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RESEARCH ARTICLE

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Feeding ethology and histo-morphological analysis of gut and stomach in the freshwater crab *Spiralothelphusa hydrodroma*

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ABSTRACT: Feeding ethology and dietary preferences were studied in *S. hydrodroma*, a freshwater crab collected from Southern region of India. Oral appendage morphology, histological analysis of gut and stomach were also carried out accordingly. After offering a series of foodstuffs to isolated male and female crabs, the crabs were found to be herbivore-biased omnivore, under laboratory conditions. Male crabs preferred 54% of egg white and female crabs preferred 35% of commercial fish feed. Food ingestion behavior was carefully recorded and represented. Feeding behavior involved the use of mouth appendages, 1st and 2nd pereiopods. Particles were trapped by the dense setation of the 2nd and 3rd maxillipeds and ingested. Histology of gut and stomach reveals the presence of mucosa, goblet cells, gastric glands, gastric pits, lamina propria and its structural adaptations depend upon its unique environment and the feeding behavior.

KEYWORDS: Aquaculture, Freshwater crab, *S. hydrodroma*, Ethology, Diet preferences.

INTRODUCTION

Freshwater crabs are a valuable ecological and economic resource that offer an alternative income source for local communities [1]. Freshwater crabs play an important role in aquatic food web and face conservation concerns due to lack of crucial information [2]. Over 1300 globally distributed freshwater crab species, only 96 species are recognized in India [3,4]. A great deal of diversity exists among the crustaceans, although many of them have a similar structure and mode of life. Their digestive processes are incredibly distinct. Across the animal kingdom, Decapods and other similar malacostracan crustaceans have the most complex digestive systems [5].

Arthropods are the most diverse animal group on earth. Morphological investigations in digestive system of insect arthropods are vast. However, investigations in digestive morphology of crustacean arthropods remains relatively sparse [6]. Feeding behavior is vital for animal productivity as it links the feed provided to the feed consumed [7]. The relationships among different foods, feeding habits, and diet plans in crustaceans are crucial for increasing seed yield and deepening our understanding of these animals in captivity [8]. Midgut morphology is essential to differentiate non-feeding and feeding larval stages [9], to realize histopathological studies [10] and to develop commercial diets for species of aquaculture interest [11]. Feeding habits are also reported to effect digestive tract structure and intestinal microorganisms [12]. Jiao *et al.*, [13] reported that histo-morphological analysis of fishes facilitates nutrient absorption and growth, as feed utilization and digestibility is being analyzed.

Food consumption in freshwater crabs is extremely diverse and thus occupy a vital place in aquatic food webs. *Spiralothelphusa hydrodroma* is an opportunistic carnivore and scavenger that feeds on water invertebrates as well as plant and animal food. Therefore, are excellent models for the study of animal feeding behavior. Studies on chemical senses during feeding have the potential to widen the process of food procurement and to develop more effective baiting strategies for successful captures [14]. Animal feeding habits establish a link between feed and behavior, which affects appetite and motivation. Feeding patterns are influenced by environmental factors that affect a perception of an animal for its food, including physical, social, and nutritional restrictions [15].

Stomach contents analysis in fish and shrimps helps to determine its feeding preferences and feeding frequency. Therefore, prey location, its capture and ingestion can be accelerated by understanding the overall feeding process [16]. Digestive tract morphology and anatomy varies on dietary preferences [17], body shape, weight, sex, feeding

behavior [18], environmental contamination [19], molt cycle, vitellogenesis, periods of famine [5]. Moreover, food preferences of brachyuran crabs is reported to be influenced by availability and abundance of resources [20] prey profitability, palatability and digestibility of the food [21], foraging time availability [22]. However, such studies are not yet reported in *S. hydrodroma*. The study thus focused on feeding ethology, dietary preferences, oral appendage morphology, and histological analysis of the gut and stomach in the freshwater crab *Spiralothelphusa hydrodroma*.

MATERIALS AND METHODS

Collection, transportation and maintenance of specimen

Adult *S. hydrodroma* specimens were collected from the Cauvery River Basin at Bhavani, Tamil Nadu, India. Animals were handpicked with gloves and placed in a thermocol box with perforations for aeration and then transported to the laboratory immediately.

Determination of morphometric indices

Male and female crabs were randomly picked to determine morphometric indices. Carapace length (CL), carapace width (CW), Total weight (TW), major chelate length (MACL) and minor chelate length (MICL) were measured using a graduated twine.

Determination of water quality parameters

Physico-chemical parameters of the aquatic habitat such as Temperature (˚C), pH, Total Dissolved Solids (mg/L), BOD (mg/L), Total Hardness (mg/L) and Turbidity (mg/L) were measured using APHA, 2017 [23].

Gut content analysis

For gut content analysis, the crabs from their natural habitat were immediately anesthetized in the laboratory. Gut was cut open by using a sterile scalpel and scissors. Gut contents were collected and observed under microscope.

Histology of gut and stomach

Histological study of gut and stomach was performed in wild caught *S. hydrodroma*. The gut and stomach was rinsed, transverse-sectioned using microtome and subjected to Harris's hematoxylin & Eosin (H&E) [24]. The specimen being covered with a glass coverslip and then fixed in a permount-mounting medium. Prepared specimens were examined under a compound microscope and photographed using a digital camera.

Dietary preferences

Captured crabs were acclimatized under laboratory conditions for 3 days and were fed with diverse feedstuffs. The experiment was carried out in male and female crabs separately for a period of 15 days. Crabs were separately offered each type of food (Commercial fish feed, Egg white, Fish head, Freshwater snail, and Rice puffs) of 1 g each. Unfed feed particles were collected after 24 hours. Collected unfed materials were weighed and re-measured to estimate the final weight. Excretory particles were not subjected to measurement. The feed intake was calculated using the formula:

Feed Intake (g) = Total feed offered (g) – feed unfed (g)

Feeding Ethology and oral appendage morphology

The role of appendages during feeding was observed in *S. hydrodroma*. The experiment was performed in a glass tank of 44×22cm with laboratory acclimatized male and female crabs. Feed was offered early every morning and continuous observations were made.

Statistical analysis

All data obtained from morphometric indices and water quality analysis were subjected to SPSS statistical software. Data were obtained as mean ± standard deviation of triplicate analysis [25].

RESULTS

Determination of morphometric indices

CL (Carapace Length), CW (Carapace Width), TW (Total Weight), MACL (Major Chelate Length), MICL (Minor Chelate Length) of male and female crabs are represented in Table-1.

Determination of water quality parameters

Physico-chemical parameters of the ecological niche of Cauvery River are represented in Table-3.

Gut content analysis

The gut of *S. hydrodroma* mainly consists of animal, plant material, phytoplanktons and zooplanktons. Phytoplanktons (*Chlorella*, *Desmids*, *Scenedesmus*, *Dinoflagellates*, *Spirogyra* and *Coscinodiscus excentricus*) and Zooplanktons (*Moina species*, copepods and other crustacean appendages) were highly represented. Fish and insects remains along with mud and detritus were also represented.

Histology of gut and stomach

The digestive system of *S. hydrodroma*, could be divided into: foregut, midgut and hindgut. Foregut begins with mouth, followed by oesophagus and stomach. *S. hydrodroma*, have a short and muscular oesophagus, lined by simple and cylindrical epithelium overlaid by a cuticle. Both the epithelium and the cuticle share similar characteristic to those of the oral region. It comprises mucosa, basement membrane and goblet cells Moreover, TS of gut shows secretory cells of chief and parietal cells along with microvilli. However, there were few goblet cells which were assumed to contain either neutral or acidic glycoproteins. Stomach of *S. hydrodroma* is sac-like, lined by a simple and cylindrical epithelium. The epithelium is underlain by a layer of connective tissues, surrounded by circular-longitudinal striated fibres. A serrated cuticle lays on the epithelium. The midgut has a simple epithelium with cubical to cylindrical cells. Cells have a central nuclei with basophilic cytoplasm. Stomach wall produces several smaller folds that project into lumen. In some zones, big haemolymphatic lagoons appears which are the result of bigger folds. A cardiopyloric valve separated the cardiac stomach from the pyloric. TS of stomach exhibits three distinct layers, mucosa, sub-mucosa and muscularis. Mucosa shows epithelium, gastric pits and mucous producing cells. Sub-mucosa lacks these cells. Muscularis is composed of several thin layers of smooth muscle fibers.

Feeding Ethology and oral appendage morphology

Freshwater crab *S. hydrodroma* is omnivorous, feeding on crab appendages, snail, and other crabs and was also found to be cannibalistic in feeding. For feeding, food is first searched upon walking and held by chelipeds. Chelipeds acts as the principal feeding tool. Third maxillipeds and chelae helps to convey food particles to mouth. In mouth, food is first cut into pieces and ground by mandibles. Chelae, third maxilliped and mandible play a major role in the feeding activity.

Dietary preferences

A variety of food items were accepted by male and female *S. hydrodroma*. However, animal materials were preferred. Experimentally, food selection patterns varied with sex. Male crabs preferred 54% egg white and females preferred 35% commercial fish food, which were their major choice of food consumption.

When offered with a choice of food items the preference was as follows:

1. **Male:** Egg white>Snail> Rice puffs> Commercial fish food>Fish head.
2. **Female:** Commercial fish food> Egg white> Fish head> Snail> Rice puffs.

Table 1. Morphometric indices of male and female crabs

Parameters	Male	Female
CL (cm)	4.36±0.8	4.63±0.3
CW (cm)	4.33±0.3	5.13±0.3
Major chela (cm)	6.70±0.8	5.90±1.2
Minor chela (cm)	5.56±1.0	4.93±1.0
Weight (g)	28.8±4.5	29.84±2.6

Mean±S.D is obtained from triplicate analysis. All values are significant at P≤0.05. CL-Carapace Length; CW-Carapace Width; TW-Total Weight; MACL-Major Chelate Length; MICL-Minor Chelate Length.

Table 2. Food analysis in gut of male and female S. hydrodroma

Plant materials	Phytoplankton	Chlorella, Desmids, Scenedesmus, Dinoflagellates, Spirogyra species, Coscinodiscus excentricus, Chroococcus indicus, Navicula radiosa
	Plant remains	Leaf remains, Root materials, Unknown plant fragments.
Animal materials	Zooplankton	Moina species, Nematode, Copepods
	Animal remains	Crustacean appendages, Fish remains
Detritus	Unidentified debris, clay-organic matter complex.	

Table 3. Physico-chemical parameters of the ecological niche

S. No	Parameters	Value
1	Temperature (˚C)	20.97±0.45
2	pH (Numbers)	9±0.2
3	Total Dissolved Solids (mg/L)	215.00±1.00
4	BOD (mg/L)	2.20±0.10
5	Total Hardness (mg/L)	176.00±1.00
6	Turbidity (mg/L)	0.90±0.10

Mean±S.D is obtained from triplicate analysis. All values are significant at P≤0.05.

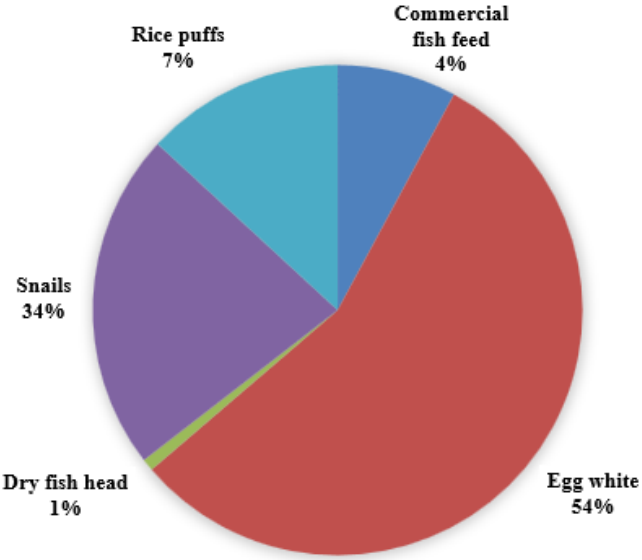

Figure 1: Dietary preferences of male *S. hydrodroma*

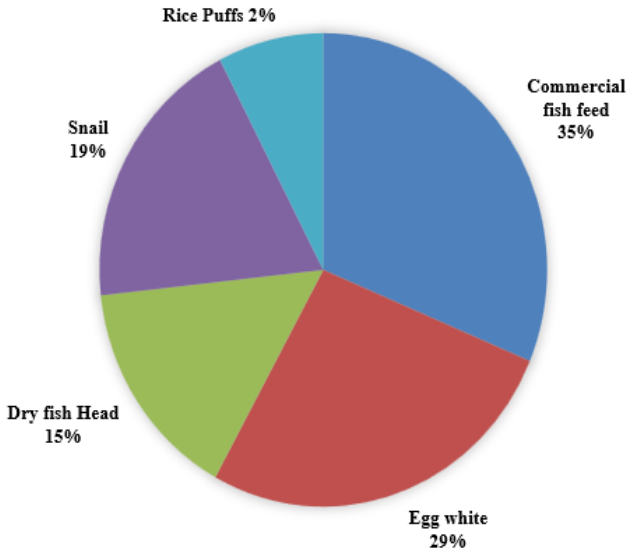

Figure 2: Dietary preferences of female *S. hydrodroma*

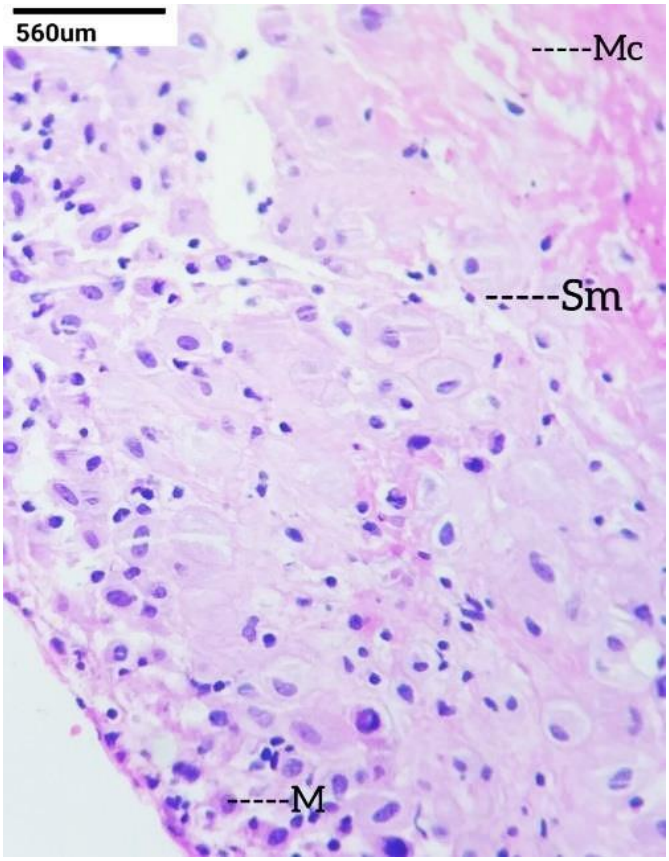

Figure 3: Photomicrograph on transverse section of stomach of *S. hydrodroma*, Showing: M-mucosa, Sm-Sub mucosa, Mc-Muscularis (X560) H&E stain**.**

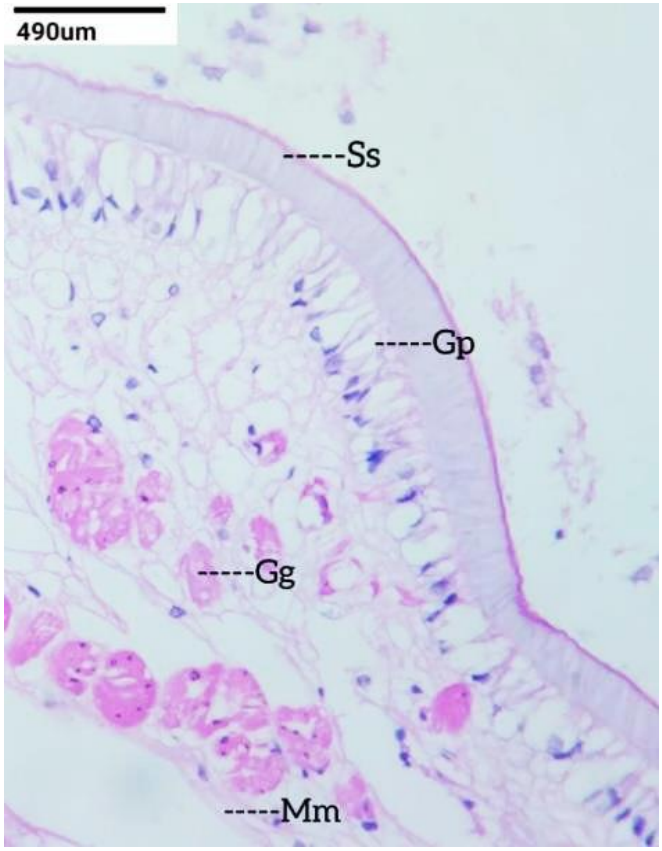

Figure 4: Photomicrograph on transverse section of stomach of *S. hydrodroma*, Showing: Ss-Secretory sheath, Gp-Gastric pits, Gg-Gastric glands, Mm-Muscularis mucosa (X490) H&E stain.

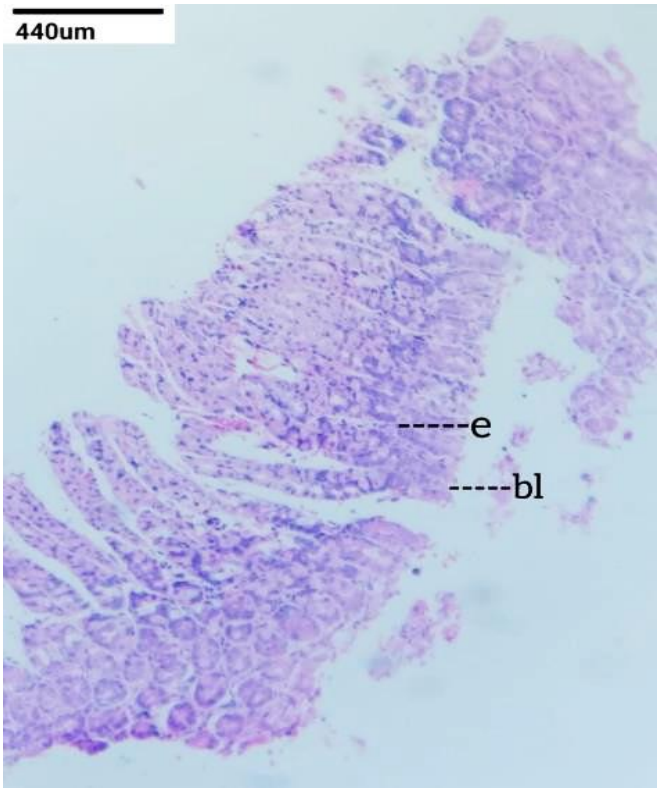

Figure 5: Photomicrograph on transverse section of gut of *S. hydrodroma*, Showing: e,-epithelium, bl-basal lamina (X440) H&E stain.

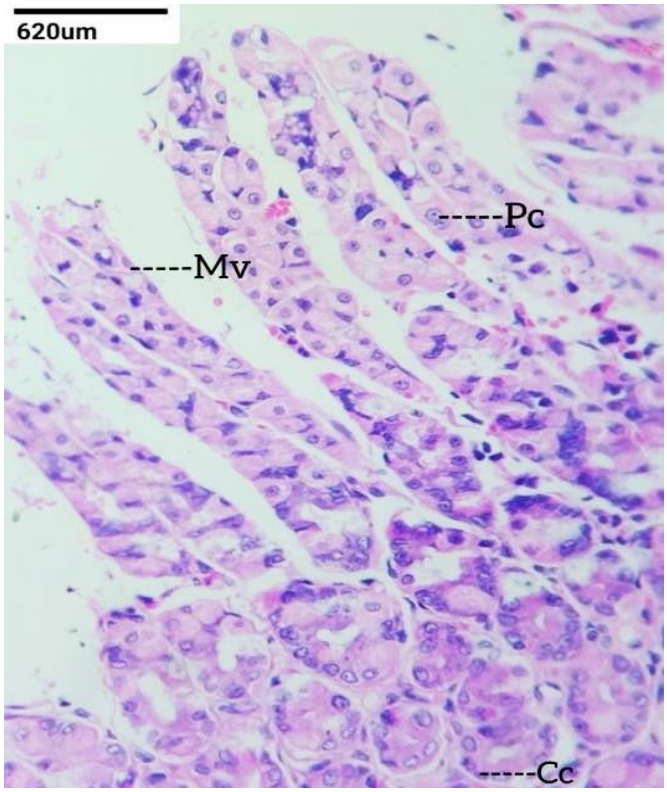

Figure 7: Photomicrograph on transverse section of gut of *S. hydrodroma*, Showing: Pc- Parietal cells, Mv-Microvillus, Cc- Chief cells (X620), H&E stain.

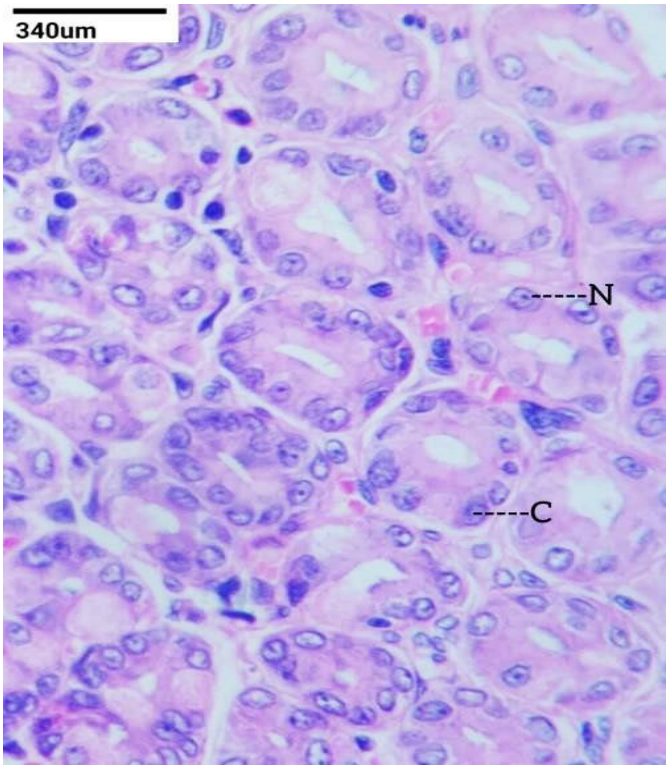

Figure 6: Photomicrograph on transverse section of gut of *S. hydrodroma*, Showing: N- Nucleus, C-Cytoplasm (X340) H&E stain.

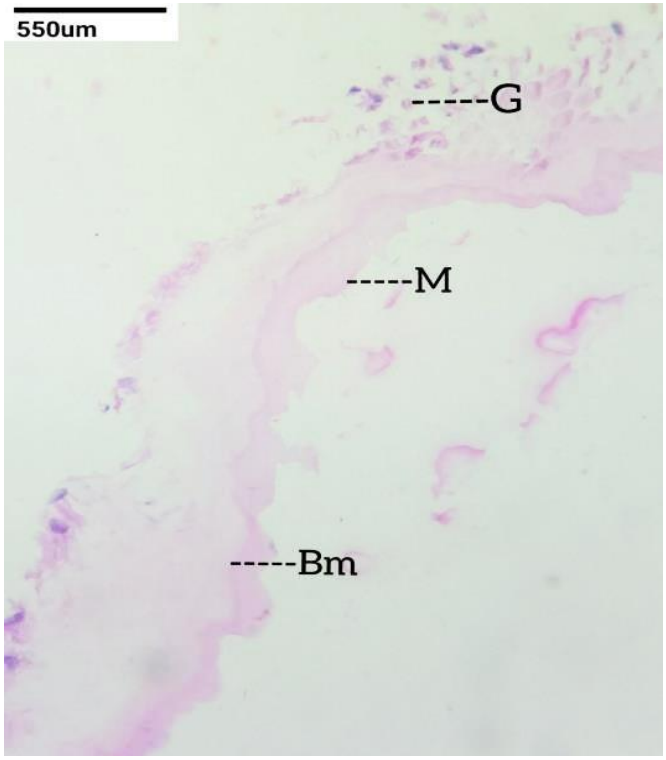

Figure 8: Photomicrograph on transverse section of gut of *S. hydrodroma*, Showing: M- mucosa, G-goblet cells, Bm-Basement membrane (X550), H&E stain.

Figure 9: oral appendage morphology in *S. hydrodroma*. a) Antennule b) Second antenna c) Mandible d) 1st maxilliped e) 1st and 2nd maxillae f) 2nd and 3rd maxilliped

DISCUSSION

Analysis of gut contents in wild captured *S. hydrodroma*, indicated that there is a wide variety of food items mostly comprising of plant materials and planktons as well as detritus, which indicates a herbivore-biased omnivores. In the present study, considerably a low turbidity value is recorded 0.90±0.10mg/L, which attributes to the low zooplankton distribution in the river. Turbidity in the water is due to suspended particles (clay, divided organic and inorganic materials, salts, colored organic matter and phyto- and zooplanktons). Omondi *et al.*, [26] reported that, turbidity reduces visibility of the predators and their feeding rhythms. Also decomposing plant materials contains greater number of associated microorganisms, such as bacteria and fungi, which provides improved nutrition [27]. There was no significant difference recorded between the quantity of food consumed by males and females, except slight variations of food items like plankton and detritus. Thus, *S. hydrodroma* in Cauvery River is highly omnivorous in nature with planktons forming the principal food item followed by plant and animal material.

According to Newman and Smith, [28] establishing typical anatomical structure and characterizing the esophagus and intestine would provide information on tissue anomalies, and associated pathology. Foregut and hindgut are derived from ectoderm, characterized by epithelia and covered by cuticle; while midgut is derived from embryonic endoderm and characterized by secretory epithelial cells with brush border [29]. Oesophagus is a simple muscular tube to drive food into cardiac stomach. The wall folds permit to change the distension degree in relation to the size and amount of ingested food. The freshwater crab generally feeds on prey such as, plant and animal materials. Esophageal muscularis therefore has a protective function for ingestion of solid material [30]. Stomach has a main role in production of hydrochloric acid to aid digestion [31]. The midgut tract of decapods is a simple tube with a columnar epithelium and located between the stomach and the hindgut tract [32]. Its role is associated with osmoregulation, transport of the ingested food and absorption of nutrients [9]. The morphological and anatomical analysis of the midgut tract is reported in adult decapods [29]. The histo-morphology of gut and stomach in *S. hydrodroma* shows great similarities to other adult Decapoda taxa. Intestinal cells of crustaceans aids in secretion of the peritrophic membrane, transport of water and ions, movement of fecal pellets through the gut [33].

Plant are the major sources of nutrition for crabs [34]. Consequently, under laboratory investigation *S. hydrodroma* is found to consumes both plant and animal matter exhibiting a strong preference for animal-based foods. Therefore *S. hydrodroma* is reported to be an opportunistic carnivore that feeds on both plant and animal matter. Although, there was a difference in preference shown by both the sexes to these foods. Thus, egg white was the most favored food item in male (54%) and commercial fish feed was the most favored food item in female (35%). However, the second most favored food item by the males is snail (34%), and in female it is egg white (29%). Moreover, the crabs were found to be cannibalistic on several occasions in the experimental tanks. The feeding intensity was higher in females than in males, that is highly correlated with the feeding intensity of mud crab *Scylla tranquebarica* [35]. Egg white is a low-calorie, fat-free and an excellent source of protein. Commercial feeds are carefully formulated to ensure that protein and amino acid requirements are met [36].

Animals can hunt for food by walking down the bottom of their habitat and then smelling the ground. When they find a food source, they use their legs to catch it, bring it to their front, and then begin to eat. Decapods primarily rely on chemoreception to locate food sources; they do not use mechano-receptors or eyesight to guide their feeding habit. Particles are trapped by the dense setation of the 2nd and 3rd maxillipeds are brushed off and ingested [37].

Chemoreceptive sensilla assists in the detection of food items. These sensilla are primarily found on appendages, including antennae, antennules, dactyls, and mouth parts

[38]. Since chemoreceptors are found on every part of a crustacean body (including its appendages) they are crucial. Similar to shrimp *Axianassa austrialis*, the first and second pereiopods as well as the mouth portions of *Spiralothelphusa hydrodroma* are the appendages directly involved in the feeding activity [39]. The particles are gathered by the third pair of maxillipeds and then transferred to the second pair. Appendages are reported to have vital role in feeding activity of thalassinidean shrimp [40].

CONCLUSION

Spiralothelphusa hydrodroma is an herbivore biased omnivore that uses chelipeds as its primary means of obtaining food. Under carefully controlled laboratory conditions, the females of this species typically prefer commercial fish feed over a specific range of food items, while the males typically prefer egg white. Consequently, their gut and stomach histology supports the suitability of these diets and the river water is less polluted and hence the crabs lives in a healthy environment.

DECLARATIONS

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Authorship contributions

Concept: A.A., Design: A.A., Data Collection or Processing: P.S., Analysis or Interpretation: A.A., P.S., Literature Search: P.S., Writing: A.A., P.S.

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Competing interests

The authors declared that there is no competing interest.

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