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Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population

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Age and growth of an Iberian cyprinodont, *Aphanius iberus* (Cuv. & Val.), in its most northerly population, La Rubina lagoon (Alt Empordà wetlands, NE Spain), was studied. The age groups for this Mediterranean population (0+, 1+ and 2+) agree with those reported for the Atlantic population (R. Guadalquivir marshes), in the southern limit of the species distribution in the Iberian peninsula. Differences in age structure between these populations and the one previously studied in La Tancada lagoon (R. Ebro delta marshes) can be attributed to age estimation bias. Male *A. iberus* data appear to fit similarly to a Gompertz growth function and to a von Bertalanffy growth function, but L_∞ was clearly underestimated. A Gompertz growth function was not a good fit for females, perhaps because female growth is clearly faster and less attenuated than male growth. The life history and growth pattern of this species is similar to that in other cyprinodontiform fishes, typified by a short longevity. The growth model of this species does not seem to fit types I and II of Sebens's classification, which are characteristic of fish.

Key words: *Aphanius iberus*; age; growth; coastal lagoon; salt marshes; Spain; Iberian peninsula.

I. INTRODUCTION

Aphanius iberus (Cuvier & Valenciennes) is a brackish water cyprinodont often considered endemic to the Iberian peninsula (Fernández-Delgado *et al.*, 1988; Moreno-Amich, 1989), but there are uncertain records of its occurrence in Morocco and Algeria (see references in García-Berthou & Moreno-Amich, 1991).

The life history of *A. iberus* has been studied for only two populations: a Mediterranean one (40°40′ N 0°44′ E) in the R. Ebro delta marshes (A. Sostoa, unpublished Ph.D. thesis, University of Barcelona; F. J. Sostoa, unpublished M.Sc. thesis, University of Barcelona), and an Atlantic one (37° N 6°25′ W) in the R. Guadalquivir marshes (Fernández-Delgado *et al.*, 1988). These two populations showed very different patterns in age and growth. For the Ebro population females aged up to 7+ and males 5+ were reported, while for the Atlantic population, Fernández-Delgado *et al.* (1988) reported just up to 2+, both for males and females.

The present study examines age and growth of another Mediterranean population, in the Alt Empordà wetlands, which is the most northerly population (42°16′ N 3°9′ E) of this species (García-Berthou & Moreno-Amich, 1991).

A. iberus is an endangered species (ICONA, 1986), and is a protected species under the Catalan and Valencian laws. We hope that this study will assist in its management.

II. MATERIALS AND METHODS

From February 1989 to January 1990, 615 specimens were captured with dip nets of 9·0 mm stretched mesh, at the southern small coastal lagoon in La Rubina salt marshes, Alt

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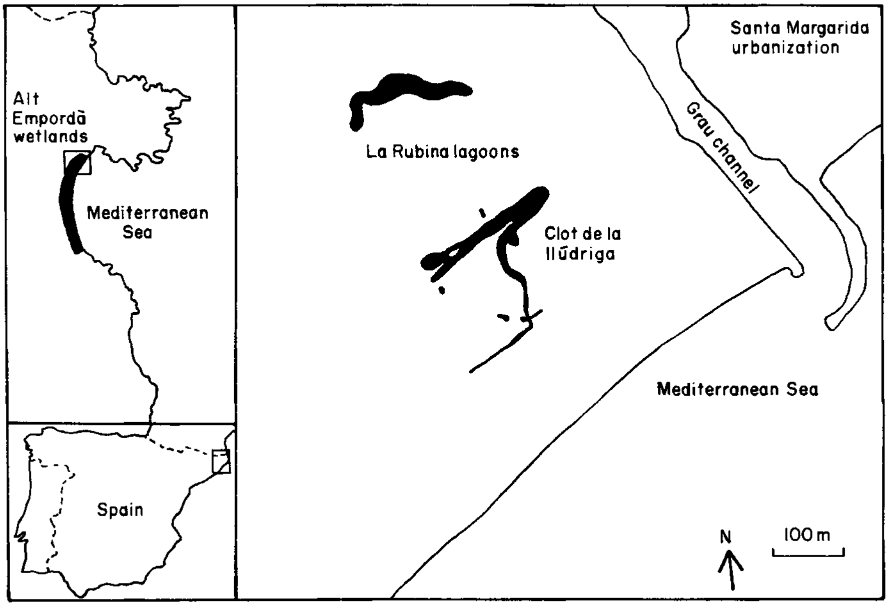

FIG. 1. Location of the study area on the La Rubina salt marshes, Alt Empordà wetlands, NE Spain.

Empordà wetlands (Fig. 1). The specimens were preserved in ice and transported to the laboratory where they were frozen. These specimens were later measured (S.L.) to the nearest 0·1 mm, weighed to the nearest 0·1 mg, and dissected. Sex was determined by external characters, and confirmed when necessary, by gonad examination under a stereomicroscope. Some individuals (total 105) from the largest samples were released after determining sex and size in the field.

Six to eight scales from the left side of the body between the lateral line and dorsal fin were removed and mounted dry between two slides for age estimation by stereomicroscope study. According to the reproduction pattern (E. García-Berthou, unpublished data) we used 1 May as the date of birth. Age validation (Beamish & McFarlane, 1983; Casselman, 1983) was attempted using two length–frequency based methods, available in the software Compleat ELEFAN (Gayanilo *et al.*, 1988). First, size groups presumed to represent age classes in the length–frequency larger samples were identified by the method of Bhattacharya (1967), and this was followed by the application of modal class progression analysis, obtaining an index which must be greater than 2 to provide meaningful separations (Gayanilo *et al.*, 1988). The second validation method was to estimate the asymptotic standard length (L_∞) by the regression model of Wetherall *et al.* (1987) modified by Pauly (1986), and then to fit the von Bertalanffy growth function to the length–frequency data (Pauly & David, 1981). Length–frequency data are restructured, by moving averages, in an attempt to identify the peaks corresponding to cohorts independently of the height or shape of the peaks themselves. The best combination of growth parameters is then automatically searched for according to a special goodness of fit index (Gayanilo *et al.*, 1988).

To transform total length (T.L., mm) data from the literature to standard length (S.L., mm), we used the following relationship calculated for the La Rubina population by least-squares linear regression:

$$\text{S.L.} = -0{\cdot}7257 + 0{\cdot}8118\,\text{T.L.} \quad r = 0{\cdot}955 \quad n = 33.$$

To describe the growth pattern, we used the von Bertalanffy and Gompertz models (Ricker, 1975; Dickie, 1978). The form of von Bertalanffy growth function was:

$$L_t = L_\infty(1 - \exp(-k(t - t_0)))$$

where L_t is standard length (mm), t is age (years), L_∞ is the asymptotic standard length (mm), t_0 is the origin of the growth curve and k is sometimes considered a stress factor (Moreau, 1987).

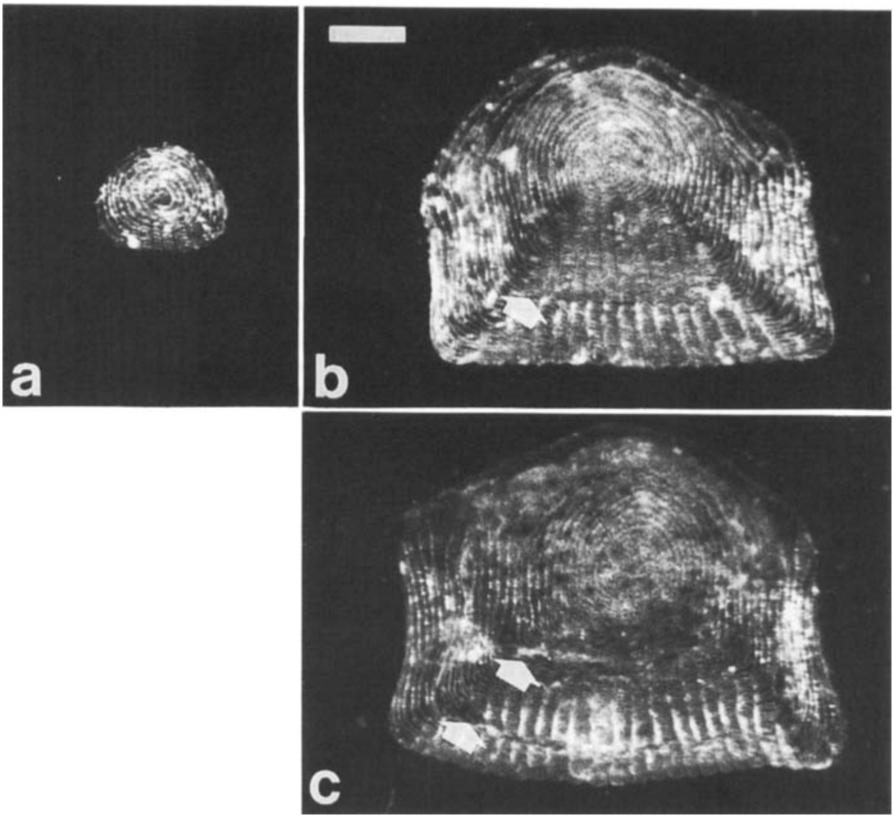

FIG. 2. Stereomicroscope photographs of scales of *Aphanius iberus* individuals captured 30 June 1990 in the southern La Rubina lagoon. White arrows show the annuli. Scale bar is equal to 0·41 mm. (a) 12·2 mm S.L. immature aged 0+; (b) 30·9 mm S.L. female aged 1+; (c) 38·2 mm S.L. male aged 2+.

The Gompertz growth function form used was:

$$W_t = W_0 \exp(G(1 - \exp(-gt)))$$

where W_t is weight (g), t is age (years), W_0 is weight at the conventional time t_0, G is the instantaneous growth rate at time t_0, and g describes the rate of decrease of G.

According to Vaughan & Kanciruk (1983), the traditional method to fit the von Bertalanffy growth function, i.e. Walford plot and least squares linear regression, gives biased estimates. These authors and Galluci & Quinn (1979) recommend using non-linear techniques to fit the von Bertalanffy growth function. For this reason, to fit both models we have used a non-linear least-squares regression with Marquardt's algorithm (Marquardt, 1963) as implemented in the program FISHPARM of the package Fisheries Science Applications System (Saila *et al.*, 1988). Models have been estimated for each sex using samples with all the age groups.

III. RESULTS

We have found three age groups: 0+, 1+ and 2+. The annulus is detected by a check on the oral side of the scale, and, as reported by Fernández-Delgado *et al.* (1988), by several circuli which cut across (cutting-over) several others on the rest of the scale (Fig. 2).

TABLE I. Decomposition of length–frequency data (S.L., mm) of the La Rubina population of *Aphanius iberus* using the method of Bhattacharya (1967) followed by the modal class progression analysis (Gayanilo *et al.*, 1988), the separation index must be greater than 2 to provide meaningfully separated groups

Sample	Group	Standard length (mm)		*n*	Separation index	Estimated age
		Mean	Standard deviation			
Female winter	1	23·31	2·08	84·63	—	0+
	2	31·00	2·69	6·37	3·23	1+
Female 29 August 1989	1	16·00	2·54	120·52	—	0+
	2	21·61	1·51	21·69	2·77	1+
Male 30 June 1989	1	22·50	1·57	19·01	—	1+
	2	30·72	1·28	17·99	5·77	2+
Male 29 August 1989	1	16·12	1·47	136·11	—	0+
	2	21·07	0·92	9·89	4·32	1+

The Bhattacharya method followed by the modal class progression analysis was useful to validate age (Table I), when using length–frequency (Figs 3 and 4) largest samples and winter (slow growth period) female pooled length–frequency data.

Age validation with the fitting of the von Bertalanffy growth function was not successful. The results disagreed with the age estimation and with the other validation method, showing four age groups. However, the asymptotic standard length estimates (33·5 mm for males, and 44·5 mm for females) were likely with maximum observed standard lengths (34·0 mm for males and 42·0 mm for females), if we consider that according to Taylor (1962) maximum observed length is approximately 95% of the asymptotic length. MacDonald (1987) pointed out that to validate age with this method we must have at least three well defined components in the length–frequency distributions, because the von Bertalanffy growth function has three parameters to be estimated. We had very few samples with three components, because we seldom captured 0+ and 2+ specimens at the same time. Moreover the components are not well defined, because *A. iberus* is a multiple spawner (Fernández-Delgado *et al.*, 1988; E. García-Berthou, unpublished data) with a long recruitment season which increases overlap between adjacent length–frequency distribution (Pauly, 1987).

The estimations of the von Bertalanffy growth function are shown in Table II. The asymptotic standard length estimates are likely for females and clearly underestimated for males, if we consider the aforementioned Taylor's relation with maximum observed standard lengths. As for the Gompertz growth function estimates (Table II), large asymptotic standard errors and a small adjusted coefficient of determination show that we cannot fit this function well to data from females.

IV. DISCUSSION

The age groups found for this Mediterranean population agree with those of the Atlantic population in the R. Guadalquivir marshes, studied by Fernández-

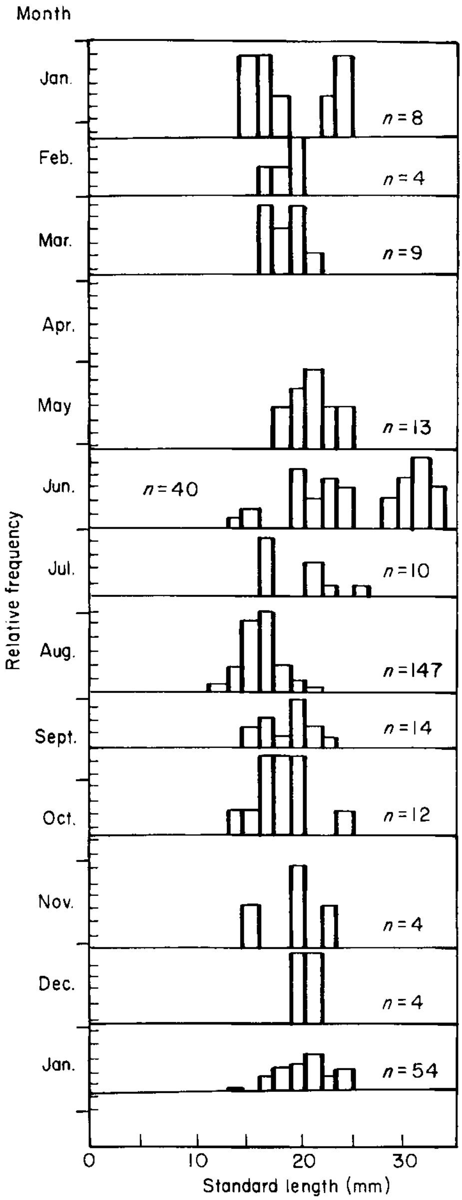

FIG. 3

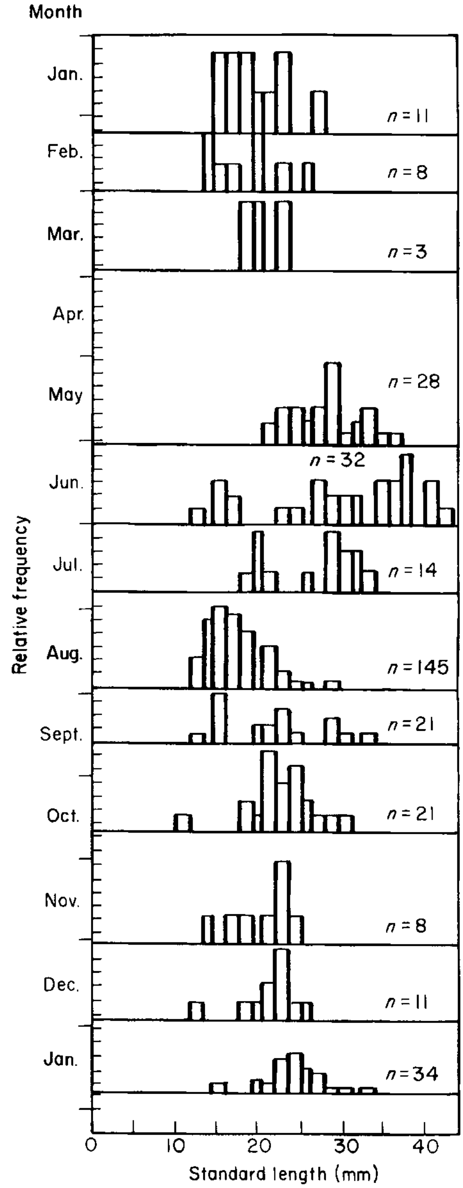

FIG. 4

FIG. 3. Length–frequency data of male *Aphanius iberus* of the La Rubina population. Bars are proportional to relative frequency.

FIG. 4. Length–frequency data of female *Aphanius iberus* of the La Rubina population. Bars are proportional to relative frequency.

Delgado *et al.* (1988). The age structure of these two populations is also very similar (E. García-Berthou, unpublished data). On the other hand, these results differ from those of the other Mediterranean population, in the Ebro delta marshes, where females were aged up to 7+ and males up to 5+.

These differences in age structure can be attributed to age estimation bias in the works on the Ebro population. The perfect agreement between the study of

TABLE II. Estimations (asymptotic standard error in parentheses) of the parameters of the von Bertalanffy (VBGF) and the Gompertz (GGF) growth functions for the La Rubina population of *Aphanius iberus*

		Males	Females
n		31	30
VBGF	L_∞	26·89 (10·49)	44·45 (21·52)
	k	0·626 (0·972)	0·419 (0·484)
	t_0	−1·622 (1·468)	−1·304 (0·740)
	R^2	0·458	0·584
GGF	W_0	0·151 (0·003)	0·303 (0·110)
	G	1·232 (0·575)	0·922 (0·428)
	g	0·934 (0·789)	1·000 (815·5)
	R^2	0·431	0·248

R^2 = adjusted coefficient of determination according to equation (12) in Kvalseth (1985).

Fernández-Delgado *et al.* (1988) and the present work (populations in the known geographical limits of the species), the validation achieved in our work, and the method used for age estimation (according to Casselman, 1983, scales are *a priori* a reliable method for aging fast-growing and short-lived fishes, such as cyprinodonts) support this hypothesis. Furthermore, we have checked some small samples from the Ebro delta (including La Tancada lagoon), and have not found individuals aged more than 2+.

Relatively short longevity is characteristic of cyprinodontiforms (Nikolsky, 1963). Several cyprinodontiforms are short-lived especially small species that live in temporary waters and die after spawning, and have eggs that can withstand living in the mud (Margalef, 1983). Some African cyprinodontiforms and some American *Cyprinodon* are annual, with most individuals reproducing in a single season (Ewulonu *et al.*, 1985; Kodric-Brown, 1988*a*,*b*). The cyprinodontiform fishes *Cyprinodon macularius eremus* Miller & Fuiman (Cyprinodontidae) and *Fundulus heteroclitus* (L.) (Fundulidae according to Parenti, 1981) are up to 3 or 4 years old (Kneib & Stiven, 1978; Samaritan & Schmidt, 1982; Miller & Fuiman, 1987).

As for growth models, our male data seem to fit similarly to a Gompertz growth function and to a von Bertalanffy growth function, but L_∞ was clearly underestimated. This could agree with Moreau (1987) who pointed out that the Gompertz function describes especially well, among some other cases, short-lived fishes of tropical flood plains. According to Ricker (1975) and Moreau (1987), the Gompertz function usually describes weight at age data well, often including the early years of increasing increments. Kneib & Stiven (1978) found a better growth description of *Fundulus heteroclitus* by means of a logistic function than with a von Bertalanffy one. We have not fitted a logistic function, because it is not usually applied to individual growth (Moreau, 1987), but the Gompertz function and logistic function are similar, as both are sigmoidal in contrast to the von Bertalanffy function.

On the other hand we cannot fit a Gompertz growth function well to females. This could be due to a different growth pattern, as female growth is clearly faster and less attenuated (Figs 3 and 4). A more general approach like the one of Schnute (1981) could help in the more reliable choice of the appropriate growth model.

Function estimates and original data (Figs 3 and 4) show an attenuating growth rate, but there is not a clear asymptote, especially for females. Thus the growth model of this species, although determinate (since a strong genetic component characteristic of vertebrates must exist), does not seem to be of types I and II of Sebens (1987), which are characteristic of fish. This special growth pattern could be related to the short longevity of this species.

Growth rate of fish is often related to water temperature (and therefore to latitude): higher rates correspond to higher mean temperatures and to less variable temperatures (Elliott, 1975, 1989; Edwards *et al.*, 1979; Casselman, 1983; Zalewski *et al.*, 1985; Lobón-Cerviá *et al.*, 1986). Samaritan & Schmidt (1982) reported latitudinal growth differences for the cyprinodontiform *Fundulus heteroclitus*. We cannot compare likely growth of *A. iberus* populations because of the age bias in the Ebro population study, and the lack of growth models in Fernández-Delgado *et al.* (1988). However, the observed maximum standard lengths (43·9 mm for the Guadalquivir population, 37·7 mm for the Ebro population, and 41·2 mm for our population) agree with that general theory (Guadalquivir water temperature 10–28°C, Ebro delta 3–28°C and La Rubina 7–26°C).

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References

Beamish, R. J. & McFarlane, G. A. (1983). Validation of age determination estimates: the forgotten requirement. *NOAA Technical Report NMFS* **8,** 29–33.

Bhattacharya, C. G. (1967). A simple method of resolution of a distribution into Gaussian components. *Biometrics* **23,** 115–135.

Casselman, J. M. (1983). Age and growth assessment of fish from their calcified structures-techniques and tools. *NOAA Technical Report NMFS* **8,** 1–17.

Dickie, L. M. (1978). Addendum: Mathematical models of growth. *Methods of Assessment of Fish Production in Fresh Waters* (Bagenal, T., ed.), pp. 130–136. Oxford: Blackwell Scientific Publications.

Edwards, R. W., Densem, J. W. & Russell, P. A. (1979). An assessment of the importance of temperature as a factor controlling the growth rate of brown trout in streams. *Journal of Animal Ecology* **48,** 501–507.

Elliott, J. M. (1975). The growth rate of brown trout (*Salmo trutta* L.) fed on maximum rations. *Journal of Animal Ecology* **44,** 805–821.

Elliott, J. M. (1989). The natural regulation of numbers and growth in contrasting populations of brown trout, *Salmo trutta*, in two Lake District streams. *Freshwater Biology* **21,** 7–19.

Ewulonu, U. K., Haas, R. & Turner, B. J. (1985). A multiple sex chromosome system in the annual killifish, *Nothobranchius guentheri*. *Copeia* **1985**, 503–508.

Fernández-Delgado, C., Hernando, J. A., Herrera, M. & Bellido, M. (1988). Age, growth and reproduction of *Aphanius iberus* (Cuv. & Val., 1846) in the lower reaches of the Guadalquivir river (south-west Spain). *Freshwater Biology* **20**, 227–234.

Galluci, V. F. & Quinn, T. J. (1979). Reparameterizing, fitting and testing a simple growth model. *Transactions of the American Fisheries Society* **108**, 14–25.

García-Berthou, E. & Moreno-Amich, R. (1991). New records of *Aphanius iberus* (Pisces: Cyprinodontidae) and review of the geographical distribution of Cyprinodontiform fishes in Catalonia (NE-Spain). *Scientia Gerundensis* **17**, 69–76.

Gayanilo Jr., F. C., Soriano, M. & Pauly, D. (1988). *A Draft Guide to the Complete ELEFAN*. Manila: International Center for Living Aquatic Resources Management.

ICONA (ed.) (1986). *Lista Roja de los Vertebrados de España*. Madrid: Publ. Ministerio de Agricultura Pesca y Alimentación.

Kneib, R. T. & Stiven, A. E. (1978). Growth, reproduction, and feeding of *Fundulus heteroclitus* (L.) on a North Carolina salt marsh. *Journal of Experimental Marine Biology and Ecology* **31**, 121–140.

Kodric-Brown A. (1988*a*). Effects of sex ratio manipulation on territoriality and spawning success of male pupfish, *Cyprinodon pecosensis*. *Animal Behaviour* **36**, 1136–1144.

Kodric-Brown, A. (1988*b*). Effect of population density, size of habitat and oviposition substrate on the breeding system of pupfish (*Cyprinodon pecosensis*). *Ethology* **77**, 28–43.

Kvalseth, T. O. (1985). Cautionary note about R^2. *American Statistician* **39**, 279–285.

Lobón-Cerviá, J., Montañés, C. & Sostoa, A. de (1986). Reproductive ecology and growth of a population of brown trout (*Salmo trutta* L.) in an aquifer-fed stream of Old Castile (Spain). *Hydrobiologia* **135**, 81–94.

MacDonald, P. D. (1987). Analysis of length–frequency distributions. *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E. eds), pp. 371–384. Ames: Iowa State University Press.

Margalef, R. (1983). *Limnología*. Barcelona: Ed. Omega.

Marquardt, D. W. (1963). An algorithm for least-squares estimation of nonlinear parameters. *Journal of the Society of Industrial and Applied Mathematics* **11**, 431–441.

Miller, R. R. & Fuiman, L. A. (1987). Description and conservation status of *Cyprinodon macularius eremus*, a new subspecies of pupfish from Organ Pipe Cactus National Monument, Arizona. *Copeia* **1987**, 593–609.

Moreau, J. (1987). Mathematical and biological expression of growth in fishes: Recent trends and further developments. *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E. eds), pp. 81–113. Ames: Iowa State University Press.

Moreno-Amich, R. (1989). Els peixos d'aigua dolça i salabrosa. *Els Aiguamolls de l'Empordà. Aspectes Ecològics, Històrics i Socials del Parc Natural* (Sargatal, J. & Fèlix, J. eds), pp. 241–250. Figueres: Ed. Art-3.

Nikolsky, G. C. (1963). *The Ecology of Fishes*. London: Academic Press.

Parenti, LL. R. (1981). A phylogenetic and biogeographic analysis of Cyprinodontiform fishes (Teleostei, Atherinomorpha). *Bulletin of the American Museum of Natural History* **168**, 335–557.

Pauly, D. (1986). On improving operation and use of the ELEFAN programs. II. Improving the estimation of L_∞. *Fishbyte* **4**, 18–20.

Pauly, D. (1987). Application of information on age and growth of fish to fishery management. *The Age and Growth of Fish* (Summerfelt, R. C. & Hall, G. E. eds), pp. 495–506. Ames: Iowa State University Press.

Pauly, D. & David, N. (1981). ELEFAN I, a Basic program for the objective extraction of growth parameters from length–frequency data with a program for pocket calculators. *International Council for the Exploration of the Sea. Demersal Fish Committee* CM 1979/G 24 (mimeo).

Ricker, W. E. (1975). Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* **191**, 1–382.

Saila, S. B., Recksiek, C. W. & Prager, M. H. (1988). BASIC fishery science programs a compendium of microcomputer programs and manual of operation. *Developments in Aquaculture and Fisheries Science* **18,** 1–230.

Samaritan, J. M. & Schmidt, R. E. (1982). Aspects of the life history of a freshwater population of the mummichog, *Fundulus heteroclitus* (Pisces: Cyprinodontidae), in the Bronx River, New York, U.S.A. *Hydrobiologia* **94,** 149–154.

Schnute, J. (1981). A versatile growth model with statistically stable parameters. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 1128–1140.

Sebens, K. P. (1987). The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics* **18,** 371–407.

Taylor, C. C. (1962). Growth equation with metabolic parameters. *Journal du Conseil International pour l'Exploration de la Mer* **27,** 270–286.

Vaughan, D. S. & Kanciruk, P. (1983). An empirical comparison of estimation procedures for the Von Bertalanffy growth equation. *Journal du Conseil International pour l'Exploration de la Mer* **40,** 211–219.

Wetherall, J. A., Polovina, J. J. & Ralston, S. (1987). Estimating growth and mortality in steady state fish stocks from length-based frequency data. In *The International Conference on the Theory and Application of Length Based Fish Stock Assessment* (Morgan, G. R. & Pauly, D. ed.), pp. 53–74. Sicilia.

Zalewski, M., Frankiewicz, P. & Brewinska, B. (1985). The factors limiting growth and survival of brown trout, *Salmo trutta* m. *fario* L. introduced to different types of streams. *Journal of Fish Biology* **27** (Supplement A), 59–73.