STUDIES ON A SEA ANEMONE, *ANTHOPLEURA NIGRESCENS* (VERRILL) FROM THE SOUTH WEST COAST OF INDIA

By

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THESIS

Submitted to the University of Cochin
in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

1978

CERTIFICATE

This is to certify that this thesis is an authentic record of the work carried out by Mr. Kuruvila Mathew, M.Sc., under my supervision in the University Department of Marine Sciences and that no part thereof has been presented before for any other degree in any University.

Kurian C.V.

Dr.C.V.KURIAN
Supervising Teacher

Ernakulam]
24th April '78]

ACKNOWLEDGEMENTS

I would like to express my deep sense of gratitude to Prof C.V.Kurian D.Sc., Dean, Faculty of Marine Sciences, University of Cochin for suggesting this problem, guiding me in this work and also for critically going through the manuscript and making necessary corrections. I am also grateful to the University of Cochin for providing me with necessary facilities to carry out the work in the Department of Marine Sciences.

I am much indebted to Dr.C.E.Cutress, University of Puerto Rico, Department of Marine Sciences, Mayaguez, U.S.A. for confirming the identification of the specimen and also for providing me with many of the literature. I would like to place on record my gratitude to Dr.G.Y.N.Ayer, Professor of Biochemistry, Medical College, Kottayam for granting me permission to work in the Biochemistry Laboratory of the college and for the valuable help rendered in connection with the biochemical estimations.

I am thankful to Dr. V.O.Sebastian, U.G.C.Fellow and Shri. T.M.Sankaran, Lecturer in Statistics in the Department of Marine Sciences for the help rendered by them in the preparation of the thesis. I may also take this opportunity to express my thanks to all my colleagues in the Department of Marine Sciences, University of Cochin for their help and co-operation in carrying out this work.

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CONTENTS

INTRODUCTION

The order Actiniaria of the class Anthozoa includes most of the animals commonly known as sea anemones. The Ceriantharia and the Corrallimorpharia are also solitary anthozoan polyps often regarded as sea anemones, but structurally they differ profoundly from Actiniaria. According to Ross (1967) as advanced cnidarians, sea anemones are essentially sedentary predators. This mode of life, however, obviously includes an extensive range of habits, the animals varying from filter feeders injesting minute organisms collected by cilliary currents to voracious non-hunting carnivores with many omnivores and non-searching scavengers occupying particular ecological niches between the two extremes.

It is interesting, however, to note that although the anemones, with their 200 genera, form a small group in one sense (as compared with a vast series such as the Mollusca), yet from an ecological point of view they are a thoroughly successful group as pointed out by Carlgren (1949). Their distribution is world wide occurring almost from pole to pole, but they are more abundant in the warm waters. They inhabit the tidal zone and the sea floor.

In deep waters they have been recorded at depths of 2,900 fathoms (Hertwig 1882) and at 2,000 metres (Carlgren 1942). In the recent trawlings where hadal zones are found they have been recorded even at 10,710 metres depth in the Mariana Trench and in the Indian Ocean deep sea surveys Actinians have been hauled from 7160 metres in the Sundra Trench as reported by Wolf (1960). The anemone population of the offshore waters is often plentiful and the species are not only numerous but often large in size (Carlgren 1949). Between the tidemarks they are met with in sandy, muddy and rocky areas. Some of the species, when occur are among the most abundant of the intertidal fauna and sometimes they carpet very wide areas. Though almost all of them are sedentary, atleast a few are pelagic (Annandale 1909). Thus the actinians form an interesting and successful group from an ecological point of view.

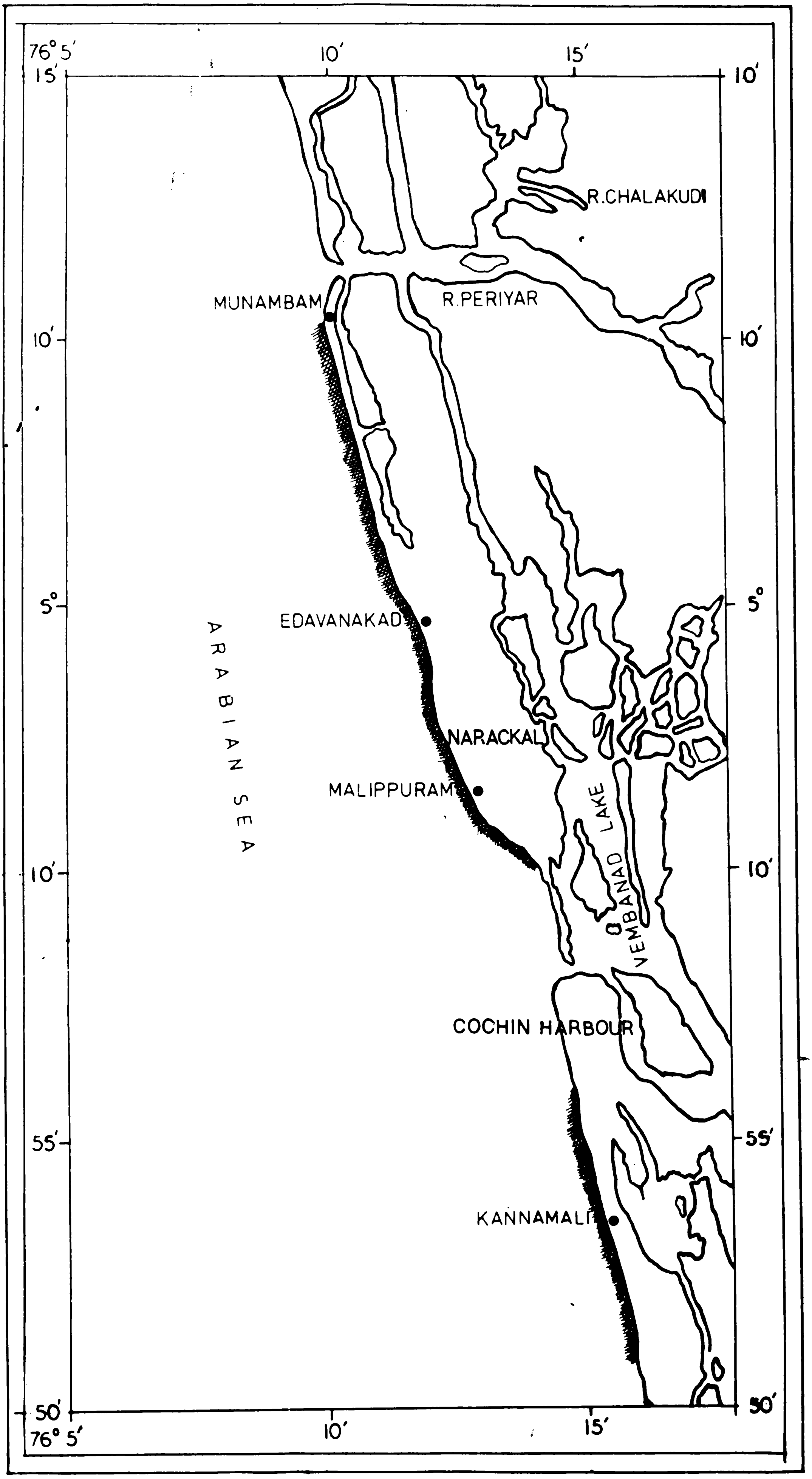
Many contributions have appeared on actinian morphology, histology, taxonomy, evolution, feeding, digestion, reproduction (sexual and asexual), regeneration, development etc. The literature on these subjects being too extensive, the scope of the previous references here is limited to the most important ones. Important taxonomic works are those of Gosse (1859), Hertwig (1879, 1882, ~~and~~ 1888), Mc Murrich (1889, 1893, 1901, 1904), Dixon and Dixon (1890), Boveri (1893), Appellof (1894), Annandale (1907, 1915), Stephenson (1918, 1922, 1935), Carlgren and Stephenson (1928), Panikkar (1935), Carlgren (1949), Perry (1951, 1952) and Hand (1955). Histological works include those of Hertwig (1879), Havet (1901), Parker (1919), Weill (1934), Robson (1953), Cutress (1955) and Hand (1961). Many of these works also pertain to a study of the structure and distribution of nematocysts since they are of very much importance in the classification of the groups. Feeding and digestion have been worked out by Parker (1896, 1905, 1919), Boschma (1925), Carlgren (1925), Elmhirst (1925), Yonge (1931, 1937, 1954), Krijgsman and Talbot (1953), Gibson and Dixon (1969) etc. Developmental studies have been made only in some forms and are those of Carlgren (1897, 1904, 1906), Duerden (1899), Appellof (1901), Bamford (1912), McBride (1914), Gemmill (1920, 1921), Weitrzykowski (1910, 1915), Panikkar (1936), Nyholm (1943, 1949) and Sembrat (1955). Asexual reproduction and regeneration ~~is~~ in a few forms has been worked out by Child (1904, 1908), Stephenson (1928, 1929), Miyawaki (1951), Ford (1964), Schmidt (1970) and Minasian (1976). Commensalism and symbiosis in a few species have been studied by Pax (1914), Orton (1922), Balls (1924) and Thomson (1923). Their association with zooxanthellae has attracted workers like Muscatine (1961), Droop (1963), von Holt (1968), Buchsbaum (1968) and Pearse (1974). Though many such studies have been made in different anemones, detailed works on the biology and ecology of a particular species are few.

The actinians have been a partialy neglected group in India. The earlier works on the sea anemones from Indian coasts are scanty. They include contributions of Stoliczka (1869), Boveri (1893), Annandale (1907, 1915), Menon (1927), Panikkar (1936, 1937, 1939, 1947), Cheriyan (1964) and Parulekar (1968). Panikkar has done considerable work on the taxonomy, morphology, bionomics and development of a few forms from the Madras coast. Other works on Indian anemones mentioned above pertain mainly to taxonomy except that of Cheriyan (1964). But hitherto no serious effort has been made to study in detail the ecology and biology of the sea anemones of the Indian coasts.

The southwest coast of India consists of rocky, sandy and clayey regions and sea anemones are present in all these regions. Many of them are deep water forms. They are also represented in the brackish water areas of Kerala and Tamil Nadu. The intertidal anemone population of the Kerala Coast is mainly constituted by the genera *Phymactis*, *Actinia* and *Anthopleura*. Of these *Anthopleura nigrescens* (Verrill) is abundant along the Narackal, Malippuram and Kannamalai areas near Cochin. (Fig. 1). Nothing much is known about this anemone apart from the paper of Verrill (1928) which mainly deals with the morphology. An animal species cannot be visualized without a searching consideration of the whole of its morphology and the biochemical and physiological aspects.

Fig.1. Map of Cochin area showing the regions where
A. nigrescens has been observed
(The areas are marked in dark shades)

FIG. 1


76° 5'
10'
15'
15'
10'
R.CHALAKUDI
MUNAMBAM
R.PERIYAR
10'
10'
5°
5°
EDAVANAKAD
ARABIAN SEA
NARACKAL
MALIPPURAM
VEMBANAD LAKE
10'
10'
COCHIN HARBOUR
55'
55'
KANNAMALI
50'
50'
76° 5'
10'
15'

Such studies on the anemones, especially from the Indian coasts have not yet received much attention. The present study was undertaken with a view to understanding some aspects of the morphology, bionomics and physiology of the sea anemone A. nigrescens. This species was selected for the study since it is abundantly available along the coast throughout the year and can be successfully reared in the laboratory without much effort. The present study deals with the following aspects of A. nigrescens:

1. Description of the species
2. Habitat
3. Food and feeding
4. Digestion
5. Studies on salinity tolerance
6. Studies on oxygen consumption
7. Studies on tolerance to desiccation
8. Asexual reproduction and regeneration

II

DESCRIPTION OF THE SPECIES

Phylum	Coelenterata
Class	Anthozoa
Sub class	Zoantharia
Order	Actiniaria
Family	Actiniidae

Genus *Anthopleura*. (Duchassaing and Michelotti 1860)

Bunodes Gosse, 1860; Carlgren, 1900
Gyractis Boveri, 1899
Cribrina Mc Murrich, 1904; Pax, 1908
Bunodactis Pax, 1920; Stephenson, 1929

Actiniidae with adhesive verrucae arranged in more or less distinct longitudinal rows. Marginal spherules present. Sphincter weak or strong, restricted to circumscript. Tentacles simple, hexamerously or irregularly arranged, their longitudinal muscles ectodermal or meso-ectodermal. Numerous perfect mesenteries, all the stronger ones fertile. Retractors of the stronger mesenteries growing from the basal disc upwards. Cnidom: Spirocysts, atrichs, basitrichs, microbasic p- mastigophores (Carlgren 1949).

Anthopleura nigrescens (Verrill 1928)

Cladactella obscura Verrill, 1928
Tealiopsis nigrescens Verrill, 1928
Anthopleura nigrescens (Cutress, 1967)

Description

Column usually nearly cylindrical, often not much higher than broad, in expansion height exceeds the diameter. Covered with regular rows of rather large verrucae arranged in vertical rows, reaching upto the base. Capable of firmly attaching to such objects as grains of sand and pieces of shells. Acrorhagi present. Tentacles arranged in 4 to 5 cycles, but not hexamerously. A well developed fossa is present. Sphincter circumscript, strong to very strong. Colour of the column purplish brown or dark brown.

Base:

Not always circular, sometimes irregular in outline. Strong adherents to stones, shell pieces etc. Usually of the same diameter as the column. Basilar muscles strongly developed. Mesenterial insertions are visible from the base as they are thin and transparent.

Column:

In well extended specimens the column is about as high as its diameter. When fully extended they have a column height of 20 mm. In contraction it appears as a hemispherical mound. Top of column is distinguished by a collar and well developed fossa. The verrucae are well developed and arranged in longitudinal rows, many of which reach the limbus. On reaching the limbus the verrucae become smaller in size. They are strongly adherent and usually they are completely covered with bits of sand particles and shell pieces. Acrorhagi present and they are with openings. In younger individuals they are not well pronounced. The colour of the acrorhagi varies, they are white, or with yellow tint. Upon sudden contraction water is ejected through the acrorhagi. They contain atrichs.

The column wall is not very thick, mesogloea and endoderm are almost of equal thickness, but the ectoderm is thicker than mesogloea. The mesogloea is fibrous. The circular muscle is distinct. Sphincter circumscript strong to very strong, and it is round to elongate- oval in cross section (Fig. 2).

Tentacles:

Their number varies in different individuals and they are not hexamerously arranged as the anemone undergoes asexual reproduction by longitudinal fission. Usually they are arranged in 4 or 5 cycles. They are upto 116 in large specimens. Tentacles are prehensils also. Their colour also varies and three forms are distinguished accordingly viz. (i) with brown colour (ii) brown with two reddish lines along the sides of each tentacle with scattered white spots and (iii) tentacles white in colour. The ectoderm of the tentacles contains spirocyst and basitrichs at the tips. Their longitudinal muscle is ectodermal.

Disc:

The disc is broad and flat. Slightly wider than column. The mesenterial insertions are visible as white lines on the disc.

Fig.2. Longitudinal section through the upper column showing circumscribed endodermal sphincter of *A. nigrescens*

Fig.3. Cross section through the column at the level of the actinopharynx showing four orders of mesenteries of *A. nigrescens*

FIG. 2

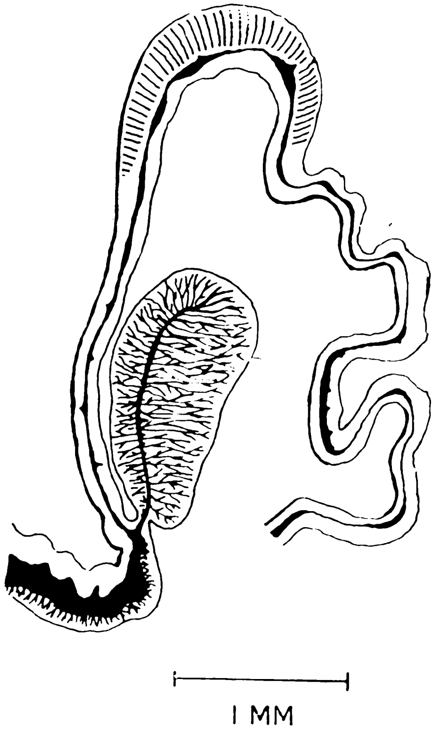
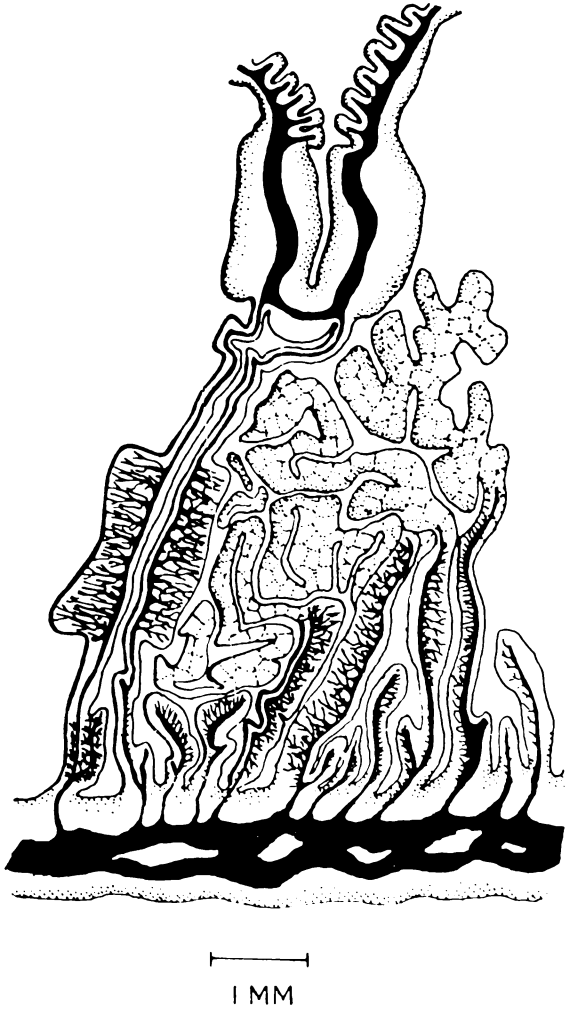


FIG. 3



They are brown in colour. Sometimes the area around the tentacle base is white. The lips may be raised above the surface of the disc and they are ribbed. Their musculature is ectodermal.

Mesenteries and internal anatomy:

The number of mesenteries varies in different individuals. They are not hexamerously arranged as this anemone undergoes reproduction by asexual method. But basically the arrangement of the mesenteries is hexamerous. The number of perfect mesenteries varies and sometimes they are more than 24 pairs. The mesenteries are arranged in 4 or 5 cycles. The first order of mesenteries is in contact with the actinopharynx for its whole length and the second only about half its length. Upto 5 siphonoglyphs are present. All the perfect mesenteries bear gonads. The sexes are separate and gonad maturity is reached only once in a year (March-April). The mesenteries grow from the base distally and thus more numerous proximally than distally. The musculature of the mesenteries well pronounced and the retractors are strong circumscript diffuse. Parietobasilar muscles also strongly developed (Fig.3).

Cnidom

Tentacles

Spirocysts	(Fig.4 a)	16.3 - 28.9 x 2.0 - 3.6 µ
Basitrichs	(Fig.4 b)	18.9 - 30.6 x 1.9 - 2.3 µ

Columns

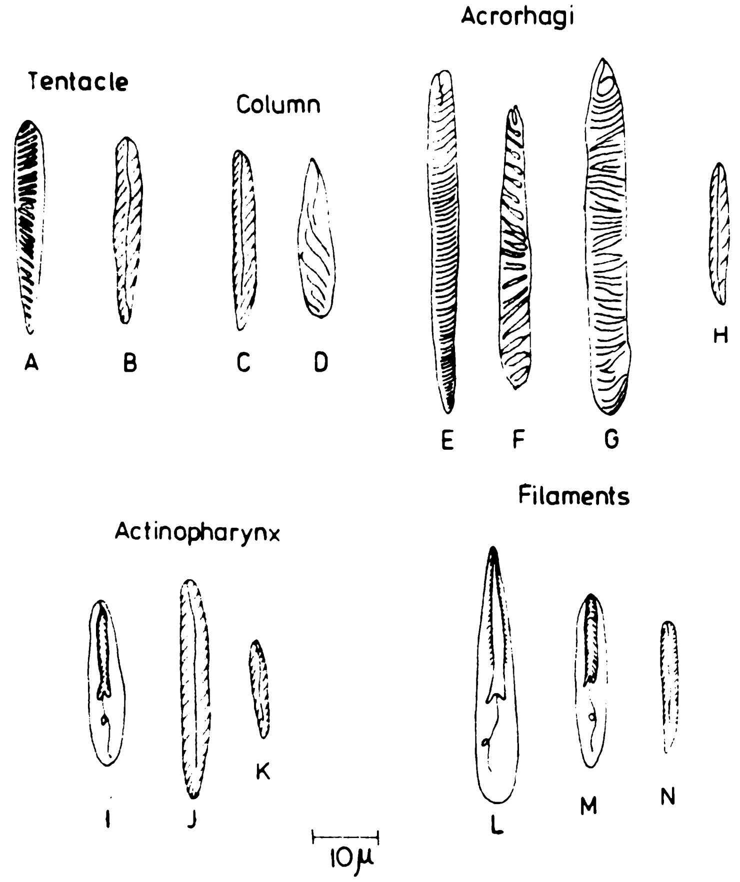
Basitrichs	(Fig.4c)	12.1 - 24.7 x 2.0 - 2.6 µ
Atrichs	(Fig.4 d)	10.4 - 18.6 x 2.4 - 4.2 µ

Fig. 4. Nematocysts of *A. nigrescens* (All drawn in the same scale)

A, B	Tentacles
C, D	Column
E– H	Acrorhagi
I– K	Actinopharynx
L–N	Filaments

(A and F. Spirocysts; B, C, H, J, K and M basitrichs; D, E and G. atrichs; I, L and N microbasic p-mastigophores)

FIG. 4


Acrorhagi
Tentacle
Column
A
B
C
D
E
F
G
H
Filaments
Actinopharynx
I
J
K
L
M
N
10μ

Acrorhagi:

Spirocysts	(Fig.4 e)	18.5 - 32.0 x 2.8 - 3.9 μ
Atrichs	(Fig.4 f)	22.5 - 48.3 x 2.5 - 3.6 μ
Atrichs	(Fig.4 g)	30.6 - 52.9 x 3.6 - 5.7 μ
Basitrichs	(Fig.4 h)	12.7 - 17.5 x 2.1 - 2.8 μ

Actinopharynx:

Basitrichs	(Fig.4 i)	10.1 - 13.6 x 1.8 - 2.5 μ
Basitrichs	(Fig.4 j)	16.2 - 34.5 x 2.6 - 3.1 μ
Microbasic P-mastigophores	(Fig. 4k)	18.4 - 26.5 x 3.2 -5.2 μ

Filaments:

Basitrichs	(Fig.4 l)	8.2 - 18.1 x 1.3 - 2.4 μ
Microbasic P-mastigophores	(Fig.4 m)	18.5 - 37.0 x 3.8 - 5.0 μ
Microbasic P-mastigophores	(Fig.4n)	15.2 - 24.3 x 4.0 - 5.1 μ

Size:

Large specimens are about 20 mm in diameter (Column and base). When extended the column reaches upto 25 mm in length. In aggregations the common size of the individual is about 12 mm in diameter and 20 mm in length when extended. In aggregation a maximum of 104 specimens per 100 sq. cm. have been observed. The smallest individuals are found in the months of April-May.

Distribution:

The species has a wide distribution and is found mainly in the Red sea, the Pacific ocean and the Indian ocean. In India Cutress collected it from Pamban near Madras (Personal communication). Along the Kerala coast they have been found only in the Cochin area.

Type locality - Nawiliwili Bay, Kauai
Type specimen - American Museum of Natural History

Habitat:

The species is found in the lower level of the balanoid zone and in the sub-littoral fringe, found mainly attached to rocks and molluscan shells. Generally they do not occur in areas of strong wave action. Mainly an aggregating form; but solitary ones are also found in some areas.

Discussion

Verrill (1928) described two species of sea anemones from Hawaiian shallow waters, *Cladactella obscura* and *Tealiopsis nigrescens*. According to Cutress (Personal communication) these are synonyms and he renamed it as *Anthopleura nigrescens*. This anemone bears some resemblance to *Anthopleura stellula* in the type and size range of the nematocysts. But *A. nigrescens* undergoes asexual reproduction by longitudinal fission whereas in *A. stellula* it is by transverse fission. It also bears much resemblance to *A. elegantissima* in the external features, in the nature of the sphincter and also in the method of asexual reproduction. In both the species sexes are separate. But it differs much in the nature and size range of the nematocyst. Considering the colour pattern of the tentacles three forms are noticed in *A. nigrescens*. But detailed anatomical studies proved that all these are of the same species and the difference may be only a local variation. In *A. stellula* also three true varities of oral disc patterns have been noticed by Schmidt (1970).

The classification of the nematocyst type is based on Cutress (1955) and for anatomical studies Stephenson (1928) is followed.

III

HABITAT

Anthopleura nigrescens (Verrill) being a sedentary anemone is found attached to hard substrata. In the present study a survey was made to assess its possible distribution on the South West Coast of India, in the Arabian Sea, extending from Cannanore in the North to Cape Comorin in the South, a distance of about 700 km. *A. nigrescens* was found only in the Cochin area, in five localities viz., Kannamali, Malippuram, Narackal, Edavanakad and Munambam, all within 8 to 20 km from the Cochin Harbour (Fig. 1). The geographical position of this area is between latitudes 9° 58' and longitudes 76° 17' a total of about 28 km length. On the Kerala coast more than eight main rivers empty their water into the sea. Some of the rivers open into the brackish water areas which are connected to the Arabian sea by narrow channels, the most important one being the well developed Cochin Harbour mouth of about 450 metres width. The two main rivers that discharge into the backwaters near Cochin are the Periyar on the north and the Pamba on the south. The backwaters also receive a complex system of canals, rain water and sewage drains, semi-perennial and seasonal rivers and their tributaries. The continental discharge of fresh water from the rivers on one hand and the influx of backwaters on the other hand bring about highly dynamic conditions reflecting the balance of forces associated with each in the Cochin area. There are two monsoons in Kerala, the South West

Monsoon and the North East Monsoon during May-August and during October-December respectively. The area receives the full benefit of the South West Monsoon while it receives some precipitation from the North East Monsoon also. These conditions make the Cochin area an interesting environment for hydrographical investigations. During the present study monthly hydrographical data were collected from the area of investigation (Narakkal) in the years 1970 and 1971 and are presented in Table 1. Surface water temperature, surface salinity, dissolved oxygen and pH were the parameters studied. Monthly rainfall data obtained from INS Garuda is also incorporated. The salient features are discussed below.

Temperature

The minimum surface water temperature during 1970 was recorded in August (28.8°C) and the maximum in March (32.0°C). During 1971 the minimum temperature was recorded in July (28.0°C) and the maximum in April (33.4°C). The lowest temperatures were recorded during July-August, this being the period of heavy rains due to the South West Monsoon in this region. In the pre-monsoon period when there is less rainfall the maximum temperature is recorded. During the post-monsoon period the temperature rises but there is slight fluctuation caused by the North East Monsoon. The slight decrease in temperature during December and January

TABLE 1

Temperature, Salinity, Rainfall and dissolved Oxygen during 1970 and 1971 at Narackal

Month	Water Temp. °C at 10.00 hrs		Rainfall mm		Salinity ‰		Dissolved oxygen ml/L	
	1970	1971	1970	1971	1970	1971	1970	1971
January	30.9	30.2	063.9	044.5	33.60	31.31	4.11	4.65
February	31.2	31.0	't'	016.1	33.85	32.24	3.85	3.88
March	32.0	31.3	091.4	000.0	34.10	33.76	3.93	3.46
April	31.7	33.4	210.6	056.6	33.81	33.90	4.53	4.86
May	31.0	32.2	436.5	575.7	27.61	21.86	5.25	4.97
June	28.5	29.1	831.0	822.8	10.25	11.38	5.70	5.47
July	29.0	28.0	589.0	782.5	14.15	12.34	5.47	5.63
August	27.8	30.8	451.6	424.5	24.62	17.62	4.90	1.52
September	28.4	29.9	310.6	350.1	28.21	24.48	4.65	4.65
October	29.7	29.7	278.0	116.8	28.45	29.12	5.10	4.38
November	29.8	29.3	082.7	039.9	31.43	31.45	4.89	4.95
December	29.5	30.0	't'	029.7	32.76	32.80	4.76	4.80

may be due to the fall in atmospheric temperature. The maximum temperature is reached during April-May. In general the surface water temperature variation is not much.

Salinity

The area is subjected to considerable variations in salinity during the course of an year. The data show that salinity is influenced mainly by the rainfall. There is considerable lowering of salinity during the South West Monsoon. With the onset of the South West Monsoon during June-July a sharp fall in salinity occurs. The minimum salinity of 10.25‰ was reached in June 1970 and 11.38‰ in June 1971. Since the rainfall during North East Monsoon (October-December) is less compared to that in the South West Monsoon, its influence on salinity is not so pronounced. From November onwards the salinity increases reaching its maximum in March-April. Maximum salinity value in 1970 was recorded in March (34.10‰) and in 1971 in April (33.90‰). The maximum salinity period correlates with the period of maximum temperature.

Dissolved oxygen

In general, seasonal changes in the dissolved oxygen content are not much. The highest values were recorded in June and July, during the South West Monsoon. During April and May also the surface water is comparatively rich in oxygen. This is the period of high salinity and temperature and hence an increase in dissolved oxygen values is not to be expected generally. Subrahmanyam (1959) and Damodaran (1973) have attributed this increase to the agitation and increased dissolution of atmospheric oxygen by strong trade winds prevailing during this period. During June and July the increase in dissolved oxygen is due to the influx of fresh water from the river mouths and also due to the decrease of temperature values. This may also be due to the high concentration of phytoplankton during these months (Damodaran 1973). The minimum oxygen value in 1970 was recorded in February (3.85 ml/L) and the maximum in June (5.70 ml/L). In 1971 the minimum oxygen value was recorded in March (3.46 ml/L) and the maximum in July (5.63 ml/L).

In August 1971 subsequent to the formation of the mud bank the water became turbid and later turned to slush. Consequently an unusual fall in the dissolved oxygen (1.52 ml/L) was noticed during this period. In general the trend is that the oxygen value of the surface water is high in June-July, and low in February-March. During the winter months, December-January, the oxygen values are comparatively higher.

pH

The pH shows a seasonal cycle. Maximum values are obtained when the salinity is high and the minimum during the monsoon months when the salinity decreases. The values range from 7.2 to 8.5

Alkalinity

Carbonate alkalinity values closely agree with the pH values. The period of high alkalinity is during the pre-monsoon months and low during the monsoon months.

Tides and Waves

The tidal ebb and flow is one of the prominent factors which affect the life of intertidal organisms. The boundaries and the width of the inhabitable area is determined by the tides. The tides here are of the semidiurnal type. The predicted heights of lower low water spring level and high water spring level are + 0.20 m and 1.05 m respectively (Indian Tide Table)

Wave action is another important factor determining shore populations and influencing their distribution. Waves and tides determine the rate of submergence and emergence of the tidal zone and also the degree of exposure of the organism to desiccation and other conditions of the intertidal region. Eventhough Narackal, Malippuram, Kannamali and neighbouring places are originally sandy beaches, studies on beach profile show that there is little erosion of the beach due to the presence of the sea wall and groins (Fig. 5 and 6). The extent of the fore shore is greater during June-July when the wave action is greater. This extension of the foreshore also has some effect on the distribution of *A. nigrescens*. The anemone is noted for its occurrence at high levels as well as at

FIG. 5

FIG. 6

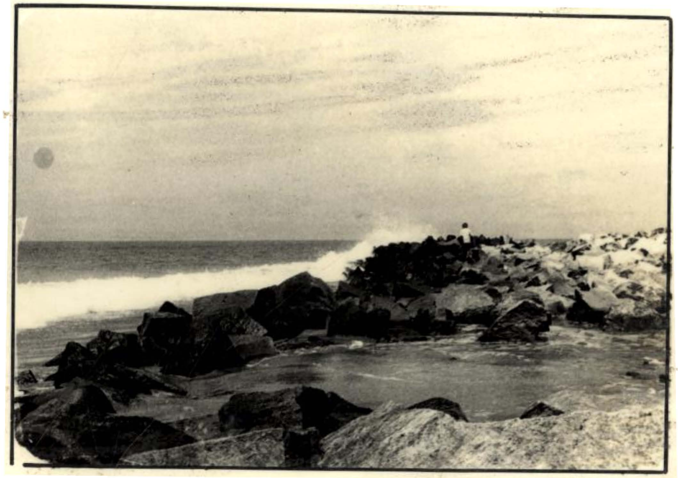


FIG. 7

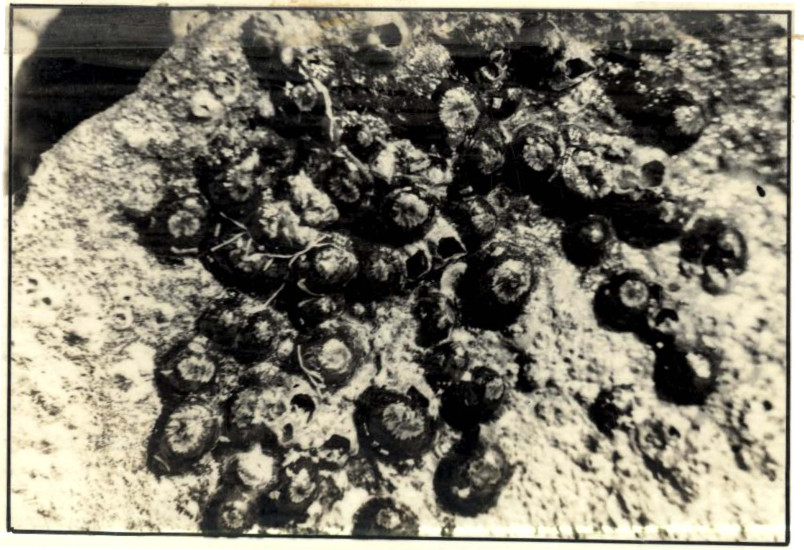


FIG. 8

low levels, but their intensity of occurrence varies from point to point. They are most abundant at the sub-littoral fringe and on certain points they are about 104/100 sq.cm. They are also found in places where it is exposed to the air for longer periods during the ebb and flow of the tide (Fig. 7). Therefore, it is clear that these anemones can withstand considerable increase of temperature and desiccation during the period of exposure. Where there is huge and direct wave action their number is lesser and on sheltered points of the groins they are more abundant. This shows that the tides and waves also play an important role in the distribution of the species.

Formation of the Mud Bank

The formation of the mud banks, locally known as 'Chakara' is x a unique annual phenomenon observed along different parts of the Kerala coast. The mud banks are generally observed in several regions between Quilon and Tellicherry where the sandy intertidal region is followed by muddy substratum. This unique feature of mud bank formation occurs along Narackal also. During the period of mud bank formation the physico-chemical conditions of the area undergo great changes, thereby affecting the life of organisms inhabiting the area. This phenomenon has been extensively studied by several workers (Bristo 1938, Damodaran and Hridayanathan 1966, Damodaran 1973). These mud banks have a peculiar property of reducing the waves and swells and thereby producing calm waters. During the monsoon the bottom mud of the inshore area gets frequently agitated and due to this the water becomes turbid. This turbidity is so high for a few days and the water in the area is turned to slush during some years, especially at Narackal. The loose mud is thrown at the LTL, thereby changing the nature of the deposit and the fauna. The animals of the lower zone are replaced by those of shallow muddy bottom. Consequent to the formation of the mud bank and due to the turbidity of the water many of the intertidal organisms including amphipods, isopods and molluscs like *Mytilus* (*Perna*) sp. and *Modiolus* sp. are destroyed. This may be due to the depletion of oxygen in the area as observed in August 1971 (1.52 ml/l) and also due to the turbidity of the water. The depletion of oxygen is attributable to the reduction of mixing with atmospheric oxygen as a result of the slimys nature of the water and the absence of wave action. Such destruction of the intertidal fauna along the West Coast of India has been reported previously by Seshappa (1953). But it was observed that *A. nigrescens* was remarkably capable of surviving in these adverse conditions. In the laboratory also when they were kept in the slush, for a few days they survived. From this observation it is evident that this animal can subsist on very low oxygen concentrations for a considerable period.

Formation of the Polychaete bed

As has been stated early the formation of the mud bank and the subsequent total absence or reduction of wave action in the intertidal zone, bring about changes in the constituents of the fauna of the area. During the South West Monsoon the water is stirred up by heavy waves and there is plentiful supply of sand and calcareous shell particles. This gives stimulus for the settlement of the larvae of the tube dwelling polychaete *Sabellaria cementarium* (Moore). The gregarious settlement of the larvae causes the formation of polychaete beds in the intertidal region giving the appearance of a coral reef (Fig. 8). The formation of these tube bed affords protection and foothold to a number of intertidal organisms. So also when these colonies spread over the rock surfaces many intertidal organisms are forced to move away or to perish (Philip and Mathew 1968). Similar observations on the bed formation by the polychaete *Sabellaria alveolata* have been made by Wilson (1971) at Duckpool along the South West Coast of England. He observed that when *Sabellaria alveolata* which forms honeycomb like colonies spread over the rock surfaces, limpets are forced to move away to make new homes on unoccupied rocks elsewhere. He has also observed that "the sea anemone *Actinia equina* while forced to move away may attach to the side walls of tubes of a growing honey comb colony and keep open a cavity for itself". Similarly at Narackal *A. nigrescens* is found in large numbers attached to the side walls of the tubes of *S. cementarium*. Thus they attain a new foothold for attachment when they are forced to move away from their original place of attachment. But after a few months when the mud bank disappears and the sea becomes turbulent again, polychaete beds gradually disappear and the anemones attached to them loose their foothold. But the stone walls now cleared of the polychaete tubes offer them substratum for attachment. Thus the formation of tube beds by *Sabellaria cementarium* at Narackal and nearby places play a prominent role in the population dynamics and ecology of the anemone *A. nigrescens*. In brief the formation of the polychaete bed which is detrimental to many sedentary animals like barnacles and encrusting bryozoans give additional facilities for the sea anemone.

Formation of the *Modiolus* bed

Modiolus undulatus, a small bivalve of the family Mytilidae which attaches to the substratum by byssus threads is another organism which forms large beds in the intertidal region of Cochin area. Immediately after the disappearance of polychaete beds *Modiolus* beds begin to appear. Very large number of them settle in thaik patches and cover many of the stone walls completely. This spreading of the *Modiolus* beds also affects the distribution of the anemones since they are forced to move away from the place of attachment. In the case of polychaete bed though *A. nigrescens* are forced to move away from their place of attachment after a while the bed itself forms a new substratum for their attachment. But in the case of *Modiolus* beds it was observed that the anemones do not get attached on this bed, or very seldom a few of them attach here and there on the bed unlike on the polychaete bed where they spread out in large numbers. This may be due to the heavy accumulation of silt and mud on these beds which the anemones do not prefer for their attachment.

Formation of *Mytilus* (*Perna*) bed

Mytilus (*Perna*) *viridis*, also a bivalve of the family Mytilidae is the edible green mussel. It is found throughout the season attached to the rocks. Their settlement in large numbers in September-October also affects the distribution of *A. nigrescens* in this area. It is found that though during the larval settlement period they are forced to move away, later when the mussels grow in size they get attached to the shells of these mussels, which offer a substratum like the polychaete bed.

Effect of pollution on *A. nigrescens*

It was observed that the area was polluted by crude oil heavily on two occasions during the period of the present investigation. This happens due to the leakage or washings from oil tankers which carry crude petroleum to the Cochin Harbour. This crude is washed ashore by the waves and get spread over the rocks of the groins. Due to this spreading of crude oil it was found that almost all organisms on the rocks were destroyed. However, the anemones were found to be surviving even in this condition. Similar instances where actinians not affected by oil pollution have been observed by North *et al* (1964) in the field and laboratory studies. They showed that 0.1% emulsion of the diesel oil spilt inactivates the tube feet of sea urchins. When exposed to the emulsion for more than one hour, they die, whereas, the sea anemone *Anthopleura xanthogrammica* seemed very resistant even to direct contact with the oil in tide pools. Further they found that it was the only animal to survive in ponded sea water effluent from a large refinery further up the California coast. Nicholson and Climberg (1971) have also recorded that *A. xanthogrammica* is a prominent occupant of the rocks at Coal Oil point which received oil from natural seepages. It seems probable that the British anemone *Actinia equina* is also quite resistant although it is absent from very polluted waters (Nelson-Smith 1972). During the present study it was observed that *A. nigrescens* remains in a contracted stage and does not open showing their oral disc and tentacles when the oil pollution occurred. Within a week the oil disappeared by getting absorbed in the sand and the life of the anemones became normal.

Fauna associated with *A. nigrescens*

Studies were also made on the fauna found associated with *A. nigrescens*. It was found that their distribution is similar to that observed by Stephenson and Stephenson (1949) and based on the distribution of organisms the intertidal region can be divided into Supra-littoral zone or the Littoral zone, Mid-littoral zone or the Balanoid zone and the Infra-littoral fringe with a wide variety of plants and animals.

The supralittoral zone is the upper part of the tidal region and submerges only during the time of High Water Spring tides. This area is inhabited by the littorinids; *Littorina undulata* and *L. granulosa* being the common species found here, mainly accumulated to the crevices of the rocks. There is no algal growth in this region and the sub-terrestrial isopod *Ligia exotica* is seen in large numbers. *A. nigrescens* does not come to this zone.

The Balanoid zone is inhabited mainly by *Balanus amphitrite* and *Chthamalus sp.* This zone may be divided into the upper Balanoid zone and the lower Balanoid zone. This zone is always wet by the spring and immersed by the high water neap tides. There is a high cone of *Balanus* in this upper zone and *Littorina* sometimes makes downward migration to this zone. In the lower Balanoid zone there is less intensity of balanoids, the limpets and oysters being the common organisms found here. The limpet *Cellana sp.* forms the *Cellana* band which is found throughout the season. The oyster *Ostrea madrasensis* forms the *Ostrea* band. The red algae *Gigratina* sp. is found seasonally. This algae and the polyzoans harbour a wide variety of animals like nematodes, polychaetes, amphipods and isopods. *A. nigrescens* is found in large numbers in the lower level of this zone. The extensive masses of the tubicolous polychaete, *Sabellaria cementarium* are found in this region seasonally.

The infra-littoral fringe or the sub-littoral fringe is exposed for appreciable periods during the lower spring tides. The division of this zone is not clear. This region is mostly occupied by the beds of the mussels *Mytilus* (*Perna*) *viridis*, *Modiolus undulatus* and *Modiolus striatulus*. But *M. undulatus* and *M. striatulus* were seen only seasonally. The mussel beds harbour a number of free living animals like trematodes, polychaetes, decapods, amphipods and ophiuroids. Algal growth is totally absent in this area. The population of *A. nigrescens* is comparatively high, the mussel beds also form footholds for their attachment. They also occupy the stones of the upper level of the infra-littoral fringe. Underneath the sheltered parts of the rock where there is less direct wave action are seen encrusting polyzoans like *Membranipora sp.* and *Aldernia sp.*, isopods like *Exosphaeroma sp.*, and *Sphaeroma walkeri* and polychaetes like *Nereis sp.* *A. nigrescens* is also found under rocks, but not in dense patches.

Thus *A. nigrescens* in the region under study is exposed to varying environmental conditions. Since Nerackal was originally a sandy beach the anemones inhabited the area only recently, after the construction of the sea wall around 1960. They seem to be thoroughly successful in adapting themselves to this new environment. The distribution of the species at present is on both sides of the Cochin Harbour; to about 12 km towards north and 15 km to the southern side. But they are not found near the bar mouth and this may be due to the high decline in salinity which reaches below 1‰ during the monsoon months. The peculiar condition during the mud bank period when the South West Monsoon is at its peak was not harmful to the anemones. Crude oil from oil tankers washed ashore during the course of the investigation on two occasions, was also not detrimental to the anemones, while this caused mortality to most of the other intertidal organisms. The high fluctuations in salinity (34.42‰ to 10.38‰) at Narackal was also not harmful to _A. nigrescens_. So also they are not affected by the lowering of oxygen (1.5 ml/L) on certain occasions. All these show that _A. nigrescens_ is a thoroughly successful actinian from an ecological point of view, and is remarkably hardy, thriving well in the new environment to where they were recruited very recently.

IV

FOOD AND FEEDING HABITS

The fact that the actinian food consists of small fishes, crustaceans, worms and the larval forms of other animal groups was known from early times, but there is little information as to the normal and average diet of a particular species. So an attempt was made to study the food and feeding habits of *A. nigrescens*.

Accounts on the feeding habits of actinians are available in the works of Parker (1896, 1905, 1919), Torrey (1904), Carlgren (1905), Hargitt (1907), Fleure and Walten (1907), Elhmirst (1926), Hyman (1940) and Nagai and Nagai (1973). The studies on the feeding habits of *Eloactis* by Hargitt (1907) showed that this anemone in confinement would injest only living *Balanoglossus* and hydroids. Studies by Torrey (1904) on *Sagartia*, by Fleure and Walton (1907) on *Tealia* and by Parker (1896) and Allabach (1905) on *Metridium* showed that these anemones besides food substances injest other indigestable matter also. These reports on the food of anemones are mainly pertaining to a study of the feeding reactions to different food substances rather than to a study of food analysis in the gastric cavity which alone would give a clear idea of the diet of the animal in question. So studies were conducted in two ways in order to understand the normal and usual diet of *A. nigrescens*, viz.

1) a detailed analysis of the gastric content on preserved material collected from their habitat and 2) an attempt to study their feeding habits and reactions to different food substances, since they could be easily reared.

Material and Methods

The material for the study was obtained from two regions of Cochin area viz. Malippuram and Narackal, approximately 3 km apart (Fig. 1). The specimens were always collected during the low tide period by gently separating the pedal disc free of the substratum. Collections were made at monthly intervals for two years (1970 and 1971). Each specimen was washed thoroughly in filtered sea water and immediately preserved in 4% neutral formalin and kept in separate labelled specimen tubes. Each specimen was fixed and kept separately because it was observed that many of them evert their gullet on preservation resulting in the food materials being thrown out. The stomach analysis was done in the laboratory from the preserved material. The anemones were cut open and the food contents were thoroughly washed out into a petri dish, care being taken to remove all food particles entangled in the mesenteric filaments. The food present in the coelenteron was found to be subjected to varying degrees of digestion and with mucus coating. But in the majority of cases they could be easily identified at least upto the genus.

There are various methods in practice adopted for the food content analysis such as numerical, gravimetric and volumetric. Hynes (1950) in his studies on the food analysis of fishes has stated that any one of the commonly accepted method of assessing the composition of the diet from the gut contents will give substantially the same result. Those food items important in the diet will always stand out clearly from those that are occasional or rare and so the variations between the different methods will not be probably greater than between the different samples of animal in question. But the most reliable is the volumetric analyis and hence it is widely followed. Mc Atee (1912) and Collinge (1927) have emphasized the importance of volumetric analysis in food studies and according to to Hynes (1950) unless the dietetic values of the food are known, volume forms a satisfactory basis for assessment. Thus by volumetric analysis it is possible to assess with sufficient accuracy the composition of the food constituents. Relative volumes of the food constituents which are often semi-digested and mixed with mucus were estimated by Pearse's method (cited by Breder and Crawford, 1922). According to this method the contents of each sample are considered as a unit, the various items being then expressed in terms of percentage by volume by rough estimate. The semidigested food particles and indigestible matter present in the gastric cavity were treated as miscellaneous items.

Food contents, after removing identifiable items were transferred to petri dishes and were examined under microscope. Volumes in percentages were estimated and recorded for different constituents of food. From individual tabulations thus made, monthly lists were prepared. The percentage composition of various organisms constituting the food during different months and years are shown in Tables 2 and 3 and in figures 9 and 10.

Laboratory experiments on the feeding reactions were carried out by giving them different items and observing their reactions.

Observations

The analysis of the gastric contents of A. nigrescens during a period of two years (1970 and 1971) showed almost similar results. In each month thirty specimens of different age groups were examined so that a total of 360 specimens were observed each year. Only very few specimens were found without food in their gastric cavity during the period of study. No variations were noticed in the food items according to the different age groups.

The percentage composition suggests that crustaceans and fishes are of greater importance in the dietetic value of A. nigrescens. Taking the average for the period under observation isopods formed 19.83% for the year 1970 and copepods formed 19.25% for the year 1971 of the gastric contents and thus ranked as the most important food items.

Table 2

Gastric contents of *A. nigrescens* during the different months of the year 1970

Contents	% occurrence in different months											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Worms (Nematodes, Planarians and Polychaetes)	9	4	-	-	3	3	-	5	5	-	8	3
Polychaete larvae	-	-	-	-	15	20	25	30	5	5	—	-
Chaetognath	8	15	10	10	5	-	-	5	-	5	3	5
Isopods	30	25	15	20	33	15	10	10	15	25	20	20
Amphipods	10	15	10	15	5	20	13	15	15	10	15	20
Copepods	16	18	25	10	12	10	15	20	25	20	15	20
Prawn (Juveniles)	-	2	3	10	-	-	-	-	2	5	13	-
Cirriped larvae	-	7	-	-	-	3	2	-	5	-	-	-
Lucifer sp.	-	-	2	5	-	-	-	-	5	-	-	4
Mollucsan larvae	-	-	5	10	3	10	17	5	10	3	-	-
Fish larvae and small fishes	12	7	20	15	5	8	-	5	3	15	12	15
Miscellaneous items	15	7	10	5	19	11	18	5	10	12	10	13

Table 3

Gastric contents of A. nigrescens during the different months of the year 1971

Contents	% occurrence in different months											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Worms (Nematodes, Planarians and Polychaetes)	4	7	4	3	-	3	-	-	3	-	5	6
Polychaete larvae	-	-	-	-	4	10	15	30	15	8	4	-
Chaetognath	9	15	20	10	8	-	-	-	10	4	3	5
Isopoda	17	25	20	15	15	15	10	10	15	10	10	10
Amphipoda	10	10	15	15	10	20	15	5	10	10	15	15
Copepoda	20	23	15	20	30	20	16	15	10	15	27	20
Prawn (Juveniles)	-	-	7	9	-	-	-	-	-	5	5	3
Cirriped larvae	-	-	-	-	-	-	5	2	5	2	-	-
Lucifer sp.	-	-	5	10	3	-	7	-	5	4	2	-
Molluscan larvae	-	-	-	-	9	18	12	15	10	5	2	-
Fish larvae and small fishes	20	5	4	12	10	10	7	13	15	20	15	20
Miscellaneous items	20	15	10	6	11	4	13	10	2	17	12	21

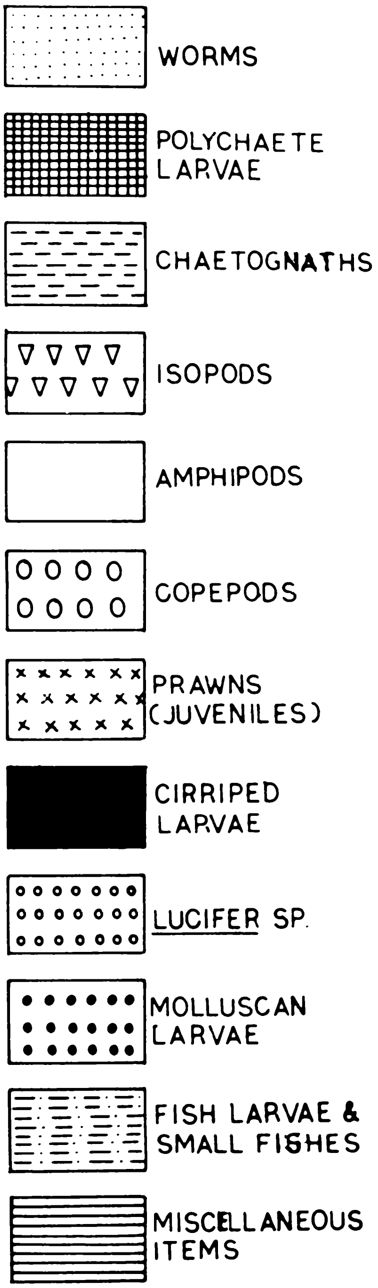

WORMS
POLYCHAETE LARVAE
CHAETOGNATHS
ISOPODS
AMPHIPODS
COPEPODS
PRAWNS (JUVENILES)
CIRRIPED LARVAE
LUCIFER SP.
MOLLUSCAN LARVAE
FISH LARVAE & SMALL FISHES
MISCELLANEOUS ITEMS

FIG. 9

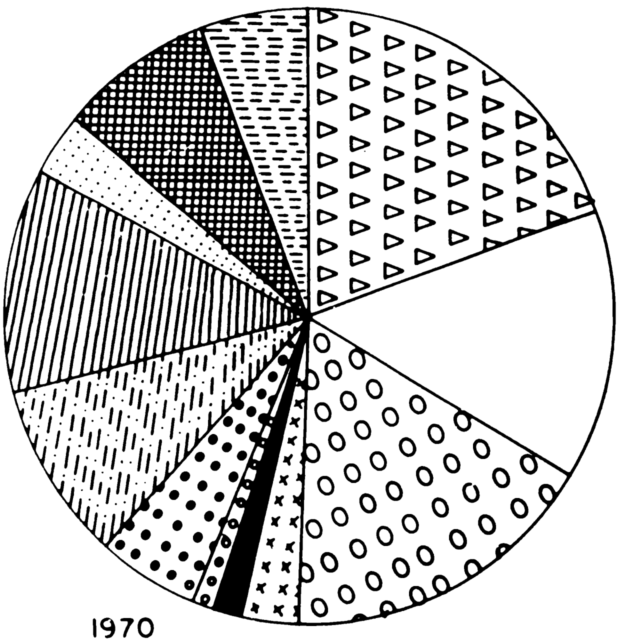
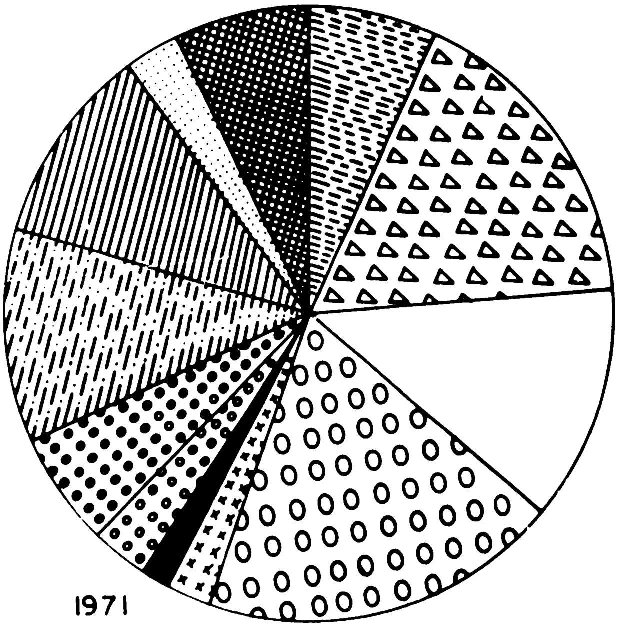

1970

FIG. 10


1971

Next to isopods and copepods, amphipods ranked the major food item, being 13.58% in 1970 and 12.5% in 1971. Isopods, copepods and amphipods were found in all months of the years under observation.

Cirriped larvae and Lucifer sp. formed only a small percentage and found only seasonally (1.42 and 1.17% respectively for the year 1970 and 1.33% and 3.25% respectively for the year 1972).

Fish larvae and small fishes occurred in all the months except during July 1970 and they formed another important food item, constituting 9.75% in 1970 and 12.59% in 1971.

Though larval polychaetes formed a major item of a food, they were found only from May to November during the two year period. But they formed 8.33% and 7.16% for the years 1970 and 1971 respectively.

Molluscan larvae also were found only seasonally and formed 5.25% in 1970 and 5.92% in 1971.

Chaetognaths and worms like nematodes, planarians and polychaetes were also found seasonally, in very small quantities.

Miscellaneous items included undigestable matter like shell pieces and sand particles and semidigested portions of animal matter. Algal matter was also observed occasionally.

From the analysis of the food contents in the gastric cavity of *A. nigrescens* it is obvious that crustaceans form the major food item of this anemone as many of them (isopods, amphipods and copepods) are found throughout the period of the present investigation and formed the major constituent of the gastric cavity. Decapods were found only during certain months. Fish larvae and small fishes were also found throughout the period. The larval forms mostly of polychaetes and molluscs are found only during certain months; but when they are present, they form a major food item. Laboratory studies showed that the anemones do not show any preference to larval food items. But during July, August and September when enormous number of larvae of the tube dwelling polychaete *Sabellaria cementarium* and the bivalve *Modiolus* sp. are liberated ~~and~~ the stomach contents are largely represented by them. Sexually matured anemones are noticed during March and April. But no variations occur in the composition of food items present in male and female during these months.

Mode of food capture

The sedentary mode of actinian life leads to extensive types of feeding habits. Many of them are filter feeders injesting very minute organisms collected by ciliary currents (Boschma 1925, Carlgren 1905, Ross 1967). In others the tentacles form the chief food capturing organs and laboratory studies have shown that it is true with *A. nigrescens* also (Figs. 11-14). The food substances which

Figs.11–14. Photographs showing *A. nigrescens* feeding on a piece of fish meat, in various stages.

FIG.11

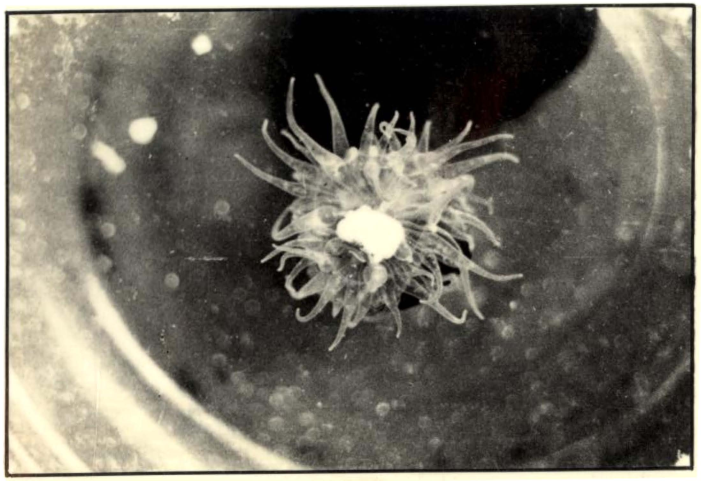


FIG.12

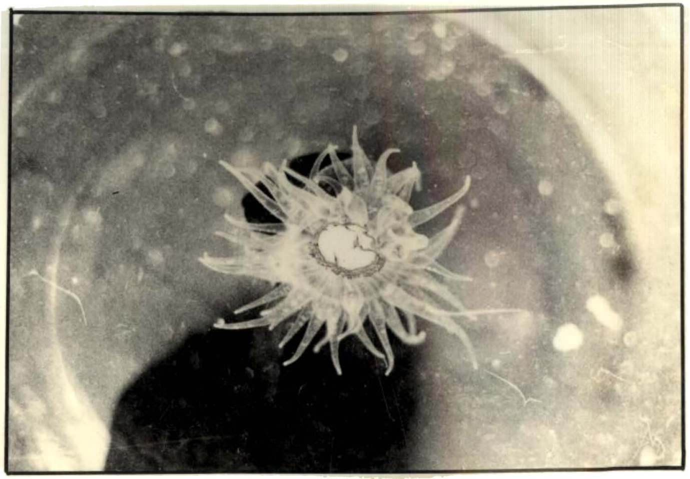


FIG.13

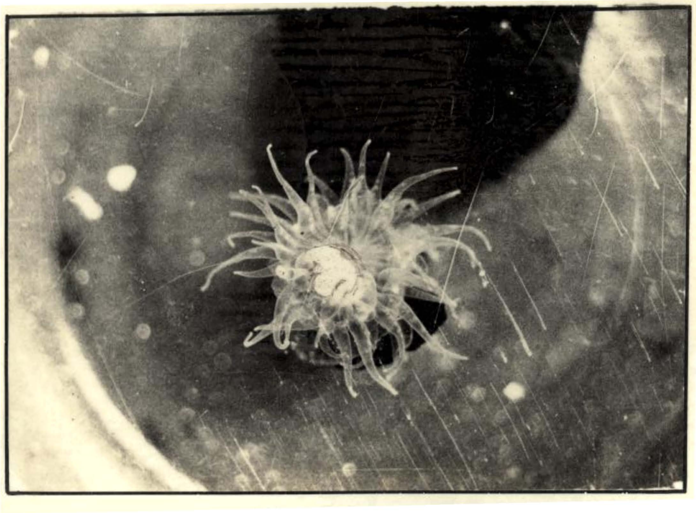
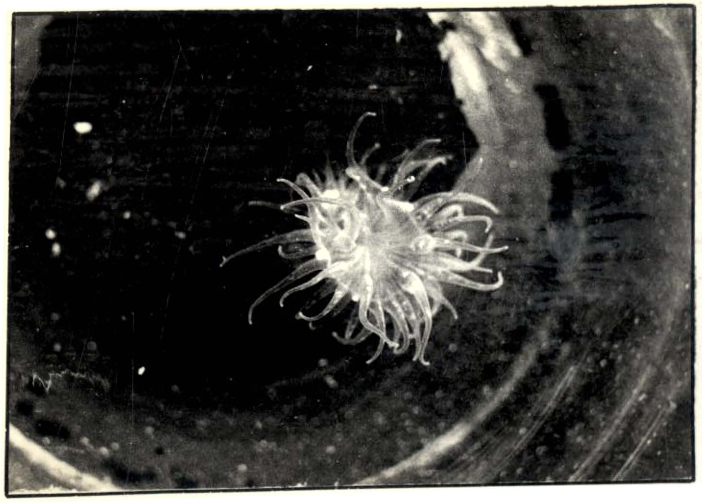


FIG.14



are given to them get adhered to the tentacles partly by mucus secretion and partly by the nematocyst threads of the tentacles. When food particles get adhered, the tentacles get shortened and bent towards the mouth. Other tentacles also join in the action especially when the food organism is considerably large. When a large number of small planktonic organisms are given all the tentables which come into contact with them are in action independently, and the prey is brought to the mouth by bending the tentacles. When the food particles touch the mouth, it opens and grasps the prey. It was also found that the tentacles are extensible and prehensile.

Feeding responses to different substances

In the laboratory active specimens of *A. nigrescens* were fed with plankton, small fishes, fish meat, molluscan meat, crab meat, boiled egg, vegetable matter like algae, carrot, potato, beet root etc. in raw and cooked condition and fruits like apple and banana. It was observed that they readily intake plankton, fishes, fish meat and crab meat. In general they preferred live planktonic organisms. Pieces of boiled chicken egg was also accepted but the reaction was slow. When the anemones were starving they readily accept dead animals and animal meat. When small fishes were given it was found that they vomit the head region and scales, though they digested other skeletal matters.

Observation on anemone subjected to starvation

A. nigrescens could live in the laboratory without food for a considerable period. When they were kept in millipore filtered sea water it was found that they could survive without food for about 135 days. But they became reduced in size and lost their body weight considerably. An anemone weighing 1.32 g lost its body weight to 0.325 g in the above period and another one of 0.856 g lost its weight to 0.211 g. But when they were well fed again they regained the normal size and weight within 25 to 30 days. This confirms the observation of Stephenson (1928) that the size of an anemone need not be dependent on its age, but rather to the availability of food.

Relation between salinity and feeding

While making studies on salinity tolerance of *A. nigrescens* it was found that in lowered salinities (below 4‰) the anemones neither intake food substances nor show any response to them. When abrupt lowering from normal salinity is made then also they do not respond to food items.

Relation between asexual reproduction and feeding

It was also found that when the process of asexual reproduction was going on (discussed in Chapter IX) only in the initial stages (Fig. 19) they intake food, but during the time of fission they do not not intake food, though they show some reaction to it. But after though they do not digest the nematocysts.

Analysis of the food from the gastric cavity of *A. nigrescens* shows that plankton forms the major food constituent and this is due to the peculiar mode of feeding of the species. Laboratory studies also prove that they show preference to planktonic organisms as food. This may be mainly due to the fact that *A. nigrescens* is a tentacular feeding animal. The food substances get adhered to the tentacles partly by the mucous secretion and partly by nematocyst threads of the tentacles. When food particles get adhered the tentacles become shortened and they bend towards the mouth. But many others are filter feeders injesting very minute organisms collected by ciliary currents, (Carlgren 1905, Boschma 1925, Ross 1967). In primitive forms like Protantheae the injestion of food takes place chiefly by ciliary currents because in these anemones the whole surface is ciliated and has ciliary currents passing from aboral to oral end (Boschma 1925). But in the majority of forms cilia are restricted to the tentacles and the ciliary currents drive small organisms towards the oral disc (Hyman, 1940). Elmhirst (1915) reported that *Peachia* obtain food by means of the incurrent stream through the conchula. In those forms with short tentacles, the tentacles hold the food against the oral disc, the mouth opens and the pharynx protrudes as bladdery lobes which reach the food and grasp it. In *A. nigrescens* the tentacles though not very long are extensible and prehensile and thus have an advanced method of food capturing mechanism. Thus they are also able to capture organisms the fission is over, even before the fusion of the body wall takes place they begin taking food substances. It was also noticed in the laboratory that starved anemones and those which were not properly fed failed to reproduce asexually while well fed ones repeatedly reproduced by asexual method. (Discussed in Chapter IX)

Discussion

Essentially the sea anemones are sedentary predators. Typical sea anemones occupy terminal position on food chains (Ross 1967). Those forms which possess symbiotic algae may supplement their normal diet of injested food (Muscatine and Hand 1958, Muscatine 1961, von Holt 1968 and Buchsbaum 1968). Though many species of *Anthopleura* (*A. xanthogrammica*, *A. elegantissima* etc.) harbour symbiotic algae and utilise it for food, in *A. nigrescens* they have not been observed. But observations by subjecting them to starve showed that they could live without food for a long period, and so further studies are required for finding out the details of the metabolism of this species.

Anemones rarely form the food of other animals, probably because their poisonous cnidae render them both unpalatable and dangerous as reported by Ross (1967). This is evidenced by the observation that carnivorous fishes do not accept *A. nigrescens* as food. But Cutress (personal communication) has observed that there are certain nudibranchs in Hawaii which mimic and prey on *A. nigrescens*, like small fishes and larger planktonic forms. If the prey is large the neighbouring tentacles also join the process. The gut analysis also shows that they feed on most of the food items available in their habitat. The state of the stomach is the most important factor of *A. nigrescens* in its reaction towards the food substances which come into contact with the tentacles. Laboratory studies show that when they are starved they take a variety of food substances other than plankton, but reject vegetable matter. Nagai and Nagai (1973) also found the same behaviour in the case of *Anthopleura midorii*.

Being sedentary forms, the sea anemones might have developed a non-selective feeding habit. Most of the sea anemones pass their lives attached to some submerged object and feed on organisms on detritus coming within the reach of the tentacle. Once settled in such a position, a typical sea anemone move very little (Ross 1967). *A. nigrescens* occurs in large numbers packed so closely and they reproduce in large numbers both sexually and asexually. Thus they are subjected to intraspecific struggle for existence and feed on animal matter without being selective feeders. Their remarkable ability for starvation, evidenced by laboratory studies, also enables the anemone to thrive well in the struggle for existence.

V

DIGESTION

Many studies have been made on digestion in anemones especially with regard to the occurrence of extracellular digestion in the coelenteron. Our earlier knowledge of the phenomenon of digestion in actinians are mainly from the works of Metschnikoff (1880, 1893) and Krukenberg (1882). Metschnikoff (1880) reported that intracellular digestion takes place in the cells lining the mesenterial filaments of _Sagartia_ and _Aiptasia_. Krunkenberg (1882) proved that the mesenterial filaments of actinians are the organs of digestion and he maintained that digestion takes place solely in these regions. According to him when the food particles come in contact with the filament a secretion of digestive fluid takes place and the food particles are more or less dissolved by extracellular digestion. Chapeaux (1893) agreed with the view of Krunkenberg and he recognised weak proteolytic enzymes in the gastric fluid of _Sagartia_, _Anemonia_ and _Heliactis_. Metschnikoff (1893) and Mensil (1907) also agreed with this view. Willem (1893) by his studies on the sea anemone _Tealia_ proved that besides extracellular digestion intracellular digestion also takes place in actinians. Jordan (1907) found that some amount of digestion occurs in the gastric cavity of the actinian, _Anemonia sulcata_. Biedermann (1911) also reported that both intracellular and extracellular digestion take place in actinians. Further he found that only a small quantity of the enzyme is secreted into the coelenteron and that too only when the food substance had contact with the mesenterial filaments and that is why enzyme is not found free in the coelenteron. Bodansky (1924) found that the unfiltered gastric fluid of *Metridium marginatum* contains trypsin and amylase. But it was too weak to be investigated. Takemura (1938) detected pepsinase and tryptase in the unfiltered gastric fluid of *Cribrina artemisia*. Ischida (1936) confirmed the presence of tryptase, pepsinase and ereptase as proteolytic enzymes in the filtered and unfiltered gastric fluid of *Actinia mesembryanthemum*. According to Yonge (1931, 1937 and 1954) some initial digestion of protein takes place extracellularly in coelenterates. Krijgsman and Talbot (1953) from their studies on *Pseudactinia flagellaria* showed some proteolytic activity in the gastric fluid. Nicol (1959) from his studies on the sea anemone *Calliactis parasitica* concluded that the mesenteric filament is the chief digestive region and contains a strong protease and the fluid shows little or no proteolytic activity. Lenhoff (1968) has reported the presence of proteolytic activity on casein in the mesenterial extracts of the anemone *Anthopleura elegantissima*.

The present study is directed towards the detection and estimation of the types of enzymes present in the gastric fluid, gullet and the mesenterial filaments which are found to be the main source of supply of enzymes in actinians.

Materials and methods

For enzymatic studies live specimens collected from Narackal were brought to the laboratory and kept for a few days and no food was given during this period. The studies were made on three regions, the gullet or stomodaeum, the mesenterial filament and the gastric fluid found at the bottom of the coelenteric cavity. The stomodaeum was taken out by opening the animal, carefully removing the attached surrounding tissues. The mesenterial filaments were also cut by using a pair of fine scissors. The gastric fluid was taken by making an incision at the base of the animal by puncturing with a fine needle or scissors, care being taken not to injure the mesenterial filaments. The fluid was allowed to flow into a small tube by gentle pressing. The stomodaeal tissues and mesenterial filaments were thoroughly washed in distilled water for removal of food particles and other foreign matter if any. After washing the stomodaeal tissues and filaments were kept in separate tubes and kept at -10°C. Homogenates were prepared from mesenterial filaments and stomodaeal tissues by grinding them in a mortar with quartz sand and with suitable quantity of distilled water (about 5 ml for 1 g of tissue). The homogenate thus obtained was centrifuged to obtain a clear supernatant. The extracts and the gastric fluid solution were then ~~fas~~ frozen at -10°C.

Experiments were carried out for the detection and estimation of amylase, proteases and lipases. Protein content was estimated for every homogenate.

Methods of Estimation

Estimation of amylolytic, proteolytic and lipolytic activities was made by the following methods.

The actinians being carnivores, feeding mainly on animal matter, amylolytic activity is expected to be very much low. However amylolytic activity is shown by some workers at least in a few forms (Ischida 1936). Amylolytic activity was estimated by the method of Natelson (1963). According to this method the homogenate was incubated at 37.5°C for 30 minutes with starch suspension (1.4%) having pH range 6.9 - 7.2. The quantity of maltose formed was then determined in a protein free filtrate. The difference in the reducing activity expressed as glucose in µg/gm protein before and after the incubation was reckoned arbitrarily as the units of amylase activity, the unit being the activity of the enzyme which will liberate maltose equivalent to 1 g. of glucose under the experimental conditions.

Proteolytic activity was estimated by the method of Hunt (1948). The homogenate was incubated for 30 minutes at 37.5°C. The experiments was carried out with different substrates in different pH media. The readings of the control and the standard were taken against the blank using the red filter with a transmission at 660 millimicrons.

The substrates used were:

1.	Bovine Albumen	pH 6
2.	Albumen	pH 7.4
3.	Gelatine	pH 8.0
4.	Casein	pH 2.0
5.	Casein	pH 10.0

The enzymatic activity is expressed as µg of tyrosine liberated per 30 minutes per gram of protein.

Lipolytic activity was determined by the method of King (1965). The incubation of the homogenate was carried out for 30 minutes at 37.5°C. Phenyl laurate was used as the substrate. pH of the media was 7.4. Optical densities were determined by using a red filter (660 nm). The activity of the enzyme is expressed as µg of Phenol liberated per 30 mts per gram of protein with Folin Ciocalteu Phenol Reagent.

Results

Amylolytic activity

It can be seen from Table 4 that amylolytic activity was seen only in the mesenterial filaments. The gastric fluid and the stomodeum did not show amylolytic activity. In general amylolytic activity is not detected in this anemone, except for a little activity shown by the mesenterial filaments.

Table 4. Showing Amylolytic activity in *Anthopleura nigrescens*

Temperature (°C)	37.5
pH	6.4
Substrate	Starch
Incubation time (hrs)	0.50
	Units of Amylase per 100 ml. of extract
Mesenterial filament	28
	30
	25
	27
	22
Gastric fluid	nil
	nil
	nil
	nil
	nil
Gullet	nil
	nil
	nil
	nil

Table 5. Showing Lipolytic activity in *Anthopleura nigrescens*

Temperature (°C)	37.5
pH	7.4
Substrate	Phenyl laurate
Incubation time (hrs)	0.50
	μg phenol liberated per 30 mts per gram protei
Mesenterial filament	1950
	1929
	2020
	2025
	1981
Gastric fluid	278
	250
	255
	263
	290
Gullet	1107
	1090
	1085
	1080

Table 6. Statement showing Proteolytic activity in *Anthopleura nigrescens*

Temperature (°C)	37.5	37.5	37.5	37.5	37.5
pH	6.0	7.4	8.0	2.0	10.0
Substrate	Bovine albumen	Albumen	Gelatine	Casein	Casein
Incubation time (hrs)	0.50	0.50	0.50	0.50	0.50
	µg Tyrosin liberated/gm protein in 30 minutes				
Mesenterial filament	187	246	75	nil	nil
	190	250	80	nil	nil
	160	247	78	nil	nil
	193	270	90	nil	nil
	180	258	73	nil	nil
Gastric fluid	70	90	41	nil	nil
	54	94	30	nil	nil
	63	82	33	nil	nil
	49	78	36	nil	nil
	57	85	39	nil	nil
Gullet	46	112	nil	55	70
	50	120	nil	62	54
	37	124	nil	58	63
	30	130	nil	61	61
	32	118	nil	57	86

Lipolytic activity

The results of the lipolytic activity are shown in Table 5. The mesenterial filaments, the stomodaeum and the gastric fluid showed lipolytic activity. The activity is well pronounced in the mesenteric filaments and the gullet. The gastric fluid showed lesser activity.

Proteolytic activity

Table 6 shows the proteolytic activity in the stomodaeum, mesenteric filament and the gastric fluid. As in the case of amylolytic and lipolytic activity, proteolytic activity is also higher in the mesenteric filaments. Observations were carried out using different substrates in different pH media. Proteolytic activity was not observed in pH media 2.0 and 10.0 for the mesenterial filament and gastric fluid but the gullet alone showed some activity. In general the maximum activity was observed in pH 7.4 for all the samples.

Discussion

The results of the present study show that digestion in this anemone mainly takes place by the enzymes secreted from the mesenterial filaments and stomodaeum. The gastric fluid also plays some role in the digestive process. This has been evidenced by previous observations also. Earlier studies by Boschma (1925), Yonge (1931), Takamura (1938) Krijgsman and Talbot (1953) and Gibson and Dixon (1969) show that small amount of digestive enzyme activity is found in the gastric fluid, and greater activity is present in crude extracts of tissues of mesenteric filament and stomodeum. Of these the mesenteric filament is the main source of supply of these digestive enzymes. Regarding the gastric fluid only a small amount of it can be collected from the sea anemones by suction or cutting, but even this small quantity shows proteolytic and lipolytic activity. As to the secretion of this enzyme there are differences of opinion. Krukenberg's (1882) opinion that the food particles should come into contact with the mesenteric filament for the secretion of the enzyme has been proved incorrect by the experiments of Jordan (1907). He enclosed fibrin in sacs of filter paper and fed them to Anemonia sulcata and digestion of the fibrin occurred and since the mesenteric filament could not come into contact with food, he concluded that extracellular proteases diffused into the sacs and caused hydrolysis of the fibrin. Since the gastric fluid showed proteolytic and lipolytic activity it may be possible that extracellular digestion may take place by which large food particles and organisms can be quickly digested. Such extra cellular proteases had been detected in the gastric fluid collected from the intact animal and secretion of proteases had been proved by using in vitro preparations in sea anemones (Krijgsman and Talbot 1953). They believed that the food particles became coated with stiff mucus in the gastric cavity of the anemone. This coating becomes impregnated with proteases and prevents against dilution by sea water. Digestion takes place within the mucous coating. Nicol (1959) concluded that since the proteases were secreted directly on to the enveloped food mass there was little chance for these enzymes to leak into the coelenteric fluids. But Ishida's (1936) studies on the anemone *Actinia mesembryanthemum* showed peptinase, tryptase, and dipeptidase in the mesenteric filaments whose glycerine extracts are entirely the same as those found in the gastrovascular fluid, because these enzymes found in the former had respectively the same optimum pH as those found in the latter. Similarly in the case of *Anthopleura nigrescens* the gastrovascular fluid enzyme activated in the same optimum pH as that of the mesenteric filament extract. So the enzymes in the gastrovascular cavity may have their origin from the cells lining the mesenteric filament. This is further evidenced from the results (Table 6) shown in the proteolytic activity of the stomodaeum and the mesenteric filament. The substrates on which the filament extract and fluid showed proteolytic activity in the same optimum pH failed to show activity on stomodaeum extract. So other substrates with different pH were used and they showed some activity.

It is clear from the present study that a protease is secreted into the coelenteron of the anemone and the observation mainly agrees with that of Ishida (1936). Regarding the proteolytic and lipolytic activity, it is mainly from the mesenterial filament, the gastric fluid and the gullet. Amylolytic activity was found associated with the mesenteric filament alone. When compared with proteolytic and lipolytic activity, the amylolytic activity is low and this may be due to the preference for the type of food on which the anemone feed; they being carnivores. Kenyon (1925), Vonk(1927) and Al-Hussaini (1949) have noticed that herbivorous fishes have much more amylase than carnivores. So the lesser amylolytic activity in anemones may be due to their preference for animal food. The lipolytic activity is high in the filament, gullet and gastric fluid and this shows that the food taken in contains considerable amount of fat. The proteolytic activity is also higher and this may be due to the fact that the anemone's food is rich in protein content. Further, studies by Gibson and Dixon (1969) on the sea anemone *Metridium senile* have shown that the proteases from the sea anemone have the same zymogen activation and active site chemistry as the proteases of the mammalian pancreas. This finding suggests a common ancestory for the digestive process of mammals and coelenterates.

VI

SALINITY TOLERANCE

Salinity is a major environmental factor in coastal and brackish water areas since salinity fluctuations affect the eco-physiology of marine organisms. In the open ocean the salinity is fairly constant and so is the ionic composition of the ocean water, and so the salinity has little influence on the organisms inhabiting this area. Therefore the study of organismic responses to salinity is largely concerned with forms inhabiting coastal or inland waters where salinity variations are very common. It has been evidenced by many workers that there exists a complex correlation between temperature and salinity. But in areas where temperature variations are not significant as seen in the case of many tropical waters, salinity is the most potent physical factor effecting the fauna. In the last two decades the effects of salinity on marine and brackish water animals have received attention by many workers and reviews on this aspect are available in the works of Beadle (1957), Moore (1958), Kinne (1958, 1963, 1964, 1971), Nicol (1960), Prosser and Brown (1961), Newell (1970) and Vernberg and Vernberg (1972).

Hydrographical studies of the coastal waters from where specimens for the present study were collected (discussed in Chapter III) have shown that the temperature variations during the course of an year are not considerable (28°C-33.4°C) while salinity varies very widely from 10‰ to 34.2‰. Therefore salinity could play an important role in the eco-physiology of *A. nigrescens*. Experiments were conducted in the laboratory to study the responses of *A. nigrescens* to different higher and lower salinity media. Observations were also made on specimens acclimatized in different salinity media. Kinne (1971) has stressed the importance of such studies since an assessment of salinity tolerance based on field observations alone is difficult, because the effect of salinity may be modified by other environmental factors.

Materials and Methods

Based on the variations in salinity in the area of investigation two main hydrographical seasons can be distinguished during a year viz. the monsoon season (June-October) characterised by low salinity due to heavy rains and the post-monsoon season (November-May) characterised by high salinity. In order to study the salinity tolerance of *A. nigrescens* during these two seasons, experiments were conducted using specimens collected during each season. In the monsoon season specimens were collected when the salinity was about 15‰. So also specimens were collected during the post monsoon season when the salinity reached about 33.5‰. Another set of experiments were conducted using animals acclimated in 42‰ salinity in order to find out whether anemones acclimated in this medium could tolerate still higher salinity conditions. (This medium was chosen because it is found that there was 100% survival in this salinity when anemones acclimated in 33.5‰ were directly transferred to it). Experiments were also conducted to study their reactions to gradual increase or decrease in the salinity. Anemones for the experiments collected from Narackal were kept in 1000 ml beakers, in the water collected from the same area for acclimation. All animals were kept in the acclimation salinity for at least seven days prior to use in any experiment and they were fed with small planktonic organisms (mainly copepods) during the period of acclimation. But when they were subjected to experiments no food was given. Healthy anemones of more or less the same size were selected for the experiments and they usually get attached to the containers within 12 to 18 hrs. Filtered sea water diluted with distilled water was used to make lower salinity media and higher salinity media were prepared by evaporating filtered sea water. 10 specimens were placed in each beaker containing 1000 ml of sea water of the required salinity.

The experiment was run in triplicate so that a total of 30 animals were exposed to each salinity. The specimens were washed with water of the same salinity to which they were to be transferred prior to the beginning of the experiment. Simultaneous control experiments were also run in the acclimation media. The mortality was determined by the failure of the column and tentacles to contract in response to mechanical stimulation with a metal probe (Sassaman and Mangum, 1970) and also by transferring them back to the acclimation medium. The experiment was conducted at room temperature 28.5°C (± 1°C). Loss of water by evaporation from the containers was compensated by adding distilled water. The water was aerated daily using an aerator. As the experiment progressed observations were made on the number of 'active' 'inactive'and 'dead' animals. The activity of the animal was recorded after every 24 hours. Expanded animals showing the oral disc and tentacles were noted as 'active' and scored 1. Those remained in a contracted state showing life by responding to mechanical stimulation were treated as 'inactive' and scored ½ and those without any sign of life to mechanical stimulation were recorded as 'dead' and scored nil (Bhatnagar and Crisp 1965, Lyster 1965). Salinities were determined by titration against silver nitrate solution using the method of Knudson (1901). The duration of the experiment was 7 days.

These salinities in which death exceeded 50 per cent at the end of the experiment were considered lethal. The formula by Lance (1963) given below has been used in the calculation of survival values:

Percent survival after exposure to various
salinities at the end of the experiment $\Big\} = a1/ b1 \times b2/a2 \times 100$

where a1 = number of survivors in the experimental medium
a2 = number of animals initially placed in the experimental medium
b1 = number of survivors in the control (acclimation medium)
b2 = number of animals initially based in the control (acclimation medium)

Experiments and Results

Tolerance of *A. nigrescens* acclimated in 33.5‰ S.

Anemones acclimated in 33.5% were transferred to salinities 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰, 35‰, 40‰, 45‰ and 50 ‰. Another set of experiments was conducted using lower salinities viz. 6‰, 7‰, 8‰, 9‰, 11‰, 12‰, 13‰, 14‰ and higher salinities viz. 42‰, 43‰ and 44‰ in order to fix the lethal salinities. The results of the experiments are given in Table 7. From the table it can be seen that animals kept in salinities 15‰ to 42‰ behaved normal for the whole period of the experiment (7 days).

But those kept in 0‰, 5‰, 10‰, 45‰ and 50‰ salinities behaved differently. In 0‰, the specimens after the transfer never expanded to show their disc and tentacles and remained in contracted state upto the second day of the experiment and then died. In the other media for the first two days all the specimens remained in a contracted state but they showed reaction to the mechanical stimuli. After the third day specimens kept in 5‰ S showed only 20% survival and by the seventh day all of them died. Anemones kept in 10‰ S after the third day showed progress in survival rate though for the first three days they remained in contracted state. By the 7th day all of them behaved normally. Those kept in 45‰ and 50‰ salinities immediately after the transfer to the experimental media remained in contracted state. On the 5th day of the experiment there was 20% survival for those kept in 45‰ S. But by that time all specimens in 50‰ S died. In the second series of the experiment anemones acclimated in 33.5‰ S gave the following results: Those kept in 42‰ S behaved normally. In 43‰ S there was 90% survival and in 44‰ S there was only 20% survival on the seventh day. So 44‰ S is found to be the upper lethal salinity. In 7‰ S there was 80% survival from the third day onwards. In salinities 8‰, 9‰, 11‰, 12‰, 13‰ and 14‰ there was 100% survival by the 7th day. In 6‰ S there was only 20% survival on the 7th day, and this is found to be the lower lethal salinity. It was also found that when there was a lowering of salinity some of the anemones reproduced asexually (discussed in Chapter IX).

Tolerance of A. *nigrescens* acclimated in 15‰ S.

After acclimation in 15‰ salinity the anemones were transferred to media of salinities 0‰, 1‰, 2‰, 3‰, 4‰, 5‰, 6‰, 7‰, 8‰, 9‰, 10‰, 15‰, 20‰, 25‰, 30‰, and 35‰, 36‰, 37‰, 38‰, 39‰, 40‰, 41‰, 42‰, 43‰, 44‰ and 45‰. No higher salinities above 45‰ were used since it was found that from the previous experiment (anemones acclimated 33.5‰) that the salinity above 43‰ was lethal. Results of the experiments are given in Table 8. It was observed that from 5‰ S to 39‰ S there was 100% survival. In 4‰ S there was 80% survival and in 40‰ S there was 60% survival. In 3‰ S there was 40% survival and in 41‰ S there was 30% survival. The lower lethal salinity was found to be 3‰ S and the upper lethal salinity was found to be 41‰ S. During this experiment no anemones were found to be reproducing asexually.

Tolerance of A. *nigrescens* acclimated in 42‰ S.

A. *nigrescens* acclimated in 42‰ were directly transferred to salinities 1‰, 2‰, 3‰, 4‰, 5‰, 6‰, 7‰, 8‰, 9‰, 10‰, 15‰, 20‰, 25‰, 30‰, 35‰, 40‰, 45‰, 48‰, 49‰, 50‰, 51‰, 52‰, 53‰, 54‰, 55‰, 56‰, 57‰, 58‰, 59‰ and 60‰. Results of the experiments are given in Table 9. Survival rate was 100% in salinities 9‰ to 51‰ and nil in salinities 0‰ to 4‰ and 55‰ to 60‰.

Table 7 Per cent survival of *A. nigrescens* at time intervals (days) when transferred to various salinities after acclimation in 33.5‰ S.

	Salinity ‰														
Days	0	5	6	7	8	9	10	11	12	13	14	15–42	43	44	45	50
1	50	50	50	50	50	50	50	50	60	60	80	100	90	50	50	50
2	0	50	50	50	50	50	50	60	80	100	100	100	90	50	50	30
3	0	40	40	80	50	60	50	70	80	100	100	100	90	70	50	30
4	0	20	30	80	90	100	80	90	90	100	100	100	90	70	30	10
5	0	20	30	80	100	100	80	100	100	100	100	100	90	60	30	0
6	0	10	40	80	100	100	90	100	100	100	100	100	90	20	0	0
7	0	0	40	80	100	100	100	100	100	100	100	100	90	30	0	0

Table 8 Per cent survival of A. nigrescens at time intervals (days) when transferred to various salinities after acclimation in 15‰S.

	Salinity ‰																
Days	0	1	2	3	4	5	6	7– 36	37	38	39	40	41	42	43	44	45
1	50	50	50	60	60	80	90	100	90	80	70	70	70	60	50	50	50
2	0	0	20	40	60	90	100	100	90	80	80	50	60	50	30	20	20
3	0	0	0	30	50	100	100	100	100	80	80	50	50	40	20	20	10
4	0	0	0	30	50	100	100	100	100	90	90	60	50	20	10	10	10
5	0	0	0	40	60	100	100	100	100	100	90	60	30	20	10	0	0
6	0	0	0	40	80	100	100	100	100	100	90	60	30	20	10	0	0
7	0	0	0	40	80	100	100	100	100	100	100	60	30	20	10	0	0

Table 9 Per cent survival of A. *nigrescens* at time intervals (days) when transferred to various salinities after acclimation in 42‰S.

	Salinity ‰																		
Days	0	1	2 4	5	6	7	8	9	10	11 48	49	50	51	52	53	54	55	56	. 57 60
1	20	40	50	70	70	90	90	90	100	100	90	80	80	60	60	50	50	50	50
2	0	0	0	30	40	80	80	80	90	100	100	80	80	70	60	80	40	20	0
3	0	0	0	30	40	60	70	90	100	100	100	90	80	70	50	40	30	0	0
4	0	0	0	20	30	40	70	90	100	100	100	100	90	50	40	40	30	0	0
5	0	0	0	20	20	40	60	100	100	100	100	100	90	50	30	20	10	0	0
6	0	0	0	10	20	40	50	100	100	100	100	100	100	50	30	20	10	0	0
7	0	0	0	10	20	40	50	100	100	100	100	100	100	50	30	20	0	0	0

The lower lethal salinity was 7‰ and upper lethal salinity 53‰. During this experiment some anemones reproduced asexually in lowered salinities.

Tolerance of *A. nigrescens* subjected to gradual lowering of salinity

A. nigrescens acclimated in 33.5% S was subjected to gradual lowering of salinity by adding distilled water every day so that an average reduction of 0.10‰ salinity was obtained every day. It was observed that when the salinity reached 4‰ there was 40% mortality. When the salinity reached 3.3‰, 50% mortality occured. Cent per cent mortality occurred when the salinity reached 3‰. So 3.3‰ was found to be the lethal salinity when *A. nigrescens* was subjected to gradual lowering of salinity.

Tolerance of *A. nigrescens* subjected to gradual increase in salinity by evaporation of the acclimation medium (33.5‰ S)

A. nigrescens acclimated in 33.5% S were placed in large troughs (5 litres) and subjected to gradual increase of salinity by evaporation of the water. The experiment was continued till all the anemones died. The average increase in salinity per day was about 0.71‰. When salinity reached 58‰ there was 100% mortality. The lethal salinity was found to be 56.02‰.

Discussion

Detailed assessments of salinity tolerance on the basis of field studies alone are difficult, because the effect of salinity may be influenced by other environmental factors such as light, temperature, water movement, dissolved gases, nutrition and interaction between co-existing organisms (Kinne 1971). Laboratory experiments conducted under controlled experimental conditions using acclimated specimens give more detailed information on the response of organisms to variations in salinity. A distinction between the 'ecological potential' exhibited in the field and the 'physiological potential' in the laboratory is therefore necessary (Kinne 1971). In general death from sub- or supranormal salinities does not occur in the open oceans except at the uppermost surface layers which may be subjected to salinity variations, due to havey rainfall or evaporation. However coastal waters such as estuaries, lagoons, beaches etc. are frequently characterised by considerable salinity fluctuations. *A. nigrescens* being an intertidal species is subjected to such conditions.

The present experiments as well as the field observations show that this anemone is essentially a euryhaline actinian, since invertebrates considered euryhaline tolerate salinity ranges of 10‰ to 30‰ or more (Kinne 1971). The observations also show that the range of salinity tolerated is also dependent on the salinity of the acclimation medium. Kinne (1964) found that in general, acclimation to low salinities tends to shift the lower lethal limits downwards and acclimation to higher salinities tends to shift the upper limits upwards. This generalisation is in agreement with the present findings. Experiments using anemones acclimated in 33.5‰S showed that the lower lethal salinity was 6‰ and upper lethal salinity 44‰. There was further decrease in the lower lethal salinity (3‰) when anemones acclimated in 15‰ salinity were used. Correspondingly the upper lethal salinity also decreased to 41‰. But when animals acclimated in 42‰ salinity were used the lower lethal salinity increased to 7‰ and the upper lethal salinity to 51‰. These observations agree with the findings of Kinne (1964).

It has been observed by several workers that the actinians could tolerate a wide rante of salinities and also that many marine actinians inhabit brackish water areas. Stephenson (1935) has reported that the anemone *Actinia equina* could tolerate wide ranges of desiccation, temperature and salinity. They could survive in very low salinities in aquaria. Pax (1920) observed that this species definitely prefers water of high salinity though he has been able to keep them for a long time in water of salinity as low as 4‰. Verrill (1898) observed that during rainfalls in the low tide, the sea water in small pools is washed away and replaced by fresh water for several hours destroying most of the marine life, but apparently without injury to the anemone *Diadumene luciae*. Selwood (1930) found that the brackish water anemone *Nematostella vectensis* could tolerate salinities varying from 0.9% to 3.27% NaCl. Orton as reported by Stephenson (1935) found that the anemone *Actinothoe lacerata* could tolerate wide ranges of salinity. He kept specimens in 36.36‰ S and by gradual evaporation the salinity reached 53.84‰ in a year. Renton, as reported by Stephenson (1935) placed *Actinia equina* in water of salinity 33‰ and this was reduced by replacing 50 cc of it twice a week by 50 cc of tap water. When the salinity reached 12.4‰ nine young ones were born. The specimen disintegrated completely when the salinity reached 6.8‰. In the case of *A. nigrescens* also it was observed that by gradual lowering of salinity they could tolerate salinities lower than those tolerated when directly transferred after acclimation in 15‰, 33.8‰ and 42‰ salinities, and that a lowering of salinity causes the anemone to reproduce asexually. In the natural habitat also *A. nigrescens* is subjected to gradual lowering of salinity by fresh water drainage from land and also due to heavy rainfall during the monsoon months. From the experiments it is evident that gradual lowering of salinity upto 3.3‰ is not lethal to the anemone. Orton's experiment as reported by Stephenson (1935) on *Actinothoe lacerata* showed that gradual increase in salinity may increase the capacity to tolerate higher salinities (36.35‰ to 53.84‰). Evans (1914) observed that *Actinia equina* when kept in aquarium for over two years without food or aeration, survived even when the concentration of the salt in the water had almost doubled as reported by Stephenson (1935). A gradual increase of salinity in the case of *A. nigrescens* also enables it to show that they can tolerate higher salinities than those tolerated by anemones subjected to abrupt changes. For anemones acclimated in 42‰ S, the higher lethal salinity was found to be 51‰, but by gradual increase the lethal salinity increased to 56.02‰. *A. nigrescens* living in small rock pools also, may sometimes be subjected to high salinities than that of normal sea water due to evaporation during the summer months. Similarly during rains the salinity of these rock pools may be reduced very much. The present experiments show that they can withstand these types of sudden or gradual variations in salinity to a considerable extent. Miyawaki (1951) when subjected the sea anemone *Diadumene luciae* to lowered salinities found that this could survive in water diluted upto 7.5‰ S. Panikkar (1936) observed that the anemone *Phytocoetopsis ramunni* and *Stephensonactis ornata*, two brackish water actinians from Madras are capable of tolerating wide variations in salinity. Cheriyan (1964) found that *Phytocoetopsis ramunni* could survive in salinity as low as 0.4‰, by gradual lowering. But no fresh water actinian has been reported so far though laboratory studies have shown that they could tolerate very low salinities.

The mechanism by which the sea anemones tolerate wide ranges of salinity is not yet fully explained or understood. Bateman (1932) is of opinion that "the concentration of salts in the tissues varies directly with the external milieu". Palmbert (1938) studied the marine hydroid *Clava multicornis* and found no evidence of osmoregulation. He stated that there was a decrease in the oxygen consumption in lowered salinities which indicated the absence of osmoregulatory mechanism. Miyawaki (1951) is of the opinion that the secretion of mucus plays a role in the resistance to low salinities in actinians. It was noticed in the present study that when *A. nigrescens* is subjected to lowered salinities the mucous secretion is very high. Panikkar (1936) stated that when variations in salinity occur the anemones withdraw into their burrows and remain in a contracted state till the conditions become favourable. *A. nigrescens* also remains in a contracted state when subjected to variations in salinity and expands only when it becomes adapted to the new salinity media. If the new salinity is unfavourable they die and disintegrate finally. However, the laboratory studies show that their adaptability to variations in salinity is very high. A recent study on the sea anemone *Diadumene leucolena* by Pierce and Leo (1974) shows that this anemone could tolerate salinities ranging from 6‰ to 33‰. They are of the opinion that the mechanism of water control is by intracellular free amino acid regulation like that found in other marine invertebrates. Pierce (1971) found that the degree of euryhalinity exhibited by marine animals is directly related to the size of intracellular free amino acid pool. *D. leucolena* is highly euryhaline and their free amino acid pool is considerably larger than that of stenohaline anthozoans in similar salinities (Kittredge *et al* 1962, Von Holt 1968, Severin *et al.* 1972), but comparable to those of other euryhaline species (Emerson 1969, Pierce 1971, Severin *et al* 1972). *A. nigrescens* being an osmoconformer in salinities ranging from 3.3‰ to 56.02‰ depending on the acclimation media, also may possess a larger intracellular amino acid pool and further observations are to be made in this line.

Remane (1958) classified euryhaline animals into four groups based on the lowest salinities to which they penetrate. The first grade of animals are those which penetrate to a salinity of 15‰. The second grade penetrates to 15‰ – 8‰ salinities and the third grade is constituted by animals capable of penetrating salinities between 8‰ and 3‰. The fourth grade of euryhaline marine animals penetrate to salinities below 3‰. According to the above classification the anemone *A. nigrescens* can be included in the third group.

It is obvious from the studies on its distribution and salinity tolerance that *A. nigrescens* is one of the most euryhaline species of actinians. But laboratory studies show that they cannot tolerate salinities below 3‰ and this may be the reason for their absence in Cochin Harbour area where the salinity goes down below 1‰ during the monsoon months. But so far they are not found migrating to the Cochin harbour area even when the salinity is as high as that of the sea water (33‰).

VII

OXYGEN CONSUMPTION

Studies on the oxygen consumption of coelenterates especially the sea anemones have been very little. It has been reported that the anemones are capable of secreting mucus as a protective device against unfavourable surroundings and it affects the respiratory rate by preventing the speed of diffusion of oxygen (Shoup, 1932). Hence the anemones are generally considered unsuitable for respiratory studies. However, authors like Trendelenburg (1909), Puetter (1911), Shoup (1932), Kramer (1937) Newell and Northcroft (1967), Belcik (1968), Sassaman and Mangum (1970) and Griffiths (1977) have reported on the oxygen uptake and metabolic rates of some species of sea anemones. But the respiratory rates given by these authors surprisingly vary very widely from one another. These reports of wide variations in the respiratory rates of sea anemones point to the need for detailed studies on this aspect. *A. nigrescens* which can be reared in the laboratory without much effort, was found to be a suitable species for respiratory studies.

Materials and Methods

Collection and acclimatization of the specimens were done as described in Chapter VI. Experiments were conducted on animals acclimated in 33.0‰ salinity and at room temperature, 28.5 (±1°C) for 10 days. The animals selected for the experiments were allowed to settle on small pieces of thin polythene sheet, one on each piece. Later the animals were placed singly in the respiratory chambers containing double filtered sea water of the acclimation salinity. In this condition they were kept without food for 24 hours. Later the respiratory chamber was connected to a reservoir containing filtered and well aerated water of the acclimation salinity. After replacing the water in the respiratory chamber with that in the reservoir, an initial water sample was taken to determine the oxygen content by Winkler method. Then the respiratory chamber was closed and the animal was allowed to respire for one hour and then another water sample was collected for oxygen determination. From this the oxygen consumed by the animal during one hour was calculated. All experiments were conducted in darkened chambers on starved animals of different size with the temperature maintained in a constant temperature bath (28.5°C). At the end of each experiment the animal was forced to full contraction, blotted and weighed.

All rates were taken at partial pressure of oxygen (pO_2) between 140 and 160 mm Hg. 20 animals of different weights were used in this series of experiments. A weight regression analysis was performed on the data using the formula:

$$O_2 = aW^b$$ (Zeuthen, 1953)

where "O_2" is the total oxygen consumed in unit time, 'W' is the live weight and 'a' and 'b' are constants.

The weight specific oxygen consumption (O_2/g/hr) or the metabolic rate of the organism was found out using a modified form of the above equation ie.,

$$\frac{O_2}{W} = aW^{b-1}$$

The parameters 'a' and 'b' were estimated by the method of least squares after converting the models into linear forms by taking the logarithms of O_2 and W ie.,

i. $\log O_2 = \log a + b \log W$ and
ii. $\log O_2 - \log W = \log a + b \log W - \log W$

Student's t test was used for testing the significance of the 'b' value.

Results

The data obtained on the oxygen consumption of *A. nigrescens* in the acclimation salinity of 33‰ are presented in Table 10. The uptake of oxygen per unit time showed an increasing tendency with increasing body weight. The regression coefficient of 'b' of the O_2 uptake against body weight was found to be 0.9666 and the parameter of 'a' as 0.2647. The regression line is shown in Fig. 15. The regression value for the weight specific oxygen consumption (metabolic rate in O_2 µl/g/hr) which is b-1 in the formula was obtained as -0.0334 and it is illustrated in Fig. 16.

The results of the statistical analysis of the data are presented in Table 11. Statistically refined values for oxygen uptake and metabolic rates are given in Table 12.

Discussion

The most important endogenous factors which influence metabolism are body size of the organism concerned and the level of its activity, as has been proved by several authors. Hence a study of the respiratory rate is possible only after understanding the effects of these endogenous factors. The relation between oxygen consumption and body size has been studied by Brody and Proctor (1932), Kleiber (1932, 1947), Brody (1945), Zeuthen (1947, 1953), Hemmingsen (1950, 1960) and Bertalanffy (1957). A vast number of papers have been published in recent years on the relation between metabolic rate and body size of poikilotherms and reviews on this aspect are available in the works of Prosser and Brown (1961) and Newell (1970). Even though it is established that oxygen consumption is a power function of the body weight, the regression coefficients ('b') reported for poikilotherms vary much. Considering these variations, Hemmingsen (1960) suggested a common 'b' value of 0.75 for poikilotherms. In the present study, the oxygen uptake

Fig. 15. Relationship between oxygen uptake (µl/hr) and body weight (mg) of *A. nigrescens* in 33‰ S (b=0.9666).

Fig. 16. Relationship between metabolic rate (O_2 µl/g/hr) in 33‰ S (b-1 = -0.0334)

FIG. 15

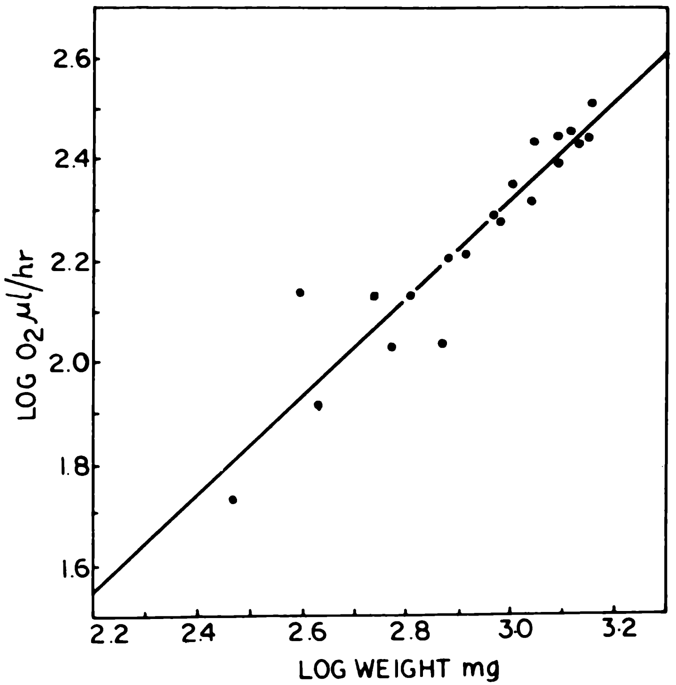

LOG O_2 µl/hr
2.6
2.4
2.2
2.0
1.8
1.6
2.2
2.4
2.6
2.8
3.0
3.2
LOG WEIGHT mg

FIG. 16

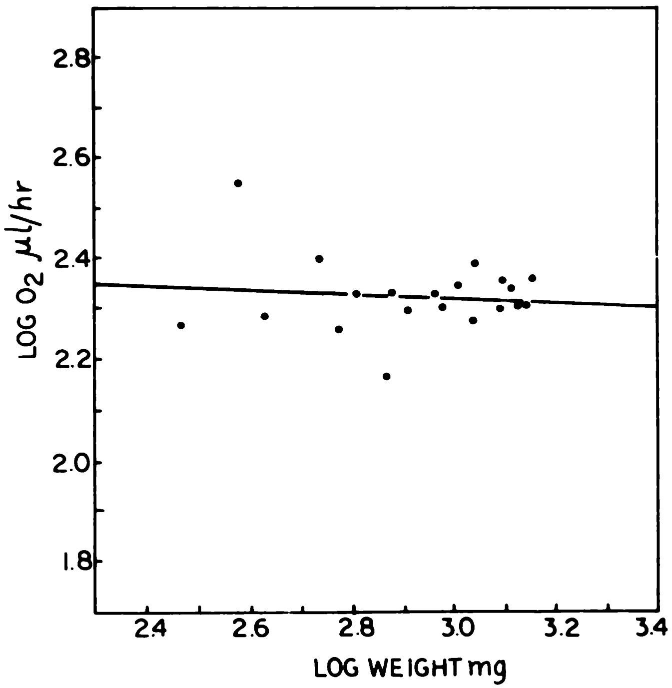

LOG O_2 µl/hr
2.8
2.6
2.4
2.2
2.0
1.8
2.4
2.6
2.8
3.0
3.2
3.4
LOG WEIGHT mg

Table 10 Oxygen uptake and metabolic rate of _A. nigrescens_ in 33‰ S.

Live Body weight mg	O_2 uptake µl/hr	Metabolic rate O_2 µl/g/hr
292.1	53.9	184.5
382.2	136.1	356.0
425.6	81.7	192.0
542.8	136.1	250.7
597.0	107.8	180.6
639.4	136.1	212.8
733.0	107.8	147.1
747.2	161.7	216.4
815.2	161.7	198.3
912.0	195.5	214.4
948.2	190.6	201.0
1015.2	224.6	221.3
1088.5	205.4	188.8
1102.3	272.3	247.1
1227.1	245.1	199.8
1234.2	280.4	227.3
1296.4	283.4	218.6
1338.1	272.3	203.5
1374.8	276.6	201.3
1412.8	326.8	231.5

Table 11 Statistical analysis of the data on the oxygen consumption of *A. nigrescens*. n = number of experiments, b = regression coefficient; r = correlation coefficient; S_b = standard error of b; t_b = student's t value; P = probability level.

n	b	b-1	r	S_b	t_b	P
20	0.9666	-0.0334	0.9335	0.0871	11.0975	0.005

Table 12 Statistically refined values of oxygen uptake and metabolic rate of *A. nigrescens* of standard weights (taken from figs. 1 and 2)

Live Body weight mg	O_2 uptake µl/hr	Metabolic rate O_2 µl/g/hr
250	55.0	220.2
500	107.5	215.1
750	159.2	212.1
1000	210.2	210.2
1250	260.7	208.5

of whole individuals showed increase with increasing body weight and the regression coefficient was found to be 0.9666. This value is not much different from 1.0 which means that the O_2 uptake in *A. nigrescens* is rather, directly proportional to body size. In this respect *A. nigrescens* differs from those reported by Zeuthen (1953) in which the metabolism is related to their surface area with the 'b' value of 0.67 (2/3 power of body weight). It also differs from those reported by Hemmingsen (1950, 1960) in which it is related not to the cell surface itself, but also to additional factors like internal convection, vascularisation, etc. with a 'b' value of 0.75 (3/4 power of body weight)

According to Zeuthen (1947, 1953) and Hemmingsen (1950, 1960) there are three phases in the relationship between body weight and respiration of poikilotherms. The first phase includes unicellular organisms with the value of 'b' as 0.75. The second phase is related to small metazoans weighing from 0.1 mg to 1.0 mg or to 40 mg with a direct body weight -O_2 uptake relationship having the 'b' value as 1.0. The third phase applies to other metazoans starting from 1.0-40 mg, the regression coefficient again being 0.75. The slope for *A. nigrescens* is very near to that for the second phase animals even though by weight it is to be included in the third group. However, Bertalanffy (1957) has reported that some insects have 'b' value as 1.0.

Comparatively very little is known about the metabolism-body weight relationship of actinians. Newell and Northcroft (1967) have found that the relationship of oxygen uptake to body weight of *Actinia equina* confirms that established by Zeuthen (1947). But at a series of temperatures ranging from 2.5 to 25°C, the 'b' values obtained by him varied from -0.12 to 0.95. A different trend in the slope is reported by Sassaman and Mangum (1970) in the actinians *Diadumene leucolena*, *Haliplanella luciae* and *Metridium senile* for which a common 'b' value of 0.652 was obtained. Mc Clendon (1917) also got the similar value for the scyphozoan *Cassiopea xamachana*. Unfortunately, references to the 'b' value of actinians are limited for comparisons. However, it can be seen that the present value obtained for *A. nigrescens* is different from the above values. The 'b' value of 0.83 reported by Sassaman and Mangum (1970) for *Metridium senile* at 22.5°C is somewhat near to the present value.

It has been established by Zeuthen (1947) that even though the total oxygen consumption of large animals exceeds that of small animals, the weight specific metabolism expressed as oxygen consumed per unit time of small animals is greater than that of large ones. But this negative exponential relationship between metabolic rate (O_2/g/hr) and body weight is not conspicuous in *A. nigrescens* since the regression coefficient of metabolic rate against body weight (b-1) is only -0.0334 as shown in Fig.16. A perusal of Table 12 shows that the metabolic rates for different body weights do not vary signifcantly from one another. This indicates that in *A. nigrescens* the metabolic rate (O_2/g/hr) is rather independent of weight. This observation is not in agreement with the findings of Newell and Northcroft (1967) and Sassaman and Mangum (1970) in some other actinians. But recently Griffiths (1977) reported that in *Actinia equina* no such correlation between size and oxygen consumption seems to exist. Shoup (1932) and Hyman (1940) have pointed out that the respiration of anemones is directly dependent on the degree of expansion of the animal. A direct relationship between total oxygen consumption and body size gives the value of 'b' as 1.0 and b-1 as 0. The values obtained for *A. nigrescens* in the present study generally agree with the above values.

The respiratory rates obtained for *A. nigrescens* vary from 220.2 to 208.5 µl/g wet wt/hr for animals weighing 250 to 1250 mg. Respiratory rates of anemones like *Anemonia sulcata*, *Adamsia rondelethi*, *Metridium senile*, *Anthopleura xanthogrammica*, *A. elegantissima* and *Tealia coriacea* reported by Trendelenburg (1909), Puetter (1911), Hyman (1940), Ramsay (1952), Nicol (1960) and Belcik (1968) are very low compared with the present values. But the rates reported by Newell and Northcorft (1967), Sassaman and Mangum (1970) and Griffiths (1977) for *Actinia equina*, *Diadumene leucolena*, *Haliplanella* *luciae* and *Metridium senile* are comparable with the present values.

While studying the oxygen consumption of *Actinia equina* Newell and Northcroft (1967) observed maximum and minimum levels of metabolism corresponding to the active and resting phases of activity. But in *A. nigrescens*, no such different levels of metabolism are noticed (Fig.15) and this finding agrees with the continuous activity characterising actinian behaviour as reported by Batham and Pantin (1950). Similar observations have been reported also by Sassaman and Mangum (1970) in the case of actinians like *Diadumene leucolena*, *Haliplanella luciae* and *Metridium senile*.

VIII

EFFECTS OF DESICCATION

Resistance to drying consequent to exposure to air is one of the most important factors which control the zonational distribution of intertidal organisms. But most of the early studies on the effect of desiccation have been related to temperature tolerance. Recent studies by Kensler (1967) and Foster (1971a, 1971b) on animals other than coelenterates have separately viewed the effect of temperature from those of desiccation. It is generally agreed that the intertidal sea anemones are subjected to severe desiccation due to exposure to air during low tide. But relatively little study has been made on them concerning this matter.

Stephenson (1935) reported that *Actinia equina* is able to withstand a considerable degree of desiccation since they are exposed to air for long periods during the ebb and flow of the tide. But beyond this statement he has not done any experimental work on this anemone to study in detail the effects of desiccation. Mashtaler (1938) demonstrated experimentally that certain actinians like *Cylista irudata* can withstand relatively long periods out of water without serious damage. Airapetyants *et al.* (1967) demonstrated the same in the case of *Metridium senile*. Recently Ottaway (1972) studied the effects of temperature, desiccation and light on the intertidal anemone *Actinia tenebrosa*. Except these works nothing much is known on the effects of desiccation in anemones. *A. nigrescens* being an intertidal anemone is subjected to varying degrees of desiccation during low tides, especially those living in the infralittoral fringe. So anemones collected from this area were subjected to drying in air in order to understand the rate of water loss and its effects on the animal.

Materials and methods

A. nigrescens collected from the infralittoral fringe were allowed to get attached on polythene sheets and kept in the laboratory for 10 days' acclimation in aquaria containing water of the same salinity as that of their habitat (33.5‰). Before the starting of the experiment, the polythene paper was cut around the base of the anemone, so that they could be weighed separately. Then they were wiped with paper towel for removing the water sticking to the body. The experiment was carried out in summer (March) when these anemones are subjected to the maximum degree of desiccation. The experiment was as follows: *A. nigrescens* (12 nos) were weighed separately and after the initial weighing they were allowed to dry in air, subjected to wind and sunlight as in the natural habitat. At every one hour interval the specimens were weighed and from this the water loss at each hour was calculated. Before each weighing the temperature and relative humidity were noticed. The temperature varied from 30 to 32°C and humidity 60 to 64% . After the experiment they were dried to constant weight at 60°C for 6 days. The difference between the initial weight and the final weight was taken as "total water" and from these data "% water loss" was calculated and plotted against time. The main criterion used to determine the vital state of animals during the process of drying was their sensitiveness to mechanical stimulation to which they reacted by defensive contraction (Airapetyants *et al.* 1968) and also by noticing the ectodermal surface which was no longer moist or wet (Ottaway 1972).

Results

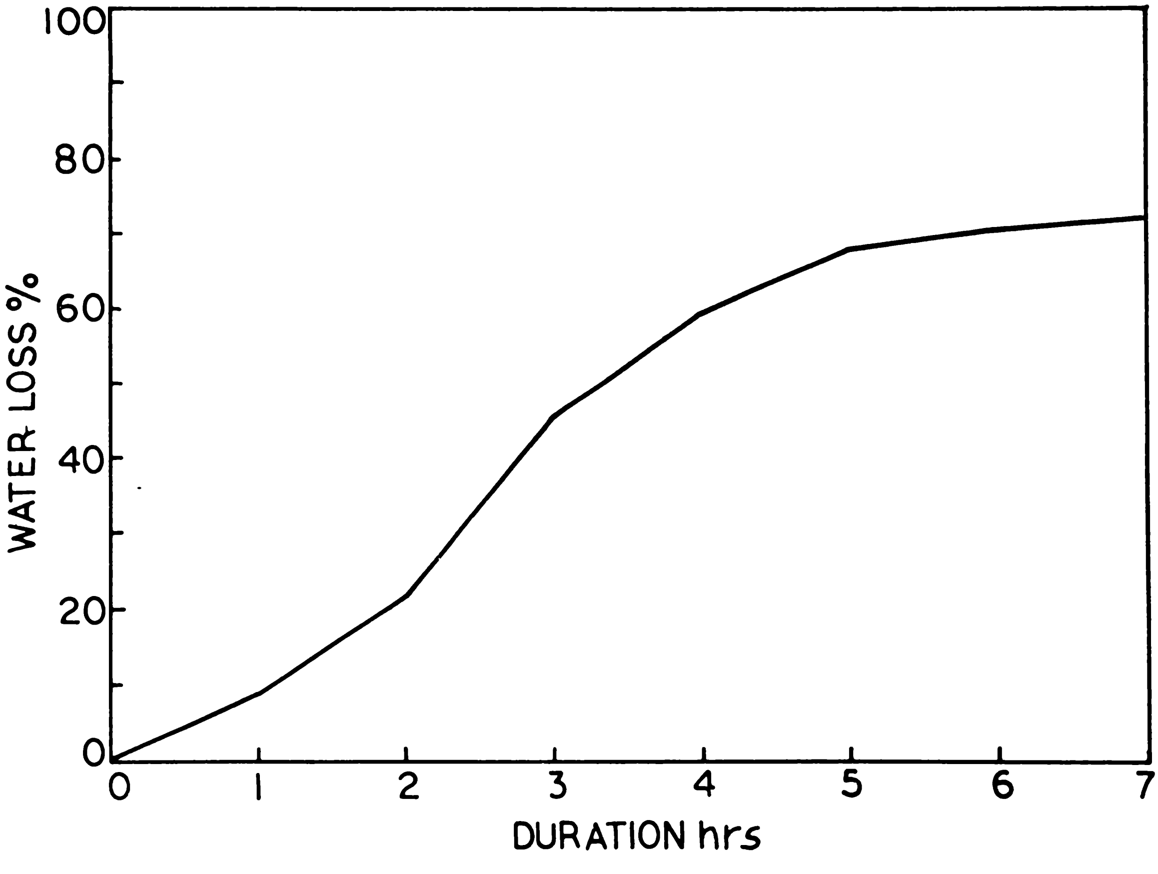
A.nigrescens when subjected to desiccating conditions retracts all the tentacles and contract the marginal sphincter, thereby allowing minimum surface area for evaporation. It was found that during exposure to air the anemone secreted the coelenteric fluid over the body surface. But the actual quantity of this discharge varied in different individuals and in different intervals. It was observed that after about three hours the anemone gets detached from the substratum. All the specimens died in the 7th hour of the experiment. The percentage of water loss in each hour is given in Table 13. 33.3% mortality occured in the 5th hour of the experiment when the average water loss reached 66.3% and 91.7% mortality occurred in the 6th hour when the average water loss reached 70.8% (Fig. 17).

Table 13. Per cent water loss on exposure to air

Live wt mg	Water loss % 1 hr	II hr	III hr	IV hr	V hr	VI hr	VII hr
308.7	7,12	12.30	17.16	10.36	3.86	1.29	0.28
388.0	9.94	13.06	21.02	13.06	8.23	3.12	1.42
414.6	7.20	11.47	17.08	13.88	4.27	2.40	0.63
457.5	12.03	12.63	20.02	11.68	5.24	2.38	0.95
468.1	10.95	14.44	32.16	15.16	9.55	3.97	1.63
573.2	5.28	10.78	26.89	11.54	6.24	2.68	1.13
596.1	7.79	12.06	20.21	9.27	5.75	2.22	1.11
634.1	8.91	14.15	31.81	16.95	9.08	3.67	1.57
698.1	8.71	13.31	27.89	14.89	8.55	4.91	1.26
910.4	8.98	11.93	23.38	15.38	8.86	3.20	0.98
957.3	9.69	12.09	28.49	17.59	10.05	3.71	1.31
1198.8	9.07	12.06	25.32	16.84	9.17	3.38	1.19

Fig. 17. Graph showing average water loss (%) of *A. nigrescens* during exposure to air.

FIG. 17



The results also show that the maximum degree of water loss was in the third hour of the experiment. The average water loss for cent percent survival was 59.26%.

Discussion

Desiccation is generally accepted as a primary factor controlling the vertical distribution of intertidal marine animals. Studies on the distribution of *A. nigrescens* showed that though they are most abundant at the sub-littoral fringe, they are also found in places where they are exposed to air for many hours during the ebb and flow of the tide. During this exposure the anemones are subjected to varying degrees of desiccation. Laboratory studies on desiccation show that though they are not adapted to remain exposed for several hours, they can undergo desiccation at least for a few hours. Actinians store some amount of water in the gastric cavity and this water can be ejected through the mouth and sometimes through the acrorhagi. According to Airapetyants *et al* (1968) the other factor contributing to this relatively prolonged resistance to unsuitable environmental conditions is the existance of a mechanism for retention of water initially, followed by "economical" excretion. During ~~this~~ the period of exposure *A. nigrescens* excreted coelenteric fluid through the stomodeum which kept the body surface moist. The actual quantity of this discharge varies for each individual. However it was found that there was a common pattern for this discharge.

The same pattern of behaviour was noticed by Airapetyants et al (1968) in the case of *Metridium senile*. Foster (1971) showed that desiccation takes place if intertidal animals are exposed even at normal temperatures. *A. nigrescens* is also subjected to desiccation if exposed at normal temperatures. But they prevent it to some extent by the secretion of the fluid over the body surface. Most of the water for evaporation come from the coelenteron but as desiccation proceeds there is loss of fluid from the epithelial cells and mesogloea as well and probably this causes the death of anemones (Ottaway 1973).

When *A. nigrescens* was subjected to desiccating conditions the anemone retracted all tentacles to the oral disc and contracted the marginal sphincter. This results in the reduction of the surface area for evaporation to the ~~max~~ minimum. In their natural habitat also when they are exposed the anemones remain contracted. It may also be noticed that many of the intertidal actinians are capable of attaching foreign bodies like molluscan shell pieces and sand grains, with the help of the verrucae. *A. nigrescens* is noted for this habit of attaching shell pieces and sand particles which is especially common in anemones that inhabit the exposed region. *A. nigrescens* generally prefers molluscan shell pieces for this attachment. The whole body surface is sometimes covered with these objects and due to this the body surface is not visible externally and this may help to prevent the evaporation of water through direct heat and wind, and thus protect them from desiccation to a certain extent. However, it may be noticed that on rearing in laboratory *A. nigrescens* throws away these foreign particles after 3 or 4 days. The acrorhagi which have got external openings may also help the anemone to keep the body surface wet by ejecting water through these organs by contraction of the column. Thus acrorhagi also function as protective organs against desiccation besides preventing rupture of the body wall on sudden contraction by ejecting out water through it.

Ottaway (1972) found that in *Actinia tenebrosa* if the rate of evaporation is high in severely desiccating conditions the animals show diminishing responses and the adults on vertical surfaces may get released from the substratum and fall to lower levels. In the case of *A. nigrescens* also it was found that as desiccation progressed the anemones detached the foothold from the surface of attachment. This happens usually after the fourth hour of exposure. Lewis (1964) also observed the same in the gastropods *Thais* and *Littorina* which relax the operculum and foot as desiccation progressed. This detachment of the foothold often enables them to fall into water which prevents the anemones from further drying up.

Ottaway (1972) observed that *A. tenebrosa* occupies positions regularly exposed to a desiccating environment during low tides, unlike other itertidal anemones. He also found that in *A. tenebrosa* the retention of juveniles within the coelenteric cavity is a common feature and this gives the juvenile *Actinia* a much better chance of setting on favourable habitat. But *A. nigrescens* though an intertidal evaporation and by storing some amount of water in the gastric cavity which is discharged out occasionally through the body openings. The secretion of the coelenteric fluid also helps them to prevent desiccation. The high amount of mucus present in the body also is a protective measure against desiccation.

Though _A. nigrescens_ in general is a hardy species which can survive high fluctuations in salinity and oxygen and also some amount of environmental pollution, as evidenced by the present studies, it is not capable of resisting desiccation for prolonged periods unlike intertidal gastropods or barnacles. This factor is reflected in its vertical distribution also (vide Chapter III).

species does not have the habit of retaining the juveniles in the coelenteric cavity like *A. tenebrosa*.

Davies (1969) found that in *Patella vulgata* 50% mortality occurred when there was 60-65% water loss and 100% mortality occurred when water loss reached 65%. These values agree in general with the results obtained for *A. nigrescens* also. Here 50% mortality occurred when the percentage of water loss reached 59.25 to 66.33 and 100% mortality occurred when percentage of water loss was 70.84. The percentage weight loss for *P. vulgata* for 100% survival was 60 and for *A. nigrescens* 59.25. These values are also comparable.

The studies on the tidal characteristics of the area show that the tides are of the semidiurnal type. *A. nigrescens* found at the sub-littoral fringe is not subjected to desiccation. But those that are attached above the sub-littoral fringe are not subjected to exposure for more than six hours because of the tide pattern. Studies on desiccation also show that by 5 or 6 hours of exposure to air more than 90% mortality occurs. Thus *A. nigrescens* is not well adapted for prolonged exposure to air. From the 5th hour onwards mortality occurred. But in the natural conditions this much duration of exposure is not lethal since the wave action and splashing of water help the anemone to keep the body surface wet. In their habitat they prevent desiccation by remaining in contracted state, by attaching foreign objects to the body surface to prevent direct

IX

ASEXUAL REPRODUCTION AND REGENERATION

Asexual reproduction and regeneration are closely allied processes and well pronounced in lowly organised animals. Where sexual methods of reproduction alone are not sufficient to keep up the race, the animals reproduce by asexual method also. Regeneration is a process mainly seen in lower groups of animals, by which the lost parts of the organism, sometimes the entire organism itself is produced. In coelenterates both asexual reproduction and regeneration are widespread. Asexual reproduction of several kinds are known to occur in actinians, and their ability to regenerate lost parts or the entire organism is remarkable. This has been worked out by different authors in some forms at least (Holdworth 1860, Broderick 1860, Child 1903, 1904, 1908, Carlgren 1904, 1907, 1909, 1925b, Stephenson 1929, Miyawaki 1951, Louis 1960, Ford 1964, Schmidt 1970 and Francis 1973). But hitherto no studies have been made on the asexual reproduction and regeneration of *A. nigrescens*.

During field collections several specimens of *A. nigrescens* which were in the process of asexual reproduction by fission could be observed by the nature of their highly extended pedal discs (Fig.18).

Fig. 18. Photograph of *A. nigrescens* showing a normal specimen and another one undergoing asexual reproduction.

FIG.18

When specimens were collected some of them showed a white vertical scar on the column wall indicating a recent division. Such anemones were found throughout the year, but their intensity varied in different months. Therefore detailed observations were made in the laboratory to study the process of asexual reproduction. During these studies they were also subjected to mutilation in order to understand their ability to regenerate.

Materials and Methods

Anemones of uniform size which showed no signs of recent division were used for the studies. They were kept in large glass beakers so that the process of asexual reproduction could be followed very easily. The water was aerated using an aerator. Salinity was maintained constant (33.5‰) and the temperature at 28.5 (±1°C). The anemones were fed with live planktonic organisms. In order to study the effect of salinity on asexual reproduction they were subjected to abrupt changes in salinity. The effect of food on asexual reproduction was also studied by subjecting the anemones to starvation. For studies on regeneration *A. nigrescens* were cut in different planes. The affect of abrupt changes in salinity on regeneration was also studied.

Observations

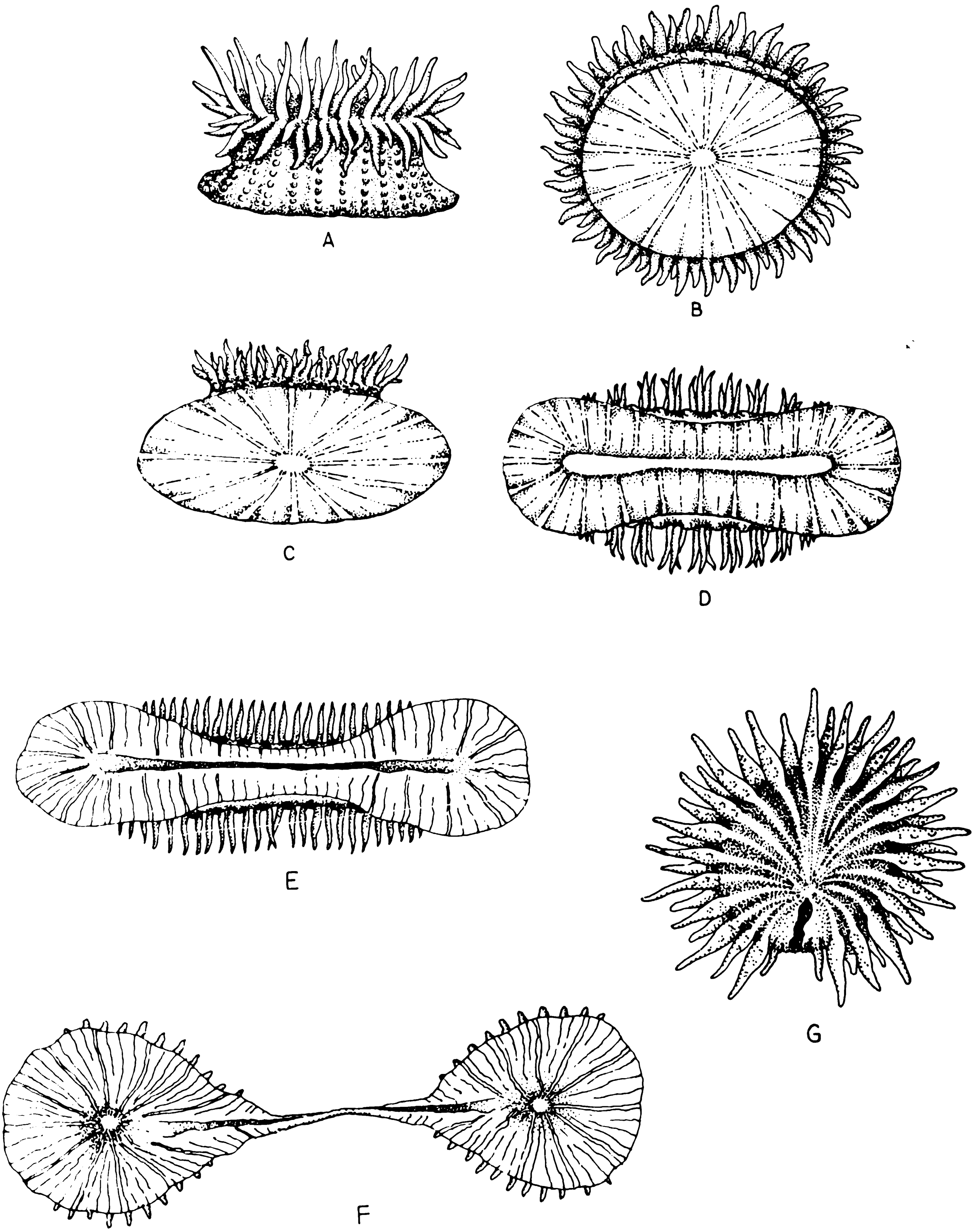
Asexual reproduction

In *Anthopleura nigrescens* asexual reproduction takes place by longitudinal fission. Fission always starts from the pedal disc region (posterior end). During the first stage of fission the circular pedal disc gets elongated and becomes oblong (Fig. 19B). In the next stage elongation is continued in a longitudinal manner (Fig. 19C). As a result of this the circular pedal disc reaches a length of 50 to 80 mm which was originally 15 to 20 mm in diameter. The time required for this transformation is 25 to 35 minutes. But sometimes this is more prolonged and may take 1 to 3 hours in certain cases. The anemone remains in this condition usually for one or two hours. In the next stage (Fig.19D) a small constriction develops in the middle region of the fully extended pedal disc. This results in the formation of two bases connected by a narrow strand of the pedal disc. Now the pedal disc extends further and the narrow connecting strand is pulled apart again, making it thinner and thinner (Fig.19E). During this process the column also gets extended to the two sides due to the pulling of the pedal disc and as a result the height of the column wall gets very much shortened to about half of its original size. Now fission starts from the pedal disc and is extended through the column wall and results in the cleaving of the body wall. The time taken for the division of the body wall, once fission has taken place in the pedal disc, is only 5 to 10 minutes.

Fig.19. Different stages of asexual reproduction in
A. nigrescens (Semi diagrammatic)

A. Adult side view
B. Initial stage of elongation of the pedal disc
C. Second Stage of elongation of the pedal disc
D. Third Stage of elongation of the pedal disc
E. Development of a constriction in the middle region of the fully elongated pedal disc.
F. Base, column wall and gullet ruptured (last stage of division)
G. Oral view of the divided specimen showing the area to be [illegible] regenerated .

FIG. 19



Though this division is quick in the column wall, near the sphincter region (at the oral disc) it is a slow process. For some time this region remains undivided and thus the dividing anemones are connected by this strong muscular region (fig. 19f). During this period it appears as if two anemones are connected by a muscle strand in the oral region. Afterwards this connecting strand gets thinner and thinner by elongation, while the already separated pedal discs finally break the connection due to its pulling to the two sides, resulting in the formation of two new individuals.

Though longitudinal division is a common feature in the natural habitat in _A_. _nigrescens_, it was found that in the laboratory conditions, if any disturbance is made at the time of the initial stages of fission, the process is retarded. Anemones that have extended the pedal discs fully during the process of asexual reproduction return to the original condition of their pedal disc when touched with hand or forced to change their place of attachment. However, they will start the process again when they are left to normal life. Stephenson (1929) has reported the occurrence of double anemones with two separate bodies united into a single column part way down and with only one base and also of anemones with two distinct bases and two bodies together. Carlgren as reported by Stephenson (1929) is of the opinion that these double anemones are the results of partial fission or the outcome of disturbance of some kind in the early stages of asexual reproduction. But no such partial fission has been noticed in *A. nigrescens*. Whenever the process of fission fails, the anemone resumes the original shape and the process is started again when conditions become normal or they remain in the original stage for a long period.

The process of fission if completed results in the formation of two individuals which are more or less identical. In *A. nigrescens* the gullet also takes part in the process of fission unlike in *Sagartia sphyrodeta* where the throat may not divide always (Stephenson 1929). Immediately after fission the broken column walls of the separated individual come closer and closer and begin to fuse together. The process of fission is completed within 18 to 48 hours. The fused region can be distinguished by a white scar on the column wall. After a few days this scar disappears and this portion also assumes the normal colour of the body wall.

Immediately after the separation of the two individuals the mouths of the anemones are not located in the centre as in typical anemones and also the area of fission at the oral disc region is devoid of tentacles (Fig.19G). As the fusion of the column wall is going on, the process of shifting of the mouth to the centre also takes place gradually. Meanwhile tentacles and acrorhagi also make their appearance as small buds where they were absent. These tentacles reach their normal size within a period of 6 to 8 days. Thus the whole process can be summarised as one of elongation of the pedal disc and rupture of the body wall including the gullet, fusion of the body wall and regeneration of the tentacles and acrorhagi.

After the fission is complete the resulting anemones are found keeping a distance of 4 to 8 cms. The distance depends upon the extended length of the pedal disc towards the two sides. However, in some cases it was noticed in the laboratory that after division, the divided anemones move away a little more and reach new places of attachment. But this movement is very seldom and usually they remain in the same place they reached when the fission was complete.

The size of the pedal discs after division are not exactly half the other size, but usually varies from 2 to 4 mm in diameter.

The time taken for fission is also found to vary in different cases. Under laboratory conditions in the case of *A. nigrescens* the time taken for the process of fission was 12 to 18 hours. In certain cases the process prolonged even for more than three days. But Stephenson (1929) has noticed in the case of *Sagartia sphyrodeta* the process which is one of rupture lasts for a matter of hours only.

Salinity and asexual reproduction

The influence of salinity on asexual reproduction in *A. nigrescens* is remarkable. While making studies on salinity tolerance of this anemone it was found that abrupt lowering of salinity induces fission. So detailed observations were made after subjecting them to abrupt changes in salinity. The influence of salinity changes on fission has been studied by Miyawaki (1951) on *Diadumene luciae*, by Louis (1960) on *Anemonia sulcata* and by Schmidt (1970) on *Anthopleura stellula*.

A. nigrescens kept in sea water (33.5‰ S) were subjected to lowered salinities. It was found that the amount of salinity variations has influence on fission. In salinities below 12‰ the process of fission did not take place. An abrupt lowering from 33.5‰ to 25‰ and then to 20‰ had immediate response. Many of these anemones subjected to such changes in salinity started fission within five to seven days, sometimes even earlier. But those subjected to salinities below 20‰ did not show any sign of fission. But these anemones when acclimated in lower salinities (25‰) and then subjected to further lowering abruptly, ~~they~~ started fission. But in any case below 12‰ S the anemones failed to undergo fission. It was also found that abrupt increase in salinity from 33.5‰ did not favour asexual reproduction. Schmidt (1970) who studied asexual reproduction in *Anthopleura stellula* reported that abrupt increase~~d~~ in salinity either destroys the fission inducing substances, or interferes with their stimulatory effects on transverse fission~~,~~ and thus the fission process is interrupted even in dividing anemones.

Field observations also reveal that a sudden lowering of salinity favours the fission process in *A. nigrescens*. Although throughout the year at least a few anemones engaged in the process of fission are observed, the maximum number of them are seen in May-June, a period when abrupt lowering of salinity is noticed in the habitat due to the onset of S.W. Monsoon (Table 1). But in July-August when the salinity is the minimum, dividing anemones are not common.

Autonomous longitudinal fission takes place in *A. nigrescens* only when the anemone reaches a certain size. But when an abrupt fall in salinity occurs suddenly it induces fission process within a period of 5 to 7 days. If these divided anemones are maintained in normal sea water, well fed and if again the salinity is reduced it was observed that the fission process again starts. However for this second division it takes more time, 10 to 15 days, and the resulting individuals are considerably smaller and in many cases only few of them could survive after fission. So further division could not be induced in these specimens.

Food and Fission

Food is another essential factor in fission process because it was observed in the laboratory that starving anemones even if subjected to abrupt lowering of salinity fail to show any sign of asexual reproduction. But when they are well fed they attain normal size and undergo asexual reproduction. It was also observed that the dividing anemone shows response to food only in the initial stages.

Regeneration

The power of regeneration is vested in almost all animals but to different degrees and is greater in animals of low organization. Actinians have remarkable power of regeneration and this aspect has been studied by several previous workers. Moszkowski (1907) studied this process in *Actinia equina* and *Actinoloba dianthus*, Child (1903a, 1903b) in *Sagartia luciae*, Stephenson (1935) in *Edwardsia callimorpha*, Abeloos (1955) in *Actinia equina*, Singer and Palmer (1969) in *Aiptasia diaphana* and Schmidt (1970) in *Anthopleura stellula*. These workers have shown that actinians are able to regenerate not only the lost parts but an entirely new organism. Usually in the above mentioned forms when the column is cut across, the aboral portion regenerates a new oral disc at any level of cut, but the oral piece fails to regenerate a new pedal disc. In the present study *A.*

nigrescens was subjected to regeneration studies by cutting them in different ways which are as follows-

a) Cutting away all the tentacles

All the tentacles and acrorhagi were removed. After 3 to 4 days small white protuberances appeared all round the margin of the oral disc (Fig.20). Within seven to nine days all of them reached the original size. The acrorhagi were regenerated only after the tentacular regeneration. The newly formed tentacles were all white in colour. But after 15 to 20 days they attained the original colour.

b) Cutting longitudinally into two equal parts

A. nigrescens was cut longitudinally into two parts and it was observed that both the pieces undergo regeneration. These pieces first get attached vertically in the normal manner and then the cut surfaces fuse together and become~~s~~ cylinderical (Fig.21). The time taken for this fusion is from 8 to 10 days. During the process, the pedal disc first assumes a rounded shape and the fusion proceeds upwards. The fused area is white in colour and semitransparent. When the fusion is complete a white longitudinal scar is left on the column wall. The fusion process very much resembles that of asexual reproduction by fission.

Fig. 20. Regeneration in *A. nigrescens* when all the tentacles are cut away

Fig. 21. Regeneration in *A. nigrescens* when cut longitudinally into two equal parts.

FIG. 20

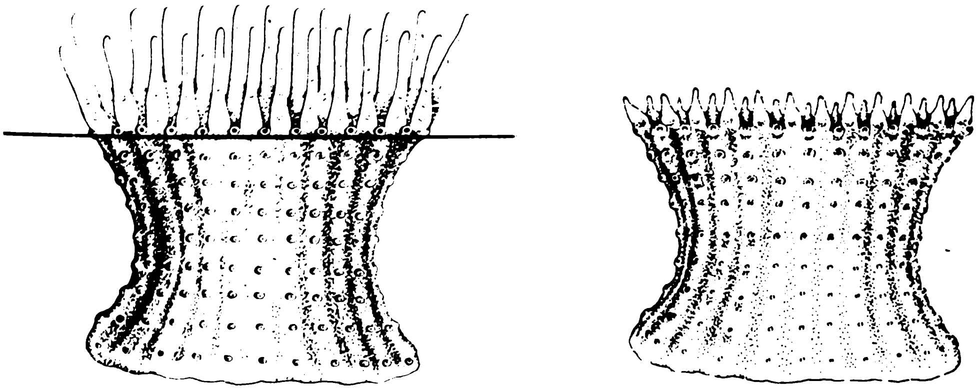
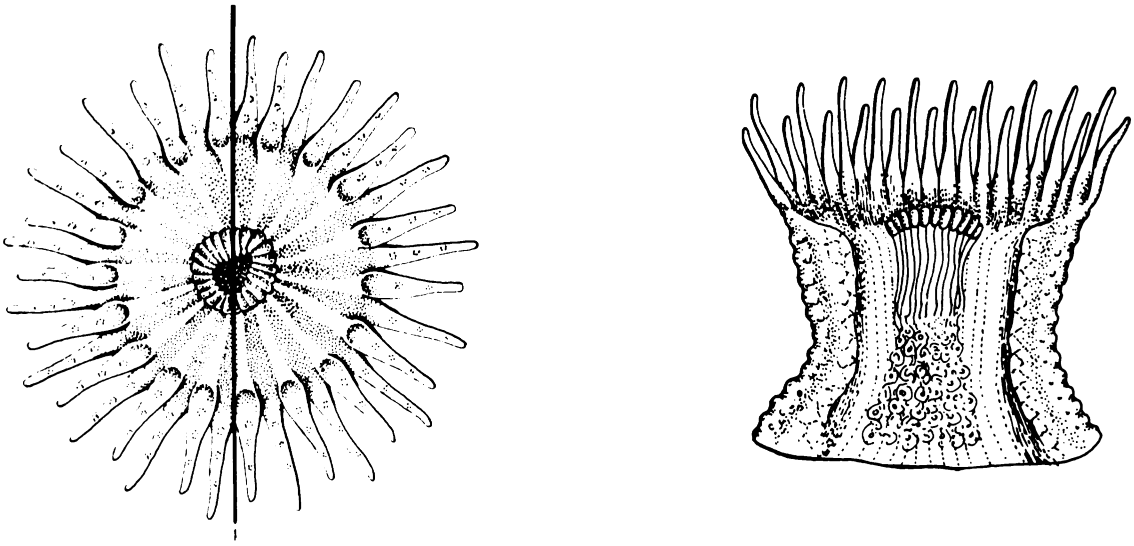
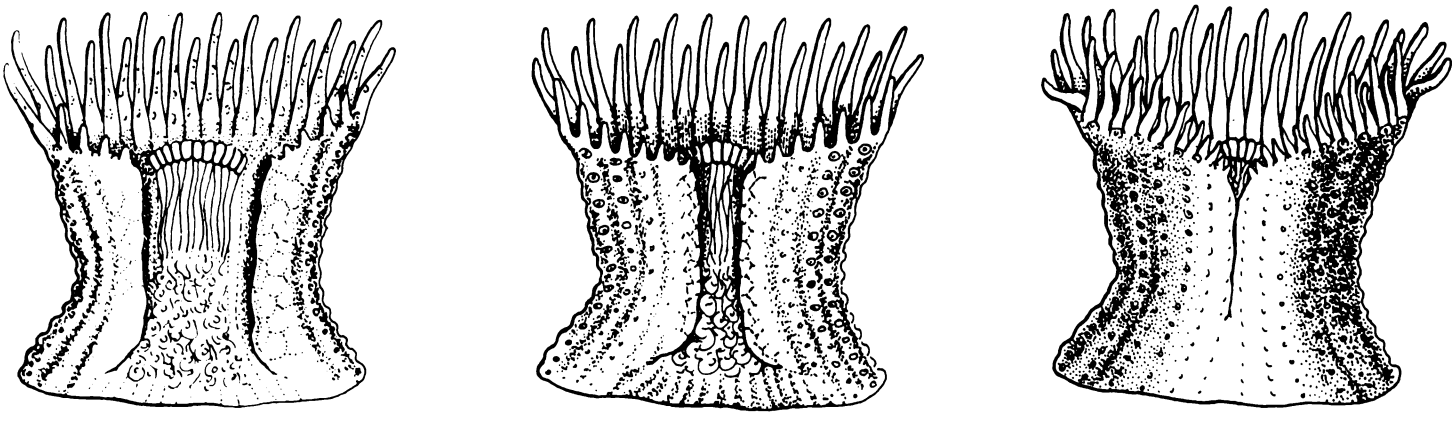


FIG. 21





c) Cutting longitudinally into four equal parts:

The specimens were cut longitudinally into equal quadrants. All the four pieces regenerated into complete individuals (Fig.22). These four pieces first get attached to the substratum and lie parallel to it for one or two days. Afterwards each piece slowly rises to the vertical position, and then begins the fusion process. The process is similar to that of a specimen longitudinally divided into two equal parts. The time taken for the completion of the fusion process is longer than that for the previous experiment. It took 12 to 15 days for the completion of regeneration. Fusion here also starts from the base.

d) Cutting longitudinally into eight equal parts

The specimens were cut longitudinally into eight equal parts (Fig.23). Here the results were different from those of the previous experiments. The pieces failed to regenerate into full anemones. But in a few cases it was observed that the regeneration process progressed nearing completion. But they could not survive. In other cases the cut pieces were alive for 2 or 3 days. They could get attached to the substratum in a parallel state but failed to come to the vertical position for attachment in few cases. Most of these specimens failed to regenerate and the very few which showed regeneration did not survive. Since the specimens cut into 8 pieces did not regenerate no further attempt was made to cut them longitudinally into more than 8 pieces.

Fig. 22. Regeneration in *A. nigrescens* when cut longitudinally into four equal parts.

Fig. 23. *A. nigrescens* cut longitudinally into eight equal parts

Fig. 24. Regeneration in *A. nigrescens* when the two sides were cut longitudinally. A-regeneration of the side piece; B-regeneration of the central piece.

FIG. 23

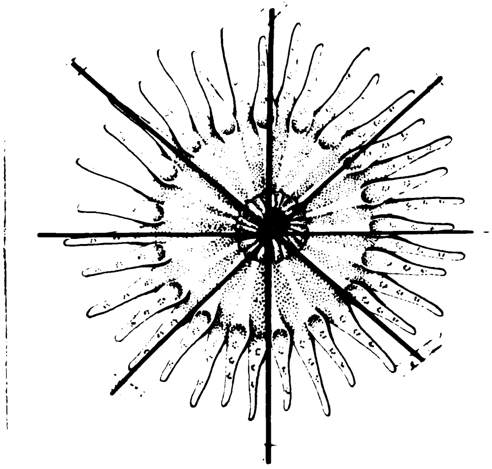


FIG 22

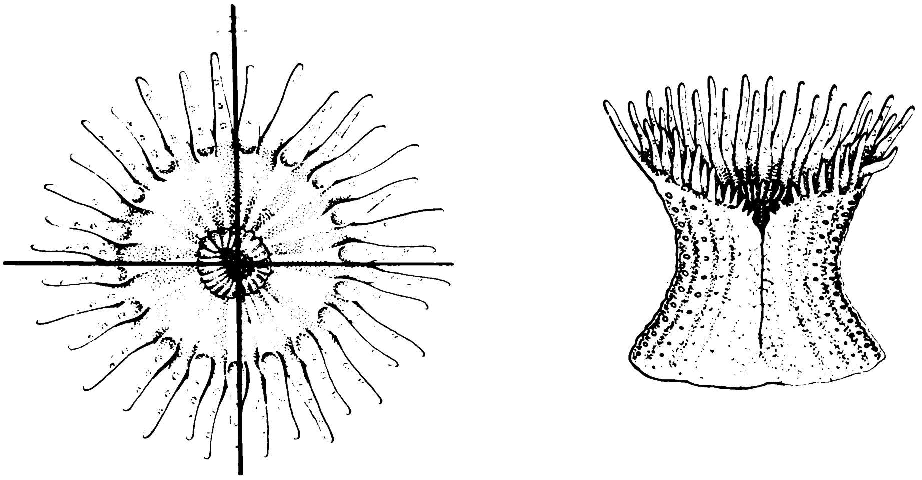
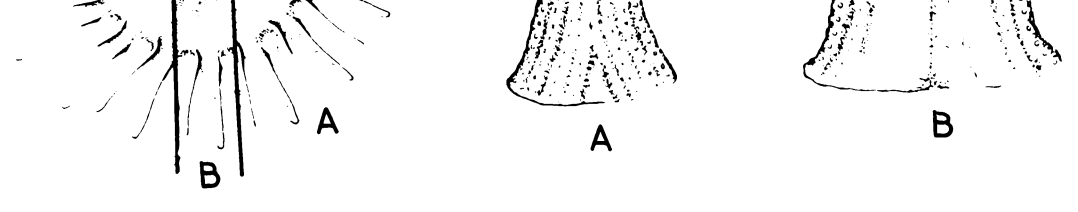


FIG. 24



e) Cutting the two sides longitudinally

The specimens were cut longitudinally into three parts, but not equally, cut being made only on the two sides as shown in (Fig.24). The side pieces were more or less of the same size and the central piece larger. But the side pieces as well as the central piece got regenerated into full individuals. The central piece took only lesser time than the side pieces, 5 to 7 days while the side pieces took 8 to 10 days. The process of regeneration in the case of the side pieces was of the same pattern as that of other cuttings previously described, but the central piece got regenerated from both sides. As a result two white scars showing the areas of fusion were left on the body wall of the anemone regenerated from the central piece, when the process was complete.

f) Cutting transversely into two equal parts

Anemones were cut transversely into two equal parts passing through the middle region (Fig.25). In this only one piece got regenerated. The anterior region failed to regenerate a base, whereas the posterior region fully regenerated a tentacular end. The anterior region was alive for a period of about 24 to 32 hours and the tentacles responded to stimul~~i~~i during this period, but slowly became inactive and got disintegrated. The posterior end was more active, got attached in the usual vertical manner and became fully extended, 6 to 10 hours after the separation. The rim of the cut surface became healed, got thickened and the process of regeneration started by growing upwards. As the process of growth of the column wall was going on over the cut surface, the formation of small tentacular buds also was observed. This usually happens after about 10 days since the cut is made. The tentacles grow in its cyclical arrangement as the oral disc, the mouth and the gullet are also regenerated. The tentacles continue their growth, but the acrorhagi develop only after 15 to 20 days, as small protrub/erances around the oral disc. The upper regenerated portion is transparent, thin and white and devoid of verrucae. Later it assumes the same colour of the posterior end and the verrucae also appear on the upper portion.

g) Cutting transversely into three equal parts

Here the anterior end (tentacular end) piece and the posterior end behaved as that of the animals which were cut transversely into two. The middle piece showed sign of life for a few hours and failed to regenerate neither the tentacular region nor the basal region. Only the lower end which alone contains the basilar tissues regenerates tentacular region (Fig.26).

h) Cutting longitudinally and then transversely into two

In this case also the anterior tentacular region failed to regenerate th/ough it was alive for about 18 hours. The two

Fig. 25. Regeneration in *A. nigrescens* when cut transversely into two equal parts.

Fig. 26. Regeneration in *A. nigrescens* when cut transversely into three equal parts.

Fig. 27. Regeneration in *A. nigrescens* when cut longitudinally and then transversely.

FIG. 25

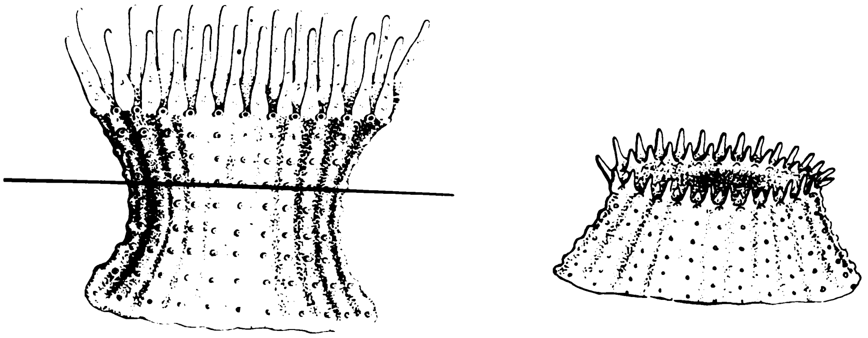


FIG. 26

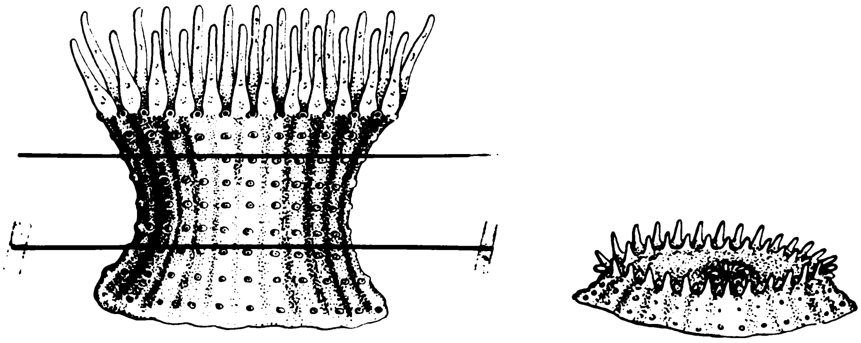
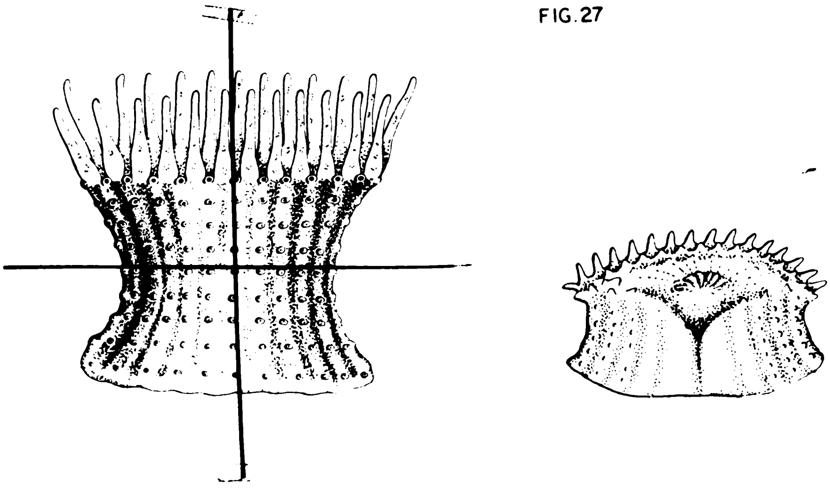


FIG. 27



posterior ends got regenerated and the details of development resemble that of the longitudinally cut pieces. But here fusion of the body wall also took place in the first stage of regeneration (Fig.27).

Discussion

Even though asexual reproduction of several kinds are known to occur in Coelenterata, in the group actinians it is mainly by the process of fission. Fission may be horizontal as in *Anthopleura stellula*, *Gonactinia prolifera* and *Aiptasia couchii* or longitudinal as in *Sagartia sphyrodeta*, *Diadumene luciae* etc. (Stephenson 1929, Schmidt, 1970). But in *Sagartia elegans* and *Diadumene cincta* asexual reproduction is of laceration by tearing whereas in *Sagartia lacerta* it is of laceration by constriction (Stephenson 1929). In the process of fission the animal divides into two only, but by laceration more than two individuals are produced, and the individuals thus produced are much smaller than those produced by fission. Thus a variety of asexual reproduction by fission is known in actinians. *A. nigrescens* reproduces by longitudinal fission which is a regular method of asexual reproduction.

Asexual reproduction by spontaneous longitudinal fission in actinians has seldom been studied. Recently Schmidt (1970) reported transverse fission in *Anthopleura stellula* and he is of the opinion that there is a horizontal gradient in the transverse fission, instead of an oral aboral gradient as suggested by Child (1903), Hazen (1903), Rasokowski (1907), Abeloos (1955) and Singer and Palmer (1969). Longitudinal division of *A. nigrescens* indicates that there is an oral-aboral gradient. But since the anemones which are cut transversely and longitudinally can regenerate, it seems that there is also a horizontal gradient besides the oral-aboral gradient and these two gradients work according to the cuts made. When longitudinal cut is made the oral-aboral gradient works and when transverse cut is made the horizontal gradient may be working.

In anemones fission is either by paratomy or by architomy. Schmidt (1970) found that in *A. stellula* fission is by architomy, unlike *Gonactinia prolifera* in which it is by transverse fission. In *A. nigrescens* fission is by architomy like *A. stellula* and it is followed by regeneration of parts that are wanting in the newly formed individual.

Stephenson (1929) has reported that the method of asexual reproduction is a specific character in actinians. Thus in *Sagartia sphyrodeta* and *Diadumene luciae* longitudinal fission alone prevails while in *Sagartia elegans* laceration by tearing alone prevails.

In *Sagartia lacerata* it is by constriction alone (Stephenson 1929). In the genus *Anthopleura* asexual reproduction as a whole is either by longitudinal fission or by transverse fission. In *A. elegantissima* (Ford 1964) and *A. thallia* (Stephenson 1929) it is by longitudinal fission whereas in *A. stellula* (Schmidt, 1970) it is by transverse fission. Thus in each case the method is specific and no species reproduces asexually by more than one method. In *A. nigrescens* it is by longitudinal fission only, though they are able to regenerate when cut both longitudinally and transversely.

Though the division is specific for each species, due to various kinds of asexual reproduction in actinians their morphology also gets considerably modified (Stephenson 1929). Many others who worked on this (Parker 1897, 1899; Carlgren 1904, 1909) also have pointed out such morphological variations. The most important morphological change that often takes place due to asexual reproduction is that the typical condition of actinian morphology, ie. the possession of two siphonoglyphs, two pairs of directives, six pairs of primary mesenteries and six primary tentacles is lost. *A. nigrescens* in which asexual reproduction is a regularly recurring feature, also deviates very often from the typical actinian morphology. Thus in all actinians where there is asexual reproduction, the adult symmetry is often more or less irregular whereas in forms which reproduce only sexually the symmetry is normally regular and hexamerous.

Ford (1964) who studied the asexual reproduction in *Anthopleura elegantissima* found that there is a unisexual aggregation of male and female as shown by their distribution on the rocks. According to him this may be due to the fact that the individuals are aggregated into large masses only as a result of growth and asexual reproduction within limitations of space. But no such unisexual aggregation of males and females were noticed in the case of *A. nigrescens*. It was found that they were usually randomly distributed. Also it was found from laboratory studies that *A. nigrescens* after division does not remain very close to form such unisexual aggregations. After asexual reproduction, they keep a distance of 4 to 8 cms away from each other due to the elongation of the pedal disc before rupture. Also it was observed in the laboratory that some of the anemones after division move away to a convenient place. Thus intermittent spaces are left out between the asexually reproduced ones and this gives space for other anemones to spread out and it may be the reason for the absence of such unisexual aggregation in *A. nigrescens*. Ford (1964) also has expressed the doubt that the unisexual aggregation in *A. elegantissima* may be due to some influence during larval settlement of males and females.

Francis (1973a, 1973b) who studied asexual reproduction in *A. elegantissima* pointed out that the production of a large number of individuals by asexual method leads to intraspecific competition.

He states that 'since these anemones live in a dependable environment, are long lived, have indeterminate growth, and reproduce sexually and asexually and since predation does not appear to be severe intraspecific competition for space is quite important'. Though such clonal aggregations are found in _A._ _nigrescens_ also intraspecific competition is not severe among them. This may be due to the fact that since _A._ _nigrescens_ is a new recruit to this area, there is enough space for them to spread out. Though intraspesific struggle among _A._ _nigrescens_ is not common, asexual reproduction by fission results in the formation of a large number of dense patches among them. But being able to reproduce sexually also they are able to spread out to new localities.

_A._ _nigrescens_ reproduces asexually throughout the year, but the time taken for successive divisions varies. Laboratory experiments proved that salinity change is a major factor ~~for~~ affecting division. Anemones which were kept in normal sea water, when subjected to abrupt lowering of salinity readily started reproduction by fission. Anemones which had undergone recent division again undergo fission when subjected to abrupt lowering of salinity. An abrupt increase in salinity had the opposite effect. So the time factor for successive division depends on other environmental factors also. Since there is not much variation in temperature at the habitat during an year it may be assumed that temperature has not much influence on the asexual reproduction of this anemone.

Studies on the relation between asexual reproduction and feeding showed that food is essential for fission since starved animals failed to reproduce. Torrey and Mery (1904) suggested that feeding could temporarily halt a fission already in progress. Minasian (1976) who examined the effect of feeding frequency on fission in *Haliplanella luciae* (Verrill) observed logarithmic rates of increase that varied with feeding level. Recent studies by Johnson and Shick (1977) on the Sea anemone *Haliplanella luciae* showed that the onset of more numerous fissions coincided with an increase on the amount of food consumed. In the present study also it is noticed that the availability of food is an important factor in the process of asexual reproduction.

Regeneration and asexual reproduction are closely related processes as by regeneration an entirely new organism can be produced. Child (1903b, 1909), Hazen (1902), Cotronei (1924) Abeloos (1955) and Singer and Palmer (1969) have distinctly demonstrated the occurrence of the oral-aboral gradient in the tentacle regeneration of actinians. But the recent study by Schmidt (1970) showed the presence of a horizontal gradient instead of the above gradient. But *A. nigrescens* can fully regenerate when cut either longitudinally or horizontally. The possession of an oral-aboral gradient is evidenced by regeneration process also besides the fission process. When the cut made is close to the oral disc the tentacles grow more quickly than when cut is made nearer to the pedal disc. This is also proved by oblique amputation made on anemones by Moszkowski (1907) Abeloos (1955) and Singer and Palmer (1969). The fact that *A. nigrescens* can regenerate when cut both longitudinally and transversely leads to the assumption that both oral-aboral and horizontal gradients are present in this species.

The experiments on regeneration of *A. nigrescens* proved that they are incapable of regenerating the pedal disc. Pedal disc regeneration has been the object of much discussion in anemones. Moszkowski (1907) is of the opinion that an inhibitory factor controls the formation of the pedal disc. But Carlgren (1909, 1926, 1929) and Abeloos (1955) state that the anemone's distal part completely lacks the capacity to regenerate a pedal disc. But Hazen (1903) and Landauer (1925, 1926) are of the opinion that the pedal disc could be formed in the distal region following artificial amputation. In *A. nigrescens* it was found that at least some portion of the pedal disc is essential for regeneration of a new anemone. This shows that the material for the process comes from the tissues of the pedal disc. However it has been reported that in *Anthopleura stellula* where the fission is transverse, the upper end regenerates a pedal disc (Schmidt 1970).

As to the stimulatory factor for regeneration in actinians nothing much is known. Since salinity has a stimulatory effect on fission it may have some influence on regeneration also. Cortonsi (1924) demonstrated that the oral disc regeneration in transversely cut cerianthids is completed more quickly in lower salinities than in higher salinities. Schmidt (1970) is of the opinion that abrupt increase in salinity prevents the pedal disc regeneration in *A. stellula* as in the case of asexual reproduction. In the case of *A. nigrescens* also when they were subjected to abrupt increase in salinity, the anemones failed to undergo regeneration. But in gradually lowered salinities the anemone's regeneration process proceeded normally. Thus it is evident that salinity plays an important role in the asexual reproduction and regeneration of *A. nigrescens*.

SUMMARY

Anthopleura nigrescens (Verrill) is an intertidal sea anemone belonging to the order Actiniaria of the class Anthozoa. It is abundant in Narackal, Malippuram and Kannamali areas near Cochin along the South West Coast of India. Apart from the paper by Verrill (1928) which pertains to the identification of the species, nothing is known about this anemone so far. The present work includes detailed studies on some important aspects of the morphology, bionomics and physiology of the species.

A. nigrescens being a sedentary anemone is found attached to hard substrata like rocks, molluscan shells etc. A survey for finding out the distribution of the species on the South West Coast of India extending from Cannanore to Cape Comorin, a distance of about 700 km showed that this species is found only in the Cochin area for about 28 km along the coast, where sea walls and groins have been constructed to prevent sea erosion.

Data on the hydrographical conditions of the area of investigation were collected once in a month for two years and the significant features are discussed. In general no significant variations were observed in the surface water temperature in a year. The minimum surface water temperature (28.0°C) was observed in July-August during the South West Monsoon and the maximum (32.4°C) in March-April in summer season. There is considerable lowering of salinity during the South West Monsoon (10.25‰). The salinity reaches its maximum (34‰) in March-April, during the summer season. The maximum salinity period correlates with the period of maximum temperature.

Seasonal changes in the dissolved oxygen are not much. The oxygen value of the surface water is high (5.70 ml/l) in June-July and low (3.46 ml/l) in February-March. Unusual lowering of the dissolved oxygen content (1.52 ml/l) is sometimes seen associated with the upwelling and mud bank formation during the South West Monsoon. The pH varies between 7.2 and 8.5. The maximum values are obtained during the summer season and the minimum during the monsoon season. Carbonate alkalinity values also agree with the seasonal changes in the pH.

The tides are of the semidiurnal type and the heights of low water spring level and high water spring level are +0.2m and 1.05 m respectively. Eventhough these are sandy beaches there is not much erosion on the beach due to the presence of the sea wall and the groins. The extent of the fore shore is greater during June-July when the wave action is greater and has some effect on the distribution of *A. nigrescens*. They are most abundant at the sub-littoral fringe. In thickly populated areas they are about 104/100 sq. cm. It was observed that the peculiar conditions prevailing in the area during the mud bank period are not harmful to this species. Crude oil from oil tankers washed ashore on several occasions was found not detrimental to this anemone while this caused mortality to most of the other intertidal organisms. It was thus found that the anemones are capable of surviving high fluctuations in salinity and depletion of dissolved oxygen in the field.

The formation of the mud bank and the beds of *Sabellaria* sp., *Mytilus* (*Perna*) sp. and *Modiolus* sp. have profound influence on the fauna of this region and hence these phenomena are discussed with special reference to their effects on *A. nigrescens*.

The distribution of *A. nigrescens* with reference to their vertical zonation of the intertidal region of the Cochin area is also discussed.

Analysis of the food contents in the gastric cavity of *A. nigrescens* showed that this anemone is carnivorous and essentially a plankton feeder. Crustaceans like copepods, isopods and amphipods formed the major food items. Laboratory experiments on the feeding habits showed that the tentacles form the chief food capturing organs of this species. Experiments on feeding reactions to different substances, the relation between feeding and salinity of the medium, effects of starvation and feeding during asexual reproduction were conducted and the results are discussed. It was found that the anemones readily intake substances like plankton, fish, crab meat, boiled eggs etc. It was observed in the laboratory that they can live without food for a considerable period. Laboratory studies revealed that the anemones neither intake food nor show any response to it in very low salinities. It was also found that they do not take food during the process of asexual reproduction.

Studies on the digestion of the species showed that the mesenterial filaments are the chief source of supply of the enzymes. Proteolytic and lipolytic activities were seen in the mesenterial filaments, stomodaeum and gastric fluid. Amylolytic activity was very little and is restricted to the mesenterial filaments.

Laboratory studies on the salinity tolerance showed that A. nigrescens is one of the most euryhaline species of actinians, tolerating low salinities upto 3‰ .

Experiments on oxygen consumption of the species acclimated in 33.0‰S showed that the uptake of oxygen per unit time increased with increasing body weight. The regression coefficient ('b') of oxygen uptake against body weight was found to be 0.9666 and the parameter 'a' as 0.2647. The regression value for the metabolic rate (O_2/g/hr) was obtained as -0.0334. The metabolic rate for different body weights did not vary significantly from one another.

Experiments on the effects of desiccation in *A. nigrescens* showed that exposure to air for 5-6 hours under laboratory conditions was lethal to this species. It was found that during exposure the anemone secretes coelenteric fluid over the body surface as a measure of preventing rapid desiccation. The average water loss upto 5-6 hrs of exposure was 66.3 to 70.8%. The average maximum water loss tolerated by this species was found to be 59.3%. The results also showed that the maximum degree of water loss occurred in the third hour of exposure.

The process of asexual reproduction and regeneration have been studied based on laboratory experiments supplemented by field data. It was found that in *A. nigrescens* asexual reproduction is by longitudinal fission which may be influenced by salinity changes. Abrupt lowering of salinity often induced longitudinal fission.

A. nigrescens can fully regenerate when cut either longitudinally or horizontally. But they are incapable of regenerating the pedal disc if at least some part of the same is not present. The longitudinal fission and regeneration of A. nigrescens indicate the possible presence of a horizontal gradient besides the oral-aboral gradient.

XI

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