Brazilian Journal of Biological Sciences, 2019, Vol. 6, No. 13, p. 413-428.
ISSN 2358-2731
https://doi.org/10.21472/bjbs.061309

Effect of administration of neurotransmitters: Serotonin and dopamine on testicular maturation in the freshwater crab *Travancoriana schirnerae* Bott, 1969 (Crustacea: Gecarcinucidae)

Navya Gopal and Arath Raghavan Sudha Devi*

Department of Zoology. Mary Matha Arts & Science College. Wayanad. Kerala 670 645. India. *Email: arsudhadevi@gmail.com.

Abstract. The current investigation aimed at the effect of administration of the neurotransmitters, serotonin and dopamine, on testicular maturation in the freshwater crab *Travancoriana schirnerae* Bott, 1969 (Crustacea: Gecarcinucidae). Adult males in active, inactive and revival phases of spermatogenesis were injected with serotonin and dopamine in multiple doses and their dissected testes were submitted to histomorphological examinations. Though serotonin treatment during active phase showed increased values for testicular index, acinar diameter and proportion of mature spermatozoa, only the testicular index values differed statistically from that of controls. Serotonin administration during inactive phase caused an increase in testicular index and acinar diameter, reduction in intra and interacinar spaces and pycnosis of germ cells and detection of division stages. A notable increase in the testicular index, acinar diameter and fresh batches of spermatozoa was perceptible in crabs injected with serotonin during revival phase. In contrast, dopamine injection showed a decline in testicular activity irrespective of the phase of spermatogenesis, as evinced from low testicular index, acinar diameter, presence of small, irregularly shaped acini with indistinct acinar boundaries, reduction in the number of mature spermatozoa and absence of division stages. To conclude, the stimulatory neurotransmittant, serotonin can be used to induce testicular maturation in species of aquaculture potential.

Keywords: Serotonin; Dopamine; Testicular maturation; Histological parameters.

Received
May 21, 2019

Accepted
August 23, 2019

Released
August 31, 2019

Full Text Article

ORCID
0000-0002-0899-9619
Navya Gopal

0000-0003-4062-8073
Arath Raghavan Sudha Devi

Introduction

Neurotransmitters are substances involved in the production and release of neurohormones which play important roles in the regulation of growth and reproduction in crustaceans (Sarojini et al., 1995; Reddy, 2000; Vaca and Alfaro, 2000; Kishori and Reddy, 2003). The known neurotransmitters in crustaceans are serotonin or 5-hydroxytryptamine (5-HT), dopamine (DA), acetylcholine (Ach), gamma-amino butyric acid (GABA), red pigment-concentrating hormone (RPCH) and octopamine (OA). Among the

neurotransmitter candidates tested for potential functions in crustacean reproduction, serotonin and dopamine draws more attention than the others. They have opposing effects on crustacean reproduction; serotonin stimulates gonadal maturation while DA hinders gonadal maturation in both the sexes (Sarojini et al., 1994; 1995).

Serotonin is a ubiquitous substance present in plants and animals, derived from the amino acid tryptophan in some enzymatic pathway. It is dispersed in the optic, cerebral, stomatogastric and thoracic ganglia and circumoesophageal connectives (Butter and Fingerman, 1983; Laxymr, 1984; Fingerman et al., 1994; Tinikul et al., 2008) in crustaceans and is known to function as a neurotransmitter, neuromodulator or as a neurohormone (Kulkarni and Fingerman, 1992). It stimulates ovarian maturation in females and testicular maturation and development of androgenic glands in males (Sarojini et al., 1993; Siangcham et al., 2013; Aprajitha et al., 2014; Akhila et al., 2016). The stimulatory action of serotonin (5-HT) on the ovary, testis and androgenic gland was found to be indirect. In females, 5-HT induces the brain and thoracic ganglion to release the gonad stimulating hormone (GSH) that stimulates precocious growth and maturation of the ovary. In males, the GSH triggers testicular maturation via the androgenic gland hormone (AGH) released by the androgenic gland (Sarojini et al., 1994; Fingerman, 1997). Other functions attributed to 5-HT include regulation of mood, appetite, rhythmic behaviour, sleep, memory (Kravitz, 1999), migration of the proximal retinal pigment, osmoregulation and mechano-reception (Fingerman, 1997).

Dopamine is a catecholamine, distributed extensively in the central nervous system of crustaceans (Kuo et al., 1995; Tierney et al., 2003). Like 5-HT, DA is known to function as a neurotransmitter, neuromodulator or as a neurohormone (Lingle, 1981; Lüschen et al., 1993). It acts as an inhibitor of GSH and a stimulator of GIH or both (Sarojini et al., 1995). Apart from reproduction, DA is concerned with the control of glucose metabolism (Simonneaux et al., 1991; Rey et al., 2001), ionic and osmotic balance in crustaceans (Morris, 2001).

Many workers have documented the antagonistic effects of 5-HT and DA on testicular maturation. Male fiddler crab, *Uca pugilator* given injections of 5-HT showed a dose dependent increase in testicular activity (Sarojini et al., 1993). Stimulation of testicular maturation following 5-HT administration was investigated by Sarojini et al. (1994) in the freshwater crayfish *Procambarus clarkii*. Like 5-HT, 5-HT releaser fenfluramine and 5-HT potentiator fluoxetine induced testicular maturation in crustaceans (Fingerman, 1997). In vivo effects of DA on maturation of the testis in *U. pugilator* were described by Sarojini et al. (1995). Influence of 5-HT and DA on testicular maturation in the freshwater prawn *Macrobrachium rosenbergii* was determined by Polijareon (2011). In Pacific shrimps, *Penaeus stylirostris* and *P. occidentalis*, Montoya and Vega (2011) observed stimulatory and inhibitory effects of 5-HT and DA on spermatogenesis. Siangcham et al. (2013) studied the effects of 5-HT and DA on androgenic gland activity in *M. rosenbergii*. A significant enhancement and delay in testicular activity was observed by Prasad et al. (2014) in *Barytelphusa guerini* following 5-HT and DA injections.

The traditional practice of eyestalk extirpation to induce maturity and spawning often associated with problems like high mortality of the broodstock and deterioration in quality and quantity of the sperm. Alternate nonsurgical methods include the use of neurotransmitters, steroid hormones, anti GIH antibody and double stranded RNA interference. Administration of stimulatory neurotransmittants like serotonin, spiperone or leucine

enkephalin to induce precocious spermatogenesis will not cause any ill effects like diminution of quality or quantity of the sperm. Moreover, it is cost effective. The selective use of neurotransmitters may be advantageous in improving the culture of commercially important crustaceans by producing quality sperms. The edible freshwater crab *Travancoriana schirnerae* Bott, 1969 (Crustacea: Gecarcinucidae) was selected as the test animal because of its local abundance, wide distribution throughout the wetlands of Wayanad and commercial value. We intend to study the effect of exogenous administration of stimulatory and inhibitory neurotransmittants: serotonin and dopamine on testicular maturation through histological means.

Materials and methods

Adult intermoult male crabs (CW 4-5 cm) were collected from the paddy fields near Mary Matha Arts & Science College campus, Mananthavady, Wayanad (Kerala, India) during June 2017 to June 2018. They were habituated to the conditions of the laboratory for 3 to 4 days and fed ad libitum with pulses and chopped beef liver. Their carapace width, moult stages and wet body weights were recorded. Moult stages were determined by noticing the changes in the carapace texture and setal development of epipodite of the third maxilliped (Anilkumar, 1980).

Serotonin and dopamine obtained from Sigma Aldrich, St. Louis, MO, USA was used for injection (1 mg/1 mL distilled water). Every month, 30 males were collected and divided into three groups of 10 each. Group I formed the control, Group II and III which received 5-HT and DA (10 µL each) injections, respectively, on 1st, 7th, 14th and 21st day through the arthrodial membrane at the base of the coxa of the third walking leg formed the experimentals. Both control and experimental crabs were sacrificed on 30th day. Testes from control and experimental crabs were carefully dissected out; their wet weights were recorded to calculate the testicular index. Tissues were then fixed in Bouin's solution and processed for histological observations. Photomicrographs were taken with a Leica DM 500 Research Microscope equipped with a DG 330/210 camera using Biowizard software.

The mean testicular index, acinar diameter, proportion of germ cells, degree of condensation of germ cells, division stages and presence or absence of fresh batches of sperms were the criteria used to examine the effect of 5-HT and DA administration on testicular maturation. The data were given as mean±SD and the differences in mean values of controls and experimentals were analysed using analysis of variance (ANOVA). A probability value of $P < 0.05$ was considered statistically significant.

Results

Morphology of the male reproductive system

The male reproductive system of *T. schirnerae* is bilateral lying below the dorsal carapace and above the hepatopancreas. It consists of paired testes, vasa deferentia, ejaculatory ducts, penises and gonopods. The testes (16-24 mm in length and 1.0-2.5 mm in width) are H-shaped, white elongate structures located dorsally in the cephalothoracic region beneath the pericardial area. From each testis emerges a creamish white, convoluted tube called the vas deferens (VD) almost occupying the entire thoracic cavity. The VD is divided into three regions: anterior vas deferens (AVD), middle vas deferens (MVD) and posterior vas deferens (PVD). The AVD is a white, thick and tightly coiled structure positioned on either side of the cephalothorax, posterior to the dorsal part of the stomach. The coils of AVD lead to a white, thick and loosely coiled tube called the MVD. The MVD of each side is continued as a thin, straight tube

called the PVD. Each PVD is connected with a slender ejaculatory duct which leads to a soft, flexible, membranous papilla, called the penis. The gonopods leaves a lateral basal opening into which the penis extends.

Histology

The testis of *T. schirnerae* is a tubular organ, formed by microscopically visible round, oval or elongate lobules or acini where spermatogenesis takes place. Each acinus is surrounded by a layer of squamous epithelium called the basal lamina. The acini are comprised of germ cells in one or more stages of spermatogenesis. Based on the stages of development, six types of germ cells were distinguished in the testis:

Primary gonia: Largest of the germ cells, round or oval in shape, found proliferating from the acinar periphery. Possessed oval nuclei (8.89±1.92 µm) with scattered chromatin granules and mildly eosinophilic cytoplasm with no clear boundaries.

Secondary gonia: Round or oval in shape, characterized by oval basophilic nuclei (6.76±0.83 µm) containing fine granular chromatin and indistinct cytoplasm. Cell boundaries not clearly demarcated.

Primary spermatocytes: Oval **c**ells with spherical nuclei (5.49±1.07 µm) enclosing peripherally arranged chromatin. Cytoplasm appeared thin and mildly eosinophilic with distinct cell boundaries.

Secondary spermatocytes: Small oval cells with distinct cell boundaries, poorly detected eosinophilic cytoplasm and nuclei (4.13±0.09) containing fine granular chromatin.

Spermatids: Round cells, smaller than the spermatozoa; possess highly basophilic condensed nuclei (3.01±0.04 µm) and hardly detectable cytoplasm.

Spermatozoa: Spherical cells (3.19±0.51 µm across); have mildly basophilic nuclei with basophilic acrosomes in the centre. Cytoplasm negligible.

The annual spermatogenic activity was divided into active, inactive and revival phases. The active phase was noticed during May-June. A perceptible decline in testicular activity was observed from September to February after the annual mating events in July-August. The spermatogenic activity got revived in March-April after a long period of rest or inactivity. The present study assessed the effect of exogenous administration of the neurotransmitters serotonin and dopamine on testicular maturation by comparing the testicular indices, number and size of acini, presence or absence of intra and inters acinar cell free spaces, mature spermatozoa and division stages and degree of pycnosis of germ cells of treated and control crabs.

Morphology and histology of testis of control crabs during active phase (May-June)

The testis appeared opaque white with a mean testicular index of 0.19±0.03 (Table 1). The testis was consistently in a state of high activity, evinced by the presence of larger acini (357.21±5.88 µm) with prominent acinar boundaries, absence of inter and intra acinar spaces, chromatin condensation and degenerative stages. Large number of acini encompassed mature spermatozoa followed by a few enclosing spermatocytes, spermatids and gonia.

Acini fully packed with mature spermatozoa comprised of 69% of the total germ cells. Primary and secondary gonia constituted 3 and 4% respectively of the total number of germ cells. Of the total primary gonia, 2% were seen compactly packed in acini. Their chromatin was evenly scattered as fine granules in the nucleoplasm and showed practically no signs of condensation. The remaining primary gonia (1%) were seen arranged towards the periphery and the centre of these acini was occupied by

active secondary gonia. Secondary gonia (4%) were seen fully packed in some acini.

Primary spermatocytes comprised 9% of the total number of germ cells, out of which 3% were seen undergoing meiosis. Secondary spermatocytes belonged to 7%, of which 3% were seen densely packed in acini, 2% occupied central positions in acini containing spermatids and the remaining 2% displayed meiotic prophase and metaphase stages. Of the total spermatids (8%), 6% were found fully packed in acini; spermatids undergoing spermiogenesis was evident in 2% (Figure 1A-B).

Morphology and histology of testis of serotonin treated crabs during active phase

Administration of serotonin significantly increased the testicular index of treated crabs (0.26±0.02) over the controls (0.19±0.03). The mean acinar diameter of the injected group was 366.84±3.597 µm, which was not significantly different from the control group (357.21±5.88 µm) (Table 1). Light microscopic examination of testis of the experimental crabs demonstrated large, fully distended acini with mature sperms (87%) which was noted to be the most remarkable feature of testis of treated crabs. However, a small proportion of gonia (3%), spermatocytes (6%) and spermatids (4%) could also be noticed in the testis. Signs of degeneration were not perceptible in the testis (Figure 1C-D).

Morphology and histology of testis of dopamine treated crabs during active phase

The testicular index (0.12±0.01) and mean acinar diameter (279.63±6.61 µm) of crabs that received dopamine were significantly smaller than the corresponding values of the controls and serotonin treated crabs (Table 1). Administration of dopamine resulted in small, irregularly shaped acini with indistinct acinar boundaries, distinct inter and intra acinar spaces.

A notable feature in the testis of dopamine treated crabs was the drastic reduction in the percentage of mature sperms (31%). About 27% of the total germ cells belonged to gonial cells, 30% spermatocytes and 12% spermatids. Dividing stages of germ cells were not perceptible in experimental crabs. In some acini, arrest of division of gonial cells was detected (Figure 1E-F).

Morphology and histology of testis of control crabs during inactive phase

A perceptible decline in testicular activity was observed after the annual mating events in July-August. The testis remained inactive from September to February as evidenced by a perceptible decline in the mean testicular index and acinar diameter values, nuclear pycnosis of germ cells, absence of mature spermatozoa and division stages.

The testicular index and acinar diameter values found further reduced to 0.11±0.02 and 221.45±0.02 µm respectively during September/October (Table 1). Inter and intra acinar spaces were prominent in the testis. Gonial cells undergoing degeneration (21%) was perceptible in many acini. However, a small number of acini displayed proliferating primary gonia (1%). A few acini contained secondary gonia (2%) along their margins and residual sperms towards the central portions. Quite a large number of acini carried loosely arranged spermatocytes (42%) with condensed chromatin undergoing degeneration. Phagocytes were noticed among acini containing the degenerating spermatocytes. A few acini have spermatids (3%) which were clumped and pycnotic in nature. Sperm degeneration (31%) was a prominent feature during this period.

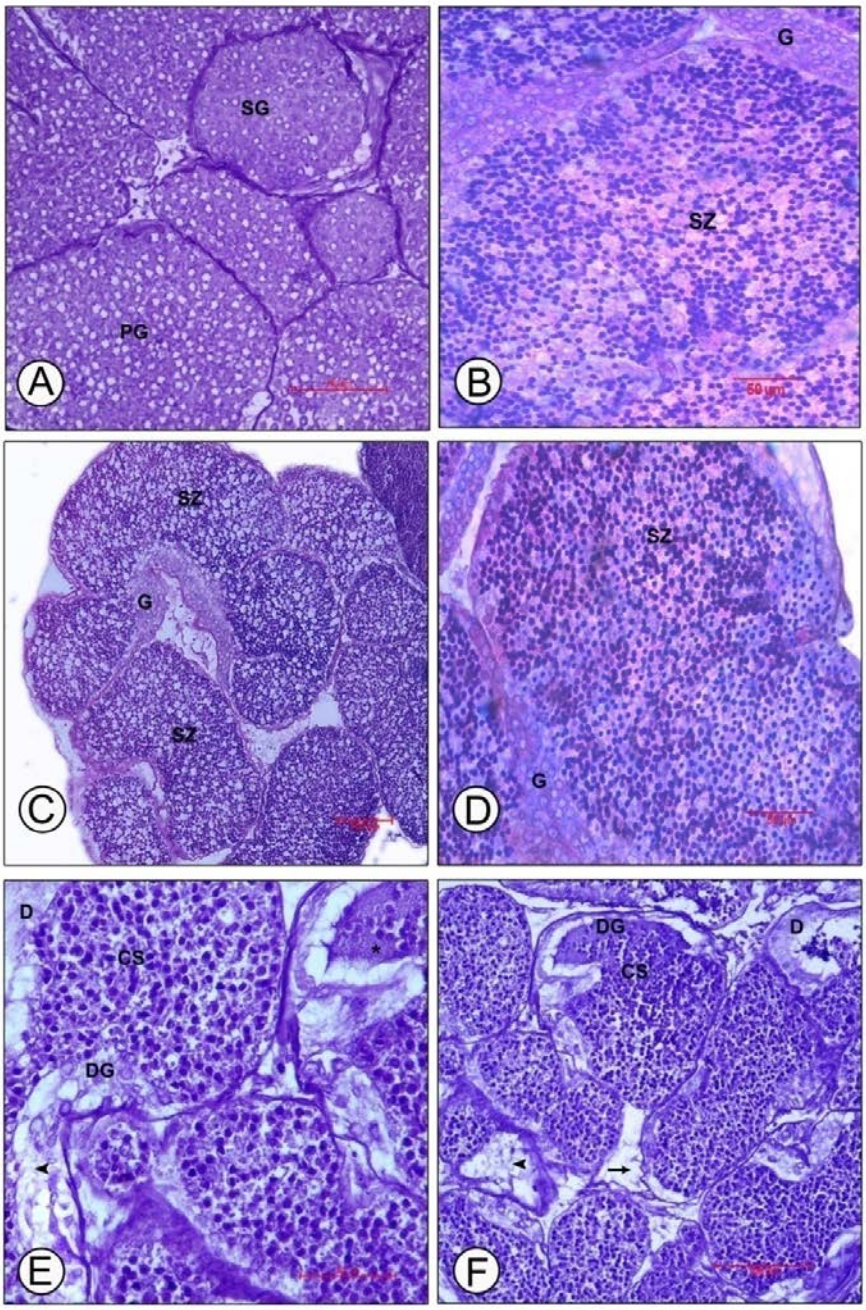

Figure 1. Light microscopic features of testis of control and treated *Travancoriana schirnerae* during active phase. A-B: Large acini with prominent acinar boundaries fully packed with gonia and mature spermatozoa in control crabs C-D: Testis demonstrating large, fully distended acini without inter and intra acinar cell free spaces and fully packed mature spermatozoa in serotonin treated crabs E: Testis of dopamine treated crabs displaying irregularly shaped acini with indistinct acinar boundaries and inter and intra acinar spaces; F: Acini with fewer spermatozoa and inactive gonial cells in dopamine treated crabs. CS: Clumped sperms; D: Debris; DG: Degenerating gonia; G: Gonial cells; SZ: Spermatozoa; Arrow indicates inter acinar space; Arrow heads indicate intra acinar spaces; Asterisk indicates degenerating spermatocytes.

Table 1. Effect of serotonin and dopamine administration on mean testicular index and acinar diameter during various phases of spermatogenesis in the freshwater crab *T. schirnerae*.

Phases of spermatogenesis	Group	Testicular index	Acinar diameter
		Mean±SD	Mean±SD
Active (May-June)	Control	0.191±0.035	357.21±5.882
	Serotonin treated	0.264±.0.028*	366.84±3.597#
	Dopamine treated	0.128±0.015*	279.630±6.616***
Inactive (Sep-Oct)	Control	0.119±0.026	221.454±0.026
	Serotonin treated	0.154±0.018*	245.508±13.413**
	Dopamine treated	0.089±0.112*	205.454±7.519**
Inactive (Nov – Dec)	Control	0.106±0.014	197.376±5.831
	Serotonin treated	0.161±0.040*	261.376±30.673**
	Dopamine treated	0.069±0.032*	181.376±3.775**
Inactive (Jan- Feb)	Control	0.086±0.016	176.502±5.096
	Serotonin treated	0.167±0.029**	279.630±6.616***
	Dopamine treated	0.063±0.014*	168.502±2.394*
Revival (March-April)	Control	0.132±0.041	203.576±5.130
	Serotonin treated	0.177±0.009*	275.214±5.883***
	Dopamine treated	0.083±0.010*	186.502±3.412***

The values are represented as Mean±SD
*P<0.05; ** P<0.01; *** P<0.001; # not significant

Spermatogenic activity was slowed down by November/December, made clear by a significant drop in GSI (0.10±0.01) and acinar diameter (197.37±5.83 µm) (Table 1). Inter and intra acinar spaces become more conspicuous. About 43% of the total germ cells were comprised of pycnotic spermatocytes and 32% pycnotic gonia. Phagocytes were frequently encountered in those acini which contained degenerating germ cells. Residual sperms (25%) clumped to form pycnotic masses in acini enclosing gonia and spermatocytes. About 10% of the total area of the testis was devoid of normal germ cells, often filled with tissue debris only.

A total pause in spermatogenic activity was detected during January/February demonstrated by small, shrunken acini and intense chromatin condensation of germ cells. The GSI (0.08±0.01) and acinar diameter (176.50±5.09 µm) were markedly reduced and cell free spaces within and between the acini become more conspicuous (Table 1). Pycnotic gonia constituted 27% of the total germ cells indicating inactivity. Forty four percentage of the total germ cells in the testis encompassed degenerating spermatocytes. Many acini contained loosely arranged residual sperms (29%). Spermatolysis was perceptible in acini containing sperm masses. Acini filled with debris were also noted in some area of the testis (Figure 2A-C).

Morphology and histology of testis of serotonin treated crabs during inactive phase

Serotonin treatment led to a surge in activity of the testis, evident from the increased number of germ cells, reduction in inter and intra cell free spaces and pycnosis of germ cells and the presence of mitotic and meiotic division stages.

In September/October, serotonin injection caused a significant rise in the testicular index (0.15±0.02) and mean acinar diameter values (245.50±13.41) compared with the control crabs (221.45±0.02 and 0.11±0.02 respectively) (Table 1). The testis of treated crabs was characterized by the disappearance of inter and intra acinar

spaces, rise in the number of active germ cells, reduction in the degree of chromatin condensation and the presence of division stages. Primary (9%) and secondary gonial cells (11%) were found gradually loosing their chromatin condensation. Cell debris was noticed amidst these gonia. Altogether, primary and secondary spermatocytes dominated (38 and 23%) the testis of treated groups, of which some were observed to undergo meiosis. Spermatids comprised 10% of the total germ cells. In some acini, they were pycnotic while in others, progress of spermiogenesis could be perceived. About 9% of the total germ cells belonged to the spermatozoa stage which includes residual as well as mature spermatozoa.

Serotonin treatment considerably elevated the testicular index (0.16±0.04) and acinar diameter (261.37±30.67) values during November/December (Table 1). In certain areas of the testis, signs of revival of spermatogenic activity was apparent, corroborated by the decreased inter and intra acinar spaces and absence of nuclear pycnosis. Medium to large acini with distinct acinar boundaries appeared densely packed with germ cells. Proliferating primary (7%) and active secondary gonia (10%) were observed in some acini. However, some regions of the testis still exhibited gonial degeneration. About 56% of the total germ cells were in the spermatocyte stage with a good number of them undergoing meiosis. Moreover, injected group showed a spectacular rise in the number of spermatids undergoing spermiogenesis (20%) indicating the production of fresh batches of sperms. The proportion of residual sperms got considerably decreased.

The testicular index (0.16±0.02) and acinar diameter (279.63±6.61) values progressively increased in treated crabs than their controls during January/February (Table 1). The percentage of gonia (12%) was further reduced and there occurred a noticeable increase in the percentage of secondary spermatocytes (38%) and spermatids (40%). Primary gonia were seen mingled with secondary gonia in some acini. Acini containing secondary spermatocytes were densely packed; a few primary spermatocytes were observed to undergo meiotic proliferation. Spermatids undergoing spermiogenesis intermingled with secondary spermatocytes filled the entire acini in some area of the testis. Mature sperms (10%) were seen scattered in some acini (Figure 2D-F).

Morphology and histology of testis of dopamine treated crabs during inactive phase

Dopamine injection induced a state of inactivity as evidenced by the presence of small, shrunken acini, reduction in the number of germ cells, prominent inter and intra acinar spaces, absence of division stages and crowding of pycnotic germ cells.

The progressive fall in mean testicular index (0.08±0.11) and acinar diameter (205.45±7.51 μm) during September/October pointed out testicular inactivity in dopamine treated crabs (Table 1). Inter and intra acinar spaces were found prominent. The testis was devoid of mitotic or meiotic activities. The acini primarily consisted of pycnotic gonia (33%) and residual sperms (43%). A gradual degeneration of spermatocytes (22%) was perceptible in many acini. Spermatids (2%) were scarcely observed and they lacked their typical round shape and possessed indistinct nuclei, showing signs of degeneration. About 14% of total testicular area was filled with tissue debris in treated crabs of this period.

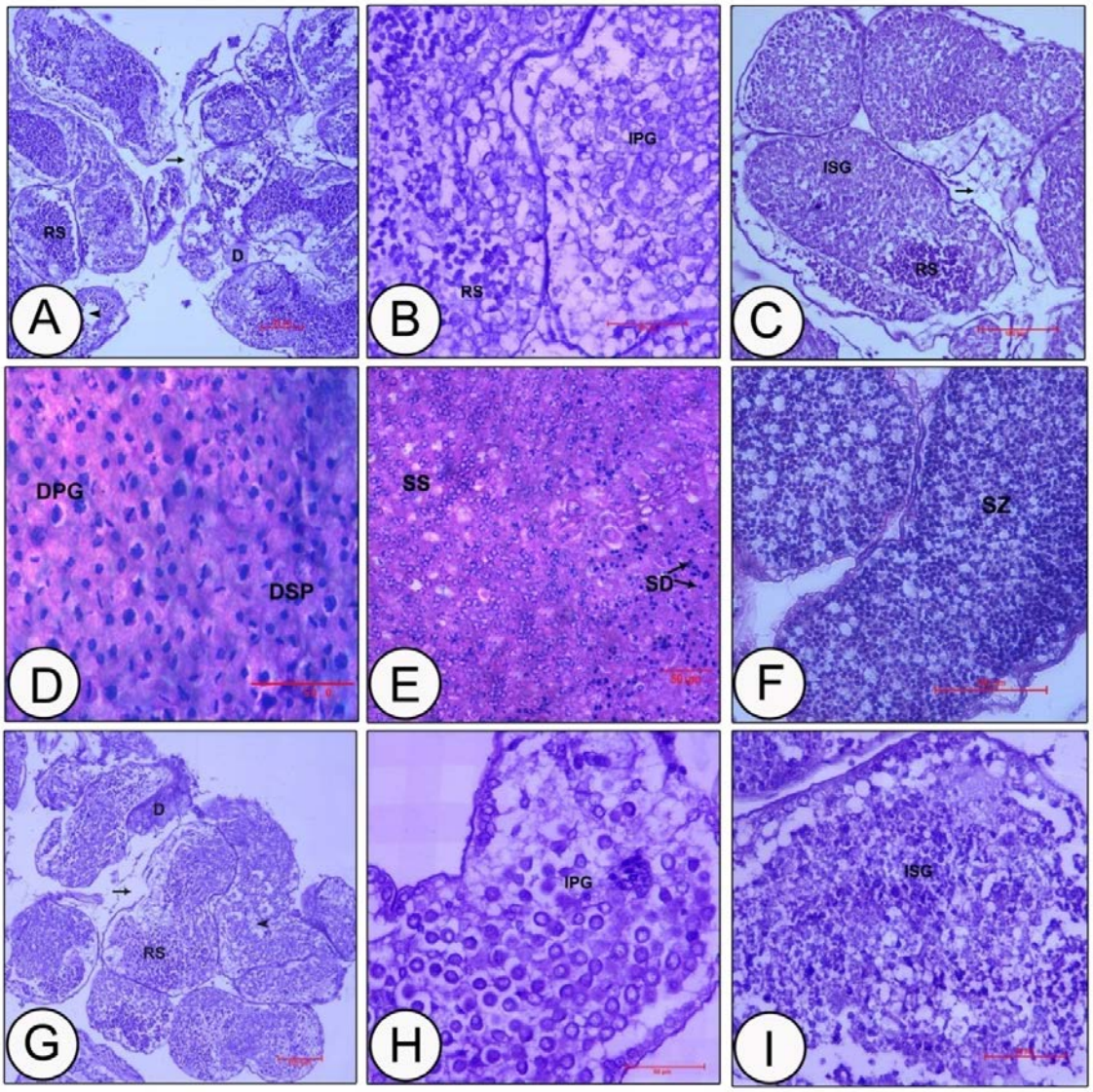

Figure 2. Testicular histology of control and experimental *Travancoriana schirnerae* during inactive phase. A: Testis exhibiting acini with conspicuous cell free spaces, pycnotic masses of residual sperms and cell debris in control crabs; B: Testicular acini of control crabs demonstrating degeneration of primary gonia; C: Secondary gonia undergoing degeneration and condensed residual sperm masses towards the periphery in controls; D. Gonial cells undergoing mitosis in serotonin injected crabs; E: Serotonin treated testis portraying active spermatids and secondary spermatocytes; F: Testis showing a spectacular rise in the number of fresh batches of sperms in serotonin treated animals; G: Testis dominated by acini with prominent inter and intra acinar spaces in dopamine administered crabs, H: Pycnosis of primary gonia in dopamine injected animals; I: Testicular acini of dopamine treated crabs illustrating degeneration of spermatocytes. D: Debris; DPG: Dividing primary gonia; DSP: Dividing spermatocytes; IPG: Inactive primary gonia; ISG: Inactive secondary gonia; RS: Residual sperm; SD: Spermatid; SS: Secondary spermatocyte; SZ: Spermatozoa; Arrow indicates inter acinar spaces; Arrow head indicates intra acinar spaces.

A steady decline in the testicular index (0.06±0.02) and acinar diameter (181.37±3.77 µm) was observed in injected crabs during November/ December (Table 1). Acinar and germ cell boundaries were indistinct. Many

acini appeared intensely basophilic due to nuclear pycnosis of gonia (36%), spermatocytes (30%) and spermatids (2%). In some acini, residual sperms (32%) were seen crowded either at the centre or periphery. Compact masses of intensely basophilic spermatozoa intermingled with secondary spermatocytes were perceptible in some areas of the testis. Considerable amount of debris were detected in acini containing degenerating germ cells. Phagocytes were noticed among debris in most of the acini.

Dopamine treatment slowed down testicular activity during January/February as apparent from a drop in testicular index (0.06±0.01) and acinar diameter (168.50±2.39 μm) values, chromatin condensation of gonia (26%), spermatocytes (28%) and spermatids (1%), dissolution of residual sperms (45%) and the presence of extensive inter and intra acinar cell free spaces. Phagocytes were noticed amidst degenerating germ cells in some acini (Table 1) (Figure 2G-I).

Morphology and histology of testis of control crabs during revival phase (March-April)

The spermatogenic activity got revived in March/April evinced by a noticeable increase in the testicular index (0.13±0.04) and acinar diameter (203.57±5.13 μm) (Table 1). The acini appeared large and germ cells showed relatively less degree of chromatin condensation. The testis appeared active with a rise in number of germ cells. Some acini containing degenerating germ cells were still present. Spermatogonia found to be the predominant cell type in the testis during March. Primary spermatogonia (40%) were either compactly packed or arranged mostly towards the periphery of acini containing secondary gonia. Large number of primary gonia were seen undergoing mitotic division. Secondary spermatogonia constituted 24% of the total number of germ cells and were detected amidst the primary gonia in some acini. Chromatin condensation was perceptible only in a few gonia. Primary and secondary spermatocytes (11 and 14%) were densely packed, undergoing active meiosis. A small number of spermatids (6%) and residual sperms (5%) were also apparent in some areas of the testis. By April, spermatids (39%) and secondary spermatocytes (36%) constituted a major proportion of the germ cells followed by small percentages of primary gonia (8%), secondary gonia (14%) and residual sperms (3%) (Figure 3A-B).

Morphology and histology of testis of serotonin treated crabs during revival phase

Testicular growth was stimulated in crabs injected with serotonin compared to the controls evidenced by a notable increase in the testicular index (0.17±0.00), acinar diameter (275.21±5.88 μm) and the presence of fresh batches of sperms (Table 1). The acini become enlarged and fully packed with active germ cells. In contrast to the testis of control crabs, chromatin condensation was completely absent in serotonin treated crabs. In some acini, primary gonia (4%) were seen mingled with the secondary gonial cells (6%). Secondary spermatocytes (41%) and spermatozoa (32%) were the prominent germ cells in the testis of treated crabs. Actively dividing primary spermatocytes constituted 7% of the total number of germ cells. Spermatids undergoing spermiogenesis constituted about 10% of the total germ cells. Residual sperms were completely absent in the injected groups (Figure 2C-D).

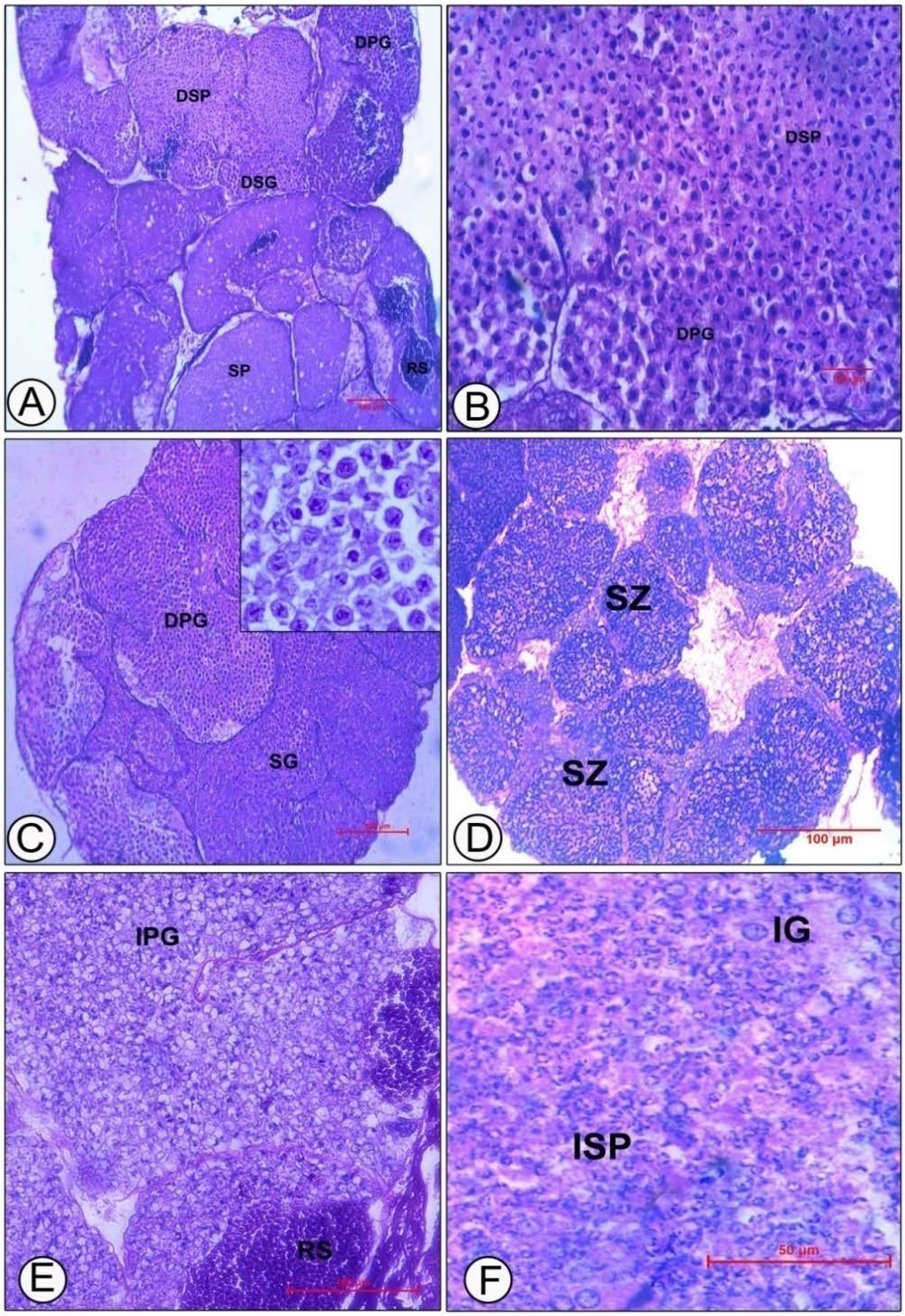

Figure 3. Section of testis of control and experimental crabs during revival phase. A-B: Acini of control crabs packed with spermatocytes, residual sperms and dividing gonia at low and high magnifications; C: Large distended acini showing primary gonial proliferation and secondary gonia in serotonin treated crabs (inset showing proliferation of primary gonia); D: Accumulation of fresh batches of sperms in acini of serotonin treated crabs; E-F: Gonial pycnosis, residual sperms and inactive germ cells in testis of dopamine administered crab. DPG: Dividing primary gonia; DSG: Dividing secondary gonia; DSP: Dividing spermatocytes; IPG: Inactive primary gonia; ISP: Inactive spermatocyte; RS: Residual sperm; SD: Spermatid; SG: Secondary gonia; SP: Spermatocyte; SZ: Spermatozoa.

Morphology and histology of testis of dopamine treated crabs during revival phase

The injection of dopamine resulted in marked degenerative changes in the testis with decreased testicular index (0.08±0.01) and acinar diameter (186.50±3.41 µm) (Table 1). Dividing stages of germ cells were rarely detected in the testis. Compared to the control and serotonin treated crabs, proliferation of primary gonia (31%) was found partially arrested in dopamine treated crabs. Secondary gonia constituted 22% of the total germ cells, frequent in acini containing cell debris. In most of the acini, primary (18%) and secondary spermatocytes (16%) appeared intensely basophilic; in a few acini, they appeared to be in a state of revival. Spermatids displaying intense basophilia, constituted 8% of the germ cells, of which 3% were seen progressing spermiogenesis. Residual sperms (3%) were observed towards the periphery of the acini showing spermiogenesis. A few acini containing loosely packed spermatozoa (1%) were also detectable (Figure 3E-F).

Discussion

The present investigation focused on the effect of neurotransmitters, serotonin and dopamine on testicular maturation in the freshwater crab *Travancoriana schirnerae*. Our observations revealed significantly higher testicular index, mean acinar diameter and proportion of mature spermatozoa in serotonin treated animals while animals treated with dopamine illustrated significantly lower testicular index, mean acinar diameter and proportion of mature spermatozoa than the untreated controls.

This study indicated that administration of serotonin elicited a significant rise in the testicular index, mean acinar diameter and total number of germ cells, reduction in the intra and interacinar cell free spaces, decondensation of germ cells and detection of division stages during inactive and revival phases of spermatogenesis over the controls. Though serotonin treatment during active phase showed a hike in testicular index and acinar diameter values and proportion of mature spermatozoa, only the testicular index value differed statistically from that of the control crabs. In support of these findings, accelerated values for testicular index, acinar diameter and number of mature spermatozoa were obtained by Sarojini et al. (1993; 1994) and Fingerman (1997) in *U. pugilator* and *P. clarkii* treated with serotonin. A similar elevation in testicular index, acinar diameter, proportion of mature spermatozoa and active proliferation of spematogonial cells was observed in *M. rosenbergii* following serotonin administration (Poljaroen et al., 2011; Siangcham et al., 2013). Prasad et al. (2014) observed a significant boost in testicular index and mean acinar diameter in a dose dependent manner in serotonin injected *B. guerini*. Treatment with leucine enkephalin, a stimulatory neurotransmitter, drastically enhanced the testicular index and mean acinar diameter values in the freshwater field crab *Oziotelphusa senex senex* (Reddy, 2003) and the eastern lubber grasshopper *Romalea microptera* (Kumar et al., 2013). Testicular index value is augmented following serotonin administration in the Gulf killifish *Fundulus grandis* (Emata et al., 1985). Banarjee et al. (2016) observed a significantly high testicular volume and index, enlarged seminiferous tubules with bunches of spermatozoa and reduced interstitial spaces of seminiferous tubules in the Japanese quail *Coturnix coturnix japonica* treated with serotonin. A similar escalation in reproductive indices was found in the white throated sparrow *Zonotrichia albicollis* on administration with serotonin (Meier and Miller, 1983).

In contrast, dopamine injection showed a decline in testicular activity irrespective of the phase of spermatogenesis in *T*. *schirnerae*, as evinced from the significantly low testicular and acinar diameter values, irregularly shaped acini with indistinct acinar boundaries, crowding of pycnotic germ cells, prominent inter and intra acinar spaces, reduction in number of mature spermatozoa and absence of division stages when compared to serotonin treated and control males. In agreement with the present observations, dopamine injection in *P*. *clarkii* displayed low testicular index and acinar diameter values, fewer mature spermatozoa and poorly developed androgenic glands (Sarojini et al., 1994; 1995). In *U*. *pugilator* and *B*. *guerini*, Sarojini et al. (1993) and Prasad et al. (2014) documented low values for testicular index and acinar diameter following dopamine treatment. Poljaroen et al. (2011) and Siangcham et al. (2013) reported significantly low testicular index, acinar diameter and germ cell proliferation and more number of immature spermatocytes in *M*. *rosenbergii* administered with dopamine. Dopamine treatment in *Z. albicollis* and *C. coturnix japonica* revealed significantly low gonadosomatic index, smaller seminiferous tubules with complete degeneration of spermatogenic activity (Meier and Miller, 1983 and Banarjee et al. 2016). Similar observations were made by the inhibitory neurotransmitter methionine enkephalin in *O*. *senex senex* (Reddy, 2003) and *R*. *microptera* (Kumar et al., 2013). On the contrary, serotonin and dopamine did not cause any effect on testicular activity in *P. stylirostris* and *P*. *occidentalis* (Montoya and Vega, 2011).

The present research clearly reported the antagonistic effects of serotonin and dopamine on reproductive performance of male *T*. *schirnerae*. The acceleration of testicular activity irrespective of the phase of spermatogenesis in serotonin treated crabs in the present study probably point out the stimulatory effect of serotonin on the testis by stimulating the release of the gonad stimulating hormone which in turn triggers the androgenic gland to produce and release the androgenic gland hormone that stimulates testicular maturation. The inhibitory action of dopamine on spermatogenesis on the other hand, is suggestive of the suppression of release of gonad stimulating hormone or trigger in the release of gonad inhibiting hormone or both. In support of this, several authors have shown the stimulatory and inhibitory effects of serotonin and dopamine in decapods such as *P*. *clarkii*, *U*. *pugilator*, *Litopenaeus vannamei*, *P*. *monodon* and *M*. *rosenbergii* (Sarojini et al., 1993; 1994; 1995; Fingerman et al., 1994; Fingerman, 1997; Fingerman and Nagabhushanam, 1992; Alfaro et al., 2004; Wongprasert et al., 2006; Poljaroen, 2011).

Conclusion

Serotonin apparently stimulates testicular activity, while dopamine hinders testicular maturation in *T*. *schirnerae*. Thus the stimulatory neurotransmitter serotonin can be used to hasten testicular maturation, leading to the enrichment of sperm quality and quantity in species of aquaculture potential.

Acknowledgements

The financial assistance in the form of INSPIRE fellowship from the Department of Science & Technology, New Delhi, India (DST/INSPIRE Fellowship/2015/IF150375) for the first author is gratefully acknowledged.

Conflict of interest

Authors declare that they have no conflicts of interests.

References

Akhila, N.; Sangeetha, S.; Deepa Rani, S.; Munuswamy, N. Impact of serotonin on moulting, ovarian maturation and spawning in a mole crab *Emerita emeritus*. **International Journal of Advanced Research**, v. 4, no. 10, p. 149-162, 2016. https://doi.org/10.21474/IJAR01/1771

Alfaro, J.; Zuniga, G.; Garcia, A.; Rojas, E. Preliminary evaluation of the effect of juvenile hormone III and methyl farnesoate on spermatophore quality of the white shrimp, *Litopenaeus vannamei* Boone, 1931 (Decapoda: Penaeidae). **Revista de Biología Marinha y Oceanografía**, v. 43, p. 167-171, 2008. https://doi.org/10.4067/S0718-19572008000100018

Alfaro, J.; Zuniga, G.; Komen, J. Induction of ovarian maturation and spawning by combined treatment of serotonin and a dopamine antagonist, spiperone in *Litopenaeus stylirostris* and *Litopenaeus vannamei*. **Aquaculture**, v. 236, p. 511-522, 2004. https://doi.org/10.1016/j.aquaculture.2003.09.020

Anilkumar, G. **Reproductive physiology of female crustaceans**. Calicut, India: University of Calicut, 1980. (Thesis of doctorate).

Aprajitha, K.; Pandy, A. K.; Singh, R.; Chauhan, U. K. Reproductive endocrinal regulation in decapod crustaceans: A review. **Journal of Ecophysiology and Occupational Health**, v. 14, no. 1/2, p. 55-65, 2014. https://doi.org/10.15512/joeoh/2014/v14i1-2/50735

Banarjee, S.; Tsutsui, K.; Chaturvedi, C. M. Apoptosis mediated testicular alteration in Japanese quail (*Coturnix coturnix japonica*) in response to temporal phase relation of serotonergic and dopaminergic oscillations. **Journal of Experimental Biology**, v. 219, p. 1476-1487, 2016. https://doi.org/10.1242/jeb.129155

Butler, T. A.; Fingerman, M. Concentrations of neurotransmitters in the central nervous system of *Uca panacea* and *Callinectes sapidus*. **American Zoologist**, v. 23, p. 954, 1983.

Emata, A. C.; Meier, A. H.; Spieler, R. E. Temporal variations in gonadal and body fat responses to daily injections of 5–hydroxytryptophan (5–HTP) and dihydroxyphenylalanine (DOPA) in the gulf killifish, *Fundulus grandis*. **Journal of Experimental Zoology**, v. 233, p. 29-34, 1985. https://doi.org/10.1002/jez.1402330106

Fingerman, M. Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. **Invertebrate Reproduction & Development**, v. 31, no. 1/3, p. 47-54, 1997. https://doi.org/10.1080/07924259.1997.9672562

Fingerman, M. The endocrine mechanisms of crustaceans. **Journal of Crustacean Biology**, v. 7, no. 1, p. 1-24, 1987. https://doi.org/10.1163/193724087X00018

Fingerman, M.; Nagabhushanam, R. Control of the release of crustacean hormones by neuroregulators. **Comparative Biochemistry and Physiology Part C: Comparative Pharmacology**, v. 102, no. 3, p. 343-352, 1992. https://doi.org/10.1016/0742-8413(92)90125-Q

Fingerman, M.; Nagabhushanam, R.; Sarojini, R.; Reddy, P. S. Biogenic amines in crustaceans: Identification,localization and roles. **Journal of Crustacean Biology**, v. 14, no. 3, p. 413-437, 1994. https://doi.org/10.1163/193724094X00010

Kishori, B.; Reddy, P. Influence of leucine-enkephalin on moulting and vitellogenesis in the freshwater crab, *Oziotelphusa senex senex* (Fabricius, 1791) (Decapoda, Brachyura). **Crustaceana**, v. 76, p. 1281-1290, 2003. https://doi.org/10.1163/156854003323009812

Kravitz, E. A. Serotonin and aggression: Insights gained from a lobster model system and speculations in the role of amine neurons in a complex behavior. **Journal of Comparative Physiology A**, v. 186, no. 3, p. 221-238, 1999. https://doi.org/10.1007/s003590050423

Kulkarni, G. K.; Fingerman, M. Quantitative analysis by reverse phase high performance liquid chromatography of 5-hydroxy-tryptamine in the central nervous system of the red swamp crayfish, *Procambarus clarkii*. **The Biologic Bulletin**, v. 182, p. 341-347, 1992. https://doi.org/10.2307/1542254

Kumar, S.; Ganji, P. N.; Song, H.; Kalm, L. V.; Borst, D. W. Exposure to exogeneous

enkephalins disrupts reproductive development in the Eastern lubber grasshopper, *Romalea microptera* (Insecta: Orthoptera). **PloS ONE**, v. 8, p. 101-371, 2013. https://doi.org/10.1371/journal.pone.0051126

Kuo, C. M.; Hsu, C. R.; Lin, C. Y. Hyperglycemic effects of dopamine in tiger shrimp, *Panaeus monodon*. **Aquaculture**, v. 135, p. 161-172, 1995. https://doi.org/10.1016/0044-8486(95)01011-4

Laxmyr, L. Biogenic amines and DOPA in the central nervous system of decapod crustaceans. **Comparative Biochemistry and Physiology Part C: Comparative Pharmacology**, v. 77, no. 1, p. 139-143, 1984. https://doi.org/10.1016/0742-8413(84)90142-7

Lingle, C. The modulatory action of DA on crustacean foregut neuromuscular preparations. **Journal of Experimental Biology**, v. 94, p. 285-299, 1981.

Lüschen, W.; Wilig, A.; Jaros, P. P. The role of biogenic amines in the control of blood glucose level in the decapod crustacean *Carcinus maenas* L. **Comparative Biochemistry and Physiology Part C: Comparative Pharmacology**, v. 105, p. 291-296, 1993. https://doi.org/10.1016/0742-8413(93)90210-C

Meier, A. H.; Miller, I. J. Temporal synergism of neurotransmitter affecting drugs influences seasonal conditions in sparrows. **Journal of Interdisciplinary Cycle Research**, v. 14, no. 1, p. 75-84, 1983. https://doi.org/10.1080/09291018309359800

Montoya, J. A.; Vega, L. A. The effect of environmental cues and neurotransmitters on male sexuality of the Eastern Pacific *Penaeus* species. **Aquaculture**, v. 316, p. 60-67, 2011. https://doi.org/10.1016/j.aquaculture.2011.03.022

Morris, S. Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. **Journal of Experimental Biology**, v. 204, p. 979-989, 2001.

Poljaroen, J.; Tinikul, Y.; Phoungpetchara, I.; Kankoun, W.; Suwansa-ard, S.; Siangcham, T.; Meeratana, P.; Cummins, S.; Sretarugsa, P.; Hanna, P. J.; Sobhon, P. The effects of biogenic amines, gonadotropin-releasing hormones and corazonin on spermatogenesis in sexually mature small giant freshwater prawns, *Macrobrachium rosenbergii* (De Man, 1879). **Aquaculture**, v. 321, p. 121-129, 2011. https://doi.org/10.1016/j.aquaculture.2011.08.022

Prasad, G. L. V.; Nagaraju, G. P.; Ko, J. E.; Naik, B. R. Effects of naloxone, serotonin, and dopamine on reproduction of the freshwater crab *Barytelphusa guerini*. **Journal of Experimental Biology**, v. 321, p. 173-182, 2014. https://doi.org/10.1002/jez.1847

Reddy, P. S. Involvement of opioid peptides in the regulation of reproduction in the prawn *Penaeus indicus*. **Naturwissenschaften**, v. 87, p. 535-538, 2000. https://doi.org/10.1007/s001140050773

Rey, E.; Hernandez.; Diaz, F. J.; Abreu, P.; Alonso, R.; Tabares, L. Dopamine induces intracellular Ca^{2+} signals mediated by α_{1B}-adrenoceptors in rat pineal cells. **European Journal of Pharmacology**, v. 430, p. 9-17, 2001. https://doi.org/10.1016/S0014-2999(01)01250-X

Sarojini, R.; Nagabhushanam, R.; Devi, M.; Fingerman, M. Dopaminergic inhibition of 5-hydroxytryptamine-stimulated testicular maturation in the fiddler crab, *Uca pugilator*. **Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology**, v. 111, no. 2, p. 287-292, 1995. https://doi.org/10.1016/0742-8413(95)00051-O

Sarojini, R.; Nagabhushanam, R.; Fingerman, M. 5-Hydroxytryptaminergic control of testes development through the androgenic gland in the red swamp crayfish, *Procambarus clarkii*. **Invertebrate Reproduction & Development**, v. 26, p. 127-132, 1994. https://doi.org/10.1080/07924259.1994.9672409

Sarojini, R.; Nagabhushanam, R.; Fingerman, M. *In vivo* evaluation of 5-HT stimulation of the testis in the fiddler crab, *Uca pugilator*: A presumed action on the neuroendocrine system. **Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology**, v. 106, no. 2, p. 321-325, 1993. https://doi.org/10.1016/0742-8413(93)90140-G

Siangcham, T.; Tinikul, Y.; Poljaroen, J.; Sroyraya, M.; Changklungmoa, N.; Phoungpetchara, I.; Kankuan, W.;

Sumpownon, C.; Wanichanon, C.; Hanna, P. J.; Sobhon, P. The effects of serotonin, dopamine, gonadotropin-releasing hormones, and corazonin, on the androgenic gland of the giant freshwater prawn, *Macrobrachium rosenbergii*. **General and Comparative Endocrinology**, v. 193, p. 10-18, 2013. https://doi.org/10.1016/j.ygcen.2013.06.028

Simonneaux, V.; Ebadi, M.; Bylund, D. B. Identification and characterization of alpha 2D-adrenergic receptors in bovine pineal gland. **Molecular Pharmacology**, v. 40, no. 2, p. 235-241, 1991.

Tierney, A.; Kim, T.; Abrams, R. Dopamine in crayfish and other crustaceans: Distribution in the central nervous system and physiological functions. **Microscopy Research Technology**, v. 60, p. 325-335, 2003. https://doi.org/10.1002/jemt.10271

Tinikul, Y.; Mercier, J.; Soonklang, N.; Sobhon, P. Changes in the levels of serotonin and dopamine in the central nervous system and ovary, and their possible roles in the ovarian development in the giant freshwater prawn, *Macrobrachium rosenbergii*. **General and Comparative Endocrinology**, v. 158, p. 250-258, 2008. https://doi.org/10.1016/j.ygcen.2008.07.009

Vaca, A.; Alfaro, J. Ovarian maturation and spawning in the white shrimp *Penaeus vannamei* by serotonin injection. **Aquaculture**, v. 183, p. 373-385, 2000. https://doi.org/10.1016/S0044-8486(99)00267-7

Wongprasert, K.; Asuvapongpatana, S.; Poltana, P.; Tiensuwan, M.; Withyachumnarnkul, B. Serotonin stimulates ovarian maturation and spawning in the black tiger shrimp *Penaeus monodon*. **Aquaculture**, v. 261, p. 1447-1454, 2006. https://doi.org/10.1016/j.aquaculture.2006.08.044