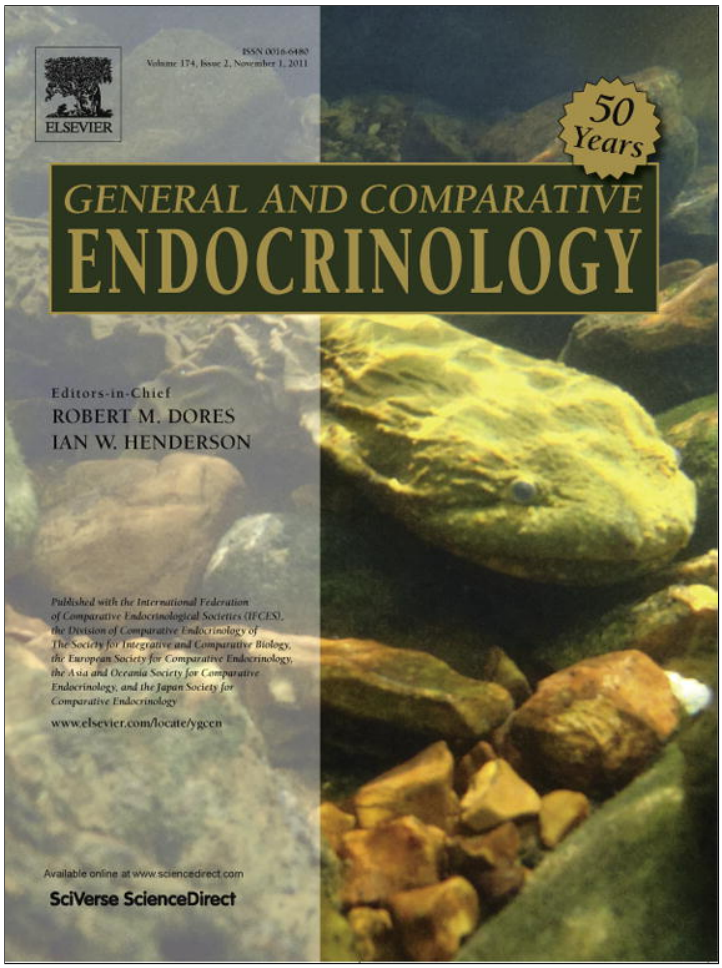

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Effects of androgenic gland ablation on growth and reproductive parameters of *Cherax quadricarinatus* males (Parastacidae, Decapoda)

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ABSTRACT

This work investigates the effects of androgenic gland (AG) ablation on the structure of the reproductive system, development of secondary sexual characters and somatic growth in *Cherax quadricarinatus* males. The AG ablation, which was performed at an early developmental stage (initial weight: 1.85 ± 0.03 g), had no effect on the somatic growth parameters (specific growth rate and growth increment), but it prevented the re-formation of male gonopores and *appendices masculinae*. However, the red patch differentiation and chelae size were similar to those in control males. All the ablated animals developed a male reproductive system. Testis structure was macroscopically and histologically normal. The distal portion of the vas deferens (DVD) was enlarged in some animals, with histological alterations of the epithelium and the structure of the spermatophore. Results suggest that the higher growth in males than in females may be due to an indirect effect of the AG on energy investment in reproduction rather than to a direct effect of an androgen. This is the first report of a potential action of the AG on the secretory activity of the distal VD and the structural organization of the spermatophore. Although the AG may play a role in the development of male copulatory organs, its association with the red patch development deserves further research. The results obtained in the present study support and complement those from intersexes of the same species.

1. Introduction

The role of the androgenic gland (AG) in sex differentiation has been extensively studied in malacostracan crustaceans, mainly by means of two alternative techniques: the implantation of this gland in females and its ablation (andrectomy) in males. The AG manipulation is feasible because in male crustaceans the gametogenic and endocrine functions are separated into two different organs, the testis and the AG, respectively [10,16]. Thus, sex differentiation can be investigated through the removal of the AG without damaging the gonads.

When these two procedures were applied to amphipods and isopods, it was clearly demonstrated that the AG controls the differentiation of the male reproductive system and is necessary for the maintenance of spermatogenesis [2,8,9,21,27–29]. For example, andrectomy resulted in partial feminization of secondary sexual characters and complete gonadal feminization in the isopod *Armadillium vulgare* [60], while it blocked the differentiation of secondary male characteristics leading to decreased spermatogenesis in *Orchestia gammarella* [7].

In astacid (Decapod) crustaceans, the implantation (or injection) of the AG tissue (or glandular extract) was performed in females of several crayfish species, such as *Procambarus clarkii* [42,61,62], *Cherax destructor* [15], and *Cherax quadricarinatus* [26,35]. This procedure was also done in other decapods, such as the giant freshwater prawn *Macrobrachium rosenbergii* [34,40,41] and the mud crab *Scylla paramamosain* [12]. In all these cases, the tissue implantation induced a variable degree of masculinization of primary (e.g., inhibition of vitellogenesis and degeneration of oocytes) and secondary (e.g., development of male gonopores or copulatory organs) sexual characters.

The AG ablation performed in decapod crustaceans also revealed the key role of this gland in male sexual differentiation. For example, *M. rosenbergii* males ablated at a very early stage of development underwent sex-reversal into completely functional females (neo-females) [1,57]. In addition, the AG ablation caused the onset of secondary vitellogenesis in the arrested ovaries of *C.*

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quadricarinatus intersex juveniles [4], and altered the histological structure of the female and male components of their reproductive system [25]. However, to our knowledge no morphological or histological characterization has ever been made of the effects of AG ablation on the external sexual characters and reproductive system of *C. quadricarinatus* males.

This freshwater crayfish is a gonochoristic species. The males have a pair of testes connected in their middle portion to the *vasa deferentia*, which open into the *appendices masculinae* at the base of the fifth pair of walking legs [17,32]. The spermatophore of this species is composed of two elements: (1) the spermatic cord, which is a continuous structure consisting of a mass of spermatozoa from the testes, surrounded in the proximal portion of the vas deferens (VD) by a thin layer named "primary layer" of the spermatophore; (2) the secondary layer of the spermatophore, which is synthesized in the middle portion of the VD and surrounds the primary layer. Two distinctive components are identified in the latter: a high quantity of round cytoplasmatic droplets, and a homogeneous matrix [32,33].

On the other hand, a possible effect of the AG on growth was also studied in *M. rosenbergii* and *C. quadricarinatus*, whose males grow faster and attain a larger size than females [11,13,20,24,48,50,55,57]. In both species, the somatic growth was reduced by the ablation and increased by the implantation of this gland [34,52]. It remains unclear whether the effect of AG on growth is direct through an androgen (still undiscovered in these species; [36]), indirect through the inhibition of the energetic investment of females in reproduction, or a combination of both [35].

In an attempt to gain a more in-depth insight of the role of the AG in some aspects of *C. quadricarinatus* biology, this study provides a long-term evaluation (300 days) of the effect of its ablation at an early stage of development on the growth, structure of the reproductive system and development of secondary sexual characters of this species males.

2. Materials and methods

2.1. Animals

The juveniles used in the present study were obtained under laboratory conditions, from a broodstock supplied by Farm Las Golondrinas, Entre Ríos, Argentina. Ovigerous females were individually placed in glass aquaria of 60 × 40 × 30 cm containing 20 L of dechlorinated tap water and under continuous aeration. The temperature was held constant at 27 ± 1 °C by ATMAN water heaters (100 W, precision 1 °C), and the photoperiod was 14:10 (L:D). Each aquarium was provided with a PVC tube (10 cm in diameter and 25 cm long) as shelter [18]. Females were fed daily *ad libitum* with *Elodea* sp. and commercial Tetradiskus® granules (approximate composition: min. crude protein 47.5%, min. crude fat 6.5%, max. crude fiber 2.0%, max. moisture 6.0%, min. phosphorus 1.5% and min. ascorbic acid 100 mg kg^{-1}) according to Sánchez de Bock and López Greco [58] and Tropea et al. [64]. When juveniles became independent at stage III [31], they were separated from their mothers. After reaching approximately 0.200 g, juvenile males were identified by observation of genital openings at the base of the fifth pair of walking legs [66]. These juveniles were maintained under the same conditions of water quality, temperature, photoperiod and feeding described for their mothers until the beginning of the experiment.

2.2. Experimental design

Male juveniles weighing 1.85 ± 0.03 g (developmental stage II of male reproductive system according to Vazquez and López Greco [67]) were selected for the experiment, and randomly assigned to one of the following treatments:

- *Andrectomy (AGA):* removal of both fifth walking legs together with the AG by cutting through the articulation between the coxa and the cephalothorax.

- *Control (C):* removal of both fifth walking legs by performing a cut in the articulation between the coxa and the base of the pereiopod.

In both treatments, after the removal of the pereiopods the cuts were anesthetized with Xylocaine (containing 2.5% Lidocaine) and electro-cauterized to avoid excessive loss of hemolymph, and to ensure the complete removal of the AG tissue. As some juveniles died during the week that followed the microsurgeries, the experiment was initiated once the number of animals of each treatment had stabilized. Organisms were maintained in 60 × 40 × 30 cm glass aquaria filled with 20 L of dechlorinated tap water with continuous aeration. PVC tubes (10 cm in diameter and 25 cm long) and onion bag mesh were used as shelter. Each aquarium was a replicate, and had 4/5 crayfish (16.7–20.8 animals/m^2). Three replicates were used for each experimental group, with a total number of six aquaria. The experiment was performed under constant conditions of photoperiod (14:10 L:D) and temperature (27 ± 1 °C by 100 W ATMAN aquarium heater). The juveniles were fed daily *ad libitum* with commercial Tetradiskus® granules and *Elodea* sp. All aquaria were cleaned and water was completely replaced once a week. The experimental period comprised 300 days, during which animals were sexed according to the position of the gonopores, weighed (precision 0.01 mg) and their mortality recorded every 2 weeks. The regeneration of the removed pereiopods, the re-formation of male gonopores and *appendices masculinae*, the development of female gonopores, and the differentiation of the red patch were also recorded.

2.3. Morphological and histological examination

At the end of the experiment, all animals were weighed (precision: 0.1 mg) and the following morphometric variables were measured (precision: 0.01 mm): cephalothorax length (CL), from the tip of the rostrum to the end of the cephalotorax; postorbital cephalothorax length (POL), from behind the eye to the end of the cephalotorax; pleon width (PWi), measured in the second abdominal segment; length, width and height of the chelae (LC, WC and HC, respectively); and length of the red patch (LRP), when present. The weight of the chelae (CWe) and pleon (PWe) of each animal were also recorded. The size of the endopod and exopod of the first and second pleopods, together with the morphology of the pleopod setae, were qualitatively analyzed under a light microscope (×40), and compared between groups, taking into account that in males only plumose setae are present while with the progress of female maturation simple (ovigerous) setae are developed in the endopod, which is larger than the exopod [53].

After being cold-anaesthetized at −20 °C for 15 min the carapace of each animal was removed and the reproductive system was inspected to determine their relative size, form and color. They were then quickly dissected, weighed, and fixed in Bouin's solution for 4 h at room temperature. The tissues were finally dehydrated and embedded in paraffin. Sections, 5–7 μm thick, were stained with Hematoxylin–Eosin and Masson Trichrome. At least three slides from each crayfish were inspected under light microscope. The structure of the testes and the VD epithelium, the relative amount of secretion and the composition of the primary and secondary layers of the spermatophore [32,33,67], were qualitatively analyzed. Also, the number of spermatic cord sections within the

distal portion of the vas deferens (DVD) and the number of spermatozoa within the spermatic cord sections (seven sections *per* male were randomly selected for this purpose) were counted to obtain a mean value *per* replicate and compare those values between treatments.

2.4. Statistical analysis

Survival was calculated as the percentage of crayfish alive at the end of the experiment. The formulae used to calculate the different variables were as follows: specific growth rate, SGR = 100 ([$\log_e$ final weight − $\log_e$ initial weight]/time), where time was expressed in days [22,23,47]; growth increment, GI = 100 ([final weight − initial weight]/initial weight) [19]; gonadosomatic index, GSI = 100 (reproductive system weight/final weight) [38,53]; relative length of red patch, RLRP = LRP/LC; relative height of chelae, RHC = HC/LC; relative width of the pleon, RWP = PWi/CL; relative weight of the pleon, RWeP = PWe/final weight; and relative weight of the chelae, RWeC = CWe/final weight.

A one-way analysis of variance (ANOVA) [59] was applied to compare final weights, SGR, GI, GSI, morphometric variables, the number of spermatic cord sections within the DVD, and the number of spermatozoa within the spermatic cord sections between experimental groups. The Fisher exact test [59] was used to compare survival and the percentage of males with red patch between experimental groups. The results *per* treatment are presented as mean ± SE [14]. All tests were carried out at a significance level of 0.05.

Table 1
Effects of AG ablation on growth and reproductive parameters of *Cherax quadricarinatus* males at day 300 after the surgical procedure.

Parameter	Treatment	
	C	AGA
Initial number	12	15
Final number	9	9
Survival (%)	75[a]	60[a]
Initial weight (g)	1.85 ± 0.02[a]	1.85 ± 0.03[a]
Final weight (g)	20.24 ± 4.62[a]	20.69 ± 1.94[a]
GI (%)	993.07 ± 234.27[a]	1016.98 ± 113.70[a]
SGR (%/day)	0.75 ± 0.07[a]	0.76 ± 0.03[a]
CL (mm)	44.65 ± 3.16[a]	46.06 ± 1.00[a]
POL (mm)	31.39 ± 2.87[a]	31.93 ± 0.44[a]
RWeP	0.29 ± 0.01[a]	0.30 ± 0.00[a]
RWP	0.40 ± 0.01[a]	0.40 ± 0.00[a]
LC (mm)	31.26 ± 2.8[a]	32.59 ± 1.24[a]
WC (mm)	9.30 ± 1.32[a]	9.39 ± 0.39[a]
RHC	0.30 ± 0.02[a]	0.29 ± 0.01[a]
RWeC	0.04 ± 0.01[a]	0.04 ± 0.01[a]
RLRP	0.45 ± 0.04[a]	0.52 ± 0.11[a]
GSI (%)	1.16 ± 0.24[a]	1.40 ± 0.06[a]
Number of SC within the DVD	159.11 ± 29.38[a]	157.72 ± 43.33[a]
Number of spermatozoa within the SC	35.58 ± 3.38[a]	32.47 ± 0,89[a]
Males with red patch (%)	77.78[a]	88.89[a]

Note: Comparisons were made between the control (C) and the andrectomized (AGA) treatments. CL, cephalothorax length; DVD, distal portion of the vas deferens; GI, growth increment; GSI, gonadosomatic index; LC, length of the chelae; POL, postorbital cephalothorax length; RHC, relative height of the chelae; RLRP, relative length of the red patch; RWeC, relative weight of the chelae; RWeP, relative weight of the pleon; RWP, relative width of the pleon; SC, spermatic cord sections; SGR, specific growth rate; WC, width of the chelae. Values (±s.e.) with the same superscript are not different ($p > 0.05$).

3. Results

3.1. Survival and growth performance

There were no differences ($p > 0.05$) in survival, final weight, GI and SGR between experimental groups (Table 1). All the morphometric variables, as well as the RWeP and the RWeC were similar ($p > 0.05$) for both experimental groups (Table 1). The red patch began to develop on day 60 in both experimental groups (average weight: 7.76 ± 1.11 g) (Table 2).

3.2. Morphological examination

In all control males, the fifth pereiopods regenerated completely by day 100, with coxae bearing elongated *appendices masculinae* in 77.8% of the animals (Table 2; Fig. 1A). The remaining 22.2% showed an *appendix masculina* at the base of only one pereiopod and a male gonopore on the contralateral side until the end of the experiment (Table 2). The development of the *appendices* was asymmetrical in 44.4% of the control males. The majority of the andrectomized males did not regenerate the fifth pereipods (Fig. 1B), while a small percentage regenerated only one walking leg of the fifth pair by day 30 (Table 2). The animals of this group had neither genital openings nor *appendices masculinae* (Table 2; Fig. 1C). A hypertrophied fourth coxa with a posterior opening and an abnormally regenerated fifth pereiopod were observed in 18.2% of the AGA males (Fig. 1D). On the other hand, the endopod and exopod of the first and second abdominal segments were of similar size for both experimental groups, resembling those of immature females. However, the structure of the setae was qualitatively intermediate between the plumose setae of the control males and the simple setae of the mature females in 33.3% of the AGA males.

Table 2
Effect of AG ablation on morphological characters of *Cherax quadricarinatus* males over an experimental period of 300 days.

Characteristic	Time (days)									
	30		60		90		120		300	
	AGA ($N = 11$)	C ($N = 9$)	AGA ($N = 11$)	C ($N = 9$)	AGA ($N = 11$)	C ($N = 9$)	AGA ($N = 11$)	C ($N = 9$)	AGA ($N = 9$)	C ($N = 9$)
Complete regeneration of one fifth pereiopod (%)	27.3	33.3	27.3	11.1	27.3	11.1	27.3	0	27.3	0
Complete regeneration of both fifth pereiopods (%)	0	66.7	0	88.9	0	88.9	0	100	0	100
Development of the red patch (%)	0	0	27.3	11.1	54.6	11.1	63.6	77.8	88.9	77.8
Presence of male gonopores (%)	0	100	0	100	0	100	0	100	0	100
Presence of one *appendix masculina* (%)	0	33.3	0	33.3	0	33.3	0	22.2	0	22.2
Presence of both *appendices masculinae* (%)	0	66.7	0	66.7	0	66.7	0	77.8	0	77.8

Note: Comparisons were made between the control (C) and the andrectomized (AGA) treatments.

3.3. Reproductive system

Both experimental groups presented well-developed male reproductive systems (Fig. 1E), except for one AGA male lacking the distal and part of the middle portions of the left VD (Fig. 1F). From an anatomical point of view, the relative size of testes did not qualitatively differ between treatments. The histological analysis of the testes revealed a normal structure of the testicular lobes in both experimental groups.

The control males presented the following histological characteristics of the DVD: (1) an epithelium composed of a monolayer of cells tightly attached to a thick muscular sheath; (2) a secondary layer of the spermatophore composed of a homogeneous matrix (which appear pale blue in Masson-Trichrome staining), and cytoplasmatic droplets (which appear bright red in Masson-Trichrome staining) of an approximately same size, both homogeneously distributed; and (3) an approximately uniform distribution of the spermatic cord sections, all of which contained spermatozoa (Fig. 2A and B).

The DVD of the ablated animals showed a remarkable enlargement from a macroscopic point of view (Fig. 1F) with certain histological abnormalities. A clearly disorganized and degenerative epithelium was observed in some cases (Fig. 2D); also, an

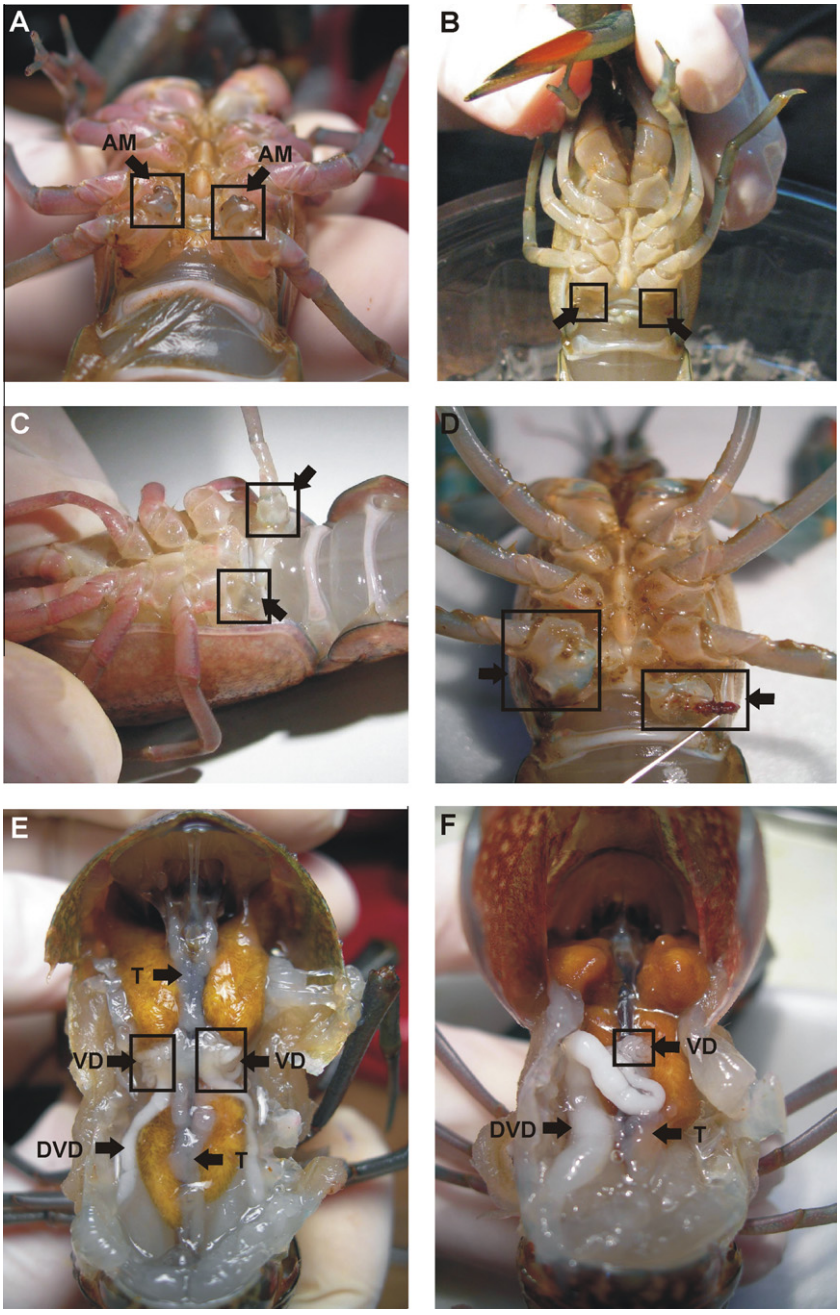

Fig. 1. Secondary sexual characters and reproductive system morphology of *C. quadricarinatus* males. (A) The arrows indicate the elongated *appendices masculinae* of a control male. (B–D) Anatomical alterations of AGA males following the AG ablation: (B) the arrows indicate the absence of the fifth pair of pereiopods and *appendices masculinae*; (C) the arrows indicate the regeneration of the left fifth pereiopod with no re-formation of the male gonopore, and the absence of the right fifth pereiopod; (D) the arrows indicate an abnormal regeneration of the left fifth pereiopod and a hypertrophied right fourth coxa with a posterior opening. (E) Normal structure of the male reproductive system. (F) Abnormal structure of the reproductive system in an AGA male, showing the absence of the distal and part of the middle portions of the left vas deferens, and an enlarged distal portion of the right vas deferens. AM: *appendices masculinae*, DVD: distal portion of the vas deferens, VD: proximal and middle portions of the vas deferens; T: testis.

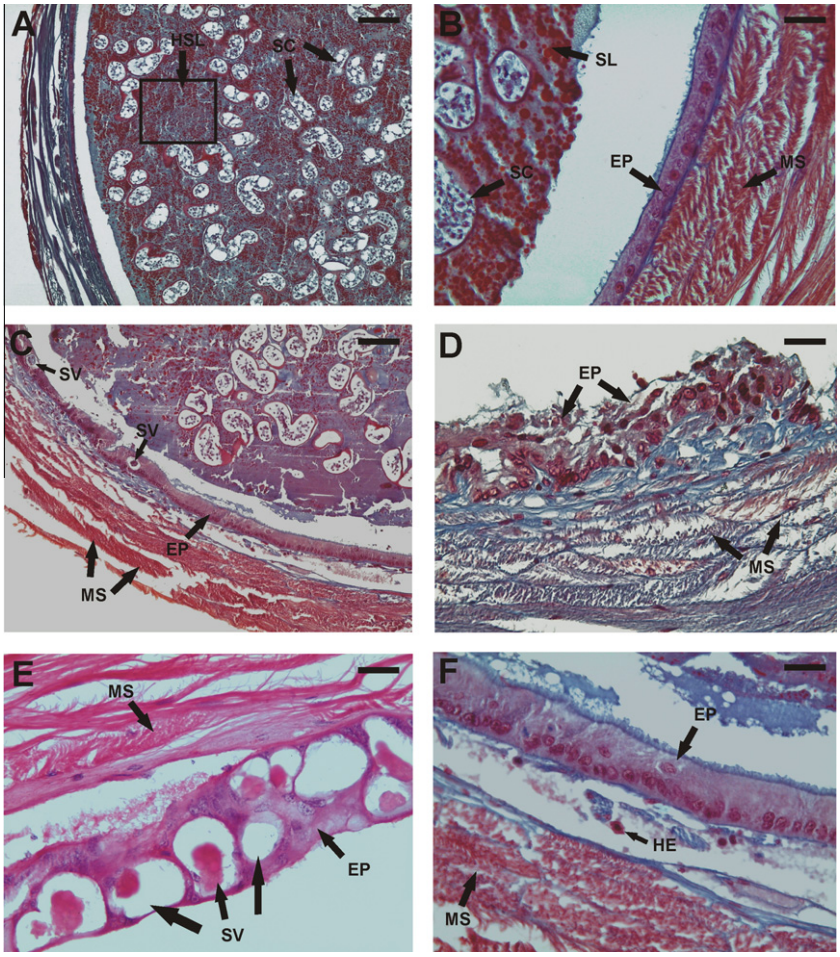

Fig. 2. Histological structure of the distal vas deferens (DVD) of *C. quadricarinatus* males stained with Masson Trichrome and Hematoxylin–Eosin. (A) General view and (B) detail of the DVD of control males. Arrows indicate the normal structure of the epithelium, muscular sheath, spermatic cord sections and secondary layer of the spermatophore. (C) General view of the DVD of an AGA male, showing an epithelium with some abnormal secretion "vacuoles" (arrows). (D–F) Detail of the alterations observed in the epithelium of the DVD of AGA males: (D) degenerative epithelium; (E) presence of abnormal secretory "vacuoles" in the epithelium; (F) presence of an hemocyte in the space between the epithelium and the muscular sheath. Scale bars: (A and C) = 45 μm; (B, D–F) = 10 μm. EP: epithelium, HE: hemocyte, HSL: homogeneous secondary layer of the spermatophore; MS: muscular sheath, SC: spermatic cord section, SL: secondary layer of the spermatophore, SV: secretory "vacuole".

abnormally increased secretory activity of the epithelium was evident in all cases (Fig. 2C and E). In certain regions of the DVD wall, the presence of hemocytes, which were identified according to the description of Martin and Hose [37], was observed in the space between the epithelium and the connective tissue surrounding the muscle fibers (Fig. 2F). The secondary layer of the spermatophore exhibited mainly three different types of alterations: (1) heterogeneous distribution of the two components of the secondary layer (Fig. 3A); (2) presence of cytoplasmatic droplets of different size, some of which were possibly composed of an amorphous debris (Fig. 3B); and (3) absence of the cytoplasmatic droplets (Fig. 3C). The distribution of spermatic cord sections in the DVD was heterogeneous since they were absent in certain regions (Fig. 3D); some of them contained no spermatozoa and others were coalescent (Fig. 3C). However, the quantitative analysis showed that both the number of spermatic cord sections within the DVD and the amount of spermatozoa within the spermatic cord sections were similar between control and ablated males (Table 1).

4. Discussion

Although *C. quadricarinatus* is considered a gonochoristic species, the presence of intersex individuals in wild and cultured populations with both male and female genital openings may indicate a high degree of sexual plasticity [5,25,39,53,56,65]. The intersex individuals have proven to be useful models to investigate the role of the AG on sex differentiation and gonad regulation. Khalaila et al. [25] and Barki et al. [4] obtained interesting results when studying the effects of AG ablation on the morphology and histology of intersex individuals that were functional males. In both studies, andrectomy caused an increase in GSI and diameter of oocytes in the female component of the reproductive system, and the activation of vitellogenesis.

The present study differs from those mentioned above because the AG ablation was performed in males of *C. quadricarinatus* (1.85 ± 0.03 g), which lack any female component, and the effects of such procedure on reproductive parameters and somatic growth were analyzed over a longer period (300 days).

In this study, no ovarian development was observed in andrectomized males. On the contrary, they presented a well differentiated male reproductive system, though certain abnormalities in the DVD could be recognized macroscopically and histologically. Moreover, they had neither male gonopores nor *appendices masculinae* in the regenerated fifth pereiopods. These results, together with those reported by Khalaila et al. [25] and Barki et al. [4], suggest that the absence of the AG removes the inhibition on the

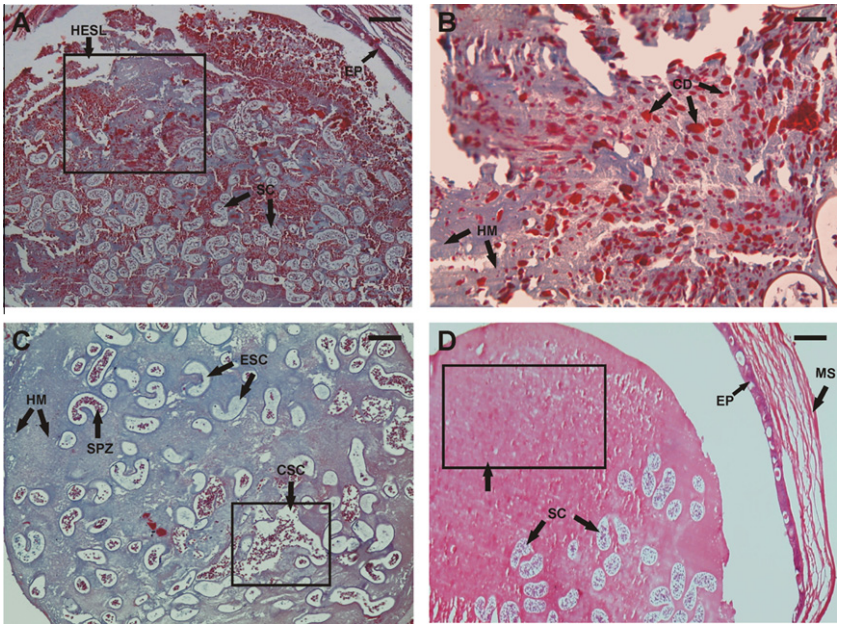

Fig. 3. Histological structure of the distal vas deferens (DVD) of andrectomized *C. quadricarinatus* males stained with Masson Trichrome and Hematoxylin–Eosin. (A–C) Alterations observed in the secondary layer of the spermatophore: (A) heterogeneous distribution of the cytoplasmatic droplets (stained red) and the homogeneous matrix (stained pale blue); (B) heterogeneous size of the cytoplasmatic droplets; (C) absence of the cytoplasmatic droplets. (C and D) Alterations observed in the distribution and structure of the spermatic cord sections: (C) some coalescent spermatic cord sections and others containing no spermatozoa; (D) region in the DVD with no spermatic cord sections (arrow). Scale bars: (A, C and D) = 45 μm; (B) = 10 μm. CD: cytoplasmatic droplets, CSC: coalescent spermatic cord sections, EP: epithelium, ESC: empty spermatic cord sections, HM: homogeneous matrix, MS: muscular sheath, SC: spermatic cord sections, HESL: heterogeneous secondary layer of the spermatophore, SPZ: spermatozoa.

development and maturation of the female reproductive system, but no shift from the male to the female reproductive system is induced when the former has already been differentiated.

However, ablation at a very early developmental stage successfully led to complete sex reversal in males of the freshwater prawn *M. rosenbergii* [1,57]. This suggests that the AG is crucial for the development of primary sex characters at an early stage of development and then it may be responsible for the development and/or maintenance of certain secondary sexual characters such as male gonopores and copulatory organs, as demonstrated for the freshwater crayfish *C. destructor* [15] and *P. clarkii* [62]. On this basis, the effect of AG ablation would depend on when it is performed.

The histological alterations observed in the AGA males may indicate that the gland also plays a role in maintaining the structure of the reproductive system throughout the animal's life, particularly of the DVD. Some of these alterations coincide with those observed by Bugnot and López Greco [6], who studied the structural alterations of the reproductive system in adult males of *C. quadricarinatus* reared in captivity. They also detected in abnormal males an epithelium with an increased secretory activity, together with the presence of granular and hyaline haemocytes. The alterations in the spermatic cord and the heterogeneous secondary layer of the spermatophore found in the AGA males of the present study may indicate that the gland is also involved in the secretory activity of the DVD and the structural organization of the spermatophore. Particularly, the absence and the abnormal structure of the cytoplasmatic droplets might have important implications since they have been proposed to play a role in the adhesive properties of the spermatophore, which has to be attached to the female sternum to ensure the fertilization of the oocytes, immediately after mating [33]. Therefore, this is the first report of a potential relationship between the AG and the formation of the spermatophore in *C. quadricarinatus* males.

The testes of the AGA males had a completely normal structure. Since spermatozoa were seen both in the testes and in the DVD, the AG removal seemed to have no effect on the initiation and completion of spermatogenesis. This is in agreement with results of Payen and Amato [46], who observed that some reptantians could undergo spermatogenesis in the absence of AGH if this process had been previously initiated by the hormone. However, some AGA males had spermatic cord sections with no spermatozoa in the DVD, suggesting that the rate of sperm release from the testis into the VD [25] or the intensity of spermatogenic activity [10] could be affected by AG ablation. The tendency toward a higher GSI value in the AGA males compared to the control males may be due to an increased secretory activity of the DVD rather than to a more developed reproductive system and/or an increased sperm production (not measured in the present study).

In *C. quadricarinatus*, the AG is believed to control certain male secondary sexual characters, such as the red patch, located on the outer edge of the cheliped, and the proportionally longer and wider propodus in relation to females. In this sense, Barki et al. [3] and Manor et al. [35] demonstrated that AG implantation in young females of this species induced the development of secondary sexual characters resembling those in normal males of the same size. However, in the present study AGA males developed the same secondary sexual characters as control males. Moreover, a few functional females with red patch on one or both chelipeds were previously observed in the authors' laboratory (unpublished). Thorne et al. [63], who also reported a low percentage of females with red patch, suggested that they had AG. Finally, Khalaila et al. [25] found that this character remained unchanged in andrectomized intersexes throughout the experimental period (50 days). On this basis, it seems that the absence of the AG from a certain developmental stage may not prevent the differentiation and maintenance of the red patch; nonetheless, further investigation is needed in order to accurately determine the relationship between the gland and this character.

In regard to somatic growth, no information was available to the moment on the effect of AG ablation on growth in *C. quadricarinatus* males. The differential growth between sexes of this species

[13,20,24,48,55] was hypothesized to be due to a greater amount of energy invested in ovarian maturation by females than in spermatogenesis by males, rather than to competitive behavior [35,64]. In addition, the AG has also been hypothesized to directly induce somatic growth in males via the secretion of a hormone [15,35]. If this was the case, then the AGA males would have shown lower growth values, as reported for andrectomized males of *M. rosenbergii* by Sagi et al. [52,54]. The fact that there were no differences in SGR and GI between C and AGA males may indicate that the AG has an indirect effect on growth, through the inhibition of ovarian development and maturation. As mentioned above, the testes and VD in the andrectomized males were as well developed as in the control. Therefore, it is expected that their energetic investment in maintaining the structure of the reproductive system will be similar, leading to a comparable somatic growth. This is supported by previous studies of AG implantation in juvenile females of *C. quadricarinatus*, showing a decrease in vitellogenin gene expression and hemolymph vitellogenin levels, followed by an increase in growth rate [3,35]. The inhibitory effect of the AG on ovarian development has also been confirmed for AG-implanted females of other decapod species, such as the mud crab *S. paramamosain* [12], the crayfish *P. clarkii* [62] and *C. destructor* [15].

The manipulation of the AG in decapod crustaceans of economic value in aquaculture seems to be an interesting way to produce greater yields and profits, particularly for species with differential growth between sexes [11,13,20,24,30,48,50,55,57]. Indeed, the neo-female technology developed in the freshwater prawn *M. rosenbergii* may represent a direct way of obtaining all-male populations (whose culture is economically advantageous) [30,44,51], without the costs and problems associated with the hand-sexing system [1,13,49,57]. This procedure seems unfeasible under the conditions of the present study, as *C. quadricarinatus* males failed in accomplishing sex reversal. Pattillo [45] ablated juveniles III (recently independent juveniles that were sexually undifferentiated) of *C. quadricarinatus* and he also failed in obtaining sexual reversion. Growth and survival of the ablated juveniles did not differ from those of control animals. However, he observed an effect of AG ablation on the development of secondary sexual characters. This coincide and reinforce the results obtained in the present study, and suggests that even performing andrectomy at a very early stage of development the reversion of primary sexual characters is difficult to achieve in this species. It must be borne in mind that the ablation in *M. rosenbergii* led to high mortality and resulted in a low percentage of neo-females (1.28%) [1]; also, the ablation at late developmental stages lead to partial feminization or no feminization at all [40,43]. Given these constraints, a huge number of males at a very early developmental stage would be necessary to achieve the desired outcome.

In brief, the information provided in the present study is complementary to that from andrectomized intersexes, contributing to shed some light on the role of the AG in somatic growth and reproductive parameters of *C. quadricarinatus*. Further research involving the manipulation of the AG in juveniles III and the evaluation of its effects for a longer period is needed to clarify the role of the gland in the differentiation of the red patch. The isolation and characterization of the AGH by molecular-based techniques could also help to understand better the relationship between the AG and the development of that secondary sexual character. Also, the sperm production of andrectomized males should be measured in future studies in order to discard a possible effect of AG ablation on the intensity of the spermatogenic activity.

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