

the accuracy of the calculated parameters, and these estimates are more variable than those obtained by direct *LS*. The *NLIN* procedure also simultaneously derives all parameter estimates while other methods require a sequential determination of specific estimates. So, in *L-T* methods the undegrable fraction, the digestion rate, and the lag time are calculated in successive steps. Moreover, the inclusion of certain assumptions in the development of the *L-T* methods would result in biased estimated. The final results depend on the accuracy of the previous estimations and these often are very subjective (e.g. the choice of the end point for the estimation of the undegrable fraction)¹.

On the other hand, the *NLIN* procedure is more flexible and it can be used to fit any model, although starting values close to the final solution are needed for the method to reduce the computations required and to increase the likelihood of finding a global and accurate solution. Additionally, this method is sensitive to variation in the data set what can lead to unrealistic estimates, unless certain constraints are specified for the parameters. This occurs with *USC* in the *NLIN* methods, where if it is not assumed that $a + b = 100$, potential degradation values higher than 100 can be obtained.

An appropriate number of data points in relation to the number of parameters to be estimated would avoid this problem. In this sense, *NLIN* methods requires more cases than *L-T* methods, but less than other methods like curve peeling.

We can conclude that *NLIN* method would be the most accurate and the least biased procedure for describing rumen digestion kinetics.

The authors would be glad to supply a listing of the program to interested readers.

REFERENCES

- 1) MERTENS, D.R. (1973). Application of Theoretical Mathematical Models to Cell Wall Digestion and Forage Intake in Ruminants. Ph.D. Dissertation Thesis, Cornell University. New York.
- 2) MERTENS, D.R. & LOFTEN, J.R. (1980). The effect of starch on forage fiber digestion techniques. *J. Dairy Sci.*, 63: 1.437-1.446.
- 3) NOCEK, J.E. & ENGLISH, J.E. (1986). In situ digestion techniques: Evaluation of rate determination procedure. *J. Dairy Sci.*, 69: 77-87.
- 4) ØRSKOV, R.E. & McDONALD, I. (1979). The estimate of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci., Camb.*, 42: 499-503.
- 5) SNEDECOR, G.W. & COCHRAN, W.G. (1981). *Statistical Methods*. 7th ed. The Iowa State University Press, Ames, Iowa.

MORFOLOGICAL STUDY OF A CASE THE HERMAPHRODITISM IN THE *PROCAMBARUS CLARKII* (CRUSTACEA, DECAPODA) FROM ALBUFERE LAKE OF VALENCIA

(ESTUDIO MORFOLOGICO DE UN CASO DE HERMAFRODITISMO EN *PROCAMBARUS CLARKII* (CRUSTACEO, DECAPODO) DE LA ALBUFERA DE VALENCIA)

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Palabras clave: *Procambarus clarkii*, hermafroditismo, gónadas.
Key words: *Procambarus clarkii*, hermaphroditism, gonades.

ABSTRAC

Approximately 7.000 specimens of the crayfish *Procambarus clarkii* from the Albufera lake (Valencia, Spain) were collected and examined, and only one apparent hermaphrodite animal was localized. This animal was studied and its gonads were fixed in Bouin's solution for 2 hours. The posterior genital lobe presented in its proximal zone an altered ovarian tissue and in its distal zone the tecticular acini found were inactive. This observation constitute a real description of a case of autentic hermaphroditism in this specie.

RESUMEN

Hemos recogido y revisado aproximadamente 7.000 ejemplares de la especie *Procambarus clarkii* y de todos ellos sólo uno era aparentemente hermafrodita. Este animal se

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An. Fac. Vet. León. 1989, 35, 193-199

examinó y sus gónadas se fijaron en solución Bouin durante dos horas. El lóbulo genital posterior presentaba en su zona proximal tejido ovárico alterado y en su zona distal acinos testiculares inactivos. Por ello este animal puede considerarse como un caso de hermafroditismo auténtico en esta especie.

INTRODUCTION

Sexual dimorphism in the chelae and abdomen of crayfish is generally recognized^{2,10}.

In relation to the aberrant secondary characters in crayfish, several thousand aberrations in *Cambarus* and *Orconectes* were described in the eastern United States⁹ but only one was found in *Procambarus* and none in *Cambarellus*. This discrepancy in occurrence among genera was explained in base to the relative lack of constancy of secondary sexual characters in *Cambarus* and *Orconectes*. This conclusion was supported by the aberrant sexual secondary characters observed in one *Cambarellus shufeldtii* of 6.000 examined and in one *Procambarus dupratzi* of some 10.000 *Procambarus* spp. examined⁷.

Procambarus clarkii (Girard, 1852) is native to the southern United States and northern Mexico but now has almost worldwide distribution⁴. It is extensively used in physiological studies and for human consumption¹. It is surprising that there have been no known observations of aberrant secondary sexual characters in this species. This could document an observation of such aberrations in *Procambarus clarkii*.

MATERIAL AND METHODS

Along the last 7 years, about 7.000 specimens of the crayfish *Procambarus clarkii*, were collected in the Albufera Lake (Valencia, Spain). The crayfish ranged in size from 15 to 35 g.

All the animal were classified according to their sexual characteristics and transferred into alimantation aquaria for posterior studies. Among the 7000 specimens studied only one apparent hermaphrodite was found.

The sexual organs from this apparent hermaphrodite and a pair of control animals were removed, anatomically studied and fixed by immersion in Bouin's solution for 2 hours. Paraffin sections (7 μ m) were stained routinely with haematoxylin and eosin.

RESULTS

The external characteristics of the hermaphrodite animal showed exterior opening of oviducts, seminal receptacle and spermiducts. The oviducts were covered by an sclerotized flap, and the seminal receptacle was flat, without relief (Fig. 1).

The anatomical study of the gonads from the hermaphrodite animal showed the presence of two anterior lobes with aspect of testicular lobes and one posterior lobe resembling an ovary.

Low power histological observations confirmed the presence of two testicular lobes, but while in the proximal zone ovarian tissue was found the distal part was occupied by testicular tissue (Fig. 2).

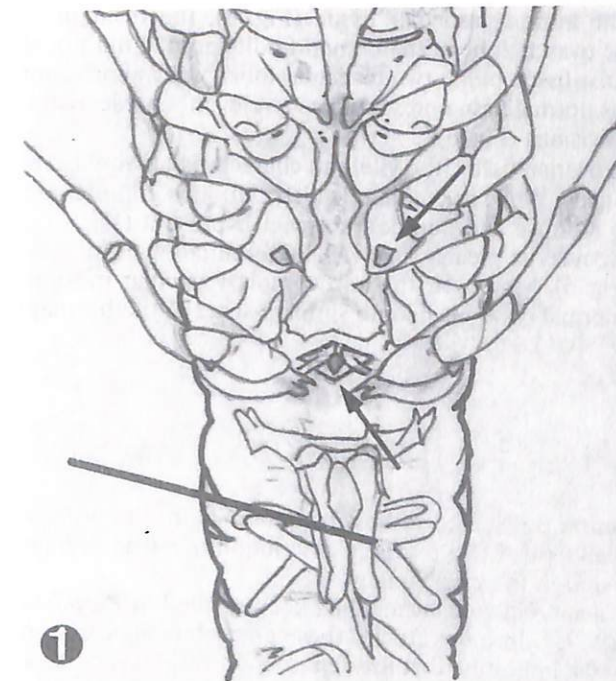


Figure 1.- Scheme from a whole animal, showing the abnormal sexual characters (arrows indicate the opening oviducts and seminal receptacle).

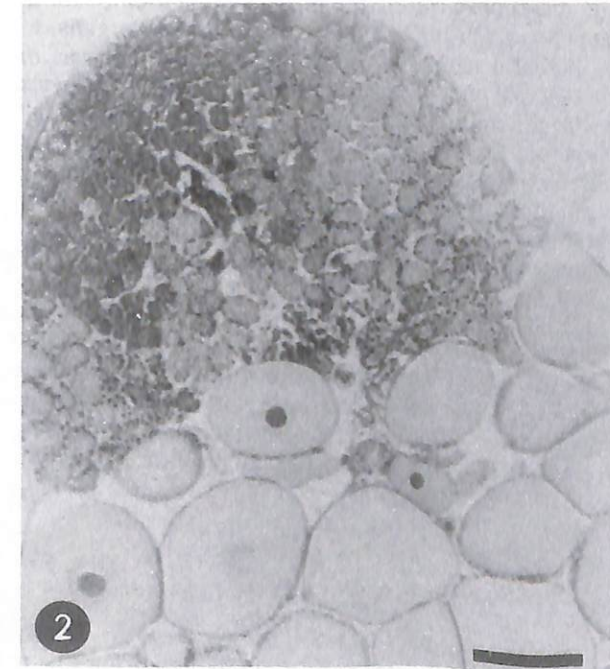


Figure 2.- Posterior lobe of the gonad containing both ovarian and testicular tissue. Bar = 200 μ .

In contrast to the normal testicular tissue (Fig. 3), the testicular tissue from the superior pole of the ovarian lobe of the hemaphrodite animal did not show active acini (Fig. 4). The testicular tissue of the two testicular lobes was in correspondence with the normal aspect. This normal tissue presented as a relevant characteristic the presence of synchronous cellular divisions (Fig. 3).

In relation to the ovarian tissue, the vitellum characteristics vary between normal and hermaphrodite animals. While in the first its distribution is granular and dense (Fig. 5), in the second case a diffuse and non-dense aspect is present (Fig. 6). The perinuclear halo of normal ovocytes is greater and well differentiated from the vitellum of the hermaphrodites (Fig. 5). Moreover, the non completely circular morphology of the nucleus, frequent in normal ovocytes had no similar aspect in the hermaphrodite (Fig. 6).

DISCUSSION

A crayfish cannot be considered to be a true hermaphrodite unless it exhibits both testicular and ovarian tissue⁹. No previous description of a true hermaphrodite (in this sense) has been found in the bibliography.

The external hermaphrodite characters had been studied in *Pacifastacus*⁶, *Cambarellus* and *Procambarus*^{7, 5}. In these articles these characters appeared in an approximate proportion of 1:10.000, indicating that this phenomenon results very exceptional in natural conditions.

The seminal receptacle of immature females of *Orconectes propinquus* was flat, essentially without relief, and an off-white sclerotized flap covered the oviduct⁸. On the other hand, in mature males of *P. clarkii*, the presence of a modification in the first and second abdominal appendages in order to serve as copulatory organs, with openings of the genital ducts on the basal segments of the fifth walking legs was mentioned⁵.

On the basis of the external characters described here, and the previous descriptions, we could suppose that the animal studied in the present work was a hermaphrodite one, and it could constitute the first description of a hermaphrodite specimen of the crayfish *Procambarus clarkii* in the Albufera Lake (Valencia, Spain).

The normal gross anatomy of the male gonads of *Procambarus clarkii* consist in two anterior and one posterior white lobes and two sperm ducts³, while our observations in the hermaphrodite animal shows that the two anterior lobes presented testicular appearance, and the posterior lobe presented ovarian aspect.

The histologic study showed that the two anterior lobes have mature testicular tissue while the other one presented both ovarian and testicular tissue. In this case both tissues presented immature aspect with ovocytes not normally developed and testicular acini not shown synchronous active cellular divisions or normal distribution.

In conclusion, in agreement with previously well established facts⁹ we can affirm that our description corresponds to a true case of hermaphroditism in *Procambarus clarkii*.

ACKNOWLEDGMENTS

We are indebted to Dr. L.A. García-Prado for kindly reviewing this manuscript and E. Abril and A. Raga for the realization of the scheme corresponding at Figure 1.

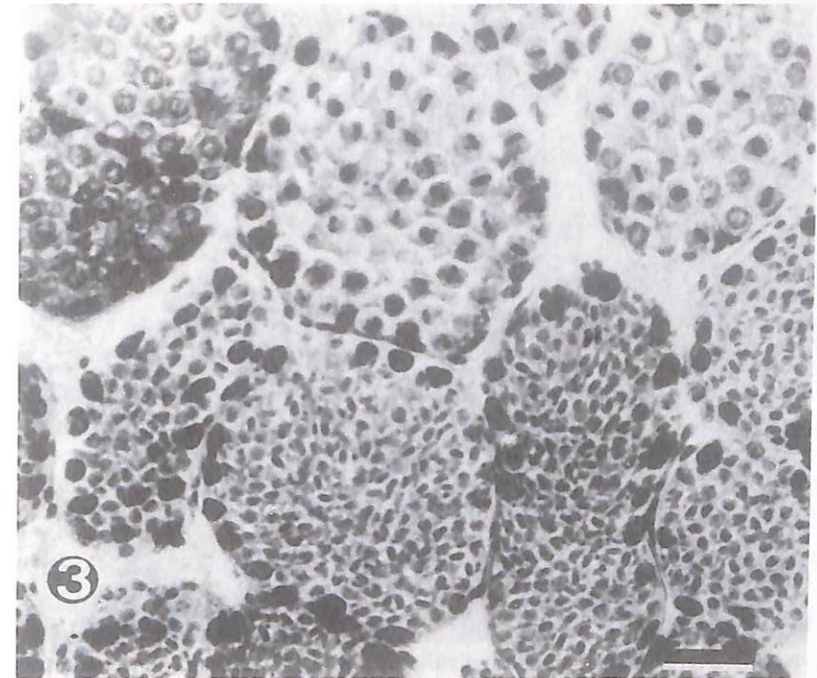


Figure 3.- Normal testicular tissue. Synchronous cellular divisions were present. Bar = 30 μ .

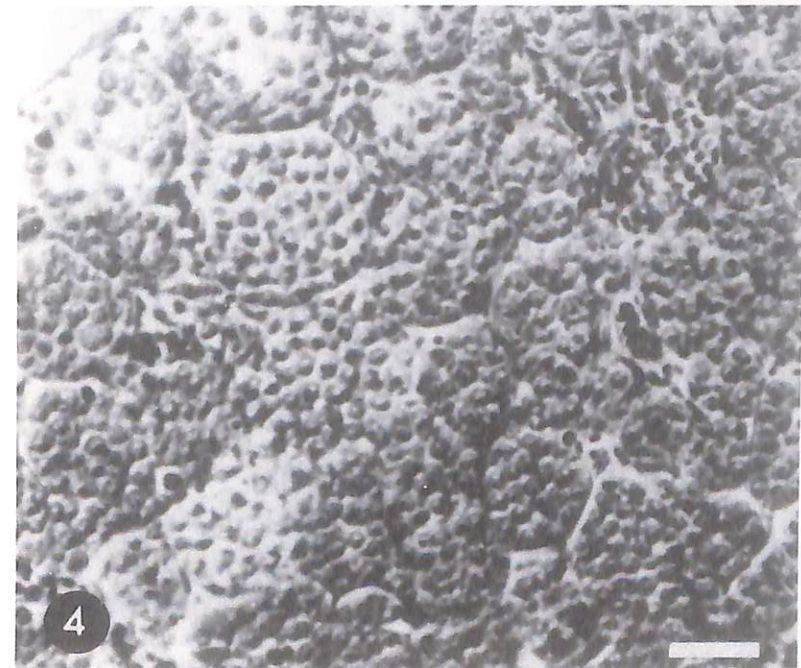


Figure 4.- Hermaphrodite testicular tissue. The acini seem to be not active. Bar = μ .

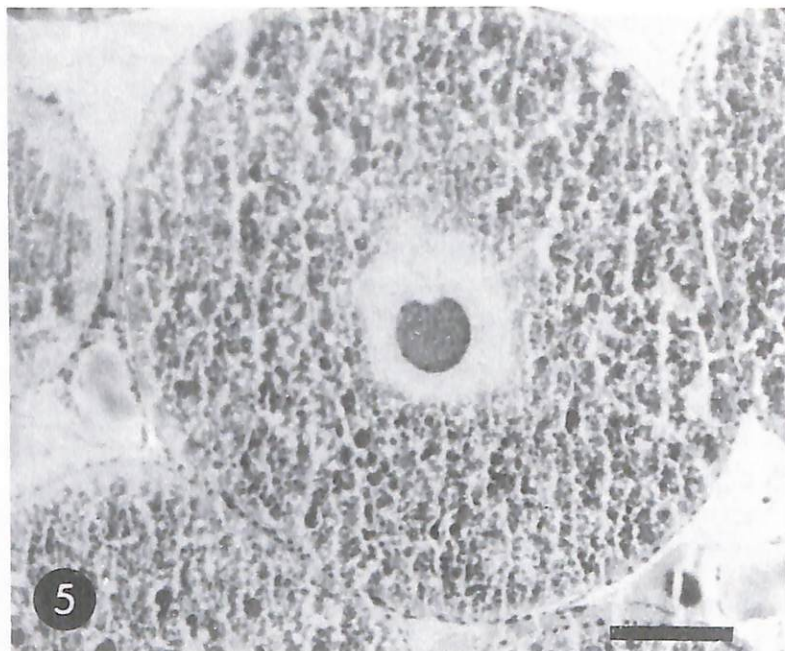


Figure 5.- Normal ovarian tissue. Vitellum distribution was granular and dense, and the perinuclear halo was great and well differentiated from the vitellum and nucleus. The nucleus presents a non completely circular morphology. Bar = 100 μ .

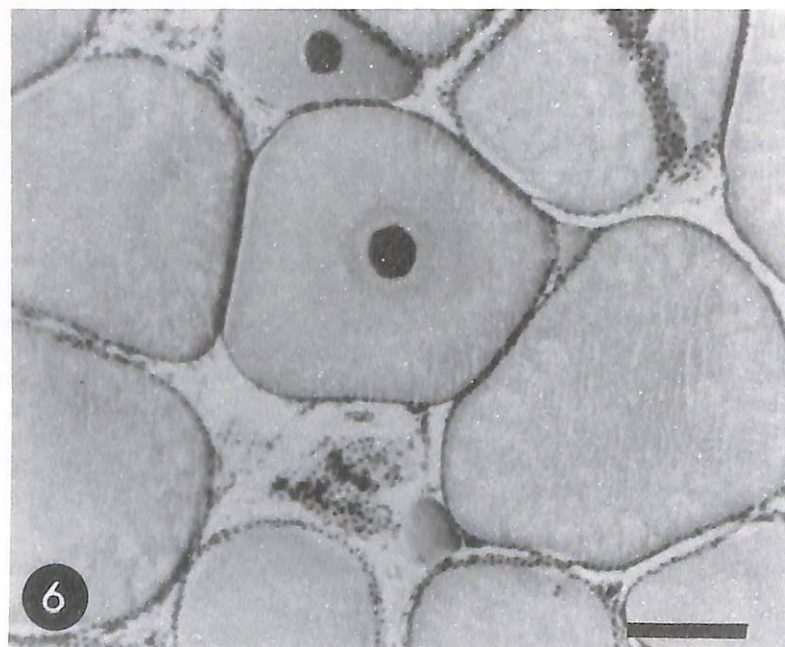


Figure 6.- Hermaphrodite ovarian tissue. Vitellum distribution presented a diffuse and non-dense aspect, and the perinuclear halo was not well differentiated from the vitellum. The nuclear morphology was always completely circular. Bar = 100 μ .

REFERENCES

- 1) AVAULT, J.W., 1972. Crayfish farming in the United States. Papers from *Int. Symp. on Freshwater Crayfish 1*: 239-250.
- 2) CROCKER, D.W. & BARR, D.W., 1968. *Handbook of the crayfishes of Ontario*. Univ. Toronto, Toronto, Ontario.
- 3) DELONG, M.D. & PAINE, J.F., 1987. Structural variation in the sperm ducts of *Procambarus* (Decapoda, Cambaridae). *Crustaceana* 53: 195-199.
- 4) HOBBS, H.H., 1972. *Crayfishes (Astacidae) of North and Middle America*. Identification Manual N.º 9. Biota of Freshwater Ecosystems. U.S. Govt. Printing Office, Washington, D.C.
- 5) HUNER, J.V. & BLACK, J.B., 1977. Aberrant secondary sexual characters in the crayfish *Procambarus clarkii* (Girard) (Decapoda: Cambaridae). *Southwest. Nat.* 22: 269-286.
- 6) MASON, J.C., 1965. Female *Pacifastacus* (Astacidae) with a male secondary sex characteristic. *Amer. Midland Nat.* 73: 501.
- 7) PENN, G.H., 1957. Aberrant secondary sex characters in the crayfishes *Cambarellus shufeldtii* and *Procambarus dupratzi* (Decapoda, Astacidae). *Trans. Amer. Micro. Soc.* 76: 93-95.
- 8) STEIN, R.A.; MURPHY, M.L. & MANGNUSON, J.J., 1977. External morphological changes associated with sexual maturity in the crayfish (*Orconectes propinquus*). *Amer. Midland Nat.* 97 (2): 495-502.
- 9) TURNER, C.L., 1935. The aberrant secondary sex characters of the crayfishes of the genus *Cambarus*. *Amer. Midland Nat.* 16: 863-882.
- 10) WEAGLE, K.V. & OZBURN, G.W., 1970. Sexual dimorphism in the chela of *Orconectes virilis* (Hagen). *Can. J. Zool.* 48: 1.041-1.042.