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Food and feeding of *Phallusia nigra*, Savigny, 1816

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Abstract
Ascidians are conspicuous and important members of shallow benthic community. Ascidians belong to phylum Chordata and class Ascidiacea. Phytoplankton and other organic particles in suspension constitute the bulk of the food of the many species of ascidians. The present study was carried out to analyze the stomach contents of solitary ascidian *Phallusia nigra* to identify the diet components on seasonal basis. The highest value of index of preponderance was shown by sand particles (54.69%) followed by micro flora (24.78%), meio fauna (13.63%), miscellaneous (4.86%) and macro fauna (2.034%). The percentage of feeding intensity was highest (27.64%) for heavy fed group and lowest value was reported for empty fed group (11.79%). *P. nigra* has been found to be an unselective suspension filter feeder which mainly feed upon large quantity of mud and micro flora (Cosinodiscus sp, Navicula sp and Prorocentrum sp) and macro fauna were less frequent than meiofauna.

Keywords: asidians, phytoplankton, feeding intensity, vizhinjam, season

1. Introduction
Ascidians are conspicuous and important members of shallow benthic community. Ascidians belong to phylum Chordata and class Ascidiacea. Phytoplankton and other organic particles in suspension constitute the bulk of the food of the many species of ascidians. Large numbers of ascidian is an indication of richly available food in water [1]. All ascidians are ciliary mucus filter feeders that remove plankton from the water current pumped through the pharynx. Little research work has been done on the value and quantity of food requirement of ascidians in India. The gut content perhaps varies according to the local water conditions [2, 3]. Large quantity of mud noted in the gut of *Ascidia sydneiensis* but the same species may contain algal cells and diatoms with little organic matter[2].
Solitary ascidian *Herdmania pallida* has been found to be an unselective suspension filter feeder which mainly feed upon large quantity of mud and micro flora such as diatoms and algae. Macro fauna were found less frequently than meo fauna in the stomach of ascidians [4]. *Phallusia nigra* is a solitary marine tunicate of the ascidian class found in tropical seas around the world. It usually lives in shallow waters attached to any hard substrate such as dead coral, pier pilings or floats [5]. An adult *P. nigra* may be 10cm in length. It is considered as a nonindigenous species. It is mainly distributed in tropical western Atlantic, Mediterranean, Indian oceans and Hawaiian Islands[5]. Filtration rates of *P. nigra* are high, allowing them to obtain large quantity of plankton from the water. It can pass 173 litters of water through its body in 24hours [6].
All ascidians are ciliary mucus filter feeders that utilize very small particulate matter primarily 0.5-2µ range [7, 8] although they do take larger particles including their own gametes [9]. When present in large numbers, their high filtration rate can have a dramatic effect on available plankton and suspended organic matter [10, 11]. The present study was carried out to analyze the stomach contents of solitary ascidian *Phallusia nigra* over a year to identify the diet components on seasonal basis.

2. Materials and methods
The collection site is Vizhinjam Bay situated in the south-west coast of India, about 16 km south of Thiruvananthapuram (Lat 8º 22'30"N - 76º56'15"E) and which is a narrow bay enclosed by a breakwater jutting into the sea on the western side. In the current study ascidian samples were collected at monthly intervals from Vizhinjam Bay. The sample collection was performed from June 2011 to May 2012. The solitary ascidian *P. nigra* is distributed from the shore in a depth of 5m to 10m depth. Species were collected during low tide by SCUBA divers. The total length, gut length and body weight of each individual were recorded. The size of the organisms ranging from 5 to 9 cm in length and 0.98g to 2.8g weight. A total of 100 specimens were examined during the study period (Plate 2). The alimentary tract was dissected out and preserved in 5% formaldehyde[12].

Systematic position of the species

Phylum	: Chordata
Sub-Phylum	: Tunicata or Urochordata
Class	: Ascidiacea
Order	: Enterogona
Sub-Order	: Phlebobranchia
Family	: Ascidiidae
Genus	: *Phallusia*
Species	: *P. nigra*

The gut contents were carefully emptied into a Petri dish and examined with the help of a binocular microscope. For evaluating the presence of food consumed, the different food items were assessed under the categories plenty, common, a

few, little, and rare and the points 40, 30, 20, 10, and 0 were allotted according to the volume of the items and these values were summed up and scaled done to percentages to indicate the percentage composition of food items for various methods. Based on the distension of the stomachs and the quantity of food present, points were given and the intensity of feeding was recorded. The intensity of feeding was assessed based on the state of fullness of the gut and the amount of food contained in it and categorized as 'poor' when the gut contained a little amount of food, moderate when the gut is half full, full when the gut is almost full, gorged when the gut is full Depending on the intensity of feeding and gut fullness, points were given into the stomach as 5, 10, 15, 20, and 25 for poor, moderate, full, good and gorged respectively. The content of the gut was squeezed into Petri dish and the contents were identified and counted under stereoscopic binocular microscope, sorted and classified into the lowest possible taxon and enumerated.

2.1 Index of preponderance

Here a combination of occurrence (qualitative method) and volume (quantitative method) of food contents is taken into account for the accurate grading of different food items [13]. This can be expressed as

$$Ii = \frac{ViOi}{\sum ViOi} \times 100$$

Where, Ii is the index of preponderance; Vi and Oi represent the percentage volume and occurrence of particular food.

3. Results

The gut content analysis of *P. nigra* revealed the presence of the following food items (Table 3. 1) which are classified into 5 groups such as micro flora (1) (Coscinodiscus sp, Thalassiosira sp, Odentella sp, Navicula sp, and Prorocentrum sp), meio fauna (2) (ostracodes, copepods, gastropods and foraminifera's), macro fauna (3) (molluscans and amphipods) sand particles (4) and some miscellaneous items (5) were observed.

Table 1: Percentage composition of food items in *P. nigra*

Food categories	Name of the food items
1. Micro flora	24.78% of the foods were algae and diatoms.
2. Meio fauna	13.63% of the food was ostracodes, copepods gastropods and foraminifera
3. Macro fauna	2.0341% of the food materials include molluscans and amphipods
4. Sand particles	54.69% of the food items were formed by sand particles.
5. Miscellaneous items	.86% in the gut content included all other items in the gut like protozoans, fish eggs, shell matter, crustacean parts and semi digested parts of copepods

The index of preponderance with percentage volume of the different food items is depicted in the pie diagram (Fig. 1). The index of preponderance from June to May was shown from Tables 3. 2 to 3. 14. The highest value of index of preponderance was shown by sand particles (54.69%) followed by micro flora (24.78%), meio fauna (13.63%), miscellaneous (4.86%) and macro fauna (2.034%). In the gut content of *P. nigra* the sand particle was higher (65.63%) in the month of June and lower (42.08%) in January. Micro flora was higher (32.09%) in the month of December and lowest (17.11%) in May. While in the case of Meio fauna reported maximum (18.65%) in February and minimum value (7.69%) was recorded in July. The miscellaneous food item was observed maximum (11.63%) in May and minimum (2.5%) was observed in the month of January. In the case of macro fauna maximum value (3.08%) was obtained in February and minimum (0.25%) in June.

The percentage composition of the feeding intensity of *P. nigra* is depicted in the pie diagram (Fig. 2). The feeding intensity of *P. nigra* for various months from June to May was given as Tables from 3. 15 to 3. 27. The percentage of feeding intensity was highest (27.64%) for heavy fed group and lowest value was reported for empty fed group (11.79%). The value obtained for active fed type was 26.04%. It is higher than poorly fed 17.81% and medium fed 16.71%. The heavy fed group showed that the feeding intensity was recorded highest (37.78%) in the month of January and lowest (10.8%) in the month of June. The actively fed type of feeding intensity in *P. nigra* was highest (33.58%) during the month of February and lowest (22.37%) in the month of May. The maximum feeding intensity (32.89%) for poorly fed type was reported in the month of June and minimum (7.88%) in the month of January.

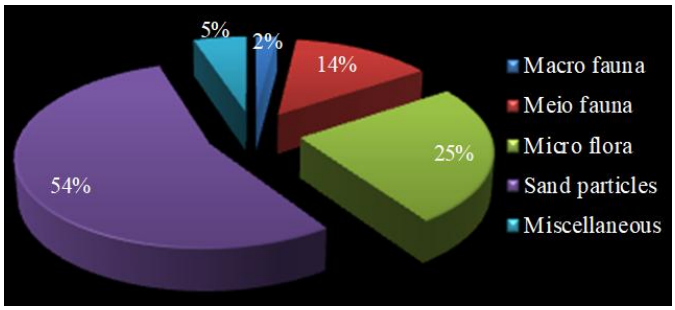

Fig 1: Percentage composition of various food items of *P. nigra*

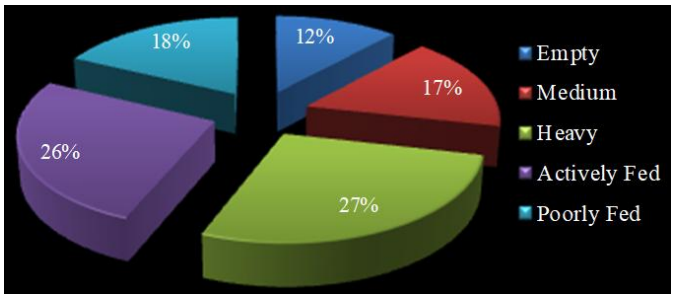

Fig 2: Percentage composition of feeding intensity of *P. nigra*

4. Discussion

In general, ascidians found to feed on diatoms, bacteria and radiolarians which are characteristics of water immediately above the substratum. Subtle difference appears to exist in food of related species living in the same area. Ascidians change its food habits with change in season[4]. In the present study also the solitary ascidian *P. nigra* changes its food habit with change in season. *P. nigra* is a black solitary tunicate feed upon large quantity of sand, micro flora, meiofauna and macro fauna. The percentage composition of food items are varied through the study period. The feeding intensity of *P. nigra* also varied in each month of food items in the habitat [14].

The percentage of sand particle is recorded to be the highest (54.44%), followed by micro flora (24.78%) meieofauna (13.63%), macro fauna (2.03%) and others (5.13%). The lowest level of macro fauna and higher level of sand particles were reported in the present study are in accordance with the observation of [4], who reported that ascidian *H. pallida* mainly feed upon large quantity of mud and micro flora and macro fauna are found less frequently than meio fauna.

The higher value for preponderance was shown by sand particles (65.63%) in the month of June and lowest value (42.08%) was observed in the month of January. The highest repletion percentage of micro flora (32.09%) was measured in December, the lowest value in (17.11%) May. Meiofauna was richly reported in February (18.65%) and lowest in July (7.69%). The preponderance value of others and unidentified items were also high (11.63%) in May and lowest level (0.64%) was observed in August. Macro fauna was highest (3.08%) in the month of February and lowest (0.25) in the month of June. Studies on feeding activity in benthic suspension feeders in the Antarctic Ocean suggest that variation in resource availability act as the main cue for their seasonal pattern [15]. However, studies on benthic suspension feeders conducted in the past decade suggest that food availability could be as important as temperature in determining seasonality in marine organisms [16, 17].

Gut content analysis of *P. nigra* consists of many amphipods and copepods along with plankters and the presence of various body parts of crustaceans are reported by [14. 3] has been reported that *H. pallida* is an unselective suspension filter feeder which mainly feed upon large quantity of mud and micro flora (Cosinodiscus sp, Naviculla sp, Prorocentrum sp and Thalassiosira sp). Macro fauna was found less frequently than meio fauna. In a previous study based on the chemical analysis carried in ascidian, *Cnemidocarp verrucosa* a year round intake and absorption of organic matter were detected [15]. This means that ascidians can handle large amounts of terrigenous materials which does not affect the absorption of organic session and can survive in a habitat with low phytoplankton throughout the whole year and especially in winter [15].

The different types of feeding intensities were observed during June 2010 to July 2011 in *P. nigra*, each specimen were examined and categorized as empty, medium, heavy, and active fed, poorly fed. The percentages of poorly fed was 19%, heavy fed 28%, and medium 17%, and active fed 23%, and empty fed was 13%. The feeding intensity for poorly fed (28.1%) was more found in the month of July and lowest (6%) was in February, heavy feed type (38.1%) was more recorded in the month of January and lowest percentage (15.2%) was observed in the month of June. Medium fed type was observed maximum (20.4%) in the month of February and minimum feeding intensity was recorded in (12.1%) July. Feeding intensity for actively fed type (35.2%) was more recorded in the month of January and minimum (17.5%) feeding intensity was recorded in the month of July. Empty feed (15.4%) was more recorded in the month of March and lowest was recorded in November (7.6%) and there was no feeding activity was observed in the month of June. The feeding intensity was varied in each month which could be due to the availability of food items in the habitat [4]. In this study also the feeding intensity of *P. nigra* varied in each month due to the variation in the abundance of food in the habitat.

P. nigra is a sessile, benthic filter feeder. The incurrent siphon takes waste sieve like pharyngeal basket that filters out food of appropriate size class before pumped from the animal via excurrent siphons. The food particles enter the brachial sac through the water current produced by the action of the cilia. The tentacles act as filters to prevent the coarse particles from entering. The food particles get entangled in the mucus sheet and they are lashed out by the flagella from the endostylar groove to the brachial sac. From there they are passed on to the dorsal lamina and along the later reach the esophagus. The digestion is extracellular. Gut content analysis on black tunicates have several prey items, including; barnacle naupli, and cyprid larvae, copepod nauplii, and gastropod veligers and setigers [18]. In addition, several species of invertebrates are commonly found living on or inside the tunic, amphipods, copepod and crab [19]. A wide range of particle size was found in stomach contents. Among these particles, large detritus and minute particles were the most abundant items during the year; this material should provide enough energy to ascidians.

Length-weight relation (LWR) of *P. nigra* was analyzed using 100 samples collected during June to May. The correlation coefficient (r) obtained from the length weight analysis of *P. nigra* was 0.81321 which clearly exhibits the strong positive correlation between length and weight of the body of *P. nigra*. The b value obtained was 2.0214. It was below the cube value of 3 and hence it implied that *P. nigra* showed a negative allometric growth.

P. nigra has been found to be an unselective suspension filter feeder which mainly feed upon large quantity of mud and micro flora (Cosinodiscus sp, Navicula sp and Prorocentrum sp) and macro fauna were less frequent than meiofauna [14]. also reported that the gut content of *P. nigra* collected from Tutucurion coast, contain many amphipods and copepods along with plankton and the presence of various body parts of crustaceans.

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