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# Age, diet, growth and reproduction of a Population of *Cobitis paludica* (de Buen, 1930) in the Palancar Stream (southwest of Europe, Spain) (Pisces: Cobitidae)

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## Abstract

A total sample of 99 specimens of the vulnerable fish species *Cobitis paludica* (de Buen, 1930), was collected on a monthly basis from a tributary of the River Guadalquivir between September 1990 and September 1991. Female longevity was 1 year longer than that of males, the former reaching age-class 5+. The diet of *C. paludica* consisted mainly of Ostracoda and chironomid larvae, benthic prey of small size and occasionally other resources, such as Macrobiotid eggs spawned during winter. Detritus in gastrointestinal content was scarce, which differs from other populations. Back-calculations of length were performed from scale annuli measurements. The relationship between standard length and scale radius was different for males and females. Females were larger than males of the same age. Both back-calculated length and theoretical length, calculated by applying von Bertalanffy's model, were larger for females. The relationship between female standard length (SL) and fecundity ($F$) was log $F = -7.465 + 5.5753$ log SL.

## Introduction

*Cobitis paludica* (de Buen, 1930) is an endemic Iberian species, catalogued as vulnerable (IUCN Conservation Categories, Blanco & Gonzalez, 1992). Knowledge of its biology is restricted (Lobón-Cerviá & Zabala, 1984; Soriguer 1996), in particular its feeding habits and diet (Hernando, 1978). However, there is information on the diet of the related European *C. taenia* (Maksunov, 1969; Spataru, 1970: Robotham, 1977). *C. paludica* is a bottom-dweller found in slow-flowing streams. The secretive nature of this fish is probably the main reason for this paucity of information.

In the Palancar Stream, *C. paludica* coexist with the cyprinids *Barbus sclateri* and *Leuciscus pyrenaicus*, which are characterised by their adaptability. A diet analysis is the starting point for obtaining knowledge of their trophic requirements and their possible interactions with other species (competition, predation, etc.).

This study of *C. paludica* was aimed primarily at determining the biology, diet variation with season and age, as well as growth rate, fecundity and insight into their reproductive cycle, in a small stream subject to an extreme low-water period.

## Materials and methods

### Study area

The study area was a 200 m section of the Palancar Stream, a small, second-order tributary on the left bank of the central watercourse of the Guadalquivir River. It is located in the Natural Park of 'Sierras Subbeticas', lies within co-ordinates 37° 27′ 46″ N; 4° 16′ 40″ – 37° 27′ 59″ N; 4° 15′ 40″ W.

### Field and laboratory methods

Monthly samples, from September 1990 to September 1991, were by electro-fishing, applying the same effort on each occasion. Due to the vulnerable status of this species, collections were kept to the minimum. Captured samples were frozen, and for each specimen, the following variables were determined: total length (TL), standard length (SL), total weight (TW), somatic weight (SW:corresponding to fresh weight after removal of the gastrointestinal tract and gonads), and gastrointestinal contents weight (GCW: determined as the difference between full and empty gastrointestinal weights).

The specimens were sexed on capture into three categories: male, female and juvenile. The wet weight of gonads (testis or ovary weight) was determined to the nearest 0.0001 g. Ovaries were preserved whole in Gilson's fluid after weighting (Bagenal & Braun, 1978). Scales below the first radius of the dorsal fin and above the lateral line were removed from each sample. These were cleaned using 7% NaOH, and placed on two slides, for age determination. The number of oocytes present in each gonad were counted using a system of subsampling by area. Their diameters were measured in mm using a profile projector. Oocytes were then separated into vitelline and transparent, and the mean diameter was calculated, together with the proportion of the different sizes present (Bagenal & Braun, 1978; Crim & Glebe, 1990). Absolute fecundity was estimated as the number of mature eggs present in the ovary immediately before spawning.

Gastrointestinal contents were viewed whole with a WILD$^{TM}$ M10 binocular microscope. The different taxa found were identified and the number of prey for each taxon was calculated. To carry out the analysis, the specimens were grouped by age and month of sampling. Assessment of the diet was based on frequency of occurrence of the different diet components (Guziur, 1976). The proportion of each type of prey was determined and expressed as pi = number of prey in each category (NP)/total number of prey (TNP). The ratios of standard length–total weight (SL–TW) and standard length–somatic weight (SL–SW) have been calculated for male, female and juvenile groups separately, using the expression:

$$W = a\ \mathrm{SL}^{\,b}.$$

The study of annual growth was based on back-calculations of length (Lea, 1910; Lagler, 1956; Bagenal & Tesch, 1978; Carlander, 1981). For this purpose, using the scales removed from each individual fish, the distance from the scale centre to each of the rings **n** (**Rn**) and to the edge (**R**) was measured to the nearest 0.001 mm using a profile projector. Growth in length was described using von Bertalanffy's equation of theoretical growth in length (Von Bertalanffy, 1938, 1957):

$$L_t = L\infty^*(1 - e^{-k*(t-t_0)}).$$

$L\infty$ and $k$ were estimated from Ford's equation (1993) and Walford (1946), as well as

$$t_0 = t + (1/k)^* \ln[(L\infty - L_t)/L\infty].$$

To make a comparison of growth we used the parameter

$$\omega = k * L\infty$$

(Gallucci & Quinn, 1979), and the index of overall fish growth performance (Munro & Pauli, 1983)

$$\phi' = \log k + 2 \log L\infty.$$

We separately calculated the instantaneous rates of growth in length $L_i$ from the mean back-calculated lengths, and the instantaneous rates of growth in weight $G_i$ from mean weights computed from the relationship TL–TW for males and females, using the expressions:

$$L_i = \ln L_{t+1} - \ln L_t,$$

$$G_i = \ln W_{t+1} - \ln W_t,$$

where $L_{t+1}$ and $W_{t+1}$ are mean length and mean weight, respectively, of the age-class $t$+1, and $L_t$ and $W_t$, mean length and weight of the previous age-class.

## Results

The relationship between total and standard length (TL–SL) was: TL = 0.113531 + 1.14441 SL ($r$ = 0.99; $p <$ 0.000).

Females reached age 5+ and attained 90 mm SL. The oldest males captured belonged to age-class 4+ and ranged from 55 to 85 mm SL. Females were not mature until they reached 65 mm SL.

The overall male–female ratio was 1:2, but this proportion varied with age-class (Figure 1). At age 1+, mature specimens were 23.8% of the total catch; of these, 80% were males and 20% females. At age 2+, mature specimens increased to 65.2%, of which 40% were males and 60% females. At age 3+, 100% of

*Table 1.* Coefficients, sample number and determining coefficient $R^2$, in SL-TW and SL-SW ratios, of *Cobitis paludica* from the Palancar Stream, Spain

| | | SL–TW | | | SL–EW | | |
|---|---|---|---|---|---|---|---|
| | *N* | log *a* | *B* | $R^2$ | log *a* | *b* | $R^2$ |
| Juveniles | 36 | −6.4763 | 3.8493 | 0.9779 | −6.2155 | 3.6288 | 0.9622 |
| Males | 17 | −4.619 | 2.7906 | 0.8004 | −4.9824 | 2.947 | 0.8354 |
| Females | 44 | −5.3484 | 3.1644 | 0.9586 | −5.4405 | 3.1664 | 0.9584 |
| Total population | 97 | −6.0438 | 3.5415 | 0.9793 | −5.734 | 3.3301 | 0.9691 |

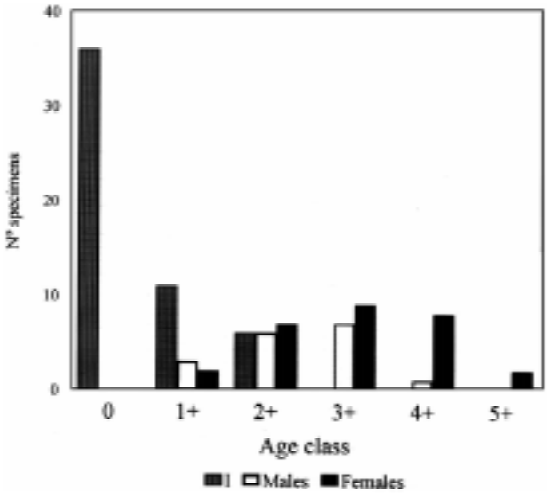

*Figure 1.* Sex ratio at each age-class of *C. paludica* in the Palancar Stream, Spain (I: Juvenile, M: Males, F: Females).

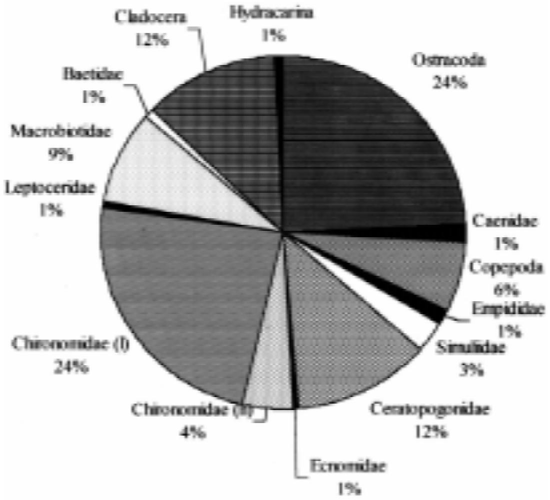

*Figure 2.* Percentage occurrence of each item in the diet of *C. paludica* from the Palancar Stream, Spain.

the captured specimens were mature, 38% males and 62% females. Males comprised only 8% of age-class 4+, and the remaining 92% were females. At age 5+, 100% are females. Thus, females became numerically more predominant with increasing age.

In respect of occurrence rates in diet, 14 taxa of invertebrates were found in the 99 gastrointestinal tracts analysed, as well as a constant low rate of diatoms and detritus. The main foods, expressed as percentage occurrence, were Diptera, chironomid and ceratopogonid larvae, Ostracoda and Cladocera, macrobiotid spawns and Hydracarina (Figure 2). A comparison of the percentage occurrence of different resources in the diet indicates that there were no significant differences ($U$ of Mann–Whitney $p > 0.05$) between months. A seasonal analysis of the numerical percentage of each type of prey (pi $*$ 100) (Figure 3) showed the amounts of Chironomidae and Ostracoda were significant, the latter predominating in autumn, while macrobiotid spawns were more frequent in winter. Figure 4 shows the percentage occurrence at different ages: Ceratopogonidae diminished with age, whereas Chironomidae and Ostracoda remained similar for all ages, except 2+; Cladocera, Copepod and Macrobiotidae became more abundant with increasing age. We frequently found chironomid nymphs in the stomachs of specimens aged 5+. Occurrence of diatoms and detritus was isolated. Vegetation remains were scarce. The $U$ of Mann–Whitney's test showed that there were significant differences ($p < 0.05$) in the diet of age-classes 0+, 1+, 2+ and 5+, whereas diet is homogeneous for age classes 3+ and 4+. In terms of the numerical proportions of each type of prey (Figure 4), those of age 0+ consumed mainly Copepods and Ceratopogonidae; those of age 1+ consumed mainly Cladocera and Ostracoda, with some chironomid larvae; those of age 2+ consumed less Cladocera and more macrobitotid spawns; those of ages 3+ and 4+ showed very similar diets, consisting mainly of Ostracoda and macrobiotid spawns; age-class 5+ was not well represented; only

*Table 2.* Relationship between SL and scale radius $R$, for males and females, of *Cobitis paludica* from Palancar Stream, Spain

| Parameter | Estimate | Standard error | $T$ Statistic | | $p$-value |
|---|---|---|---|---|---|
| **Males** | | | | | |
| Intercept | 4.14678 | 0.027185 | 190.933 | | 0.0000 |
| Slope | 0.297356 | 0.0355547 | 8.36842 | | 0.0000 |
| Analysis of Variance | | | | | |
| Source | Sum of Squares | Df | Mean Squared | $F$-ratio | $p$-value |
| Model | 0.117656 | 1 | 0.117656 | 70.03 | 0.0000 |
| Residual | 0.0268812 | 16 | 0.0016807 | | |
| Total (Corr.) | 0.144537 | 17 | | | |
| Correlation Coefficient= 0.90223 | | | | | |
| $R$-squared= 81.4019 percent | | | | | |
| **Females** | | | | | |
| Intercept | 4.34353 | 0.0172471 | 251.841 | | 0.0000 |
| Slope | 0.485973 | 0.0266336 | 18.2467 | | 0.0000 |
| Analysis of Variance | | | | | |
| Source | Sum of Squares | Df | Mean Squared | $F$-ratio | $p$-value |
| Model | 1.28301 | 1 | 1.28301 | 332.94 | 0.0000 |
| Residual | 0.146436 | 38 | 0.00385357 | | |
| Total (Corr.) | 1.42944 | 39 | | | |
| Correlation Coefficient= 0.947395 | | | | | |
| $R$-squared= 89.7558 percent | | | | | |

*Table 3.* Comparison of regression lines of *Cobitis paludica* from Palancar Stream, Spain. Dependent variable: lnSL. Independent variable. ln $R$. Level codes: sex. Number of complete cases: 58 and number of regression lines: 2

| Parameter | Estimate | Standard Error | $T$ Statistic | $p$-value |
|---|---|---|---|---|
| Constant | 4.34535 | 0.0157401 | 257.952 | 0.0000 |
| ln$R$ | 0.485973 | 0.0243064 | 19.9936 | 0.0000 |
| sex= males | −0.196749 | 0.0338949 | −5.80468 | 0.0000 |
| ln$R$*sex=males | −0.188437 | 0.054825 | −3.43706 | 0.0011 |

two specimens were caught, and analysis showed a fairly even mix of prey found in the other age classes.

Table 1 shows the growth rate variables in SL–TW and SL–SW ratios for the total population of *C. paludica*, and separately for juveniles, males and females. The **b** and **b**′ values in SL-TW and SL-SW ratios, respectively, were larger for juveniles than for mature samples, and larger for females than males.

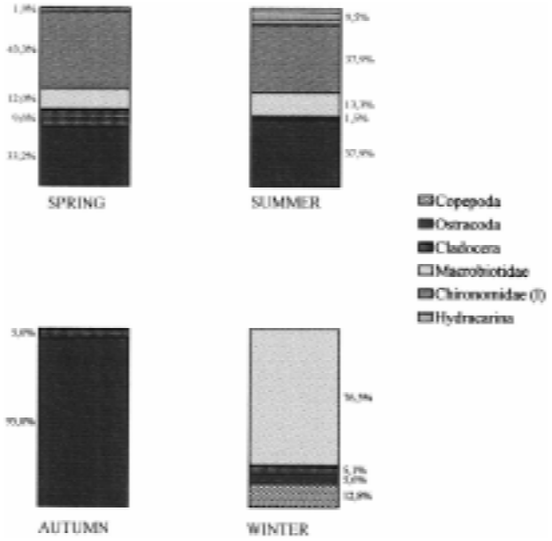

*Figure 3.* Seasonal variation in the numerical proportion of each type of prey in the diet of *C. paludica* from the Palancar Stream, Spain.

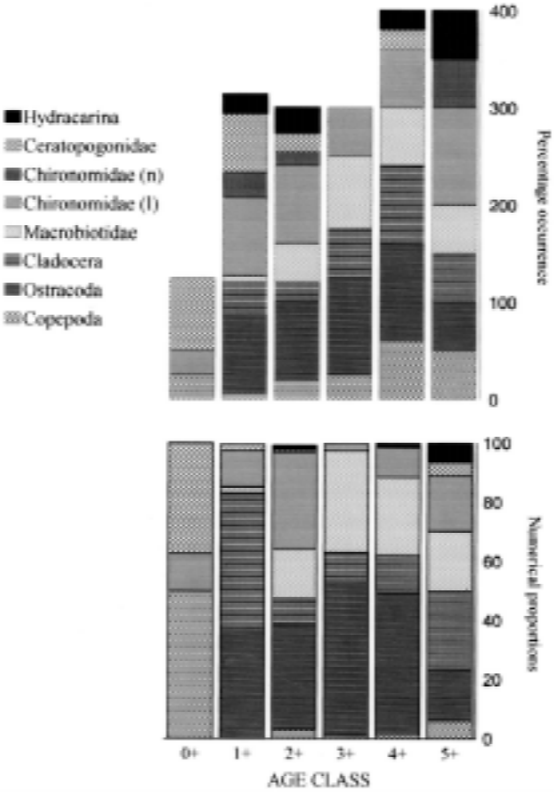

*Figure 4.* Variation with age of the percentage occurrence and numerical proportion of each item in the diet of *C. paludica* from the Palancar Stream, Spain.

The relationship between standard length and scale radius in males and females was fitted to the equations:

$$\ln SL = 4.14678 + 0.297536 * RT \text{ for males,}$$

and

$$\ln SL = 4.34353 + 0.485973 * RT \text{ for females}$$

The relationship between these variables, in both sexes, is statistically significant at the 99% confidence level (Table 2). There were significant differences in this relationship between the two sexes (Table 3), and the expressions for back-calculating the lengths are:

For females: $Ln = Ls * (Rn/R)^{0.48597}$

For males: $Ln = Ls * (Rn/R)^{0.29754}$

Table 4 show the mean standard lengths obtained for each group and age, as well as length increments between successive age-classes.

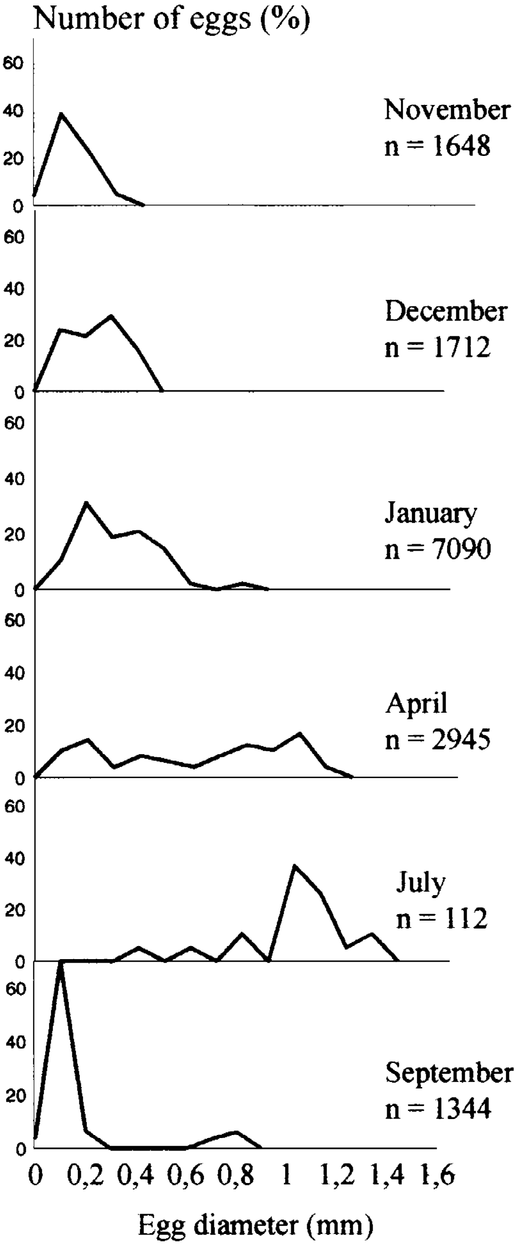

*Figure 5.* Monthly distribution of oocyte diameters of *C. paludica* from the Palancar Stream, Spain.

Back-calculated lengths from male and female scale measurements were similar only for ages 1+ and 2+ (ANOVA $p > 0.05$). However, for ages 3+ and 4+ they were significantly different (ANOVA $p < 0.05$). When the possible differences of size are considered as a function of sex and age (by MULTIFACTOR ANOVA), in age classes 1+ and 3+, significant differences of size in function both of age and sex were seen;

in age class 4+, differences were only significant in respect of sex, while in age class 2+ there were no significant differences. The length increment at intervals I, II, III and IV was greater in females than in males.

Constants in Von Bertalanffy's (1938) equation of theoretical growth in length, $k$, $L\infty$, and $t_0$, as well as variables $\omega$, $\phi'$ for each sex, are shown in Table 6. Lobón-Cerviá (1982) finds that computed infinite lengths are smaller than sizes attained by the population. In our case, this is not so. None of the captured specimens reach the calculated $L\infty$.

The equations of theoretical growth in length for this species in the Palancar Stream are as follows:

FEMALES: $L_t = 110.094^* \, (1 - e^{-0.2088665(t+0.896726)})$

MALES: $L_t = 66.83^* \, (1 - e^{-0.439856(t+0.867196)})$

Theoretical lengths of male and female (Table 6) show that, from the third year of life (2+), females are larger than males.

The instantaneous growth rates for length ($L_1$) and weight ($G_1$), of males and females separately, shown in Table 7, indicate a decrease in both growth rates with age. Also, these values are much smaller for males, especially younger males, but tend to become more similar with age.

A monthly GSI analysis could not be carried out because no females were captured in May and June. Monthly analysis of the frequency distribution of oocyte diameters (Figure 5) showed a mode in November, corresponding to translucent oocytes of approximately 0.1 mm in diameter. In winter, oocytes increased in diameter, with the proportion of vitelline eggs increasing progressively. In April, oocytes attained a suitable size for spawning and these became predominant in July, with no translucent oocytes occurring. In September, translucent oocytes accounted for the dominant mode again. There were also small numbers of intra-ovarian oocytes of size suitable for spawning, which were re-absorbed.

Absolute fecundity of females captured in April was calculated from the expression: $\log F = -7.465 + 5.5753 \log \mathrm{SL}$; $r = 0.9998$ ($p < 0.01$).

## Discussion

Given the vulnerable status of the species, the number of specimens captured is less than that desirable for a definitive study. The ratio of sexes in the population of *C. paludica* in the Palancar Stream is very different from that found in this species in the Jarama River, where it is close to parity (Lobón-Cerviá & Zabala, 1984), and in *C. taenia*, in Italy (Gambetta, 1934; Lodi, 1967; Marconato & Rasotto, 1989) and England (Robotham, 1981). As with females of the populations of *C. taenia*, the longevity of female *C. paludica* in the Palancar Stream is a year longer than that of males. By age 4+, females were clearly predominant. The reduced proportion of males as they age appears to be a result of earlier mortality, although hermaphroditism has been reported in other populations (Lodi, 1967).

Regarding their diet, *C. paludica* in the Palancar Stream consumed only a limited variety of resources. The most frequently found prey in their diet were chironomid larvae and Ostracoda, similar to the population of *C. taenia* studied by Moretti (1948) in Italy. Detritus and diatoms in gastrointestinal contents of this population was limited. However, these components predominate in the diet of this species in the marshlands of the Guadalquivir River (Hernando, 1974), and are also usually predominant in the diet of *C. taenia* (Skora, 1966; Maksunov, 1969) or only when other resources are scarce (Robotham, 1977). Prey of relatively large size, such as Ephemeroptera (Caenidae and Baetidae) and Trichoptera (Ecnomidae and Leptoceridae), were limited. Only at age 0+ are there significant differences in the diet in respect of age, although these were only differences in the consumption of isolated resources, such as macrobiotid spawns, and not in monthly variations in the diet.

Male and female growth rates were different. Parameters **b** and **b**$'$ in SL–TW and SL–SW ratios were much higher for juveniles, and males showed lower values than females. The back-calculated length of females was greater than that of males. The population growth rate in the Palancar Stream was similar to that of the population in the Jarama River (Lobón-Cerviá, 1982), being somewhat lower for females and higher for males in the Palancar population. $L\infty$ and $k$ values in Von Bertalanffy's growth equation can be used as indicators for making comparisons of growth (Misra, 1980). The value of $k$ was greater for females than for males in this population, which was not the case with the population in the Jarama River (Lobón-Cerviá & Zabala, 1984), where values for both sexes were also greater than those estimated for this population. Considering the index of overall fish growth performance, which is recommended by Moreau et al. (1986) because it has a smaller variation and can be used to compare similar taxa, once again, the growth of the

*Table 4.* Standard back-calculated lengths obtained for *Cobitis paludica* from Palancar Stream, Spain

| Age | N | L I | L II | L III | L IV | L V |
|---|---|---|---|---|---|---|
| **Males** | | | | | | |
| I+ | 3 | 36.75 +/−4.9 | | | | |
| II+ | 6 | 37.28 +/− 5.6 | 48.54+/−3.9 | | | |
| III+ | 7 | 37.49 +/−3.4 | 48.32+/−2.7 | 54.85 +/−2.4 | | |
| IV+ | 1 | 36.77 | 43.97 | 53.94 | 58.94 | |
| Mean | | 37.38+/−3.22 | 47.83+/−2.9 | 54.75+/−2.3 | 58.94 | |
| Δ | | | 10.5 | 6.92 | 4.19 | |
| **Females** | | | | | | |
| I+ | 2 | 39.40+/−6.0 | | | | |
| II+ | 6 | 31.38+/−5.8 | 46.48+/−4.7 | | | |
| III+ | 6 | 30.40+/−3.7 | 46.62+/−6.2 | 60.79+/−4.2 | | |
| IV+ | 6 | 36.36+/−4.4 | 51.31+/−6.3 | 62.40+/−5.3 | 70.32+/−3.1 | |
| V+ | 3 | 35.90+/−6.0 | 49.45+/−5.8 | 61.56+/−5.17 | 70.89+/−5.9 | 77.87+/−5 |
| Mean | | 35.90+/−6 | 49.45+/−5.8 | 61.50+/−5.2 | 70.87+/−5.2 | 77.87+/−5 |
| Δ | | | 13.6 | 12.1 | 9.4 | 7.0 |

*Table 5.* Values of constant terms in Von Bertalanffy's equation of theoretical growth in length, $k$, $L\infty$, and $t_0$, together with parameters $\omega$ and $\phi'$ for each sex, of *Cobitis paludica* from Palancar Stream, Spain

| | $K$ | $L\infty$ | $t_0$ | $\omega$ | $\phi'$ |
|---|---|---|---|---|---|
| Females | 0.208866 | 110.094 | −0.896726 | 22.99 | 3.403395 |
| Males | 0.439856 | 66.83 | −0.867196 | 29.52 | 3.2932535 |

*Table 6.* Theoretical standard lengths obtained for *C. paludica* from von Bertalanffy's model (Palancar Stream, Spain)

| | Males | Females |
|---|---|---|
| $L_0$ | 21.19 | 18.80 |
| $L_1$ | 37.43 | 36.00 |
| $L_2$ | 47.90 | 49.98 |
| $L_3$ | 54.63 | 61.61 |
| $L_4$ | 58.97 | 70.50 |
| $L_5$ | | 77.97 |

population studied was lower than the Jarama River population, for both males and females, and higher than that obtained by Slavik (1995) for *C. taenia*. As is the case of other populations studied (Robotham, 1981; Lobón-Cerviá & Zabala, 1984; Slavik, 1995), growth rates reached high values during the first years of life and low values in the later years, the decrease in growth being due to sexual maturity.

The vitelline oocyte mode of a size suitable for spawning in the month of July indicates a long reproductive period, already noted by Lobón-Cerviá & Zabala (1984) in this species, and by Marconato & Rasotto (1989) in *C. taenia*. These results cannot be taken to indicate the existence of two spawning periods, but they may indicate that spawning is sequential, starting in late April. Fecundity was similar to that described for this species in the Iberian Peninsula, with values ranging from 281–1397 oocytes for standard length interval 60–80 mm. Females of a smaller size in the Palancar Stream were not as fecund as those of the same size in the Jarama River (Lobón-Cerviá &

*Table 7.* Instantaneous rates of growth in length ($L_i$) and weight ($G_i$), for *C. paludica* males and females from Palancar Stream, Spain

| | $L_i$ | | $G_i$ | |
|---|---|---|---|---|
| Age interval | Females | Males | Females | Males |
| I–II | 0.320 | 0.238 | 0.987 | 0.481 |
| II–III | 0.218 | 0.135 | 0.814 | 0.307 |
| III–IV | 0.142 | 0.074 | 0.287 | 0.197 |
| IV–V | 0.094 | | 0.147 | |

Zabala, 1984), whereas larger females had a higher fecundity than in the Jarama River.

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