CHAPTER 2

MATERIALS AND METHODS

The distribution of *Holothuria (H.) scabra* from Palk Bay and the Gulf of Mannar of southeast coast of India was studied to assess the availability of the resources along both the coast. Generally fishing of holothurians is not very regular due to poor visibility thereby making skin diving impossible. The tallu valai at Tuticorin centre was operated round the year while seasonal skin diving was carried out at Kilakarai and Tirupalakudi.

Among these centres, Tuticorin was selected as the main centre as the holothurian fishery is conducted throughout the year and the specimens were caught by skin diving from October to March and by tallu valai for the rest of the period.

For the present work, specimens were collected from Tuticorin landing centre at fortnightly intervals from March 1988 to February 1990. Specimens were abundantly collected by skin diving near Van theevu and Kaswar theevu. On an average 30–40 specimens were collected in a month from the landing centres and transported to the laboratory in water containing 8% magnesium chloride to avoid evisceration. The total length (TL) of the samples was measured dorsally from mouth to anus to the nearest
0.5 cm by means of flexible tape, when the individuals were in turgid condition. The eviscerated individuals were discarded. The total weight (TW) to the nearest 5g; drained weight (DW) (following the opening of the body and the removal of coelomic water) to the nearest 5g; gonad weight (G) to the nearest 0.1g; gutted weight (GW) (following removal of gonads, alimentary canal and respiratory tree) to the nearest 5g were also determined according to the method described by Conand (1981).

2.1 Food and Feeding habits

The length and wet weight of the digestive tract were recorded and the alimentary canal was preserved in 10% formalin to study the food and feeding habits. The gut contents were analysed qualitatively and quantitatively and the details of the methodology adopted are given below.

2.1.1 Qualitative analysis

The gut content samples from different regions of the digestive tract viz. oesophagus, stomach and intestine were collected separately and observed for different food items.

2.1.2 Quantitative analysis

For quantitative analysis, gravimetric method was followed
(Roberts, 1979). The weight of each grade food item was expressed as percentage of the weight of the total gut contents. The gut content from different regions of the digestive tract were collected carefully, washed and dried in oven at 65°C for 24 hours. The different particle sizes were segregated by passing them through seven different grades of standard sieves of 90, 125, 250, 500, 710, 1003 and 1680 μm, using an automatic sieve shaker. Each fraction was weighed and expressed as percentage of the total gut content weight.

2.1.3 Condition of feed

To ascertain the feeding conditions during various months, the degree of fullness of the stomach was noted before the stomach was cut. As no work has been reported in holothurians regarding feeding intensities, the stomach was classified as 'full', '3/4 full', '1/2 full' and '1/4 full' depending on the relative fullness and the space occupied by stomach contents. The stomach was designated 'full' when it was completely filled with food and its wall appeared very thin and transparent. It was considered '3/4 full' when it was in a partly collapsed condition, in which case the wall was usually thick. Stomachs termed 'empty' contained practically nothing in them. From the total number of holothurians examined in a month, the percentage occurrence of full, 3/4 full, 1/2 full, 1/4 full and empty
stomachs was estimated. The holothurian stomachs classified as full, 3/4 full and 1/2 full were considered to have actively fed, whereas those with 1/4 full had fed poorly.

2.1.4 Relationship between length and weight of gut and length and weight of animal.

The total length, total wet weight of the holothurian and that of the digestive tracts were measured. A relationship was made between the total length, digestive tract length, total weight and digestive tract weight.

2.2 Biochemical Analysis of Nutrients

The holothurian samples were collected from the bottom sediments and the sediment samples were taken from H. (M.) scabra by scraping only the top few millimeters of sediment where the individual lies. On return to laboratory the animals were immediately dissected and the sediment in their oesophagus, stomach, intestine was collected. Nearly 25-35 individuals were examined. The sediment samples, gut materials and faeces were rinsed with fresh water, dried and sieved to provide samples for the determination of organic carbon, carbohydrate and nitrogen.
2.2.1 Estimation of organic carbon

The total organic carbon and organic matter was analysed according to the method of Walkey-Black (1934).

Procedure: 0.5 to 1.0 g of sample was taken in a 500 ml conical flask and 10 ml of potassium dichromate solution and 20 ml concentrated sulphuric acid were added. After 20-30 minutes 175 ml of distilled water followed by 6 to 7 drops of diphenylamine indicator were added. The flasks were shaken well and titrated against ferrous ammonium sulphate solution. A blank control was also made. The colour becomes deep violet blue when ferrous ammonium sulphate was added drop wise with shaking. At the end point the colour becomes sharp green.

Calculation:

Percentage of oxidizable organic carbon = Blank titre - Actual titre x 0.3 x M = %

Weight of soil

where 'M' is the concentration of ferrous ammonium sulphate solution.

The percentage of organic carbon was converted to total organic carbon by multiplying with the factor 1.33 and to percentage organic matter by multiplying with the factor 2.
2.2.2 Carbohydrate

Carbohydrate was analysed by Anthrone method (Roe, 1955).

Procedure: To 2 g of dry sediment, 10 ml of 15% TCA was added and kept for one hour after which it was filtered. 0.5 ml of the filtrate was taken and made up to 2 ml and 4 ml of anthrone solution was added. The mixture was heated in boiling water bath for 10-15 minutes and cooled in the dark for half an hour. The blue colour developed was measured at 620 nm. Glucose (100 mg in 100 ml) was used as standard. The concentration of carbohydrate was calculated from a standard graph and expressed in μg/g.

2.2.3 Nitrogen

Nitrogen was estimated by kjeldahl method as followed by Tanaka (1958a).

Procedure: One gram of sample was taken in a digestion flask, 10 g of potassium sulphate, 0.7 g mercuric oxide and 20 ml sulphuric acid were added. The flask was heated gently at an inclined angle until frothing subsided and a clear solution was obtained. Boiling was continued for an additional half hour. If frothing is excessive, a small amount of paraffin wax can be added.

On cooling, about 90 ml of distilled water was added and
recooled, then 25 ml of sulphide solution was added and mixed. A small piece of boiling chip was put to prevent bumping. 80 ml of sodium hydroxide solution was added while tilting the flask so that two layers were formed. The digestion flask was connected to the condensor unit, heated, the distilled ammonia was collected in 50 ml boric acid indicator solution. 50 ml of the distillate was collected after which, the receiver was removed and titrated against standard acid solution.

Calculation

Percentage of nitrogen

\[
\text{content of sample} = \frac{\text{ml. of} \times \text{normality of} \times 0.014 \times 100}{\text{acid standard acid}} = \% \\
\frac{\text{Weight of sample (g)}}{}
\]

Percentage of Protein content = Nitrogen content \times 6.25.

2.3 Size frequency distribution

The frequency distribution study was made to find out the growth of \( H. (H.) \) scabra using length was determined. The relationship between the characters considered as independent variables were computed by regression and correlation analyses. (Snedecor & Cochran, 1967).
2.4 Reproductive cycle

The gonads were preserved in 10% neutral buffered formalin for macroscopical and microscopical examination. The macroscopic observation was based on the form, colour and consistency of the gonad. Five maturity stages were recognised and classified accordingly by Krishnaswamy and Krishnan (1967). Earlier, Krishnaswamy and Krishnan (1967) recognised four stages of maturity in *H. (M.) scabra* viz. immature, mature, gravid and spent. In the present work mature stage has been divided into early mature and late mature to demarkate the stages more precisely, thereby making a total of five stages. In the case of females, the oocyte diameter was measured by means of an ocular micrometer, in order to establish their frequency distribution. These approaches permitted five stages of maturity to be defined for the present species.

For histological studies, standard methods were followed (Clark, 1981). A piece of gonad was fixed in Bouin's fluid and neutral buffered formalin at 10% dilution. Sections of 6 μ thickness were cut and stained with Delafield's hematoxylin and Mallory's Triple Stain (MTS).

2.4.1 Gonad Index

The gonad index (GI) was expressed as the ratio of gonad
weight to drained weight. The mean value was calculated for each sample and the standard deviation was computed (Conand, 1981). The gonad index was correlated with monthly sea water temperature and salinity recorded from Tuticorin coast. A relationship was established between the total length, total weight, gonad weight, gutted weight and maturity stages.

2.4.2 Size at first maturity

The size at first maturity is an important factor for stock management. The method followed was that described by Conand (1981). The percentage of individuals in maturity stages III, IV and V were recorded in size classes of total weight (TW) and gutted weight (GW) using the entire sample. Samples with stage I and II were excluded since the number of indeterminate individuals were found to be maximum at this time. The classes at which 0% to 100% of the individuals matured were determined on the curve. The point on the curve at which 50% of size classes are sexually matured (TW 50) may be taken as an index of size at first sexual maturity. This method assumes that the population consists of a single age class or that in a population containing several age classes, the older animals at stage III, IV and V will be larger than those attaining sexual maturity for the first time. The total length, total weight and gutted weight at first
maturity were calculated from the regression equation.

2.4.3 Fecundity

Fecundity was estimated by taking a weighed piece of mature ovary, counting the mature ova present therein and computing them to the total weight of the ovaries. Fecundity was related to total length, total weight and weight of gonad by logarithmic regression equation.

2.5. Burrowing Behaviour

Burrowing behaviour of *H. (M.) scabra* was studied in round bottom plastic troughs (25 cm height, 60 cm diameter and 50 liter capacity). *H. (M.) scabra* ranging in size from 20 - 24 cm previously acclimatized to laboratory conditions were introduced into these troughs containing 50 - 60 mm thick layer of beach sand and filtered sea water filled to capacity. The behaviour of the animals was observed till they had burrowed completely and this observation was repeated three times.

A similar experimental set up was used to study the influence of light on burrowing behaviour. The holothurian *H. (M.) scabra* was exposed to different light conditions viz. 12 hour light and 12 hour dark, 24 hour light and 24 hour dark. Three holothurians were introduced at 0900 A.M in each trough and
observation made at three hourly intervals for 48 hours. Both buried and semiburied specimens were considered as buried. During the dark hours, troughs with black cloth and for light, artificial light (400 - 500 lux) was provided. The experiment was conducted in triplicates.

2.6 Locomotion

Locomotion was observed in the aquaria (100 litre) containing 50 - 60 mm thick sand layer at the bottom.

2.7 Fishery

The holothurian landings were observed from Rameshwaram to Mallipattinam along the Palk Bay coast and from Pamban to Tuticorin along the Gulf of Mannar coast in the southeast coast of India. Five centres viz. Tirupalakudi, Rameswaram, Vedalai, Kilakarai and Tuticorin were selected for studying aspects of the fishery.

Holothurians are fished round the year. Along the Palk Bay coast, fishing is conducted from March to October and along the Gulf of Mannar from October to March. Observations on the particulars of the Catch Per Unit Effort were made by observing the number of specimens collected per unit per day.
The fishing of holothurians is carried out by skin divers at a depth of 4-20 m, by using a net bag in which the holothurians are stored and brought to the shore. In recent years, aluminium plates are used for the feet as improvised flippers to give greater utility for the skin divers. Tallu valai operation at a depth of 4-8 m also collected this specimen. The same specimen is also encountered as bycatch in bottom trawlers. The catch recorded by operating different gears, total landings in the respective centres and the method of processing of beche-de-mer are discussed in detail in the respective chapters.
PART I.

BIOLOGY