## BIOLOGY OF PROCAMBARUS CLARKII (GIRARD, 1852) IN LAKETRASIMENO

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## ABSTRACT

The aim of this study was to gather more informations about the biological characteristics of *Procambarus clarkii* and to investigate the reasons of its rapid expansion in Lake Trasimeno. Specimens of both sexes (544 males and 624 females) were collected from October 2000 to November 2001. During the sampling period physical factors such as water temperature and hydrologic level of Lake Trasimeno were recorded. The study was conducted monthly in order to assess this species' reproductive period. 1,168 specimens were analyzed for colour, sex, weight, and length. The sexual maturation of gonads was investigated in females. The results of this study evidenced that the population is in expansion, showing that Lake Trasimeno is an optimal habitat for this species.

**Key-words:** Red-swamp crayfish, *Procambarus clarkii*, growth, reproduction, Lake Trasimeno.

#### BIOLOGIE DE PROCAMBARUS CLARKII (GIRARD, 1852) DANS LE LAC TRASIMENO

## RÉSUMÉ

Le but de cette étude est d'avoir plus d'informations sur la biologie de *Procambarus clarkii* et d'enquêter sur les raisons qui sont à la base de son expansion rapide dans le Lac Trasimeno. Exemplaires des deux sexes (544 mâles et 624 femelles) ont été capturés d'octobre 2000 à novembre 2001. Pendant cette période ont été relevés les paramètres physiques comme la température de l'eau et le niveau du lac. L'étude a été menée mensuellement afin d'évaluer la période de reproduction de cette espèce. 1 168 exemplaires ont été analysés selon la couleur, le sexe, le poids, la longueur et le degré de maturité des gonades a été relevé pour les femelles. Les résultats de cette étude ont mis en évidence que la population de *Procambarus clarkii* est en expansion et il semble que le Lac Trasimeno soit un habitat optimal pour cette espèce.

Mots-clés : Procambarus clarkii, accroissement, reproduction, Lac Trasimeno.

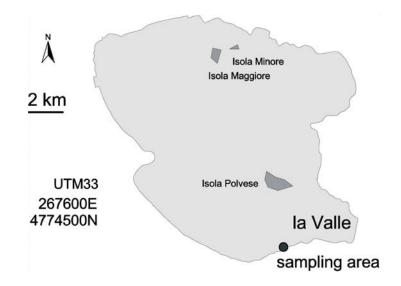
## INTRODUCTION

The red-swamp crayfish *Procambarus clarkii* (Girard, 1852) is a native species of South-central United States (Louisiana) and North-eastern Mexico (HOBBS, 1989). It is highly tolerant to unfavourable conditions (e.g. poor water quality, temperature fluctuations, low oxygen concentrations and drying) and as a consequence has an extraordinary production rate in farming (HUNER and LINQVIST, 1995). So far it has been introduced into many other countries all over the world (HENTTONEN and HUNER, 1999). The only native crayfish species in Umbria (central Italy) is *Austropotamobius pallipes italicus* (Faxon 1914), which was abundant and well distributed in the past, but in decline since the last twenty years (PETESSE *et al.*, 1990). No allochthonous crayfish has been reported in Umbria until 1999 (BARBARESI and GHERARDI, 2000), even if in the last 15 years fishermen of Lake Trasimeno recorded isolated captures of *Procambarus clarkii*, considering this species as extremely rare. Since the beginning of 2000, the red-swamp crayfish population has rapidly increased and has become object of professional fishery in the marshy zone of the lake called "La Valle", and is since then sold in the local fish markets (DÖRR *et al.*, 2001). The aim of this study was to record the morphometric and biological characteristics of *P. clarkii* population in LakeTrasimeno to investigate the factors inducing its acclimatization and rapid spread.

## MATERIALS AND METHODS

#### Study area

Lake Trasimeno, situated in the province of Perugia (Umbria, central Italy), is the largest lake of the Italian peninsula (126 km<sup>2</sup>) (Figure 1). This laminar and mesotrophic lake is characterized by an average depth of 4.7 m and a theoretical water return time of 24 years. Trasimeno is considered as pSIC and ZPS according to BIOITALY (Biotopes Inventory of Italy, Italian ratification of the UE Directives HABITAT 92/43). A Mediterranean climate, with a maximum rainfall in autumn and a minimum in summer, characterizes this biotope. The dominant aquatic vegetation includes, *Phragmites australis*, *Typha angustifolia* and *Typha latifolia*. The macrophyte community is dominated by vegetal associations like *Caricetum ripariae*, *Potamogenetum lucentis nymphacetosum*, *Hydrocharicetum*, and *Potamogenetum ceratophylletum demersi* (ORSOMANDO and PEDROTTI, 1985). Potential aquatic predators are pike (*Esox lucius*), largemouth bass (*Micropterus salmoides*), perch (*Perca fluviatilis*), and eel (*Anguilla anguilla*).



## Figure 1

Lake Trasimeno. Sampling area.

#### Figure 1

Lac Trasimeno. Site de l'expérience.

## Sampling methods

*P. clarkii* specimens were collected monthly from October 2000 to November 2001 by a professional fisherman within 48 hours using 2 fyke nets at a depth of about 1.5 m. Once in the laboratory, for each specimen we recorded the parameters as follows: sex, total length (TL) (from the tip of the rostrum to the rear edge of telson), carapace length (CL) (from the tip of the rostrum to the posterior margin of the cephalothorax), chelae length (ChL), measured with a digital caliper within 1 mm accuracy, and body weight (W) using an electronic balance within 0.1 g accuracy.

Body colour, according to BEINGESSER and COPP (1985) and MANCINI (1986), permitted to distinguish between red adults and greenish to mottled brown juveniles.

The moulting state (soft shell, hard shell) was determined for both sexes, while the reproductive state only for females (internal egg stages and hatched juveniles).

Water temperature ( $\pm$  0.1°C) and lake level ( $\pm$  1 cm) were registered daily from the hydrological station of the province of Perugia for the whole sampling period. Chemical parameters were recorded weekly during summer, the period of internal egg maturation, as follows: dissolved oxygen concentration (surface and bottom) was determined using an OXI 320; pH was measured with a WTW pHmeter 720 and conductivity with a WTW LF 320.

Ovarian egg colour was used as an indicator of successive growth in oocytes according to PENN (1943), and DE LA BRETONNE and AVAULT (1977): white colour indicates the ovary is at rest (immature), yellow the ovary has started maturation, orange the ovary is almost mature, and dark brown the oocytes are ready to be expulsed.

## **Statistical analysis**

To test the differences among morphometrical variables, one-way ANOVAs were used, followed by Tukey test; comparisons between sexual variables were made using t-tests. The minimum level of significance under which the null hypothesis was rejected is  $\alpha = 0.05$ . Statistical analyses were carried out with R software (R DEVELOPMENT CORE TEAM, 2004).

Length data of carapace (CL) were grouped in 0.5 cm size class increments to estimate the growth rate of each size class with the Von Bertalanffy's growth model (VON BERTALANFFY, 1938): devised by PAULY AND MORGAN (1987):

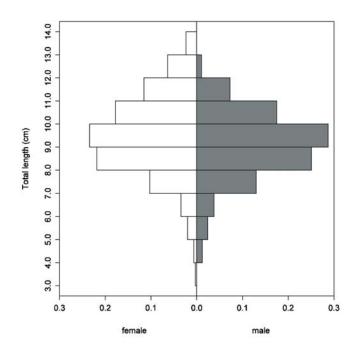
$$L_{t} = L \quad (1 - e^{-k(t-t_{0}) + Ck/2\pi \{\sin 2\pi (t-t_{s}) - \sin 2\pi (t_{0} - t_{s})\}})$$

where  $L_t$  is the length of the carapace at a given time t;  $L_{\infty}$  is the theoretical possible length of the carapace; t is the given time;  $t_0$  is the time when the organism would have a length = 0;  $t_s$  is the start of the oscillation with respect to t = 0, C is a dimensionless costant expressing the amplitude of the growth oscillations, and k is the intrinsic growth rate. For practical purposes,  $t_s$  was replaced by WP (= $t_s$  + 0.5), representing a winter point that indicates the moment in which the growth rate is the lowest within the annual cycle. The ELEFAN I mathematical procedures used for estimating Von Bertalanffy's growth parameters are included in the FiSAT II software package (GAYANILO and PAULY, 1997).

## RESULTS

A total of 1,168 *P. clarkii* were collected from October 2000 to November 2001. Descriptive statistics of 4 morphometric variables for the whole sample are reported in Table I. The mean body length was 9.32 cm and the mean individual weight was 22.2 g. The smallest specimens found in the fyke net was 3.9 cm TL (female) and weighted 1.1 g, while the longest specimen was 14.0 cm TL (female) and the heaviest weighted 76.7 g

(male). Crayfish over 7.5 cm TL represented 87% of the whole sample. Adult males had TL between 5.7 and 13 cm, but only a little fraction (7%) of males was greater than 11 cm; adult females grew even larger, ranging from 5.9 to 14 cm. Differences between TL of males and females are statistically significant (t = 5.1; p < 0.001) (Figure 2). In females chelae length varied between a minimum of 0.8 cm (1.9 cm CL) and a maximum of 5.8 cm (7.3 cm CL). Chelae of males grew larger, ranging from minimum of 1 cm (1.8 cm CL) to a maximum of 7.8 cm (7.0 cm CL) as reported in Table I. Moreover, green juvenile males and females had smaller chelae than the red adults (one-way ANOVA F = 86,5; p < 0.01) as shown in Table II.



## Figure 2

Frequency histogram of total lengths for males (grey) and females (white).

#### Figure 2

Histogramme de la fréquence des longueurs totales des mâles (gris) et femelles (blanc).

#### Table I

Descriptive statistics of the sample. TL=total length, CL = carapace length, ChL = chelae length, W = weight.

## Tableau I

Description statistique de l'échantillon. TL = longueur totale, CL = longueur de la carapace, ChL = longueur de la pince, W = poids.

	Female			Male			
	min	max	Mean ± std	min	max	mean $\pm$ std	
TL	3.9	14.0	9.6 ± 1.8	4.3	13.0	9.1 ± 1.4	
CL	1.9	7.3	$4.9 \pm 0.9$	1.8	7.0	$4.7 \pm 0.8$	
ChL	0.8	5.8	3.0 ± 1.0	1.0	7.8	3.7 ± 1.2	
W	1.1	74.6	25.8 ± 14.8	1.2	76.7	23.6 ± 12.5	

#### Table II

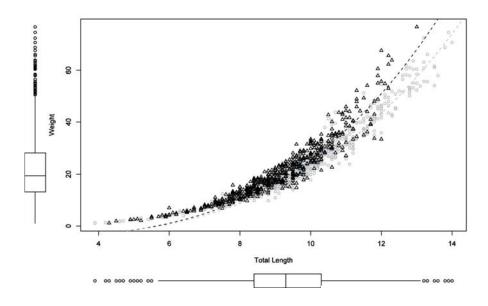
Mean  $\pm$  standard deviation of the chelae lengths in cm for each body colour gradient and both sexes. ChL = chelae length.

#### Tableau II

Moyenne  $\pm$  de la déviation standard de la longueur des pinces en cm pour chaque couleur du corps des deux sexes. ChL = longueur de la pince.

Body Colour		Green-gray Green		Dark-green	Brown-green	Red
Female	ChL	1.91 ± 0.56	1.85 ± 0.63	$2.62 \pm 0.78$	$2.65 \pm 0.75$	$3.35 \pm 0.94$
	Specimens	39	31	12	93	369
Male	ChL	2.54 ± 1.00	$2.50 \pm 0.75$	2.54 ± 1.00	3.16 ± 1.27	4.10 ± 1.01
	Specimens	68	36	12	35	356

There is a significant linear relationship ( $R^2 = 0.97$ ) between total length and carapace length, where TL = 1,83\*CL + 0.76. The length-weight relationships for females and for males were determined by regression analysis. For both sexes *b* values were greater than 3:  $b_{female} = 3.38$  ( $R^2 = 0.97$ ) and  $b_{male} = 3.54$  ( $R^2 = 0.95$ ) (Figure 3).



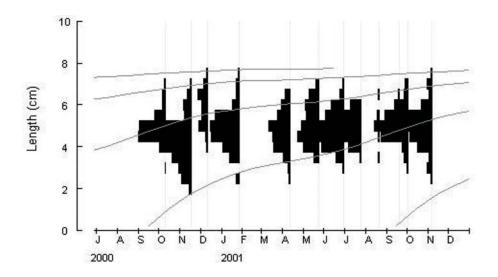
#### Figure 3

Length-weight relationship overlapping males (black) and females (light grey). Box and whiskers plots above and to the left of the axes represent the distribution of length and weight variables. Box contains the 50% of the values, whiskers show the min-max range and white circles are the outliers.

#### Figure 3

Relation longueur – poids des mâles (noir) et femelles (gris clair). Les boîtes à moustache à gauche et sous les axes représentent la distribution des variables du poids et de la longueur. La boîte contient le 50 % des valeurs, les traits l'intervalle min-max et les cercles les valeurs extrêmes.

Population growth rate (*k*), estimated using the Von Bertalanffy model, is 0.85, the mathematical asymptote of the curve ( $L_{\infty}$ ) is 8.08 cm (CL), oscillation amplitude C = 0.5 and the winter point WP = 0.25. In Figure 4 the growth curves that highlight three main size classes in Lake Trasimeno are represented. The offspring released from the females after the reproductive period of 2000 had a CL of ca. 2 cm at the end of the same year (December 2000) and grew up to over 5 cm CL until November 2001. Individuals of the second size class had a CL of ca. 5.5 cm in December 2000 and reached a CL of ca. 7 cm in November 2001. In the third size class individuals with a CL between 7 cm and 7.25 cm in December 2000 arrived at a CL close to 7.5 cm in November 2001.



#### Figure 4

Growth curve for *P. clarkii*. Histograms represent the distribution of the lengths of the crayfish sample; the grey curve the VBGF model for each size class.

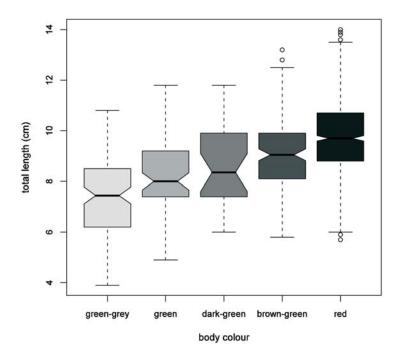
#### Figure 4

Courbe d'accroissement de *P. clarkii*. Les histogrammes représentent les longueurs de l'échantillon des écrevisses ; chaque courbe grise correspond à une classe de taille différente.

Analysis of TLs and ChLs, separated by body colour, showed significant differences (one-way ANOVA F = 81.7; p < 0.001) between red and all green classes. In particular the green gradient seems to be correlated to the growth steps of crayfish (Figure 5).

#### Moulting

With the increased water temperature in April 2001 (mean 14°C) moult started in both sexes, reaching a local maximum of 28.6% for females and 27.9% for males, but diminished in May. Only females moulted a second time (mean 27.6%) between June (23.6°C) and July (25.2°C). No moults were recorded for both sexes in August, September and October, but they started again in November 2001 (11.2°C) for females and males with over 60% for the latter (Figure 6).

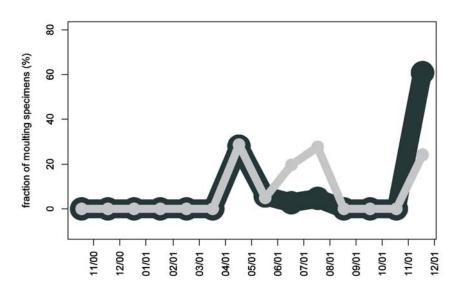


## Figure 5

Boxplots of the TL separating crayfish by body colour.

## Figure 5

Boxplots de la LT séparant les écrevisses pour la couleur du corps.



## Figure 6

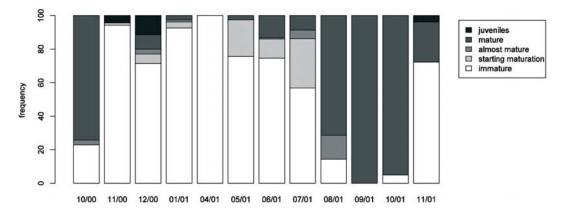
Fluctuation of moult for females (white) and males (grey).

## Figure 6

Fluctuation des mues pour les femelles (blanc) et mâles (gris).

## Reproduction

Females were classified for sexual maturity from the internal egg stages. The smallest female found with mature ovarian eggs measured 7.6 cm (TL). The smallest female that carried eggs or juveniles had a size of 9.3 cm TL (4.7 CL). Maturation of ovarian eggs, characterized by yellow colour, started in May after the first moulting period (April) and continued until July. From August to October nearly all adult females had mature oocytes (dark brown) and the expulsion was imminent. Juveniles were found in November and December 2000 and the same hatching period was confirmed for November 2001 (Figure 7).



#### Figure 7

Internal egg stages during the sampling period.

## Figure 7

## Degré de maturité des gonades pendant la période de récolte.

The period of maximum ovarian egg maturation was late summer, when water temperature ranged between 24.4 and 28.6°C, dissolved oxygen between 4.08 (41.7%) and 23.59 mg/l (185.4%), pH between 7.81 and 9.37, conductivity between 1,345.5 and 1,434.0  $\mu$ S/cm (Table III), and the mean lake level was around 50 cm under the hydrometric reference level.

#### Table III

# Summary of chemical and physical measurements of the sampling area during the summer, the period of ovarian egg maturation.

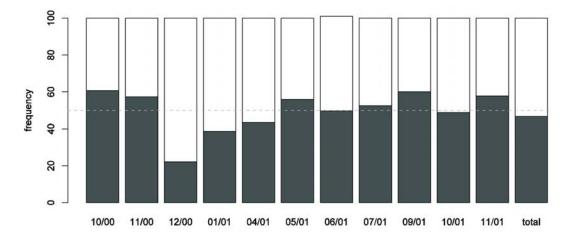
#### Tableau III

Résumé des mesures chimiques et physiques de la site de l'expérience pendant l'été, la période de la maturation des gonades.

Environmental parameters	Level	Mean	Std	Min	Max
Water Temperature (°C)	surface	26.27	1.00	24.55	28.65
Water Temperature (°C)	bottom	26.06	0.97	24.45	28.60
Dissolved Oxygen (mg/l)	surface	7.63	3.87	4.08	23.59
Dissolved Oxygen (mg/l)	bottom	4.60	3.51	0.08	13.62
Dissolved Oxygen (%)	surface	89.31	36.97	41.68	185.40
Dissolved Oxygen (%)	bottom	55.48	45.50	0.05	172.35
pH (pH unit)	column	8.46	0.53	7.81	9.37
Conductivity (µS/cm)	column	1,402.30	25.54	1,345.50	1,434.00

#### Sex ratio

Sex ratio for the whole population was 1.15:1 in favor of the females. The latter were more abundant in December 2000 and January 2001, which is the period following the hatching of the juveniles (Figure 8). In February and March 2001 no specimen was captured; in both months mean water temperatures were respectively 7.8 and 12.4°C (the coldest period in the year). These low temperatures seem to inhibit the activity of *P. clarkii* in Lake Trasimeno.



## Figure 8

Variations in the sex-ratio of females (white) and males (grey) during the sampling period.

#### Figure 8

Variation de la sex-ratio pour les femelles (blanc) et les mâles (gris) pendant la période de la récolte.

## DISCUSSION

The comparison of our results with published data indicates that P. clarkii in Lake Trasimeno grows longer than in warmer locations. No individuals of wild populations studied by PENN (1943) in Louisiana exceeded 11.6 cm TL and 47 g body weight. In Doňana National Park (SW Spain) and in Săo Miguel (Azores, Portugal), P. clarkii did not exceed 10.5 cm and 9.86 cm in total body length, respectively (BRAVO et al., 1994; COSTA et al., 1996). HUNER and ROMAIRE (1978) studied populations in stable deep waters from the Atchafalaya Basin in Louisiana and found that body size was directly influenced by habitat quality. Those stable habitat conditions, combined with low population densities, produced large crayfish, ranging from 8.4 cm TL (~ 18 g) to 13.5 cm TL (~ 84 g). Specimens collected in Lake Trasimeno had a TL equal to 14 cm with a weight over 76 g. The crayfish population was characterized by a theoretical maximum length greater than 15 cm. Therefore it appears that P. clarkii lives considerably longer here than it usually does in its original habitats where most males die at the end of their first year, and females become rarely older than two years (PENN, 1943; HUNER, 1988; BRAVO et al., 1994). The three main size classes we recorded suggest, that most animals reach the age of three years, and that the largest specimens may be up to four years old. FRUTIGER et al. (1999) found, that in an eutrophic pond in Zwitzerland (with stable habitat conditions) P. clarkii could even grew up to five years. Moreover, in Lake Trasimeno P. clarkii shows an allometric

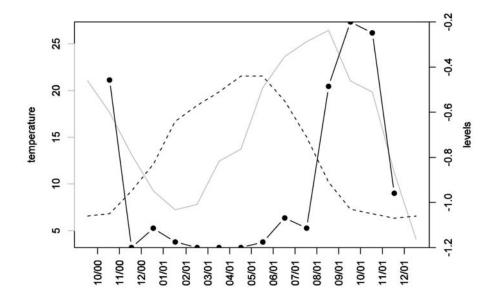
growth for weight more than for length (b > 3) in both sexes. The combination of rapid growth by green juveniles and abundance and large size of sexually mature females might explain the rapid expansion of *P. clarkii* in this shallow and mesotrophic lake.

The green colour gradient, varying from green-grey (the smallest size class) to brown-green (the largest size class), seems to be correlated to the growth steps of sexual immature cravitish. Furthermore, juvenile males and females, of all green body colour gradients, had smaller chelae than the red adults, as reported in literature (GHERARDI et al., 1999; HUNER and BARR, 1991). ACKEFORS (1999), reported that egg production can be completed within six weeks, incubation and maternal attachment within three weeks and maturation within eight weeks. Optimal temperatures are 21-27°C and growth inhibition occurs at temperatures below 12°C (ACKEFORS, 1999). In Lake Trasimeno ovarian eggs started maturation in June 2001 (15% of the mature females at a water temperature of 20°C), and increased to nearly 100% in August, September and October 2001, so that the juveniles released from the pleopods in the warmer months had better environmental conditions for feeding and growing. Furthermore the attainment of sexual maturity and reproduction of smaller adults, and the progressive release of juveniles by females, have been considered to be a strategy to assure their continued existence when predator pressure is high (HUNER and ROMAIRE, 1978; JONES, 1995). GUTIERREZ-YURRITA and MONTES (1999) found that females with a not isometric relationship of W versus TL, when weight increased more than length (exponent > 3), as in the case of the population of Lake Trasimeno, timing of juvenile release from pleopods could vary greatly, from 10 to 35 days. Females releasing their offspring within a period of one month is indicating that, by the time the last juveniles are released, the first to have been released from the pleopods should have moulted two or three times and are so advantaged for their further development and survivorship. Females thereby provide two or three cohorts of recruits to the population (HUNER and AVAULT, 1976; GUTIERREZ-YURRITA, 1997).

The moult cycle of a *P. clarkii* population in an old sewer stream in the Central Meseta of Spain was similar to that observed in Lake Trasimeno. Significant moulting activity was present in both males and females, at the start of spring and near the end of winter. Moulting activity decreased in summer although it did not cease, and a maximum was observed in winter when the water temperature was below 10°C (GUERRA and NIŇO, 1995). In Lake Trasimeno both sexes had synchronized moults. HUNER (1978) reports that the optimum water temperature for molting is around 20°C. Thus, in our case data would indicate that moulting activity is linked to the reproductive cycle rather than directly to the temperature, as observed in the Central Meseta of Spain. In fact, in Lake Trasimeno females and males moulted in the pre-spawning and post-spawning period when mean temperatures were respectively 14°C and 11.2°C.

The sex ratio for the whole sample was near a 1:1 ratio, as reported by other authors (PENN, 1943; HUNER, 1978), but varied throughout the seasons. Females were less abbondant in the months with maximum ovarian egg maturation. In October and November 2000, females accounted for 39.33% and 42.68% of the samples, respectively. In December 2000, however, their portion raised to 77.77% before decreasing again from January to April 2001, from 61.36% to 56.57%, respectively. As described by FRUTIGER *et al.* (1999) for a small eutrophic pond in Zwitzerland, the most likely explanation for this decrease of female numbers in autumn is that most of the mature females became ovigerous in this season and thus stayed in their borrows (BRAVO *et al.*, 1994; FRUTIGER *et al.*, 1999). This is likely explained by the reproductive behaviour of *P. clarkii;* low numbers of females in samples correspond with high numbers of ovigerous females, that are difficult to catch. In our study, only a few females carrying juveniles were found in November and December 2000 and in November 2001. This also suggests that spawning and reproduction takes place mainly in autumn.

Female (CL > 4.5 cm) reproduction and juvenile development and survivorship require not only a hormonal induction by the photoperiod (GUTEIRREZ-YURRITA and MONTES, 1998) but also a low hydroperiod longer than 4 months with a temperature over 18°C and a pH between 7 and 8 (GUTIERREZ-YURRITA, 1997). In the Doňana National Park water regime and water temperature were the major factors influencing spawning of P. clarkii females in four different aquatic ecosystems. In two of them, with a hydroperiod of over 6 months, Lucio del Bolin (7 months) and Lucio del Palacio (12 months), a second period of ovarian maturity, after the first one in spring, was observed in autumn (November), when the mean water temperature was 16.9°C. It was also in these ecosystems that the greatest percentage of females with the ovary at rest were observed during summer and in the months with lower temperatures (GUTIERREZ-YURRITA and MONTES, 1999). In Lake Trasimeno ovarian eggs started maturation when the water temperature was around 20°C and the lake level began to decrease. By contrast, the internal egg maturation was reduced when temperature turned under 20°C and lake level reached the lowest value (Figure 9). So the reproductive period seems to be strongly linked to water temperature and lake level. In particular, the reproductive period in Lake Trasimeno is autumn, these results suggesting that P. clarkii is well acclimated with the hydrological cycle of the lake.



#### Figure 9

Fluctuations of water temperature (light grey), lake levels (dotted line) and percent fraction of females with mature ovarian eggs (black with dots). The percent fraction ranges between 0 to 100.

#### Figure 9

Fluctuations de la température de l'eau (gris clair), des niveaux du lac (ligne en tirets) et la fraction en pourcentage des femelles avec des gonades mûres (noir avec des points). L'intervalle du pourcentage est de 0 à 100.

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