CHAPTER 6
FOOD AND FEEDING HABITS OF ASCIDIANS

"In every walk with nature one receives far more than he seeks.

John Muir"
INTRODUCTION
Nutrition is one of the vital requirements of living beings in nature for the continuation of their dynamic needs like growth and reproduction for survival. Ascidians directly depend upon their surrounding marine environment for their food requirements and are highly adopted in their food and feeding habits, utilizing most of the readily available food. Ascidians are considered as primary and secondary consumers of the marine ecosystem as they feed on mostly phytoplankton, zooplankton and suspended organic particles of their surrounding environments by the filter feeding (Millar, 1971; Ruppert et al., 2004; Karthikeyan et al., 2011). Food collection methods of ascidians were examined by several researchers (Orton, 1913). Fol described the method elaborately (Fol, 1872; Orton, 1913). Later on, Roule (1884), Willey (1894) and Herdman (1904) described the same process. Experiments were carried out on diverse species of ascidians like Asciella aspera, Phallusia mammillata, Ascidia mentula, Ascidia virginea, Ciona intestinalis, Clavelina lepadiformis, Diplosoma gelatinosum and Morchellium sp. to study the mode of feeding in ascidians (Orton, 1913). Besides the experiments, feeding habit of Molgula sp., Paramolgula gregaria (Lesson), Microcosmus sulcatus (Coquebert) (Costa, 1960; Millar, 1971), and Herdmania pallida (Karthikeyan et al., 2009) were examined and many other species were also been studied.

Ascidians inhale the water and the food particles (Planktons, nutrients etc.) through the branchial siphon (incurrent flow) to the branchial cavity and against the wall of the gill. Inside the branchial sac, water current produced by the cilia situated on the margins of the gill slits (Orton, 1913). Like most of the filter feeders, it filters a large amount of food and filters the water equivalent to its body volume per second (Ruppert et al., 2004) and it has been found that few centimeter long Phallusia nigra can filter 173 liters water for feeding in 24 hours (Ruppert et al., 2004). Experiments on Ciona intestinalis exhibit the role of starvation to manage the filtration rate increases and decreases after sometimes may be due to the fullness of the gut (Petersen and Riisgard, 1992). Petersen and Riisgard (1992) also found that the maximum filtration rate found in 4 to 21ºC temperature. Above 21ºC, the filtration rate declined with the increase of temperature. Filtration rate of the ascidians increases along with the body size of the ascidians (Sumerel, 2009). Ascidians can regulate their feeding by varying frequency of contraction and it is also reported that the frequency to increase at the lower food concentration (Millar, 1971). Ascidians
can halt feeding by closing the branchial siphon, by arresting ciliary beats or by stopping production of flow of mucus from endostyle (Ruppert et al., 2004). Suspended food particles are pushed towards endostylar groove and then immediately lashed out of this groove onto the wall of the pharynx (Orton, 1913). All the food particles are trapped by the mucus net secreted by the endostyle (Orton, 1913; Ruppert et al., 2004). This mucus covered particles which are transported across the branchial sac to the dorsal lamina (Orton, 1913; Ruppert et al., 2004). This mucus trapped food particles then rolled into a thread like cord by dorsal lamina and conveyed into the oesophagus (Orton, 1913; Ruppert et al., 2004). In ascidians, mostly U-shaped gut loop contains oesophagus, stomach, intestine, rectum and anus. Stomach contains the secretory cell lining and act as the site of extracellular digestion. In most of the ascidians, a pyloric gland has been seen on the outer wall of the intestine which secret digestive enzymes, pH regulatory substances and helps in the removal of blood borne toxins. The undigested material passes through the anus (Ruppert et al., 2004). These creatures also can be in predatory in nature e.g. *Megalodocopia hians* Oka, 1918 while other deep sea ascidians are not true predators. As *Megalodocopia hians*’s pharynx did not have the ciliary perforation which is commonly known as stigmata (Berrill, 1950; Kott, 1969; Okuyama et al., 2002). They use the water current for their feeding (Okuyama et al., 2002). Okuyama et al. (2002) found that in in-situ condition, *Megalodocopia hians* usually opens its extraordinary large oral apertures to engulf the drifting particles in the water current as most of the animals face their oral apertures in the same direction, keeping their apertures open. Okuyama et al. (2002) found a water current of 0.03~0.32 m/s in their surrounding water. Due to this reason, *Megalodocopia hians* might catch relatively large materials drifting in the water current and engulf them without distinction. In the gut content of *Megalodocopia hians*, diatoms, detritus and small crustaceans etc. were found which supports the evidence of non-selective feeding habit (Okuyama et al., 2002) due to their morphology of pharynx (Monniot and Monniot, 1978; Okuyama et al., 2002). Some researchers believe that photosymbiotic didemnid ascidians use the excess photosynthate materials as their food by the prochlorons beside their filtrate food materials (Ruppert et al., 2004). For the culture of ascidians, studies on the natural diet of ascidians are very important. Although for cultured ascidians a variety of algae (diatom) are provided as food (Joly et al., 2007). Size of the food particles varied for same species in juvenile and adult stage, as < 10 µm is suitable for juveniles of *Ciona*
intestinalis (Joly et al., 2007; Tyree, 2001) while adult can lived on 20 – 60 µm particles (Joly et al., 2007). Berrill (1947) identified Rhodomonas as more suitable food for Ciona intestinalis. Most of the ascidians in natural conditions feeds upon plankton and suspended organic materials in the surrounding water current of them. Costa (1969) found bacteria, diatoms and radioralians in the branchial sac of the Microcosmus salcatus which are the characteristic animals, present in the water immediately surrounding its subatratum (Millar, 1971; Karthikeyan et al., 2011). Hence, the feeding habit of ascidians will be varying with its surrounding water constituents (Kott, 1974; Millar, 1957; Karthikeyan et al., 2011). As ascidians are sessile in nature, they are non-selective feeders regarding a particular sized animals or water components. The food particle size depends upon its pharynx perforation and its ciliary border which take active part to filter the water inhaled by ascidians. It has been found that the gut content of Ascidia sydneiensis contains a large amount of sediment particles however, the same species may contain large number of algal diatoms with little amount of inorganic particles depending upon its habitat (Millar, 1971; Karthikeyan et al., 2011). Subtle difference appears to exist in the food of related species living in the same area as found in Phallusia nigra and Ascidia interrupta (Goodbody, 1966; Millar, 1971). In this case, difference in the arrangement of the oral tentacle may be responsible. Although, there might be some variation in food content of the water since the species occupy somewhat different ecological niche (Millar, 1971). All the ascidians are ciliary-mucus filter feeders that utilize very small particulate matter primarily in the 0.5 – 2 µm range (Flood and Fiala-Medioni., 1981; Coma et al., 2001; Tatian et al., 2002;Bone et al., 2003). They do take larger particles including their own gametes (Young, 1988). When present in large numbers, their high filtration rate can have a dramatic effect on available plankton and suspended organic matter (Hily, 1991; Coma et al., 2000; Riisgard and Larsen, 2000). The feeding behaviour of ascidians is important to understand their ecological role and their food value and to determine the edible and non-edible species categorization. This chapter deals with the food particles of two species of ascidian such as Polycarpa pigmentata (Herdman) and Phallusia mammillata (Cuvier, 1815) belongs to the Orders Stolidobarnchia and Phlebobranchia to understand their position in the ecological niche in the same locality as well as in different locality along with their feeding variation depending on the seasonal variation.
AIMS AND OBJECTIVES

- Observation of feeding behavior of Ascidians in two different orders, Stolidobranchia (Polycarpa pigmentata) and Phlebobranchia (Phallusia mammillata)
- Variability in feeding practices of ascidians according to areas of A&N Islands
- Estimation of ecological role of a species depending upon feeding habit

STUDY AREAS

Craggy Island

Craggy Island (Lat.: 13°13.516’N; Long.: 93°03.406’E) is situated in the Andaman Sea of North Andaman region of Andaman and Nicobar Islands (Fig. 1). It is the only island at the close vicinity of Saddle Peak National Park. The total landmass of this island is near about 0.08 sq. km. and covered with littoral forest. The island fringe is covered by healthy reef environment with the maximum depth of 31 m and average depth of 13 m.

Oliver Island

Oliver Island (Lat.: 12°59.684’N; Long.: 92°57.257’E) is situated in the Andaman Sea of Middle Andaman region of Andaman and Nicobar Islands (Fig. 1). It is one of the 96 sanctuaries of these groups of islands and notified in 1987. The total landmass of the island is 0.16 sq. km. This small island is mostly covered by mangrove and littoral forests. The island lies in the creek, and characterized by muddy areas. The maximum depth of shelf region of this island is 27 m with the average of 13 m. Most of the reefs are reported at the depth of 6 to 17 m.

North Bay

North Bay (Lat.: 11°43.006’N; Long.: 92°45.465’E) is situated in the Andaman Sea of South Andaman region of Andaman and Nicobar Islands (Fig. 1). It is situated at the foothill of Mount Harriet National Park. The southern side of this area harbours protected by live reef cover while eastern side of the area is covered by rocky substrate. The depth of the nearshore reef areas varies from 2 to 9 m while a maximum depth of 37 is reported with sandy bottom. The shallow reef areas of North Bay is one of the favorable destination for the tourist due to facilities of watersports.
like SCUBA diving, Snorkeling, Jet-ski riding, Glass-bottom boat riding, Banana riding etc.

Fig.1. Study areas- Craggy Island, Oliver Island and North Bay of A&N Islands
MATERIAL AND METHODS
The study was made on the two species of solitary ascidians viz., *Polycarpa pigmentata* and *Phallusia mammillata*. Sampling of both the species of ascidians were made from the three regions namely North Andaman, Middle Andaman and South Andaman by simple hand picking method employing Self Containing Underwater Breathing Apparatus (SCUBA) diving during September 2013 to August 2016. The collection of data was also made on the basis of three seasons such as Post-monsoon (September to December), Monsoon (May to August) and Pre-monsoon (January to April). Collected specimens were fixed in 4% formaldehyde-seawater solution directly without relaxation of the specimen to avoid any extracellular digestion. Fixed specimens were measured, weighted and dissected within 4 days to avoid any further disintegration of the food particles. The gut loop was also weighted separately for the precision of the result by using weigh balance (MH-200). Dissection of fresh specimens were avoided due to contraction of the specimen as *Polycarpa pigmentata* is covered with tough leathery test and very difficult to dissect during contract stage while this problem is not obvious for *Phallusia mammillata* as they used to contract the branchial sac region quickly during disturbed condition which is faster than the test. The dissected alimentary canal was preserved in 4% formaldehyde-seawater solution for the further study. The stomach content was classified into some categories such as gorged or distended, full, ¾ full, ½ full, ¼ full, trace and empty depending upon the relative fullness of stomach by eye estimation. Eye Estimation method and Points (volumetric) method used to determine the volume of the food content (Zacharia and Abdurahiman, 2004). The gut content of specimens was emptied in a petridish before examination under the binocular microscope (Labomed CZM 4) to estimate the quality and quantity of the food. Sedgewick Rafter slide was used to quantify the components of food material. Identification of the food components upto lowest possible taxon was carried out with the conjunction of Al-Yamani *et al.* (2011) and Padmavati *et al.* (2008).

For quantitative analysis:
i) Frequency of occurrence was calculated by using the following formula (Zacharia and Abdurahiman, 2004).

\[
O_i = \frac{J_i}{P}
\]
Where, \( O_i = \) Frequency of Occurrence, \( J_i = \) Number of individuals containing prey \( i \) and \( P = \) Number of individuals with food in their stomach

ii) Abundance of food particles was recorded using the ACFOR scaling method. The methodology includes: \( A = \) abundant (species present in 30% or more of quadrant squares), \( C = \) common (species present in 20-29% of quadrant squares), \( F = \) frequent (species present in 10-19% of quadrant squares), \( O = \) occasional (species present in 5-9% of quadrant squares) and \( R = \) rare (species present in 1-4% of quadrant squares).

iii) The Gut Repletion Index (GRI) was calculated using the standard formula (Ekpo et al., 2014).

\[
\text{GRI} = \frac{\text{Number of non-empty guts}}{\text{total number of specimens examined}} \times 100
\]

iv) Seasonal variation of the feeding intensity was calculated by Gasto Somatic Index (G.S.I.) (Kumar et al., 2015) using the formula.

\[
\text{G.S.I.} = \frac{\text{Weight of gut}}{\text{Weight of ascidian individual}} \times 100
\]

The diet was analyzed for each season and a cluster analysis was recorded with the help of Ward’s method, using the Past Statistic Program to define groups within similar diet (Viana and Vianna, 2014). One way ANOVA was applied to measure the differences in diet composition among seasons and feeding habit in relation to area (Agbali and El-Mor, 2015). Factorial ANOVA was also performed to understand the significant variation between the feeding patterns. The used factors were species, season and area.

RESULTS

Food particles of \( P. \text{pigmentata} \) and \( P. \text{mammillata} \) contains zooplankton (crustacean appendages, nematode, bivalve, tintinids) (Fig. 2 and 3a-c, f-h), fish egg (Fig. 3d-f) phytoplankton (\textit{Rhizosolenia}; and \textit{Thalassiotrix}) (Fig. 4), sand and soil particles and others (unidentified). In \textit{Polycarpa pigmentata}, frequency of occurrence was recorded...
maximum (0.39) for zooplankton in North Andaman during January to April of each year while minimum (0.06) was recorded for fish egg from North Andaman during January to April every year. Zooplankton was displayed maximum (0.38) frequency of occurrence during May to August from Middle Andaman and fish egg displayed the minimum (0.05) during the same time period from the same area. Zooplankton was found maximum (0.39) according to frequency of occurrence from South Andaman while (0.06) was recorded for fish egg from the same area during January to April of every year. Zooplankton was abundant, during all the seasons at all the study areas. Phytoplankton was found as abundant from South Andaman (0.31) during September and December, from Middle Andaman (0.30) as well as North Andaman (0.30) during May to August and September to December respectively (Fig. 5). Except these three time period, phytoplankton was commonly found. Soil particles were frequent, throughout the seasons at all areas while others were found as occasional except during January to April from South Andaman, September to December from Middle Andaman, from North Andaman it was frequent. Fish egg found as occasional in every season from every area except from North Andaman during May to August and September to December and from Middle Andaman during September to December.
Fig. 2: Food particles: a – d. Crustacean appendages; e – h. nematode.
Fig. 3: Food particles: a & b. Bivalves; c – e. Fish eggs; f – h. Tintinids.
Fig. 4: Food particles: a – d. *Rhizosolenia*; e – h. *Thalassiotrix*.
In *Phallusia mammillata*, maximum Frequency of Occurrence (0.61) was observed for soil particle in South Andaman during May to August of every year and minimum (0.02) for others was observed from the same area during the same season and also noticed the minimum (0.02) for fish egg from the same area during January to April of every year.

Soil particles shown maximum (0.57) frequency of occurrence during May to August from Middle Andaman and fish egg observed as minimum (0.03) in every season from the same area. Soil particles were found as maximum (0.60) according to frequency of occurrence during May to August from South Andaman while minimum (0.02) was recorded for fish egg and others from the same area during September to December of every year. Soil particles were abundant, in every season at all the study areas. Zooplankton was found as common in every season from all the area except...
from North Andaman during January to April as it was found with the frequency of 0.17. Except January to April from Middle Andaman, phytoplankton was frequently found, where it was observed as occasional (0.09). Others were frequent (0.12 and 0.10) during January to April from South Andaman and Middle Andaman respectively, while it was occasional (0.07 and 0.08) during September to December from South Andaman and Middle Andaman respectively and also during May to August (0.07) and January to April (0.05) from North Andaman (Fig. 6). Rest of the time it was observed as rarely found food particles. Fish egg found as rare from every season from every area during the study period.

**Fig. 6:** Frequency of Occurrence of food particles in *Phallusia mammillata* in September, 2013 to August, 2016
In *Polycarpa pigmentata*, the highest value of Gut Repletion Index (GRI) (88) was observed during May to August in South Andaman while the lowest value of GRI (74) was found during September to December of every year from North Andaman. The variation of GRI value in *Phallusia mammillata* was observed from 98 to 100 as they were found rarely with empty stomach. The lowest value of GRI (98) was observed during May to August and September to December from South Andaman while from North Andaman during May to August and in rest of the cases it was observed at highest value of GRI (100) (Fig. 7).

![Gut Repletion Index (GRI)](image)

**Fig. 7**: Gut Repletion Index (GRI) value of the *P. pigmentata* and *P. mammillata* in September, 2013 to August, 2016

The GSI value of *Polycarpa pigmentata* was observed between 0.79 and 2.17 according to the season and places. The highest value (2.17) of GSI was found during May to August from South Andaman and the lowest value (0.79) of GSI during May.
to August from Middle Andaman. The GSI value of *Phallusia mammillata* was recorded between 2.81 and 12.67 in accordance with season and study areas. The highest value (12.67) of GSI was found during January to April from South Andaman and the lowest value (2.81) of GSI during May to August from Middle Andaman (Fig. 8).

![Graph showing GSI values](image)

**Fig. 8:** Gastro Somatic Index (GSI) value of the *P. pigmentata* and *P. mammillata* in September, 2013 to August, 2016

Two factor (species and season) ANOVA displayed significant variation in GSI value between the species as the *P*-value was 0.0016 (*P* < 0.05) but seasonally they did not vary significantly as the *P*-value was 0.5152 (*P* > 0.05).

According to two factor (species and area) ANOVA a significant variation also found as the *P*-value was 0.0001 (*P* < 0.05) between two species while, there is a significant differences as the *P*-value was 0.0326 (*P* < 0.05) between two species on the basis of area.

However, GSI of *Polycarpa pigmentata* varies significantly in area wise with the *P*-value was 0.0103 (*P* < 0.05) but not varied significantly seasonally as the *P*-value was 0.0759 (*P* > 0.05). Whereas, GSI of *Phallusia mammillata* did not vary significantly area wise as well as season wise as the P-value was 0.1588 and 0.7989 (*P* > 0.05) respectively.
Two factor (species and season) ANOVA displayed no significant differences in GRI value between species ($P > 0.05$) and also seasonally as the $P$-value was 0.6481 ($P > 0.05$).

Two factor (species and area) ANOVA did not find significant variation in GRI value between the species ($P > 0.05$) as well as among the areas as the $P$-value was 0.1033 ($P > 0.05$).

However, GRI of *Polycarpa pigmentata* found no significant variation in area wise as the $P$-value was 0.1219 ($P > 0.05$) as well as seasonally as the $P$-value was 0.729 ($P > 0.05$). Similarly, GSI of *Phallusia mammillata* was not varied significantly area wise as well as season wise as the $P$-value was 0.2962 and 0.2962 ($P > 0.05$) respectively.

**DISCUSSION**

Feeding habit of ascidians is playing a great role in the species diversity as well as niche ecology of marine ecosystem. Being filter feeder, ascidians are mainly feeds on suspended particles in water and the planktonic community (Millar, 1971). In the present study, two species of the ascidians such as *Polycarpa pigmentata* and *Phallusia mammillata* represents the two different orders of the class Ascidiacea. It is documented from the present study, *Polycarpa pigmentata* feeds mainly on planktonic communities, both the phytoplankton and zooplankton and the suspended particles or sediments were found in less proportion in their gut content throughout the year and invariably in all regions. Whereas, *Phallusia mammillata* feeds mainly on suspended particles or sediments and throughout the year it has been seen that the sediments are more than 50% of the food material from all the studied area. In *P. mammillata* the planktonic proportion was much more less than observed in *P. pigmentata*. Feeding habit of both the species shown a distinct differentiation in feeding niche. The feeding habit of both the species also justified their body orientation in the reef habitat, as most of the time *P. pigmentata* was found in the upright positioned branchial siphons in the sediment free habitat but, *P. mammillata* mainly found under the rocks or inside the crevices, where their branchial siphon is very close to the sediment enriched bottom. Gut Repletion Index (GRI) value was always higher in *P. mammillata* throughout the year from all the locations, whereas, in case of *P. pigmentata*, the value was lesser and the species has been observed with empty stomach in every season while *P. mammillata* was always with stomach
content in most of the time during the study period. This differentiation was observed may be due to the difference in the feeding habit of both the species. It can be possible, being suspension feeder and they were always filtering the waters to absorb the nutrients from the sediments continuously. On the other hand, GRI value is less in *P. pigmentata* may be due to their planktonivorous nature, they may get the sufficient nutrients or as all the *P. pigmentata* is found in pristine environment, they close their siphons during turbidity in the environment. Gastro-Somatic Index (GSI) value was higher in *P. mammillata* while the GSI value of *P. pigmentata* was observed very less throughout the year. The differentiation between two species may be countered due to two reasons such as the test/tunic of the *P. pigmentata* is opaque, thick, tough and more leathery in nature while, tunic of the *P. mammillata* is translucent, cartilaginous in nature so, the body of *P. pigmentata* is always higher than *P. mammillata* and *P. pigmentata* had empty stomach as well as not fully engorged stomach but *P. mammillata* always had non-empty stomach or engorged stomach. These two factors increase the GSI value in *P. mammillata* as well as decrease the GSI value in *P. pigmentata*. 