



# International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129

P-ISSN: 2394-0506

(ICV-Poland) Impact Value: 5.62

(GIF) Impact Factor: 0.549

IJFAS 2018; 6(2): 184-189

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www.fisheriesjournal.com

Received: 09-01-2018

Accepted: 13-02-2018

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## Effect of eyestalk ablation on mandibular organ activity in the freshwater crab *Travancoriana schirnerae*

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and Sneha Dineshan**

### Abstract

The present investigation analyzed the effects of eyestalk ablation on mandibular organ activity in the freshwater crab *Travancoriana schirnerae*. The MOs are paired, pale yellow, coma-shaped glands located at the base of the posterior adductor muscle of the mandible, barely distinguishable from the adjacent tissues. The gland contained two distinct cell types: type I and type II. In unilaterally ablated crabs, the gland was hypertrophied with patches of compactly arranged type I cells. The moderately basophilic cytoplasm of type I and II cells exhibited high granularity indicating signs of activity. The MO of bilateral eyestalk ablated crabs displayed large, highly basophilic secretory vesicles. The control MO had a degenerated appearance, lacking distinct cell types and secretory vesicles. The secretory vesicles perceptible in MOs of bilaterally destalked females may represent the synthesis of methyl farnesoate resulted from destalkation which caused the removal of the source of mandibular organ inhibiting hormone.

**Keywords:** Eyestalk ablation, mandibular organ, histology, *Travancoriana schirnerae*

### 1. Introduction

The mandibular organs (MOs) are paired, ductless, vascularized glands of ectodermal origin, positioned at the base of the posterior adductor muscles of the mandibles in decapod crustaceans. Le Roux <sup>[1]</sup> first identified the gland in the cephalothorax of the crab *Carcinus maenas*. Earlier research supported the view that the MO played decisive roles in growth and reproduction <sup>[2, 3, 4, 5, 6, 7, 8, 9, 10]</sup>. Byard *et al.* <sup>[11]</sup> and Hirsch and Al Hajj <sup>[12]</sup> reported that the structure of MO in the lobster *Homarus americanus* and the male spider crab *Libinia emarginata* bear a strong resemblance to steroid synthesizing cells. Besides growth and reproduction, the hormone methyl farnesoate (MF) synthesized and secreted by the MO played vital roles in protein synthesis <sup>[13, 14]</sup>, metamorphosis <sup>[15, 16]</sup>, behaviour <sup>[17, 18]</sup> and stress <sup>[19, 20]</sup>.

In decapods, the function of MO is negatively influenced by a mandibular organ inhibiting hormone (MO-IH) secreted by the sinus gland (SG) of the eyestalk <sup>[21, 22, 23, 24, 25, 26]</sup>. In *H. americanus*, injection of eyestalk ablated animals with SG extract revealed a hasty reduction in hemolymph titre of MF <sup>[27]</sup>. In vitro incubation of MOs with eyestalk extract inhibited MF synthesis in *L. emarginata* <sup>[24]</sup> and *Cancer pagurus* <sup>[28]</sup>. Borst *et al.* <sup>[29]</sup> have shown in *C. pagurus* that the in vitro exposure of MO with X-organ- sinus gland (XO-SG) extract synthesized less MF compared to the MO incubated alone.

Extirpation induced stimulation in MO activity has shown in crabs like *C. maenas*, *Pisidia longicornis* and larvae of the shrimp *Palaemonetes varians* <sup>[1, 30, 31]</sup>. Hirsch<sup>[32]</sup> investigated the fine structural changes in the MO of *L. emarginata* following eyestalk extirpation. In the field crab *Oziotelphusa senex senex*, Nagaraju *et al.* <sup>[33]</sup> have documented a noticeable increase in the weight of the MO after bilateral destalkation.

Though a number of studies detailed the structure and activity of MO in marine decapods <sup>[2, 34, 32, 9, 10]</sup>, very few detailed the histology and activity of MO in freshwater crabs <sup>[35]</sup>. The current light microscopic study looks for some histological evidence for eyestalk ablation induced augmentation in activity of MO of an edible freshwater crab, *Travancoriana schirnerae*, widely distributed in the wetlands of Wayanad, Kerala, India. This crab species forms a cheap source of animal protein for the poor, malnourished local tribes.

The relationship between eyestalk ablation (ESA) and enhanced MO activity is particularly exhilarating from the practical perspective as ESA accelerates growth and reproduction which can be exploited in aquaculture practices of freshwater crabs of commercial importance.

## 2. Materials and Methods

Adult intermoult crabs having carapace widths (CW) 4.5-5 cm were collected from the paddy fields of Ondayangadi (5 km northeast of Mananthavady in Wayanad district of Kerala state, India) for a period of one year (October 2015 to October 2016). The individuals were left to acclimatize to the conditions of the laboratory for three days before ablation. Crabs (n=45) were divided into three sets of 15 each: controls with intact eyestalks, -E1 with single eyestalk excised and -E2 with both the eyestalks removed. They were maintained in large cement tanks, fed ad libitum with cooked beef liver and fish feed. The CW and wet weights were recorded for all the specimens collected. The moult stages were determined by observing the nature of carapace and setae of pleopods.

### 2.1 Eyestalk Ablation

The eyestalks were excised using fine scissors. Soon after surgery, the incision was burnt with a hot needle and blotted with clean cotton in order to prevent infection. The distress was alleviated by applying ice to the lesion. The second eyestalk was detached after 24 h from the first.

For histological studies, the MOs were dissected out, fixed in Bouin's fluid, dehydrated in ethanol series and embedded in paraffin wax. Sections, 5µm thickness, were cut and stained with Heidenhain's hematoxylin-eosin. Images were captured using a DG 330/210 camera, attached to a Leica DM 500 Research Microscope. All the measurements were taken using an image analysis system of Biowizard software.

## 3. Results

### 3.1 Morphology

The MOs were paired, coma-shaped, pale yellow glands located at the base of the posterior adductor muscle of the mandible, barely distinct from the neighbouring tissues. The gland (0.6-1.0 mm long and 0.2-0.5 mm wide) was bordered by a thin layer of connective tissue which attaches it to the tendon of the adductor muscle.

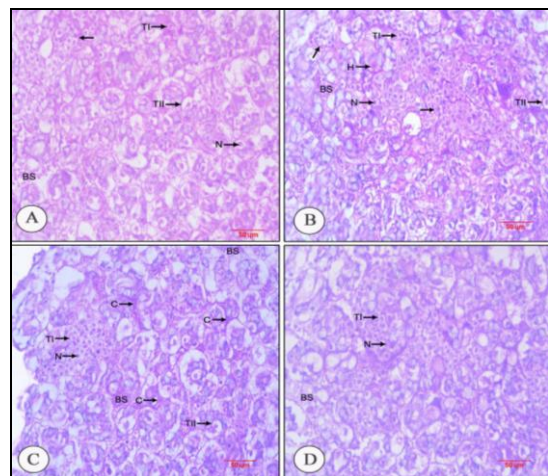
### 3.2 Histology

The MO consisted of polygonal cells arranged in cords. Their nuclei were positioned mostly within the centre of the cells. These cells have mildly basophilic cytoplasm with round, ovoid or elongate nuclei containing one or two nucleoli and aggregated chromatin. The cells were arranged loosely around the hemolymph channels and sinuses. Hemocytes could be detected in these sinuses and channels.

Two distinct cell types could be distinguished in the MO- type I and type II. Type I cells were characterized by large, oval or round nuclei and a small amount of mildly basophilic granular cytoplasm with high nucleocytoplasmic ratio. In type I cells, the basophilia of the nuclei was very strong that the nucleoli could not be seen. Type II cells, predominant of the cell types, were characterized by small nuclei with large amount of cytoplasm and low NPR. Their nuclei were oval or elongate and cytoplasm moderately basophilic. Some type II cells enclosed single large secretory vesicles which occupied nearly the whole cellular volume.

### 3.3 Histology of MO of Unilaterally Ablated Crabs

Unilateral ablation caused considerable changes in the morphology and histology of MO. The gland was pale white to yellow with a bulged appearance (length  $529.80 \pm 1.22$  µm and width  $357.43 \pm 1.26$  µm). The organ was highly vascularized with large number of sinuses and capillaries all through (Figure 1A). The most notable feature in MOs of unilaterally ablated crabs was the proliferation of type I cells which appeared as compactly arranged masses interspersed among large type II cells (Figure 1B, C). These masses were either small with 8-18 cells or rather large with 46-54 cells (Figure 1D). These cells ( $8.91 \pm 0.03$  µm width) exhibited large basophilic nuclei ( $5.27 \pm 0.02$  µm) with small amounts of mildly basophilic granular cytoplasm. They mostly have centrally placed round or oval nuclei with indistinct nucleoli (Figure 1B, C). Nuclear hypertrophy was more pronounced in MO of -E1 crabs signifying increased activity of cells. Type II cells ( $15.82 \pm 4.18$  µm) formed the major cell types in MO of unilaterally ablated crabs. These cells possessed centric or acentric nuclei ( $5.42 \pm 1.02$  µm) and their cytoplasm displayed a moderate basophilia. The amount of cytoplasm was found varied in these cells. Their multinucleolated (1-3) nuclei appeared more basophilic with nucleoli occupying central or peripheral positions (Figure 1A-C). The occurrence of large blood sinuses and numerous fine capillaries connected to these sinuses form another noteworthy character of MO of -E1 crabs. Capillaries were seen encircling the cells (Figure 1C). Hemocytes could be perceptible within the hemal sinuses (Figure 1B).



**Fig 1:** Light micrograph showing mandibular organ of unilaterally destalked *Travancorianaschirnerae*.

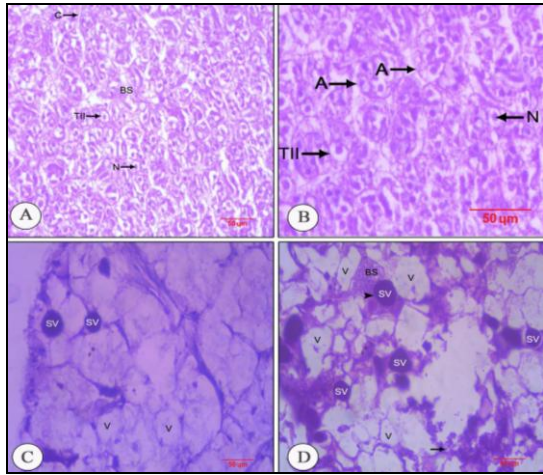
(A) Mandibular organ displaying type I and II cells, (B) Mandibular organ with small and large patches of proliferated Type I cells, (C) Blood sinuses and capillaries, (D) Another MO illustrating patches of type I cells.

BS: Blood sinus; C: Capillary; H: Hemocyte; N: Nucleus; T1: Type I cell; T2: Type II cell; Arrow indicates proliferation of type I cells.

### 3.4 Histology of the MO of bilaterally ablated crabs

The gland became hypertrophied and measured  $582.36 \pm 0.90$  µm in length and  $386.48 \pm 1.60$  µm in width in -E2 crabs. The gland accommodated densely packed cells with distinct cell boundaries (Figure 2A). Intercellular spaces were diminished compared to control crabs. Type II cells formed the prominent cell types in MO of bilaterally ablated crabs. These cells were noticeably large and measured  $16.0 \pm 4.07$  µm in diameter.

Their cytoplasm appeared moderately basophilic and granular. Nuclei ( $5.15\pm 0.46$ ) were large, round or oval, centrally or peripherally placed with intensely basophilic chromatin granules (Figure 2A). In some -E2 crabs, an acinar pattern for arrangement of type II cells was evident (Figure 2B). These acini carried type II cells with distinct nuclei while their cell boundaries were hardly discernible. The gland was characterized by a low percentage of type I cells.



**Fig 2:** Light micrograph portraying mandibular organ of bilaterally destalked *Travancorianaschirnerae*.

(A) Mandibular organ exhibiting densely packed large type II cells, blood sinuses and capillaries, (B) Acinar pattern of arrangement of type II cells, (C) Type II cells with secretory vesicles and vacuolations; Note the indistinct cell boundaries of type II cells, (D) Release of secretory vesicles into blood sinus illustrating the holocrine mode of release of secretion.

A: Acinar wall; BS: Blood sinus; C: Capillary; N: Nucleus; SV: Secretory vesicle; TII: Type II cell; V: Vacuole; Arrow

indicates cell debris; Arrow head indicates secretory vesicle in blood sinus.

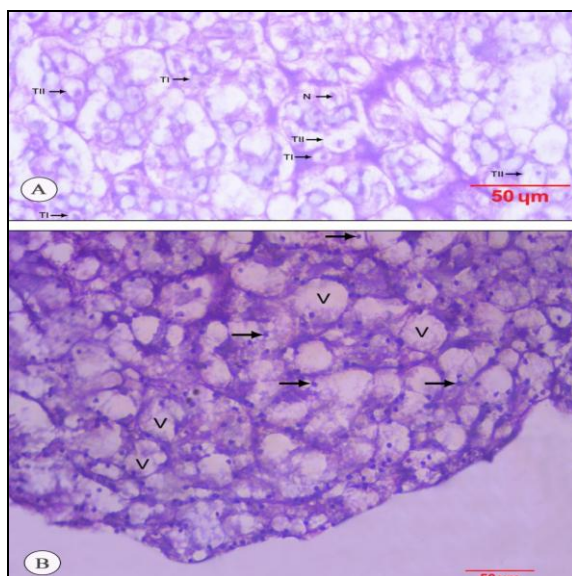
Appearance of intensely basophilic secretory vesicles was the most important observation of -E2 crabs. The MOs in some -E2 crabs enclosed several large secretory vesicles ( $31.50\pm 6.17\mu\text{m}$ ). Each type II cell carried a single, large, round to oval, highly basophilic secretory vesicle with distinct limiting membrane. The type II cells laden with secretory vesicles have indistinct cell boundaries (Figure 2C). Occasionally, the boundaries of a few cells carrying the secretory vesicles were found broken, releasing their contents into the blood sinuses indicating a holocrine mode for release of secretion (Figure 2D). Vacuolization was more conspicuous in these glands; cell debris were also detected (Figure 2C, D). Type II cells with sharp cell boundaries were hardly perceptible in such glands. Type I cells were seldom detected. Blood sinuses and capillaries were noticed throughout the gland (Figure 2A).

### 3.5 Histology of MO of control crabs

By contrast, the MO of control crabs exhibited a degenerated appearance ( $471.08\pm 1.33\mu\text{m}$  in length and  $327.98\pm 1.36\mu\text{m}$  in width) (Figure 3A, B). The cells were loosely arranged with prominent intercellular spaces. The gland appeared small, shrunken and atrophied in nature. There were no distinct blood sinuses and capillaries. The gland was characterized by large vacuolated areas indicating cellular degeneration (Figure 3B). Intact cells with clear cell boundaries were scarcely detected. Pycnotic nuclei were seen scattered inside the gland (Figure 3B). No secretory vesicles or proliferation of type I cells were apparent in the gland. Type II cells ( $9.05\pm 0.69$ ) with mild basophilia were found occasionally. Type I cells ( $7.06\pm 0.54$ ) were scarcely noted in the gland. Cells mostly have transparent and vacuolated cytoplasm (Figure 3A).

**Table 1:** Comparison of cells types in mandibular organ of control, unilateral and bilateral destalked *Travancorianaschirnerae*.

Group	Type I cells			Type II cells		
	Cell diameter ( $\mu\text{m}$ )	Nuclear diameter ( $\mu\text{m}$ )	NPR	Cell diameter ( $\mu\text{m}$ )	Nuclear diameter ( $\mu\text{m}$ )	NPR
Control	$7.06\pm 0.54$	$3.35\pm 0.34$	$0.47\pm 0.60$	$9.05\pm 0.69$	$2.80\pm 0.88$	$0.31\pm 0.12$
-E1	$8.91\pm 0.03$	$5.27\pm 0.02$	$0.59\pm 0.01$	$15.82\pm 4.18$	$5.42\pm 1.02$	$0.34\pm 0.12$
-E2	$8.43\pm 0.30$	$4.47\pm 0.17$	$0.53\pm 0.01$	$16.0\pm 4.07$	$5.15\pm 0.46$	$0.32\pm 0.11$



**Fig 3:** Light micrograph illustrating histology of mandibular organ of control crabs

(A) Control MO depicting cells with indistinct boundaries, (B) Control MO portraying scattered pycnotic nuclei and vacuolated areas.

N: Nucleus; TI: Type I cells; TII: Type II cells; V: Vacuole; Arrows indicate pycnotic nuclei.

### 4. Discussion

The current investigation observed hypertrophy and structural changes in the MO of unilateral and bilateral eyestalk ablated crabs compared to intact controls. Similar observations were made by Hinsch [32] and Le Roux [30, 31] in crabs *L. emarginata* and *P. longicornis* and the shrimp *P. varians*. Byard *et al.* [11] described the eyestalk ablation induced hypertrophy and changes in ultrastructure of the MO in *H. americanus*. Taketomi and Kawano [36] reported some changes in the MO cells following destalkation in *Penaeus japonicus*. Bazin [37] reported hypertrophy of the MO and a higher density of smooth endoplasmic reticulum in MO cells of eyestalk extirpated *C. maenas*. Allayie *et al.* [38] noticed destalkation induced stimulation in MO activity of the ghost crab *Ocypode macrocera*. The observed hypertrophy and structural changes

in the present study indicate signs of activity of the MO induced by the removal of the inhibitory principle, MO-IH.

The most remarkable feature in MO of -E1 *T. schirnerae* was the proliferation of type I cells which appeared as small or large patches of compactly packed cells with large nuclei. In the same species, Sudha Devi and Smija [39] and Smija and Sudha Devi [40] have shown cell proliferation during revival phase in the androgenic gland and and late intermoult in the Y organ. Phoungpetchara *et al.* [41] documented hypertrophy with proliferation of type I cells in the androgenic gland of the giant freshwater prawn *Macrobrachium rosenbergii* undergone bilateral ablation. The current observation on proliferation of type I cells in the MO of -E1 crabs is indicative of increased activity resulted from destalkation.

In *T. schirnerae*, bilateral eyestalk ablation effected synthesis of MF, evidenced by the presence of secretory vesicles. Eyestalk removal has been shown to increase MF levels in a wide variety of crustaceans, including the crayfish *Orconectes virilis* [42], *C. pagurus* [29], *Callinectes sapidus* [43], *O. senex senex* [44] and *L. emarginata* [24]. Tsukimura and Borst [27] noticed increased levels of MF following eyestalk ablation in *H. americanus* and a decrease in MF levels in eyestalk ablated (ESA) animals treated with SG extract. Eyestalk removal also enhanced farnesoic acid-O-methyl transferase activity, which intercedes the conversion of farnesoic acid to MF in the MO of the crayfish *Procambarus clarkia* [23]. Likewise, Li *et al.* [45] have shown an ablation induced spur in activity of farnesoic acid-O-methyl transferase in *H. americanus*.

The current investigation also revealed cellular hypertrophy and increased cytoplasmic granularity in MO cells of destalked crabs. Evidence for cellular hypertrophy was shown in *Portunus sanguinolentus*, wherein the Y organ cells showed increased cytoplasmic and nuclear volume following bilateral extirpation [46]. Eyestalk ablation induced hypertrophy was observed in the androgenic gland cells of protandric prawns [47] and isopod crustaceans [48, 49]. Several authors reported the ESA associated cellular hypertrophy in Y organ cells of crabs *C. irroratus* [50], *Pachygrapsus marmoratus* [51] and *C. antennarius* [52]. In a similar way, the Y organ in the freshwater prawn *Palaemon paucidens* [2] and *O. limosus* [53] demonstrated marked cytological changes following ESA.

Based on the size of cell and nuclei, two distinct cell types were identified in the MO of *T. schirnerae*. Several studies have indicated and confirmed the presence of two cell types in the MO. For instance, Hinsch [32, 4] identified light and dark cells in the MO of male and female spider crabs. The mandibular organ cells from males of the Indian prawn *Fenneropenaeus indicus* also contained two cell types: dense and less dense cells [54]. Dorn [55] observed light cells in mature males and females and dark cells in late larvae of *Oncopeltus fasciatus*. On the other hand, Borst *et al.* [56] identified three cell types in the MO of *H. americanus*: mitotically active A cells, undifferentiated B cells containing large number of small vacuoles and C cells containing many mitochondria and vacuolar structures. It is suggested that the two cell types observed in the present study were not structurally or functionally distinct cell types, rather they seemed to represent different stages in the secretory activity of the gland.

The results of our study clearly indicated a holocrine mode of release for MO secretion, the MF. Holocrine pattern for release of secretion was also noted in many decapod crustaceans such as *M. rosenbergii* [57, 58], *Eriocheir sinensis*

[59], *Eriphia verrucosa* [60] and *P. trituberculatus* [61]. The cytoplasmic vacuolation and cellular degeneration in the Y organ of *P. sanguinolentus* demonstrate evidences for its holocrine mode of release of secretion [46]. Simione and Hoffman [50] have reported a holocrine mode for release of secretion in the Y organ of *C. irroratus*. Conversely, there was no evidence for holocrine mode of release of secretion in *P. crassipes* [62].

The current investigation also revealed a prominence of hemal sinuses and capillaries with hemocytes in -E1 and -E2 MO. This high vascularization of MO of -E2 crabs facilitates the release of secretory substances (MF) from MO cells into the sinuses and capillaries for transport to the hemolymph. Comparable results were obtained in the Y organ of *P. sanguinolentus* which displayed large number of sinuses and capillaries during premoult [46].

## 5. Conclusion

The observed cellular and glandular hypertrophy with proliferation of type I cells, annihilation of intercellular spaces, cytoplasmic granularity, prominence of hemal sinuses and capillaries and the presence of secretory vesicles in MO of -E2 crabs, all point towards high synthetic nature of the gland. The results of this study invigorate the fact that the activity of MO is under the control of an inhibitory principle, the MO-IH from the XO-SG complex of the eyestalk and removal of this principle stimulate the MO to synthesize and secrete MF.

## 6. Acknowledgements

This work was undertaken with the aid of funds provided by the University Grants Commission, Govt of India.

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