Executive summary of the project titled Role of hormones on growth and reproduction in the freshwater crab *Travancoriana* *schirnerae’*

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Effect of eyestalk ablation on fine structure of the mandibular organ of the freshwater crab *Travancoriana* *schirnerae’*

**Fine structural study of the mandibular organ of the freshwater crab *Travancoriana schirnerae***

*Travancoriana schirnerae* is a commonly distributed edible freshwater crab widely distributed in the wetlands of Wayanad, Kerala, India and forms a cheap source of protein for the poor, malnourished local tribes. Considerable evidence indicates that eyestalk ablation (ESA) removes mandibular organ inhibiting hormone (MO-IH), which in turn accelerates the activity of the mandibular organ in a variety of crustaceans (Landau et al., 1989; Chaves et al., 2001; Borst et al., 2002). The present fine structural study seek to provide some ultrastructural evidence for eyestalk ablation induced enhancement in the production of methyl farnesoate (MF), secreted by the MO, in this species. The relationship between ESA and enhanced production of MF is particularly exciting from an applied perspective as ESA accelerates growth and reproduction which can be exploited in aquaculture practices of freshwater crabs of commercial importance.

For electron microscopic studies, the MOs excised from adult intact and eyestalk ablated crabs 30 days postoperation were fixed in 3% glutaraldehyde overnight. The tissues were then washed twice in phosphate buffer, postfixed in osmium tetroxide and dehydrated in graded series of ethanol. The tissues were transferred to propylene oxide (2 changes 10 min each) and to a mixture of propylene oxide and araldite followed by pure araldite overnight in a rotator. The tissue was finally embedded in araldite and kept in an oven at 60°C for polymerization. After polymerization, semithin and ultrathin sections were cut with glass knives under a Leica UC6 Ultramicrotome. Semithin sections were stained with 1% toluidine blue and observed under a light microscope. For electron microscopic observations, ultrathin sections collected on copper grids were stained with uranyl acetate followed by lead citrate and observed under a Tecnai G2 Spirit Biotwin Transmission Electron Microscope. Interested areas were captured using a Megaview-III CCD camera.

This study analyzed the eyestalk ablation induced fine structural changes in the mandibular organ of the freshwater crab *Travancoriana schirnerae*. The MOs were paired, coma shaped, pale yellow glands located at the base of the mandibular tendons. Fine structural studies demonstrated a cord-like pattern of MO cells. Channels of hemolymph divide the MO into cords. The hemolymph channels were interconnected with sinuses and these sinuses were seen surrounded by MO cells. Each cord is composed of many rectangular cells. Within the channels and sinuses were two distinct types of hemocytes: granulocytes and agranulocytes. The granulocytes have oval, elongate or irregularly shaped nuclei with chromatin condensed around the peripheral karyoplasm. Dispersed in the cytoplasm of granulocytes were electron dense granules of varying sizes and shapes.

Ultrastructural observations revealed that the organ cells of intact crabs possessed cytoplasmic organelles like numerous rod, oval or circular mitochondria with electron dense matrices and tubular cristae and ribosomes which provide evidence that they were concerned with steroid biosynthesis. Fine structural changes in the organ cells after 30 days destalkation were the development of giant forms of elongate, dumbbell, ring (with 1-3 concentric rings) or cup shaped mitochondria with proliferation of cristae, presence of large number of dilated cisternae of rough endoplasmic reticulum with fibrillar contents, numerous free and polyribosomes, aggregates of smooth endoplasmic reticulum vesicles, well developed Golgi complexes and electron dense secretory vesicles which apparently were accumulations of methyl farnesoate. Several of the cup shaped mitochondria were found stacked one inside the other. The large concentric rings of mitochondria may possibly suggest their role in cleavage of the cholesterol side chains as reported for mammals. The secretory vesicles may represent the production of methyl farnesoate caused due to lack of mandibular organ inhibiting hormone resulted from eyestalk extirpation.

**Histology of the Mandibular Organ of unilateral and bilateral eyestalk ablated female crabs**

Adult intermoult male and female crabs having a carapace width (CW) of 4.5-5 cm were collected from the paddy fields of Ondayangadi, nearly 5 km northeast of Mananthavady in Wayanad district of Kerala state, India. The individuals were left to acclimatize to the conditions of the laboratory for 3 days before ablation. Both males and females were divided into three groups of ten each - control with intact eyestalks, -E1 with one eyestalk extirpated and -E2 with both the eyestalks removed. They were maintained in large cement tanks, fed ad libitum with cooked beef liver and boiled egg. The carapace width (CW) and wet weights were recorded for all the specimens collected. The moult stages were determined by observing the carapace texture and setal development of epipodite of the third maxilliped in the case of males and pleopods in females.

Eyestalk ablation: The surgical excision of the eyestalks was done using fine sterilized scissors. The wound was cauterized with a red-hot needle and blotted with sterilized cotton after the surgery. The distress was alleviated by applying ice to the lesion. The second eyestalk was detached after 24 hours from the first to minimize the shock.

The present investigation analyzed the effect of unilateral and bilateral eyestalk ablation on mandibular organ activity (MO) in males and females of the freshwater crab *T. schirnerae*. The gland contained two distinct cell types: type I and type II, arranged in cords. In all the experimental crabs, the gland got hypertrophied with compactly packed cells arranged in cords. The cells possessed abundant cytoplasm and their moderately acidiophilic cytoplasm exhibited high granularity. In MO of ESA males, numerous patches of proliferated type I cells were discerned, indicating signs of activity. Large secretory vesicles were perceptible in MOs of destalked females whereas no evidence of secretory vesicles was noted in MOs of destalked males. The MO of control crabs have a degenerated appearance in both the sexes. The secretory vesicles noticeable in MOs of destalked females may represent the synthesis of methyl farnesoate caused due to the absence of mandibular organ inhibiting hormone resulted from eyestalk extirpation.

**Moult related changes in the histology of sinus gland in the freshwater crab Travancoriana schirnerae**

X-organ-sinus gland (XO-SG) is an important neurosecretory system of the crustaceans. This structure serves as a storage and release of neurohormones which include the moult inhibiting hormone, gonad inhibiting hormone, hyperglycemic hormone, salt and water balance regulating hormone, distal retinal pigment hormone and chromatophorotropins. Very few authors analyzed the structure of sinus gland in freshwater decapods. The present study provides moult related changes in the histology of the XO in the freshwater crab *Travancoriana schirnerae*.

Results showed that the SG was located in the eyestalk between medulla externa and medulla interna. It was made up of several small axonal endings and supportive cells. The histology of the SG showed striking differences according to the moult stage of the animal. The axonal endings appeared as round basophilic structures. Inside the axonzal endings, small highly basophilic round granules were noted. Based on the size and distribution of granules, five types of axonal endings were distinguished

During intermoult the axonal endings appeared as round basophilic structures. Inside the axonal endings, small highly basophilic round granules were noted. Based on the size and distribution of granules, five types of axonal endings were distinguished: large Type I, Type II, Type III, Type IV and Type V. A few supportive cells (5.9-6.8 µm) were found scattered among the axonal endings. They showed irregular outline and their nuclei appeared somewhat larger and highly basophilic with large chromatin granules. Cytoplasm moderately basophilic and granular in nature. Large moderately or highly basophilic secretory vesicles (20-30 µm) were noticed. A holocrine mode of release of secretion was noted.

In postmoult, the SG gland had a degenerated appearance. Axonal endings were generally reduced in postmoult. Type IV (3.6-4.8 µm) axonal endings with homogenously dense granules were more distributed. Medium sized type II (2.8-3.3 µm) axonal endings with homogenously dense granules were sparsely distributed. Compared to intermoult, supportive cells (6-7.6 µm) were more dispersed. Blood sinuses were not prominent. Type I axonal endings were reduced in number.