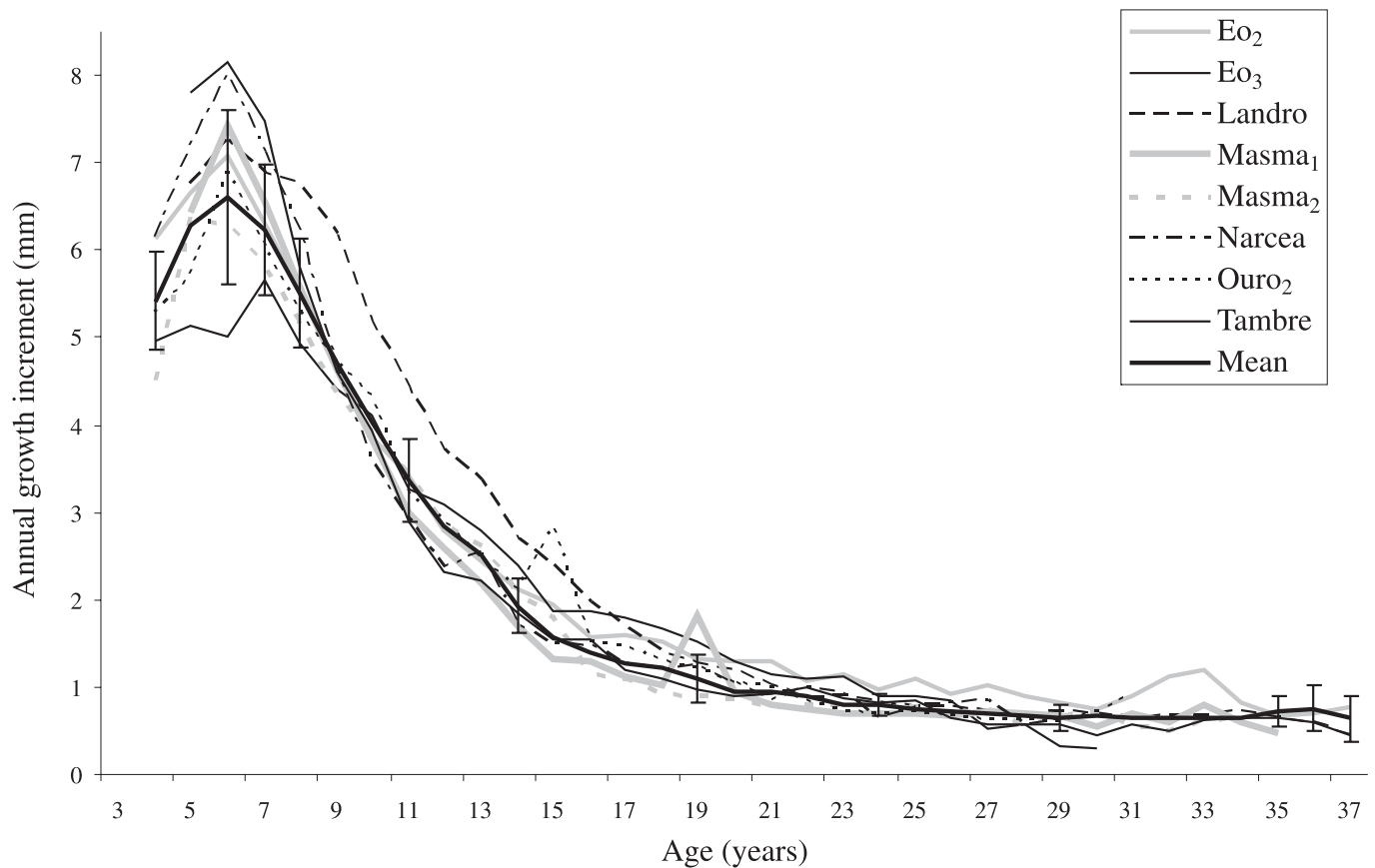
Growth increments

As shown by a plot of average growth increments versus age (see Fig. 1; for clarity only nine populations are represented), values could not be calculated for ages prior to 3 years because of shell erosion. The locations of maxima in such graphs varied widely: sometimes between the ages of 4 and 7 years, but most often between 5 and 6. Thus, when all samples were lumped, maximum growth also appeared between the ages of 5 and 6 years ("Mean" in Fig. 1). In general terms, for any population, growth increments almost invariably decreased from age 6 to about age 20 and then remained constant.

Maximum lengths and ages

L_{\max} and A_{\max} values for each (sub)population are given in Table 1. The maximum life-span (A_{\max}) varied markedly across samples (its coefficient of variation (CV) was 16.47%): there are populations for which an A_{\max} value of only 35 or 40 years can be recorded (Narcea and Ouro₁, re-

Fig. 1. Annual growth increments for *Margaritifera margaritifera* populations in selected Spanish rivers: Eo (samples 2 and 3), Landro, Masma (samples 1 and 2), Narcea, Ouro (sample 2), and Tambre. Each point represents the average growth between 2 consecutive years. Weighted mean values (“Mean”) were calculated using all 16 samples. Several standard error bars overlap. Numbers of shells measured are given in Table 1.



spectively), whereas in others this value reaches 64 or even 65 years (Ouro₂ and Tambre, respectively). L_{\max} values also varied among populations, ranging from 90.5 mm (Arnego) to 117.32 mm (Landro).

VBGF model

Growth parameters derived from von Bertalanffy's equation and their confidence intervals are displayed in Table 2. By averaging all r^2 values we obtained 99.45%, which indicates good performance of the model, i.e., VBGF accounts for 99% of the variance in shell length exhibited by these populations. Estimates of t_0 were very small, ranging from -0.397 to 1.613 years (Trimaz and Landro, respectively). Therefore, this parameter can be confidently set to zero with very little effect on the model's performance.

Estimates of k showed great variability. Overall, k was higher than 0.10, with only two exceptions (0.097 and 0.089 for Eo₂ and Eo₃, respectively). The highest growth rate (0.144) was for the Tambre population. Relevant differences appeared among populations when confidence intervals for k were compared, i.e., between Arnego and Ouro₂, Arnego and Ulla, Landro and Narcea, Tambre and Eo (any sub-population), and Tambre and Trimaz. Furthermore, it was possible to detect such differences even within the same river (Eo₁ and Eo₃).

L_{∞} values are also strikingly different among populations. Significant differences can be discerned from the confidence intervals in Table 2. Representative VBGF curves resulting from these parameters are plotted in Fig. 2 for five populations. It can be appreciated that as age increases, the relative differences in size also increase for populations with different growth functions.

Several parameters appear to be correlated (see Tables 1 and 2). There is a positive relationship between L_{∞} and L_{\max} (Spearman's correlation, $r_s = 0.5166$, $p < 0.05$). A positive correlation between k and A_{\max} ($r_s = 0.5719$; $p = 0.02$) was found, suggesting that k may be a determinant of A_{\max} (see Fig. 3).

Hyperbolic model

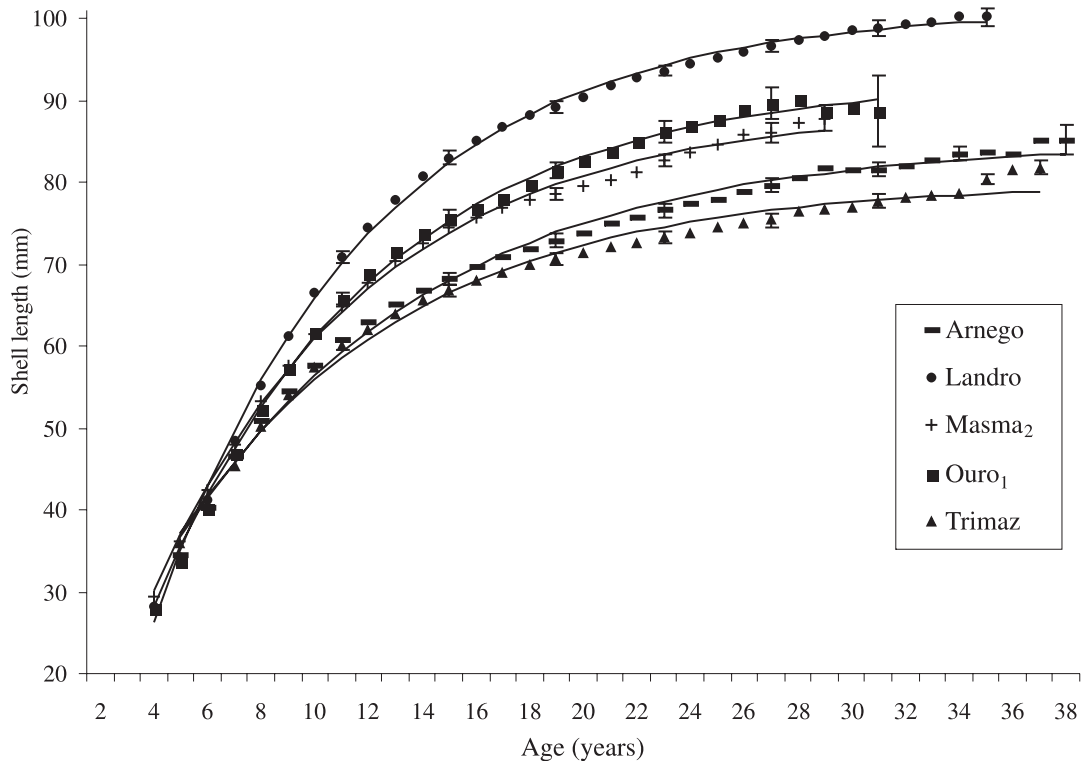
The same length-at-age data sets used for computing VBGF parameters were also employed to estimate the corresponding HGF parameters and confidence intervals (Table 3). L_{∞} is the sum of the corresponding values for L_0 and L_1 . As in the previous case, excellent fits to all data sets were obtained with HGF; r^2 values were always higher than 98%, and their average (99.60%) slightly exceeds that of the VBGF model.

A wide variation was found for k' among populations: Arnego, Masma₁, and Trimaz showed the lowest values (around 6 mm·year⁻¹), whereas Landro, Narla, and Tambre

Table 2. Parameter estimates for a von Bertalanffy growth function for 16 Spanish *M. margaritifera* (sub)populations.

Population	r^2 (%)	t_0 (years)	k (year ⁻¹)	L_∞ (mm)
Arnego	99.41	-0.347 (-0.984 to 0.220)	0.105 (0.097 to 0.114)	84.968 (83.904 to 86.147)
Eo ₁	99.17	0.672 (-0.054 to 1.293)	0.112 (0.100 to 0.125)	96.967 (95.133 to 99.093)
Eo ₂	99.46	0.280 (-0.164 to 0.677)	0.097 (0.090 to 0.105)	96.457 (94.836 to 98.253)
Eo ₃	99.88	0.141 (-0.089 to 0.358)	0.089 (0.085 to 0.093)	97.756 (96.406 to 99.215)
Landro	99.87	1.613 (1.436 to 1.781)	0.126 (0.121 to 0.130)	101.198 (100.506 to 101.916)
Mandeo	99.55	0.680 (0.341 to 0.988)	0.121 (0.113 to 0.130)	84.787 (83.715 to 85.938)
Masma ₁	97.79	-0.204 (-1.717 to 0.948)	0.107 (0.086 to 0.129)	91.781 (89.000 to 95.449)
Masma ₂	99.80	0.653 (0.410 to 0.879)	0.123 (0.116 to 0.130)	89.034 (87.956 to 90.192)
Mera	99.36	-0.007 (-0.717 to 0.609)	0.104 (0.093 to 0.115)	90.407 (88.801 to 92.259)
Narcea	99.32	0.257 (-0.204 to 0.656)	0.103 (0.092 to 0.115)	101.335 (98.433 to 104.745)
Narla	99.66	1.149 (0.843 to 1.430)	0.130 (0.122 to 0.137)	95.187 (94.296 to 96.129)
Ouro ₁	99.80	0.962 (0.738 to 1.170)	0.119 (0.113 to 0.126)	92.669 (91.619 to 93.786)
Ouro ₂	99.85	0.992 (0.808 to 1.165)	0.131 (0.125 to 0.137)	89.362 (88.501 to 90.270)
Tambre	99.70	1.120 (0.829 to 1.389)	0.144 (0.136 to 0.153)	91.184 (90.328 to 92.090)
Trimaz	99.00	-0.397 (-1.240 to 0.325)	0.115 (0.103 to 0.128)	79.943 (78.789 to 81.253)
Ulla	99.51	0.503 (0.016 to 0.943)	0.127 (0.118 to 0.136)	85.371 (84.517 to 86.292)

Note: Values in parentheses are approximate lower and upper bounds for 95% confidence intervals.

Fig. 2. Estimated von Bertalanffy growth curves for *Margaritifera margaritifera* populations from the rivers Arnego, Landro, Masma, Ouro, and Trimaz. Data points represent computed mean length-at-age values. Several standard error bars overlap.

exhibited values around 10 mm·year⁻¹. By comparing confidence intervals for L_1 and t_1 it was possible to reveal noteworthy differences in k' , e.g., between Tambre and Trimaz, Narcea and Ulla, or Ulla and Arnego, or between Landro and others. Large differences were also found within a river (subpopulations Eo₁ and Eo₃). Thus, k' may be taken as the growth rate when searching for differences among and within drainages.

L_∞ values also ranged widely among populations, from 90.281 mm (Trimaz) to 120.199 mm (Narcea). Many differ-

ences in L_∞ among populations were notable (see confidence intervals for L_0 and L_1 in Table 3). In general terms, L_∞ values were higher than those obtained with VBGF. Representative growth curves calculated with HGF are plotted in Fig. 4.

As before, a positive association was also found between L_∞ and L_{\max} (see Tables 1 and 3), although it was not significant ($r_S = 0.4592$; $p = 0.07$). Likewise, no correlation was detected between k' and either A_{\max} or L_∞ . However, k' is positively correlated with L_{\max} ($r_S = 0.5651$; $p = 0.02$). It is

Fig. 3. Scatter plot of growth rate (k) against maximum age (A_{\max}) for 16 Spanish *M. margaritifera* populations.

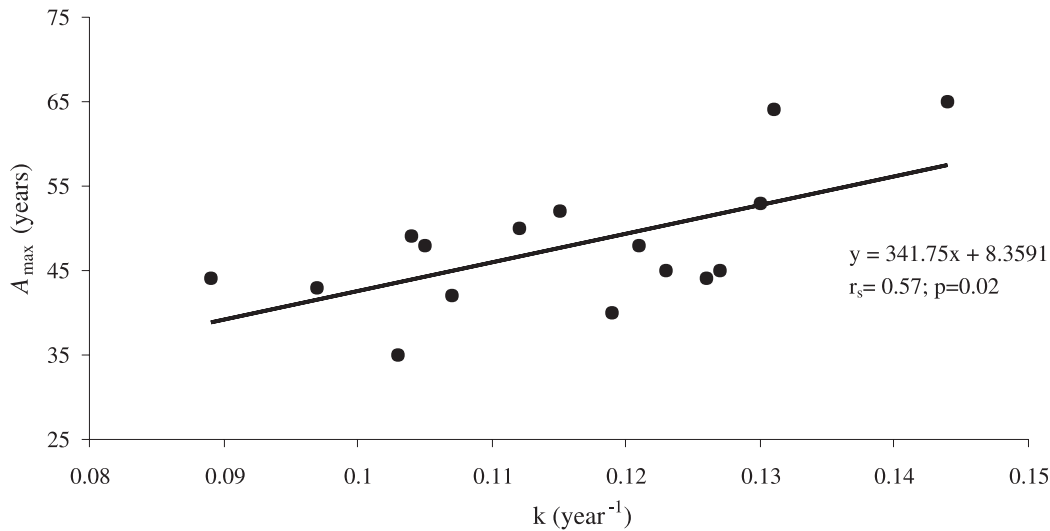


Table 3. Parameter estimates for a hyperbolic growth function for 16 Spanish *M. margaritifera* (sub)populations.

Population	r^2 (%)	$k' = L_1/t_1$ (mm·year ⁻¹)	L_0 (mm)	L_1 (mm)	$L_\infty = L_0 + L_1$ (mm)	t_1 (years)
Arnego	99.86	5.790	40.954 (40.250–41.652)	56.918 (56.003–57.857)	97.872	9.83 (9.196–10.522)
Eo ₁	99.69	7.262	43.414 (42.055–44.752)	70.619 (68.624–72.753)	114.033	9.725 (8.689–10.928)
Eo ₂	99.63	6.057	41.931 (40.514–43.325)	74.156 (71.804–76.746)	116.087	12.243 (10.850–13.884)
Eo ₃	99.83	6.559	37.790 (36.762–38.805)	81.032 (78.527–83.802)	117.822	12.354 (11.212–13.660)
Landro	99.87	10.703	39.744 (38.741–40.739)	76.300 (75.143–77.465)	116.044	7.129 (6.703–7.588)
Mandeo	99.28	6.779	39.824 (38.104–41.510)	58.134 (55.945–60.435)	97.958	8.575 (7.368–10.050)
Masma ₁	98.44	5.871	44.943 (42.213–47.581)	62.863 (58.703–68.057)	107.806	10.707 (8.208–14.352)
Masma ₂	99.81	7.170	41.993 (41.025–42.949)	62.057 (60.437–63.786)	104.05	8.655 (7.863–9.557)
Mera	99.67	6.229	41.263 (40.021–42.487)	65.082 (63.110–67.219)	106.345	10.449 (9.302–11.792)
Narcea	99.19	7.183	44.574 (42.209–46.878)	75.625 (70.914–81.528)	120.199	10.528 (8.445–13.385)
Narla	99.78	9.279	42.601 (41.472–43.717)	65.244 (64.002–66.495)	107.845	7.031 (6.493–7.627)
Ouro ₁	99.72	8.533	39.446 (38.154–40.721)	67.619 (65.809–69.507)	107.065	7.924 (7.143–8.818)
Ouro ₂	99.74	8.534	40.787 (39.620–41.940)	61.691 (60.033–63.417)	102.478	7.229 (6.529–8.029)
Tambre	99.84	9.237	44.309 (43.413–45.120)	58.228 (57.200–59.265)	102.537	6.304 (5.858–6.793)
Trimaz	99.52	5.961	40.691 (39.473–41.889)	49.590 (48.176–51.043)	90.281	8.319 (7.366–9.437)
Ulla	99.80	7.191	42.080 (41.211–42.939)	54.448 (53.457–55.450)	96.528	7.572 (7.013–8.189)

Note: Values in parentheses are approximate lower and upper bounds for 95% confidence intervals.

noteworthy that L_0 and L_1 are not correlated ($r_s = -0.0882$; $p = 0.75$), i.e., initial size (at 6 years) appears to be independent of L_{\max} .

Discussion

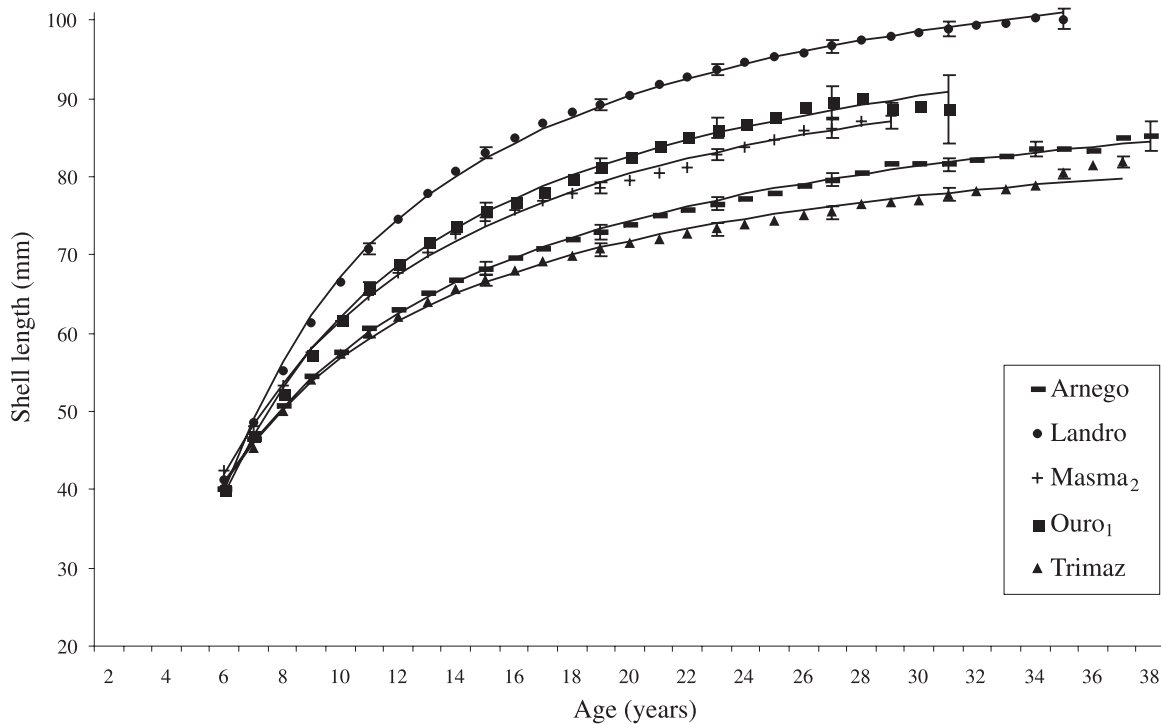
Comparison of growth models

Despite a wide array of criticisms, the model of growth in length (and sometimes in mass) most commonly used for fishes and molluscs is the three-parameter equation developed by von Bertalanffy (1938). Applying VBGF to the populations studied here, regular growth and considerable variation in the main growth parameters (k and L_∞) were revealed (Table 2 and Fig. 2). Thus, the model appears to be appropriate for length-at-age data from *M. margaritifera*. Indeed, averaging the coefficients of determination (r^2 , which are very high in all samples) yields a value of 99.45%,

which indicates good performance of the model. The hyperbolic model (starting at 6 years of age) also appears to be appropriate for the same data (Table 3 and Fig. 4). Excellent fits of HGF to all data sets were observed; the mean r^2 value is 99.60%, only slightly exceeding that from the VBGF model. Correlations from the two models are similar. Both functions make it easy to find and discuss new aspects of growth.

Both models seem to fail when applied to the youngest age classes. This is the reason why we decided to apply HGF only from the sixth year onwards. A better fit was possible with VBGF, but it yields equally erroneous estimates for the initial 3 years. However, this is only somewhat speculative, because shell abrasion precludes extending the data set to the initial years. Furthermore, the life history of *M. margaritifera* indicates that this lack of fit is not an artifact (see below).

Fig. 4. Estimated hyperbolic growth curves for *M. margaritifera* populations from the rivers Arnego, Landro, Masma, Ouro, and Tambre. Data points represent computed mean length-at-age values. Several standard error bars overlap.



The two models also clearly diverge in behaviour at the other end of the curve, corresponding to the oldest age classes. The differences have been further investigated by comparing the theoretical asymptotic lengths (L_{∞}) with the maximum lengths observed in each population. The length of the longest specimen found in each population is shown in Fig. 5. Populations are ordered from left to right according to the age of the oldest specimen found; for clarity, they are divided into three major groups ($A_{\max} < 45$ years, $45 \text{ years} < A_{\max} < 50$ years, and $A_{\max} > 50$ years). These values are compared with the predicted L_{∞} values (including confidence intervals) from both models.

Both L_{\max} and L_{∞} display reasonable good association in either model. However, it should be noted that the observed L_{\max} considerably exceeds the L_{∞} from VBGF in virtually all populations (the only exception is that which reaches the youngest age). This seems to suggest that VBGF systematically underestimates the expected L_{\max} values. In contrast, the theoretical values from HGF are larger than the observed ones in nine of the studied populations, fall within the confidence interval of the estimated L_{∞} in five instances, and are smaller in only two populations. For the group of populations in which the oldest specimen found is relatively young (less than 45 years), observed L_{\max} values tend to be considerably lower than those predicted by HGF. However, for those in which the oldest shell found is more than 50 years old, L_{\max} is much closer to L_{∞} . This strongly suggests that HGF yields much more accurate estimations of L_{\max} in the *M. margaritifera* populations studied.

Extreme longevity and its plasticity

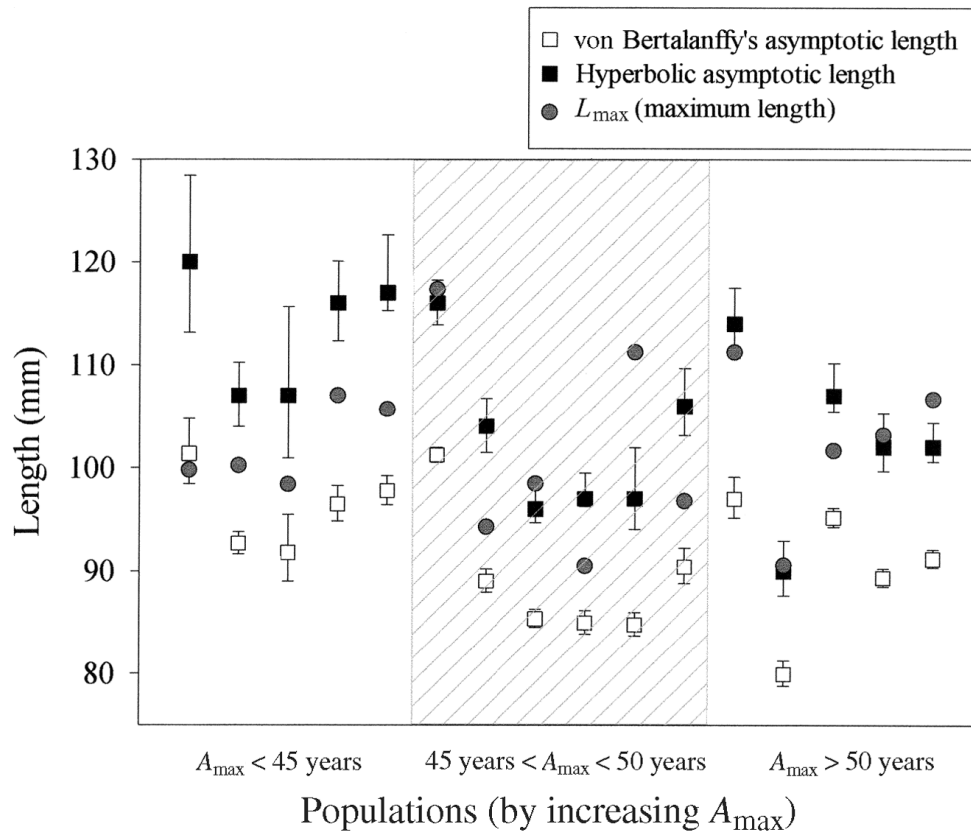
The variation in maximum life-span among Spanish populations of *M. margaritifera* is comparable to that reported for

others living farther north. Across much of Europe, values range from 30 to 132 years (Bauer 1992). In Ireland, recorded values range from 55 to 110 years (Beasley and Roberts 1999), with a CV of 18%, very similar to that found in Spain. Among Arctic populations, remarkable variation exists over a wide area, with values ranging from 41 to 190 years (Ziuganov et al. 1994, 2000; CV = 45.34%). Even the northern Scottish populations, located in a rather small region, exhibit large variation, from 28 to 123 years (Hastie et al. 2000; CV = 46%). Nevertheless, our data from the watersheds examined show that no Spanish specimen lives beyond 65 years; in contrast, in northern Europe it is usual to find individuals older than 70 years.

There is also a considerable variation in L_{\max} across the range of *M. margaritifera*. Spanish populations attain only a small size (not reaching 118 mm) and show little variation in L_{\max} (CV = 7.30%). In contrast, Scottish (Hastie et al. 2000), Irish (Beasley and Roberts 1996), and Russian (Ziuganov et al. 1994, 2000) specimens grow beyond 150 mm and may reach 162 mm, with wider variation (CV = 11.05%, 10.75%, and 20.81%, respectively). In accordance with the present findings, no *M. margaritifera* longer than 118 mm have been reported anywhere in the Iberian Peninsula (Locard 1899; Azpeitia Moros 1933; Nobre 1941; Alvarez Claudio et al. 2000; Ziuganov et al. 2000; Velasco et al. 2002).

Extraordinary phenotypic plasticity in growth rate (as computed with VBGF) has been reported for other naiads. While in *Elliptio complanata*, k is limited to between 0.15 and 0.16 (Kesler and Downing 1997), in *Lampsilis radiata* it ranges from 0.08 to 0.21 (Day 1984; Bailey and Green 1988), and in *Unio crassus* it may be as low as 0.1 or as high as 0.30 (Hochwald 2001). The strong negative correlation between k and latitude for *M. margaritifera* across Eu-

Fig. 5. Relationships between maximum length (L_{\max}), maximum age (A_{\max}), and asymptotic length predicted by the growth models for Iberian *M. margaritifera* populations ordered, from left to right, by increasing A_{\max} .



rope has been attributed to various physicochemical factors but mainly to water temperature, because growth rate and temperature are related to metabolism (Bauer 1992). This observation is supported by independent studies on the same species (Beasley and Roberts 1999; Hastie et al. 2000), although it has also been criticized (Ziuganov et al. 2000). Data from 25 Bavarian populations of *U. crassus* also suggest a relevant dependency of k on water temperature in brooks (Hochwald 2001). Likewise, growth increments of *M. margaritifera* in a largely undisturbed river in southern Sweden appear to be correlated with summer air temperature (Mutvei and Westermarck 2001). Nevertheless, it is difficult to explain the large and significant differences among Spanish populations on the basis of water temperature alone, because our study area is fairly small and apparently has a rather uniform climate (at present there are no rigorous data on water temperature in the drainages studied).

To explain the variation in k values, genetic differences among individuals or populations and physicochemical factors must also be considered. We are not aware of any estimate of heritability (a measure of genetic variability in quantitative characters) of morphological, physiological, or life-history traits for any unionoid. Indeed, field data on heritability are very difficult and sometimes impossible to obtain. Life-history traits (such as growth parameters) are quantitative traits under strong selection, and genetic variation in such traits should be expected to be low compared with traits less directly connected to fitness (Stearns 1992).

Growth curves for *M. margaritifera* such as those analyzed here are based on data from individuals living in small habitat patches. Thus, microscale environmental effects should not be expected to have a major impact on means or variances. Therefore, growth of *M. margaritifera* presumably can be more affected by environmental factors (such as water quality) than by genetic factors, at least within drainages and probably across wide regions.

The growth curves for Spanish populations show rapid growth during the first 6 years of life (taking into account the A_{\max} values, this period covers approximately 9%–17% of the life-span), followed by a consistent decrease. Such a reduction in growth rate with age indicates a change in energy allocation, presumably with gradually increasing proportions going to reproduction. During this early stage, the model that best fits the growth of adults (HGF) shows deviations, which can now be interpreted not as a shortcoming of the model, but as a useful indication of a biological process, a different mode of growth in juvenile *M. margaritifera*. Indeed, the increase in length of juvenile *M. margaritifera* follows a rather linear trajectory (Wächtler et al. 2001). It can be hypothesized, therefore, that for 6 years Spanish *M. margaritifera* remain deeply buried in the river bottom, their growth following a function distinct from that of adults. The absence of live individuals younger than 6 years in all samples studied is likely due to their infaunal habitat requiring a special (i.e., certain type of) collecting effort. The scarcity of empty shells belonging to these earliest age classes

can be explained by the corrosive effect of acidic water on such fragile calcareous structures, which likely dissolve without ever reaching the bottom.

The distinctness of Spanish populations

While comparing 48 populations of *M. margaritifera* across Europe, Bauer (1992) found significant relationships among several growth parameters, including a strong negative relationship between k and A_{\max} . This holds true for some populations located in small areas or uniform regions. Thus, we computed the coefficient of Spearman's rank correlation for data from Irish (Beasley and Roberts 1999), Scottish (Hastie et al. 2000), and Russian (Ziuganov et al. 1994) populations. We found a significant negative correlation only for the Scottish populations ($r_s = -0.7275$, $p < 0.01$). As shown above, this correlation exists for Spanish populations, but here it has the opposite sign. Are there any remarkable relationships between growth parameters (principally k and A_{\max}) and reproductive output (or some fitness component) of *M. margaritifera* in the Iberian Peninsula? An affirmative answer clearly would distinguish these populations from those in the rest of Europe.

Basic data on reproductive biology, such as approximate age at maturity or production of glochidia, are missing for Iberian populations of *M. margaritifera*. Estimates of age at maturity in northern populations of this species yield values from 12 or 13 years (Young and Williams 1984) to 12–20 years (Ziuganov et al. 1994). Therefore, it is likely that Iberian populations, which reduce growth at 6 years of age, reach sexual maturity at an earlier age than northern populations. In this context, the positive correlation between k and A_{\max} found in Spanish populations further suggests that they are genetically differentiated from those inhabiting the rest of Atlantic and Arctic Europe.

Delayed senescence is a common trait of all populations of *M. margaritifera*, but the average life-span of northern populations is considerably longer than that of Iberian ones. The probability of surviving from one year to the next, a manifestation of senescence (Freeman and Herron 1998), falls steadily with age in populations in both areas, but does so more slowly in northern populations. Iberian populations exhibit the highest growth rates, together with the lowest maximum age and maximum length known for *M. margaritifera*. It has been argued (Bauer 1992) that *M. margaritifera* with a high k (>0.1), low A_{\max} , and low L_{\max} produce fewer offspring than those with a low k , high A_{\max} , and high L_{\max} . Moreover, it has long been recognized that in a variety of organisms, mortality increases with growth rate (k) but decreases with body size (Gunderson 1980; Roff 1984, 1992; Rose 1991). Thus, Iberian populations, lying at the southern edge of the species' range, appear to be close to their physiological (and reproductive) limits.

Acknowledgements

We are grateful to all the forest guards (specially Mr. Jesús Latas) for assisting with locating sampling places. This study is financially supported by the Xunta de Galicia (project No. PGIDIT02RFO26101PR).

References

- Altaba, C.R. 1996. Counting species names. *Nature* (Lond.), **380**: 488–489.
- Altaba, C. R. 2000. La última oportunidad de *Margaritifera auricularia*, nuestro bivalvo de agua dulce más amenazado. *Quercus*, **170**: 16–23.
- Altaba, C.R., López, M.A., and Monserrat, S. 2001. Giant pearl mussel's last chance. In *Die Flussperlmuschel in Europa: Bestandssituation und Schutzmaßnahmen. Ergebnisse des Kongresses, Freiburg and Hof, Germany, 16–18 October 2000*. Edited by G. Bauer. Albert-Ludwigs-Universität, Freiburg, Germany, and Wasserwirtschaftsamt Hof, Germany. pp. 224–229.
- Alvarez Claudio, C., García-Rovés, P., Ocharán, R., Cabal, J.A., Ocharán, F.J., and Alvarez, M.A. 2000. A new record of the freshwater pearl mussel *Margaritifera margaritifera* (Bivalvia, Unionida) from the River Narcea (Asturias, north-western Spain). *Aquat. Conserv. Mar. Freshw. Ecosyst.* **10**: 93–102.
- Azpeitia Moros, F. 1933. Conchas bivalvas de agua dulce de España y Portugal. *Mem. Inst. Geol. Min. Esp.* **38**(1): 1–458; **38**(2): 1–763.
- Bagenal, T. 1978. *Methods for assessment of fish productions in fresh waters*. Blackwell Scientific Publications, Oxford.
- Bailey, R.C., and Green, R.H. 1988. Within-basin variation in the shell morphology and growth rate of a freshwater mussel. *Can. J. Zool.* **66**: 1074–1708.
- Bauer, G. 1983. Age structure, age specific mortality rates and population trend of the freshwater pearl mussel in north Bavaria. *Arch. Hydrobiol.* **98**: 523–532.
- Bauer, G. 1986. The status of the freshwater pearl mussel in the south of its European range. *Biol. Conserv.* **38**: 1–9.
- Bauer, G. 1988. Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in Central Europe. *Biol. Conserv.* **43**: 239–253.
- Bauer, G. 1992. Variation in life span and size of the freshwater pearl mussel. *J. Anim. Ecol.* **61**: 425–436.
- Bauer, G., and Wächtler, K. 2001. Environmental relationships of naiads: threats, impact on the ecosystem. Indicator function. In *Ecology and evolution of the freshwater mussels Unionida*. Edited by G. Bauer and K. Wächtler. Springer-Verlag, Berlin. pp. 311–316.
- Beasley, C.R., and Roberts, D. 1996. The current distribution and status of the freshwater pearl mussel *Margaritifera margaritifera* L. 1758 in north-west Ireland. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **6**: 169–177.
- Beasley, C.R., and Roberts, D. 1999. Towards a strategy for the conservation of the freshwater pearl mussel *Margaritifera margaritifera* in County Donegal, Ireland. *Biol. Conserv.* **89**: 275–284.
- Bertalanffy, von, L. 1938. A quantitative theory of organic growth. *Hum. Biol.* **10**: 181–213.
- Cerrato, R.M. 1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. *Can. J. Fish. Aquat. Sci.* **47**: 1416–1426.
- Chesney, H.C., Oliver, P.G., and Davis, G.M. 1993. *Margaritifera durrovensis* Phillips, 1928: taxonomic status, ecology and conservation. *J. Conchol.* **34**: 267–299.
- Clarke, A.H. 1981. *The freshwater molluscs of Canada*. National Museum of Natural Sciences, Ottawa, Ont.
- Comfort, A. 1957. The duration of life in molluscs. *Proc. Malacol. Soc. Lond.* **32**: 219–241.
- Day, M. 1984. The shell as a recording device: growth record and shell ultrastructure of *Lampsilis radiata radiata* (Pelecypoda: Unionidae). *Can. J. Zool.* **62**: 2495–2504.

- Freeman, J., and Herron, J.C. 1998. Evolutionary analysis. Prentice Hall, New York.
- Gallucci, V.F., and Quinn, T.J. 1979. Reparameterizing, fitting, and testing a simple growth model. *Trans. Am. Fish. Soc.* **108**: 14–25.
- Goong, S.A., and Chew, K.K. 2001. Growth of butter clams, *Saxidomus giganteus* Deshayes, on selected beaches in the state of Washington. *J. Shellfish Res.* **20**: 143–147.
- Gunderson, D.R. 1980. Using r - k selection theory to predict natural mortality. *Can. J. Fish. Aquat. Sci.* **37**: 2266–2271.
- Haas, F. 1969. Superfamilia: Unionacea. *Tierreich*, **88**: 1–663.
- Hastie, C.C., Young, M.R., and Boom, P.J. 2000. Growth characteristics of freshwater pearl mussels, *Margaritifera margaritifera* (L.). *Freshw. Biol.* **43**: 243–256.
- Hendelberg, J. 1961. The freshwater pearl mussel, *Margaritifera margaritifera*, L.: on the localization, age, and growth of the individual and on the composition of the population according to an investigation in Pärälven in Arctic Sweden. *Rep. Inst. Freshw. Res. Drottningholm*, **41**: 149–171.
- Hochwald, S. 2001. Plasticity of life-history traits in *Unio crassus*. In *Ecology and evolution of the freshwater mussels Unionida*. Edited by G. Bauer and K. Wächtler. Springer-Verlag, Berlin. pp. 127–142.
- International Union for the Conservation of Nature and Natural Resources. 1990. Invertebrate Red List. International Union for the Conservation of Nature and Natural Resources, Gland, Switzerland.
- Kesler, D.H., and Downing, J.A. 1997. Internal shell annuli yield inaccurate growth estimates in the freshwater mussels *Elliptio complanata* and *Lampsilis radiata*. *Freshw. Biol.* **37**: 325–332.
- Locard, A. 1899. Conchyliologie portugaise : coquilles terrestres, des eaux douces et saumâtres. *Arch. Mus. Hist. Nat. Lyon*, **7**: 1–303.
- Motulsky, H. 1996. The graphpad guide to nonlinear regression. Available at <http://www.graphpad.com> [accessed 10 March 2003].
- Mutvei, H., and Westermark, T. 2001. How environmental information can be obtained from naiad shells. In *Ecology and evolution of the freshwater mussels Unionida*. Edited by G. Bauer and K. Wächtler. Springer-Verlag, Berlin. pp. 367–379.
- Mutvei, H., Westmark, T., Dunca, E., Carell, B., Forberg, S., and Bignert, A. 1994. Methods for the study of environmental changes using the structural and chemical information in molluscan shells. *Bull. Inst. Oceanogr. Monaco*, **13**: 163–168.
- Nobre, A. 1941. Fauna malacologica de Portugal. II. Moluscos terrestres e fluviais. *Mem. Estud. Mus. Zool. Univ. Coimbra*, **124**: 1–277.
- Primack, R.B. 1995. A primer of conservation biology. Sinauer Associates Inc., Sunderland, Mass.
- Primack, R. 1998. Essentials of conservation biology. 2nd ed. Sinauer Associates Inc., Sunderland, Mass.
- Reis, J. 2003. The freshwater pearl mussel [*Margaritifera margaritifera* (L.)] (Bivalvia, Unionida) rediscovered in Portugal and threats to its survival. *Biol. Conserv.* **114**: 447–452.
- Ricker, W.E. 1979. Growth rates and models. In *Fish physiology*. Vol. VIII. Bioenergetics and growth. Edited by W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 677–743.
- Roff, D.A. 1984. The evolution of life histories parameters in teleosts. *Can. J. Fish. Aquat. Sci.* **41**: 989–1000.
- Roff, D.A. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.
- Rose, M.R. 1991. Evolutionary biology of aging. Oxford University Press, New York.
- SAS Institute Inc. 1995. JMP Statistics and graphics guide. Version 3.1. SAS Institute Inc., Cary, N.C.
- Stearns, S.C. 1992. The evolution of life histories. Oxford University Press, New York.
- Tevesz, M.J.S., and Carter, J.G. 1980. Environmental relationships of shell form and structure in unionacean bivalves. In *Skeletal growth of aquatic organisms*. Edited by D.C. Rhoads and R.A. Lutz. Plenum Press, New York. pp. 295–322.
- Velasco, J.C., Araujo, R., Bueno, R., and Laguna, A. 2002. Discovered the southernmost known European of the freshwater pearl mussel *Margaritifera margaritifera*, L. (Bivalvia, Unionida) in the Iberian Peninsula (Agueda River, Salamanca). *Iberus*, **20**: 94–108.
- Wächtler, K., Dreher-Mansur, M.C., and Ritcher, T. 2001. Larval types and early postlarval biology in naiads (Unionida). In *Ecology and evolution of the freshwater mussels Unionida*. Edited by G. Bauer and K. Wächtler. Springer-Verlag, Berlin. pp. 93–126.
- Watters, G.T. 1994. Form and function of unionoidean shell sculpture and shape (Bivalvia). *Am. Malacol. Bull.* **11**: 1–20.
- Young, M.R., and Williams, J.C. 1983. The status and conservation of the freshwater pearl mussel in Great Britain. *Biol. Conserv.* **25**: 35–52.
- Young, M.R., and Williams, J.C. 1984. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland. I. Field studies. *Arch. Hydrobiol.* **99**: 405–422.
- Young, M.R., Cosgrove, P.J., and Hastie, L.C. 2001. The extent of, and causes for, the decline of a highly threatened naiad: *Margaritifera margaritifera*. In *Ecology and evolution of the freshwater mussels Unionida*. Edited by G. Bauer and K. Wächtler. Springer-Verlag, Berlin. pp. 337–358.
- Ziuganov, V., Zotin, A., Nezhlin, L., and Tretiakov, V. 1994. The freshwater pearl mussels and their relationships with salmonid fish. VNIRO Publishing House, Moscow.
- Ziuganov, V., Beletsky, V.V., Neves, R.J., Tretiakov, V.A., Mikhno, I.V., and Kaliuzhin, S.M. 1998. The recreational fishery for Atlantic salmon and the ecology of salmon and pearl mussels in the Varzuga River, northwest Russia. Virginia Polytechnical Institute and State University, Blacksburg.
- Ziuganov, V., San Miguel, E., Neves, R.J., Longa, A., Fernández, C., Amaro, R., Beletsky, V., Popkovitch, E., Kaliuzhin, S., and Johnson, T. 2000. Life span variation of the freshwater pearlshell: a model species for testing longevity mechanisms in animals. *Ambio*, **29**: 102–105.