Journal of Invertebrate Pathology 102 (2009) 160–166

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/yjipa

Structural alterations in the male reproductive system of the freshwater crayfish, *Cherax quadricarinatus* (Decapoda, Parastacidae)

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ARTICLE INFO

Article history:
Received 27 March 2009
Accepted 6 August 2009
Available online 12 August 2009

Keywords:
Cherax quadricarinatus
Red claw
Reproductive system
Melanisation

ABSTRACT

No diseases affecting reproductive performance have been previously reported in freshwater crayfishes. This study aims to characterise one reproductive system abnormality found in males of *Cherax quadricarinatus* reared in captivity. Fifteen adult males of *C. quadricarinatus* (70–110 g) were purchased from San Mateo S.A. farm (Entre Ríos, Argentina) each season during 2007. Macroscopic analysis showed that 26.6% of the animals sacrificed in winter presented brownish distal vasa deferentia. Histological analysis showed different levels of structural abnormality in the epithelium of the vasa deferentia and spermatophore. Granular and hyaline haemocytes were identified within the vasa deferentia but no significant differences were found in the sperm count between normal and brownish vas deferens. Histological analysis of the crayfishes sacrificed in autumn also showed these modifications in 22% of the animals, however, they did not show the brownish colour under macroscopic analysis. The similarities between the male reproductive system syndrome in shrimps and the abnormalities found in *C. quadricarinatus* are notable. An unspecific response to thermic stress is a possible explanation of these structural alterations.

1. Introduction

The main objective in aquaculture is to achieve maximum reproduction readiness all the year, and to develop specific techniques for reproduction and growth in captivity. Hence, many studies concerning reproduction performance have been conducted. Among other factors, it has been demonstrated that diseases affect the reproductive system, decreasing the reproductive performance. The darkening of the spermatophore, also named melanisation, was found in the reproductive system of males of the penaeid shrimps *Litopenaeus setiferus* (Alfaro et al., 1993; Bray et al., 1985; Brown et al., 1979; Chamberlain et al., 1983; Leung-Trujillo and Lawrence, 1987; Pascual et al., 1998; Rosas et al., 2004; Talbot et al., 1989), *Litopenaeus vannamei* (Alfaro and Lozano, 1993; Chamberlain et al., 1983; Diamond et al., 2008; Dougherty and Dougherty, 1989; Parnes et al., 2006; Perez Velazquez et al., 2001), *Penaeus stylirostris* (Chamberlain et al., 1983) and *Pleoticus muelleri* (Díaz et al., 2001), and in the caridean shrimp *Macrobrachium rosenbergii* (Harris and Sandifer, 1986). It is characterised by a reduction in sperm count and sperm viability leading to male infertility (Chamberlain et al., 1983; Leung-Trujillo and Lawrence, 1987; Pascual et al., 1998; Rosas et al., 2004 for revision). It is thought to be produced by stress, bacterial infection, inappropriate diet or problems of endocrine nature associated with environmental stress (Alfaro and Lozano, 1993; Alfaro et al., 1993; Chamberlain et al., 1983; Diamond et al., 2008; Dougherty and Dougherty, 1989; Leung-Trujillo and Lawrence, 1987; Rosas et al., 1993; Rosas et al. 2004). This syndrome is usually referred as "male reproductive tract degenerative syndrome" (Diamond et al., 2008; Talbot et al., 1989; Rosas et al., 2004 for revision) and its aetiology remains unknown.

Cherax quadricarinatus von Martens (1868) is a freshwater crayfish native to Australia and Papua New Guinea, which has an increasing importance for culture. In this species, the female reproductive performance has been intensively studied (Barki et al., 1997; Barki and Karplus, 1999; Jones, 1995; Karplus et al., 2003; Levi et al., 1999). On the contrary, reproduction in males has been poorly studied (Bugnot and López Greco, 2009; López Greco et al., 2007; López Greco and Lo Nostro, 2008) and no diseases affecting reproductive system have been reported so far (Edgerton et al., 2002). In this context, this study aims to characterise one structural alteration of the reproductive system found in males of *C. quadricarinatus* reared in captivity.

2. Material and methods

Fifteen adult males of *C. quadricarinatus* (70–110 g) were purchased from San Mateo S.A. farm (DGDAyRN N° 12/96) in Chajarí (30° 46′ S; 57° 58′ W, Argentina) during January (summer), May (autumn), July (winter) and September (spring) 2007. These animals were collected from grow out earthen ponds (100 m

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0022-2011/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2009.08.004

long × 25 m wide × 1 m depth). Mean temperatures in the ponds were 10, 18, 20 and 25 °C for winter, autumn, spring and summer, respectively. Once in the laboratory, the animals were weighted, cold anaesthetized at −20 °C during 15 min, and then they were dissected removing the carapace. Macroscopic analysis of the reproductive system was made: size, colour and consistency of the spermatophore were recorded. For sperm count and sperm mortality determination, a modified protocol developed by Leung-Trujillo and Lawrence (1987) and vital dye exclusion technique (methylene blue 10%) were employed in accordance with Bugnot and López Greco (2009). One-cm section of distal vas deferens (DVD) was disaggregated in 1 ml of physiological solution for crustaceans (van Harreveld, 1936). This section corresponds to the approximated size of the spermatophore transferred to the female during mating (López Greco et al., 2007; López Greco and Lo Nostro, 2008). Then, spermatozoa were counted in a Neubauer camera. Sperm count was expressed as spermatozoa/DVD section (Bugnot and López Greco, 2009). For statistical analysis, paired samples *t* test was employed.

Histological analysis was carried out for five males in summer and nine males in autumn, winter and spring. The vasa deferentia were fixed in Bouin solution for 4 h at room temperature and processed for routine histological analysis in accordance with López Greco et al. (2007). Histological sections were cut with a Carl Zeiss microtome (5–6 μm thick) and then stained with Haematoxylin Eosin and Masson Trichrome. The sections were examined with a Carl Zeiss, Axioimager A1 light microscope and photographs were taken with digital camera Nikon Coolpix 7.1 megapixels. Structure of vas deferens was evaluated observing size, density and composition of the sperm cord in the secondary layer, and structure of primary and secondary layer of the spermatophore in accordance with López Greco et al. (2007) and López Greco and Lo Nostro (2008).

3. Results

The animals sacrificed in spring, summer and autumn did not show any alterations during the macroscopic analysis. On the contrary, 4 of 15 animals sacrificed in winter (26.6%) presented brownish distal vas deferens (Fig. 1). This colour was found in different intensities in one or both vasa deferentia (VD). These VD were also harder in consistency than normal ones; they did not have the usual elasticity of the muscular sheath and broke easily under manipulation. No significant differences were found in sperm count and sperm mortality between brownish and normal vas deferens ($p > 0.05$).

The sperm count of brownish vas deferens was $1.52 \pm 0.25 \times 10^8$ spermatozoa/DVD solution, while for normal vas deferens it was $1.77 \pm 0.4 \times 10^8$ spermatozoa/DVD section. Under light microscopic analysis the sperm did not show any morphological abnormalities.

Histological analysis of the brownish VD showed different levels of alteration of the epithelium and spermatophore. A hypertrophied epithelium showing an initial separation of the muscular sheath is observed from early stage of structural alteration (Fig. 2). High quantity of secretion is observed due to this hypertrophy. As concerns the spermatophore, no droplets of secretion from the vas deferens found in the secondary layer of a normal spermatophore can be observed (Fig. 3). Instead, the secondary layer presents droplets that seem to be formed of an amorphous debris. Many granular and hyaline haemocytes were identified using Martin and Hose's classification (1992).

Fig. 4 shows a histological section of a brownish VD with an intermediate level of alteration. The spermatophore has an advanced level of disorganization and it cannot be recognized,

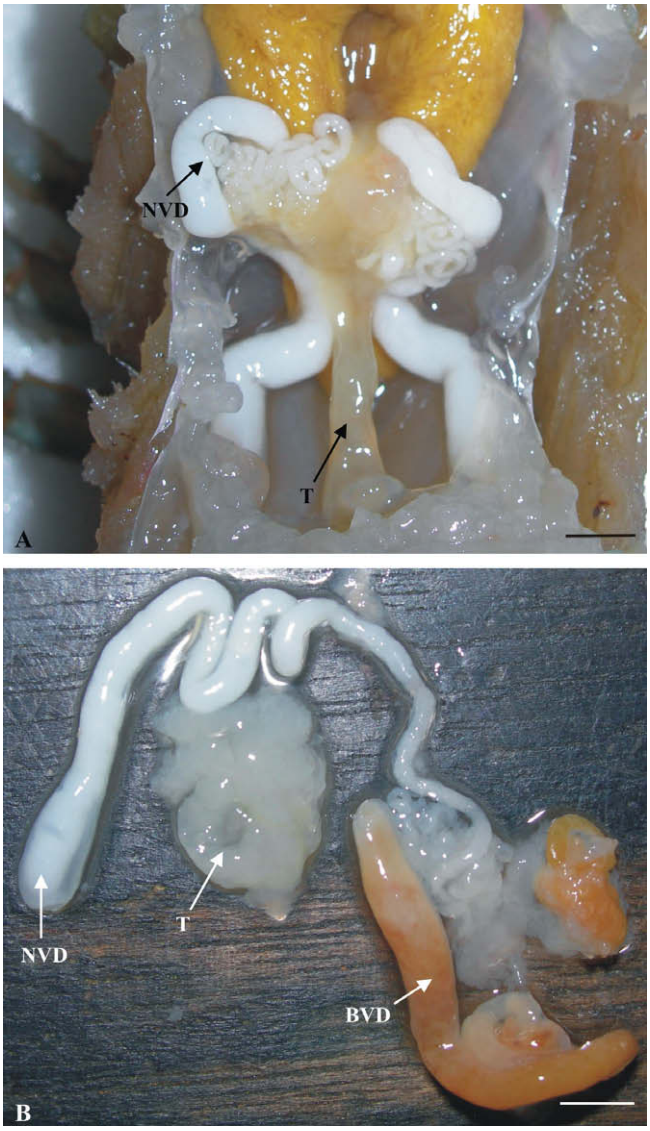

Fig. 1. Male reproductive system of *C. quadricarinatus*. (A) Male sacrificed in summer, showing both normal vasa deferentia and (B) reproductive system of a male sacrificed in winter showing one normal and one abnormal brownish distal vas deferens. BVD: brownish vas deferens, NVD: normal vas deferens, T: testis. Scale bar: 10 mm.

the epithelium is more separated from the muscular sheath and more disorganized, and the muscular sheath is beginning to disaggregate (Fig. 4A and B). In this case, nodular structures formed by haemocytes were observed in the lumen of the VD (Fig. 4A and C). They present a dense central core, and an internal and external layer which are formed by dense and more distended disposition of granulocytes, respectively. An advanced level of disorganization of the vasa deferentia is shown in Fig. 5. Both the epithelium and the spermatophore are not distinguishable, and the muscular sheath presents an advanced level of disorganization. Aggregated granulocytes are seen in the lumen (Fig. 5C).

Histological analysis of vasa deferentia of 2 out of 9 animals sacrificed in autumn (22%) also showed the alterations described above, although they did not show brownish colour. Two levels of alteration were found. In one case, the spermatophore is disorganized, but the epithelium remains unaltered (Fig. 6); and in other case, the spermatophore presents a higher level of disorganization, and the epithelium is separated from the muscular sheath (Fig. 7A and B). Haemocytes were also observed (Fig. 7C).