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E. D'Agaro, E.A. Ferrero & P. G. Giulianini

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Induction of ovarian maturation by means of dietary hormonal treatment in Austropotamobius pallipes

E. D'Agaro [1], E.A. Ferrero [2], P. G. Giulianini [2]

[1] Dipartimento Scienze Animali, Università di Udine, Italy
[2] Dipartimento Biologia, Università di Trieste, Italy

Corresponding author: Edo D'Agaro. Dipartimento Scienze Animali. Via Sebenico 17/4, 33010 Pagnacco, Italy –

Tel: +39 0432 650110 – Fax: +39 0432 660614 – Email: dagaro@dspa.uniud.it

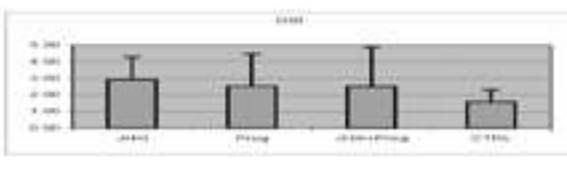
RIASSUNTO – Induzione della maturazione ovarica mediante l'impiego di ormoni di sintesi nel gambero *Austropotamobius pallipes. Lo scopo della presente ricerca era quello di indurre la riproduzione nel gambero autoctono Austropotamobius pallipes in ambiente controllato. A tal fine, 33 femmine adulte di Austropotamobius pallipes sono state alimentate durante un periodo non riproduttivo (febbraio-aprile) con diete contenenti sostanze ormonali di sintesi (ormone giovanile III, 17α-idrossiprogesterone, 17α-idrossiprogesterone + ormone giovanile III) per stimolare la maturazione ovarica. Vennero utilizzate per l'esperimento due gruppi d animali: il primo in cui le femmine si erano già riprodotte l'anno precedente e il secondo in cui non si eran riprodotte. Alla fine dell'esperimento, l'indice gonadosomatico e il diametro degli oociti aumentarono significativamente negli animali trattati con l'ormone giovanile III rispetto al controllo. Venne inoltre osservata la produ*

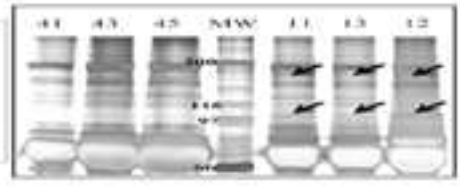
zione di vitellogenine emolinfatiche del peso di 105 e 164 Kda rispetto al controllo. L'ormone giovanile III somministrato con l'alimento ha esercitato un effetto positivo sullo sviluppo ovarico di tutte le femmine nella specie A. pallipes durante un periodo non riproduttivo.

Key words: ovarian reproduction, hormones, Austropotamobius pallipes.

INTRODUCTION – The freshwater crayfish *Austropotamobius pallipes* is an annual species with low fecundity and a long embryonic development. Restocking programmes for this species have recently been prompted in many countries in Europe because of its ecological importance in the freshwater ecosystem.
The role and interactions of neurotransmitters which intervene in crustacean reproduction have been identified but they are not still completely understood. Ovarian development appears to be under the control of two hormones: the vitellogenesis-inhibiting hormone and the gonad stimulating hormone (Fingermann, 1997). Methyl farnesoate, secreted by the mandibular organs, also stimulated the oocyte growth in crayfish *Procambarus clarkii* (Laufer *et al.*, 1998). The administration of juvenile hormone III (the epoxidized form of methyl farnesoate) seems to have an effect on ovarian development in penaeids (Tsukimura and Kamemoto, 1991) and crabs (Zapata *et al.*, 2003; Reddy *et al.*, 2004) but not in crayfish *P. clarkii* (Rodriguez *et al.*, 2001). The hormone 17a-hydroxyprogesterone produced an increase of the ovarian size in crayfish *P. clarkii* (Rodriguez *et al.*, 2002) and the concentration of vitellogenin in shrimps (Quinitio *et al.*, 1994) and crabs (Shih, 1997). The objective of the present study was to measure the effects of two hormones juvenile hormone III, 17α-hydroxyprogesterone and their combination incorporated in the food on the ovarian maturation.

MATERIAL AND METHODS – Adult females were all collected from the same river in the Friuli Venezia Giulia region in October 2003. In January 2004, 33 females (mean weight: 13g) were selected for the experiment and randomly allotted into two experimental groups (8 tanks, throughout system) with five or three replicates for each tank: recently spawned females (A) and no spawned females (B). Eggs were delicately stripped from the spawned females in December. The experimental design for each group was as follows: 1) Control group; 2) 17α-hydroxiprogesterone (2.5 µg/animal/day); 3) juvenile hormone III (2.5 µg/animal/day); 4) 17α-hydroxiprogesterone (2.5 µg/animal/day) + juvenile hormone III (2.5 µg/animal/day). Chemicals were bought from Sigma (Milano). They were first dissolved in ethanol and than incorporated into the diets via lipids. Feed, consisting in a pelleted diet (crude protein, 29% on d.m. and crude fat 8.3% on d.m.), was distributed 6 times per day, by means of automatic feeders at the feeding rate of 2% of body weight per day. Mortality and water quality parameters were checked every week. Mean values of temperature and photoperiod were 13±0.2°C and L:D 10:14 h. The experiment lasted 61days and 101 respectively for the group A and B. Hemolymph samples of 100-150 µl were withdrawn from the pericardial sinus of each animal using a 1-ml syringe. Each sample was centrifuged for 40 s at 10000 rpm and 50 µl of the supernatant was transferred into a tube containing 20 µl of anti-protease mixture (Roche diagnostics) and kept on ice. Hemolymph samples were run on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Dissected ovaries were weighted at the nearest mg and Gonado-Somatic Index (GSI) calculated. The mid-part of the left gonad of each crayfish was then fixed in 2.5% glutaraldehyde, 1% paraformaldehyde, 7.5% saturated picric acid solution in





0.1 M cacodylate buffer pH 7.4 overnight and post-fixed in 1% osmium tetroxide in the same buffer, serially dehydrated in ethanol and embedded, via propylene oxide, in Embed812/Araldite. Sections 2 µm thick were collected on slides, baked for 5 min at 80°C and stained with 0.5% toluidine blue in 0.1% carbonate solution at pH 11.1 at the same temperature. Experimental results were analysed using a two-tailed Student t-test to check significant differences among means.

RESULTS AND CONCLUSIONS – The dietary hormonal treatments in *Austropotamobius pallipes* resulted in an increase of the GSI mean in all groups of animals compared to the control. However, only the GSI mean of animals treated with juvenile hormone III was significantly different from control GSI (P=0.02, Figure 1a). Concerning the results obtained with JHIII + progesterone treatment, the JHIII and progesterone hormones have been shown to exert their action on parallel endocrine targets with no additive effects. The 10% polyacrylamide SDS-PAGE separation of hemolymphatic proteins showed specific bands with molecular masses of about 164 and 105 KDa in animals fed with juvenile hormone III (Figure 1b; 11, 12, 13) that were not present in control animals (Figure 1b; 41, 43, 45). Measurements of oocyte diameters (control, 680.0 mm (n=41); JHIII, 813.9 µm (n=70)) at the most advanced stage of maturation in ovarian sections of treated animals compared to the controls, confirmed the morphometric and biochemical data and identify the juvenile hormone III as the most effective dietary hormonal treatment. Our data are in good agreement with those obtained in *Procambarus clarkii* with methyl farnesoate (Laufer *et al.*, 1998) and therefore we concluded that the epoxidized form of methyl farnesoate (juvenile hormone III) can stimulate ovarian growth in *Austropotamobius pallipes* independently of the environmental stimuli.

Figure 1. a) Effect of dietary hormonal treatment on GSI of Austropotamobius pallipes females (means±SD). b) SDS-PAGE of hemolymph of females treated with JH III (11, 13, 12) and control females (41, 43, 45).