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OCCURRENCE AND BIOLOGY OF THE SOLITARY ASCIDIAN *ASCIDIELLA ASPERSA* IN TAMIL NADU COASTAL WATERS*

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ABSTRACT

The ascidian which normally occurs in temperate waters, was found in the Madras area and studied by culturing it in the laboratory. The external and internal characteristics have been studied, as well as the development from the fertilized egg, the tadpole-larva and the young juvenile after its attachment to the substratum.

The tolerance to salinity, temperature and pH have been studied. Besides, the food and feeding preference of the attached adults have been noted. This species grows 8 to 10 mm per month on the average; it becomes sexually mature when it is 5-6 cm in total length, which is attained when the animal is approximately 6-7 months old. In the laboratory, the animals do not live for more than 15 months or after they reach 14 cm in overall length; an individual during its 1½ years of life-span releases eggs thrice.

INTRODUCTION

THE OCCURRENCE of the solitary ascidian *Ascidiella aspersa* (Müller, 1776) was noticed only after the adults of this exotic species developed from tadpole-larvae; introduced along with those of many other commoner ascidians in plankton collections taken outside Madras Harbour during April/May 1976, when put into aquaria in the laboratory. The identification of this interesting monascidian, hitherto known only from the Mediterranean boreal seas, and hence a new record to Indian waters, prompted a research project in the ensuing years through 1986, to study it in greater detail and to compare its bionomics with that already known from European waters.

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The results of these studies are presented briefly in this paper.

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Ascidiella aspersa (Müller, 1776)

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Systematic position

Ascidiella aspersa (Müller, 1776) is placed under the genus *Ascidiella* Roule 1884, as the nerve ganglion is located immediately behind the dorsal tubercle and the internal longitudinal vessels are smooth with the branchial papillae reduced/absent.

The genus is placed under the Family Ascidiidae Herdman 1880, along with other genera with firm test, and which show 6-8 branchial siphon-lobes and 8 atrial lobes; the alimentary canal is ideally situated to the *left* of the branchial sac; the latter has well developed, smooth/papillate, internal longitudinal vessels; the stigmata are straight; the heart is a straight tube; the dorsal lamina is a continuous ribbed membrane, sometimes with a toothed margin; the reproductive organs are *within* the loop of the gut; the oviduct and sperm-duct follow the intestine all the way to open by the side of the anus; eggs are small (0.15-0.18 mm), and numerous; all ascidians are oviparous-producing small, typical tadpole-larvae.

The family is placed under the Suborder Phlebobranchiata Lahille 1885, along with similar families whose bodies are not normally divisible into thorax and abdomen; the branchial sac typically shows internal longitudinal vessels/incipient vessels forming bifurcating branchial papillae, but never with folds; gonad in gut-loop or laterally displaced relative to branchial sac.

The Suborder, in turn is included under the Order Enterogona (= Hemigona Perrier, 1898) along with similar Suborders whose oviduct and sperm-duct follow the last part of the intestine (rectum) to open beside the anal opening; the neural gland is normally situated ventral to the neural ganglion; the tentacles are simple.

Description

External characters: Adult rather elongated, ovate or even elliptical in shape (Pl. I A); attached to substratum by its base, and when it settles as a metamorphosing tadpole larva on the glass wall of aquarium, firm cartilagenous test (which is covered with fine papillae) spreads out on glassy substratum as a sheet of translucent tissue with blood vascular system very clearly visible.

Specimens collected from sea, usually dark, dirty-grey in colour and larger individuals invariably encrusted with a rich epifauna consisting primarily of a variety of bryozoans, hydroids, small gastropods, lamellibranchs and juveniles of various other ascidians, including those of same species.

The pure aquarium tank culture specimens, on the other hand, are milky-white, translucent with most of internal organ systems perfectly traceable.

Branchial siphon terminal, with atrial one about one-third of body-length towards basal extremity on dorsal aspect of animal; when fully expanded in life, individual measures upto 12 cm in total length, *i.e.*, from tips of oral siphon lobes to basal plate which anchors metamorphosed animal for life to substratum.

Internal characters: Adult animal when opened, shows tentacles wide apart, flattened basally and upto 40 in number. Dorsal tubercle has a C-shaped slit with both horns rolled inwards (Fig. 3) internal longitudinal bars numbering upto 80 on each side and more numerous than tentacles in same individual; no secondary papillae at junctions of transverse and inner longitudinal vessels nor any intermediate papillae; in very young individuals inner longitudinal vessels occur as bifid papillae (Fig. 4). Digestive apparatus occupies lower half or more of body cavity; stomach proper lies at base of branchial sac; digestive tube (intestinal part) has a long loop which rises sharply to atrium which is situated towards dorsal aspect of animal. Gonads show the ovary lying on *left side* within loop of intestine, while testicular part spreads as large caecae over both sides of intestinal loop. Renal vesicles large, situated chiefly over *left side* of intestine and part of adjacent mantle.

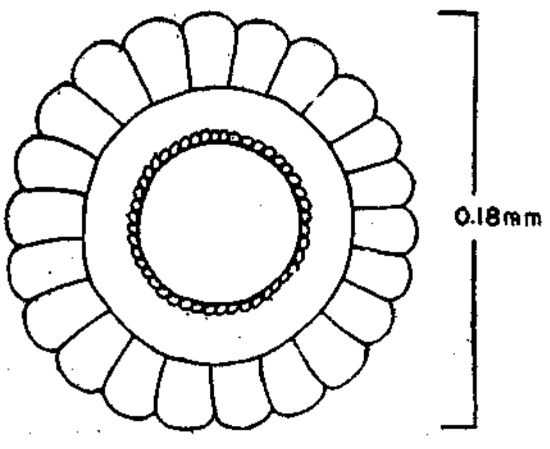

FIG. 1. Floating egg of *Ascidiella aspersa*.

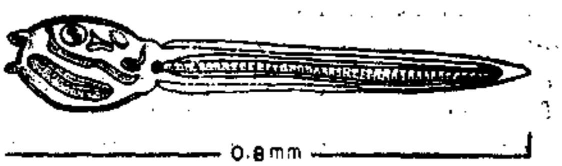

FIG. 2. Tadpole-larva of *Ascidiella*.

Distribution: This species has a known distribution from the Crimean Peninsula in the Black Sea, Marmora Sea, throughout the Mediterranean, Adriatic and Aegean Seas, and has colonised both sides of the English Channel, west coast of Scotland, Irish Sea, Shetland Isle, Heligoland (German Bight), Gullmars Fjord, off the Skagerraks. It would therefore, appear to have a chiefly mediterranean-boreal type of distribution. Millar (1970) mentions that it prefers warm sheltered sea-areas. The present reporting is the first for this species in Indian waters, specifically from Tamil Nadu coastal waters.

Development: A number of ascidian eggs and tadpole-larvae were taken in regular plankton tow-nettings outside Madras Harbour, over shallow depths (5-50 metres) during April/May 1976; this prompted very careful examination of later samples, during March-May and again during October-December in subsequent years, through 1986.

The plankters were brought living to the Laboratory and introduced into well-aerated aquaria (150 l cap. each), fed with plentiful phytoplankton and smaller zooplankton and allowed to develop to adulthood. Only those identified as *Ascidiella* were retained in the tanks and the other commoner species were progressively weeded out. The adults of this species were then allowed to breed and their eggs and tadpole-larvae studied in detail. Growth-rate to adulthood, senescence and death were observed. The animals were subjected to various physiological experiments and behavioural characteristics and responses to different stimuli noted. Data were also recorded on feeding, locomotion (for the tadpole-larvae), breeding, general development, etc.

Shore-collecting, specially among the large rocks and 'Triads' making up the breakwaters of the Harbour, yielded colonies of this medium-sized ascidian; trawled material was obtained of this species on small and medium rocks, shells, etc., brought up from the sea-floor in depths between 5 and 50 m offshore.

While a number of Tunicates (particularly prominent were *Herdmania pallida* Lahille, *H. ennurensis* Das, *Styela areolata* Heller, *Ecteinascidia thurstoni* Herdman and various species of the genera *Polyclinum* and *Perophora*) developed to adulthood from the eggs and tadpole-larvae of these species introduced into the aquaria. All (except those adults belonging to this species) were weeded out carefully, till the tanks were stocked with a pure culture of *A. aspersa*. This culture was allowed to develop further and was provided with plenty of fresh phytoplankton and small zooplankton for further studies.

Egg: Fertilized egg very characteristic; 0.16 to 0.18 mm in diameter, with large outer follicular cells and highly vacuolated protoplasm; outer follicular cells strongly adherent to a thin chorion which encloses a very large perivitelline space; a sphere of small inner 'test' cells enclose egg proper towards centre of perivitelline space (Fig. 1). Egg lighter than ambient seawater; floats as plankton. 'Test' cells less than ½ the diameter of outer follicular ones.

Tadpole-larva: Measures between 0.8 and 0.9 mm; body with two dorso-lateral and one ventro-medial papillae which are very small and used later to attach the metamorphosing larva to sea-bed, etc.; body contains a prominent cerebral vesicle consisting of an otolith and an ocellus with three tiny lenses. A pair of peribranchial invaginations into body from dorsal ectoderm and a clearly visible endostyle, in a semi-horizontal position, lies within

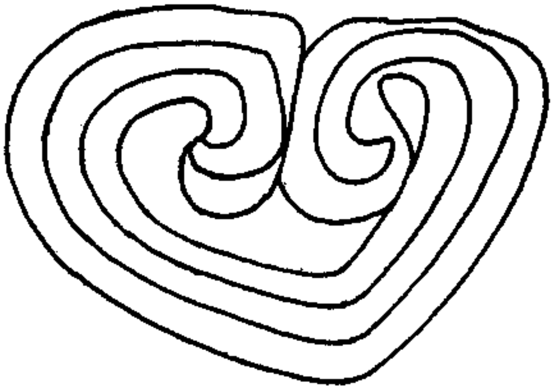

FIG. 3. Dorsal tubercle of *Ascidiella*.

body proper; attached to body is a long tail made up of an axis of 42 notochordal vacuolated cells, surrounded by large tail-muscle cells (about 20 cells on each side) arranged in three rows; these tail-cells give the larva a tremendous turn of speed (10 mm/sec.) to drive it through water. Figure 2 illustrates main features of tadpole-larva. A thin 'test' membrane covers the body and tail.

Settling and metamorphosing: At 25°C, the fertilized eggs, which freely float in the water, develop and hatch a tadpole-larva each; the time-lag between fertilization and hatching of the tadpole-larvae is approximately 22 hours on an average; the tadpole-larva remains an active member of the plankton for approximately 14 hours, after which period it settles on a suitable substratum. It was found that tadpole-larvae had settled in large numbers on air-stones and covered each stone with a thriving colony of young individuals (Pl. I B). Perhaps the reason for this may be (*i*) tadpole-larvae prefer a rough surface to a smooth one for settlement and (*ii*) water currents in tank's lower part lead to air-stone, carry tadpole-larvae there, and hence this might result in colony formation on stone. The larva settles such that its three small, body-papillae are opposed to the settling surface.

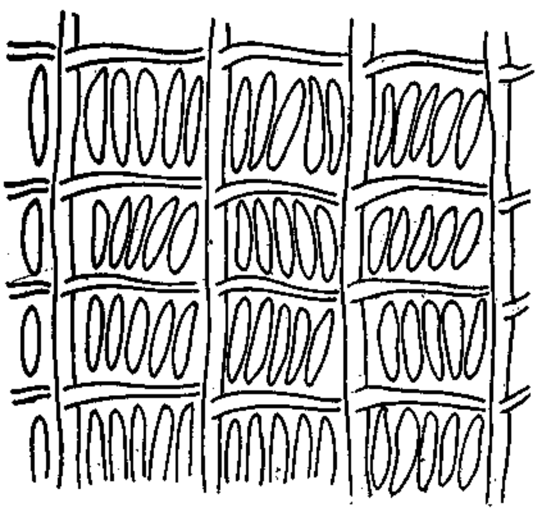

FIG. 4. Branchial sac of *Ascidiella*.

The larva's papillae flatten out into a thick epidermal plate made up of many layers of 'test' cells (the basal plate), which firmly attaches the settling larval body to the surface.

A series of quick developments now occur. The body of the larva undergoes a twisting turn, such that its tail-portion is rotated through approximately 30° relative to its original vertical position/basal plate; the tail-portion is mostly completely resorbed during the subsequent developments in the body proper, but sometimes a sizeable remnant of the tail is thrown off: the significance of this action (particularly when it represents a food reserve for growth for the juvenile, which has yet to establish a feeding action with the outside environment) is not understandable. Inside the transparent juvenile the branchial sac appears with its definitive stigmata, along with a straight tube-like beating heart and attendant circulatory system; the embryonic endostyle orients itself below the brancial sac; simultaneously, the definitive stomach appears below the branchial sac along with its tubular intestine; two widely separated peribranchial siphons also appear on the dorsal aspect of the juvenile opposite to the ventrally oriented endostyle; the definitive oral and atrial siphons establish communication with the external water-mass, and the oral siphon draws in the first stream of food-bearing seawater into the body's branchial sac under the influence of the rapidly beating branchial sac ciliated surfaces and the endostylar systems; the food taken in consists of very small phytoplankters and protozoans.

All these changes occur within 24 hours of settlement of the tadpole-larva. At the moment of starting its first feed of phytoplankters, etc., the juvenile measures just 1.2 cm; it now starts rapidly growing and laying down its other body-systems, specially the renal glands and gonads in the loop of the gut.

Locomotion: The adult animal, due to its very nature of being attached or anchored for life to the substratum, once the tadpole larva settles and metamorphoses, is precluded from displaying any kind of locomotion; however, when forcibly detached from the substratum, the animal is capable of re-attachment to the substratum by putting out new test-processes and pulling itself into a more or less vertical posture. In this connection, it may be pointed out that this species normally occurs in fairly sheltered habitats — among rocks, shells, etc., and taking into account its very strong cartilagenous test—processes by which it anchors itself, it would appear very unlikely to easily get detached from the substratum, even when it settles as a 'fouler', among clusters of other well-known foulers, on the bottoms of fast moving ships.

The tadpole-larva on the other hand, is highly mobile once it hatches from the floating egg; and is capable of moving, by lashing movements of its muscular tail, at an average speed of 10 mm/second; the tadpole has a body which measures approximately one-fourth the total length of the larva, with the tail making up the balance; the weight of the body and its size would appear to make it hydrodynamically stable, at least sufficient to give direction to the propulsive impetus of the lashing tail. After actively swimming for a period averaging 14 hours, the larva settles preferably on a rough substratum, failing which it will settle even on a smooth surface, *e.g.* the glass walls or bottom of an aquarium.

fingers; the maximum and swiftest reaction is achieved if the oral lobes are so touched; the animal does not tire of repeated touches every time it attains full expansion.

Using acids/alkalis gave similar contractile reactions on the part of the experimental animals. Protracted use of these strong chemicals and Menthol, resulted in death of the experimental animal.

Reaction to disturbances in the prevailing water-currents in the aquarium, in the immediate vicinity of the animal, was equally swift and the animal remained contracted till the normal currents re-established themselves.

Reaction to illumination: The animals are very sensitive to sudden changes in illuminaton in their immediate vicinity: Strong light from a torch if suddenly shown on them results in the affected individuals quickly contracting. It is well-known that the siphonal lobes are well provided with ocelli and 'pigment-spots' which are light sensors. If the siphonal lobes excised, the animals operated on are rendered unable to react to light-changes.

TABLE 1. *Tolerance of* Ascidiella aspersa *to different salinity (‰) in the environment*

Salinity (‰)	50% Mortality level	Well-being level	Remarks
24 to 27	All animals died		The animals died immediately
28	All animals survived		Some moribund; majority thriving
29 to 35	All animals survived	All well	All specimens in thriving condition
36	Few animals died		Some moribund; 45% died
37	All animals died		Died within 6 hours
38 to 40	All animals died		Died immediately

Reaction to touch/disturbance/irritation: The demersally settled animal contracts by approximately one-third of its fully expanded normal length (tip of oral siphon to basal plate), when touched with a glass rod or

The tadpole larvae are also very sensitive to strong light, moving away with great rapidity into the shadows; the tadpole larvae have ocelli in the body which enable them to perceive changes in illumination.

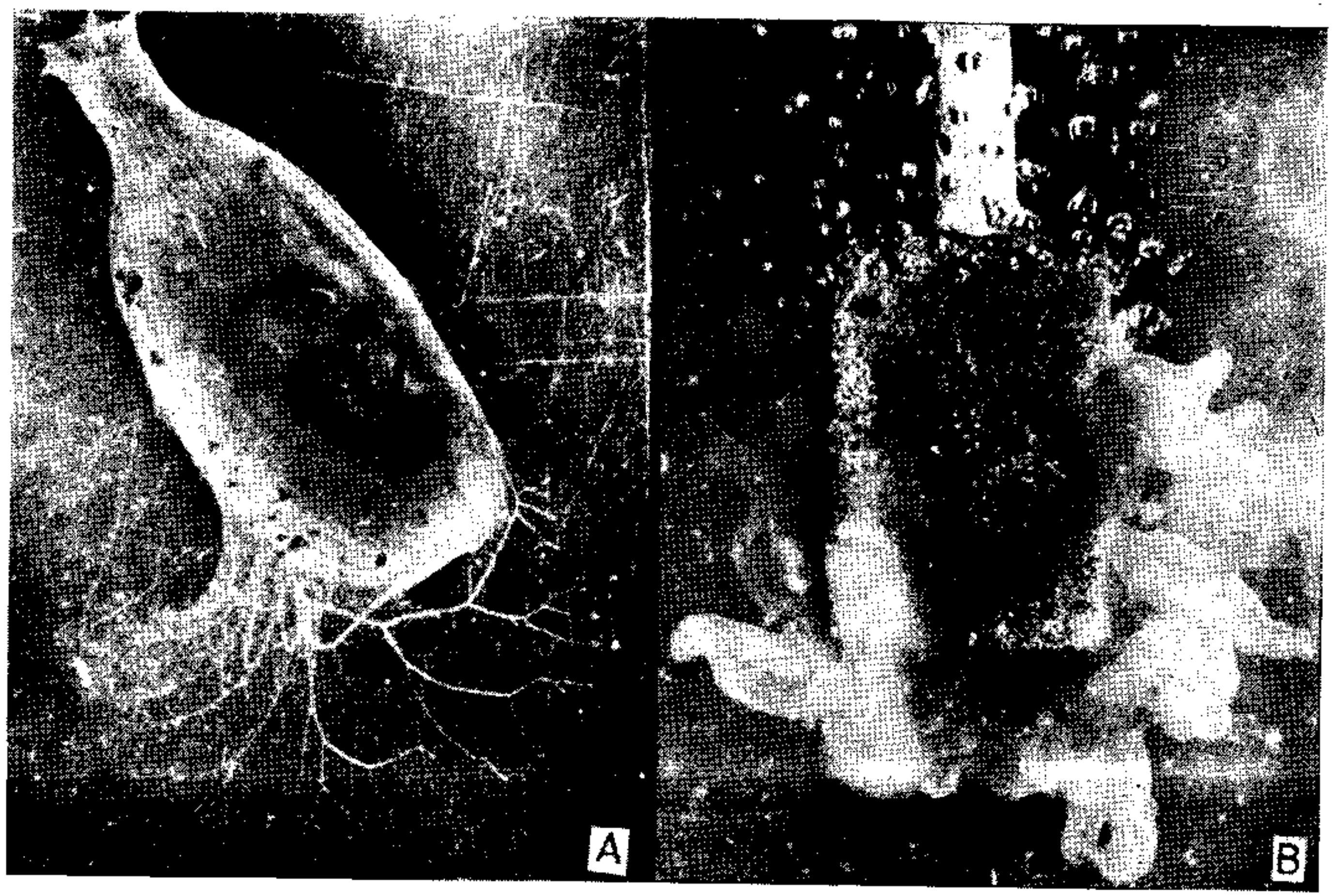

PLATE I A. *Ascidiella aspersa* (10 cm) and B. juvenile *Ascidiella* (1.5 mm) settled and metamorphosed on the aerator pumice-stone of the aquarium.

Reaction to salinity : The animals in the aquarium were found to thrive in seawater of a salinity ranging from 29 ‰ to 35 ‰ (Table 1); above and below these limits, 50% mortality occurred; it would therefore appear that this species is primarily a marine form in our waters; we have been unable to collect it from rock-pools in the inter-tidal zone, despite the fact that other species do occur in the same rock-pools; in European waters, however, this species has been reported from brackish waters in addition to a purely marine habitat.

Reactions to temperature : The animals thrive in ambient temperatures between 15°C and 26°C, becoming moribund above and below these limits (Table 2).

TABLE 2. *Tolerance of* Ascidiella aspersa *to different temperature (°C) in the environment*

Temperature (°C)	50% Mortality level	Well-being level	Remarks
13	All specimen died		The animals died immediately
14	70% specimens died		30% animals moribund; died
15	70% specimens survived		30% animals moribund; died
16—26	All specimens survived	All well	All animals thrived well
27	60% specimens died		60% animals died; others moribund
28 and 29	All specimens died		Animals died immediately

Reactions to pH: The animals are active in medium of pH values between 7.8 and 8.5 (Table 3), becoming rapidly moribund if these limits are crossed.

Food : The tadpole-larvae do not feed, in the usually accepted meaning of the term, although it is reasonable to expect that they must get sustenance by osmosis, not only of oxygen, but of other molecular-level inputs to maintain their extreme range of activity; this would be a fruitful field of research in the future.

The settled or metamorphosed individuals, on the other hand, showed active feeding activity once the oral and atrial siphons established communication with the environment— a steady stream of seawater bearing oxygen and food (Phyto- and small zooplankters) enters the body and after circulation in the branchial sac, and denudation of the food-content by the mucus-ciliary streams of the stigmata and endostylar-system, the water-current leaves *via* the atrial siphon, carrying away the waste-products of the animal. The atrial siphon pulses a strong jet from the atrial opening so that the waste-water is carried far from the animal: the jet of an individual 12 cms long can be distinctly be felt 120 cms away. The oral siphon is very flexible in young individuals (Pl. I A) and muscular contractions bend it in any direction at will; a gentle stream of water-food is drawn in from the immediate vicinity of the oral siphon and passed into the body; the atrial siphon is much shorter than the oral one, and expels a jet of waste-water away from the animal's body.

The chief diet of this species are small phyto-plankton and zooplankton. An individual measuring 8 cm total length was found to reject medusae, ctenophores and other plankton measuring over 2 mm in length/width/diameter; irrespective of the size of the ingesting individual, organisms of the plankton measuring less than 1.0 mm were preferred; this preference was displayed in no uncertain way: the animal's oral lobes play an active role in preventing large plankters from entering the body by simply bending over and blocking the orifice of the siphon and allowing only the very small plankets to enter with the water-stream. The eye-spot or pigment-spot system at the base of the oral siphonal lobes were found to be responsible for this selective activity : if they were excised or blocked out carefully, the oral lobes no longer prevented larger organisms from entering the siphon ; and this led to choking of the siphon, eventual starvation and death of the operated individual.

Growth, maturity and longivity

The increment of total length of the growing juvenile is now approximately 8 to 10 mm per month of growth ; it attains sexual maturity when it reaches a total length of approximately 6 or 7 cm when it is about 7-8 months old.

The animals keep growing till they are about 12 cm in total length ; around this size, many of the individuals appear to be undergoing a process of senescence and most of them die and disintegrate.

TABLE 3. *Tolerance of* Ascidiella aspersa *to changes in pH in the environment*

pH	50% Mortality level	Well-being level	Remarks
7.5	All animals died		The animals died immediately
7.6	All animals died		The animals died immediately
7.7	80% animals died		20% animals moribund ; later died
7.8	All animals survived	All well	All animals survived, thrived
7.9 to 8.5	All animals survived	All well	All animals thrived well
8.6	70% animals died		30% moribund ; later died
8.7 and 8.8	All animals died		All animals died immediately

Introduction of small quantities of Carbon-Black into the food stream *via* the oral siphon, showed that it took about 5 hours for the food to pass through the alimentary system of an animal of 13 cm total length, before the remains were voided *via* the atrial siphon.

Breeding : When the metamorphosed juvenile ascidian attains a total length between 6.0 and 7.0 cm, it appears to become sexually mature and releases both eggs and sperm *via* the atrial siphon into the surrounding sea-water ; the individual is a hermaphrodite ; it could not be ascertained whether self-fertilization occurs, or whether eggs from one individual are fertilized necessarily by sperm drawn from another.

There appeared to be two main periods of the year for such release of sexual products : March-May and October-December.

In the laboratory's aquaria we have not succeeded in keeping this species alive for more than 15 months, when many of them attained 14 cm overall length. Assuming that in nature also they survive this long, it would seem that the individuals live for a period of about 1½ years and during this period, they release gonad-products at least thrice in this life-span.

Regeneration

This species shows great powers of regeneration of lost portions of the body. The excision of parts of the cartilagenous test in different locations of the body, with the exception of the siphonal tubes/areas, resulted in the quick filling up of the wounds so caused : a wound 1.0 mm deep and 1.5 mm square was filled up with hardly any trace in 72 hours; excision of parts of the siphons took much longer; upto a week or 8 eight days.

Removal of the greater part of the branchial sac, but leaving at least part of the stigmata-bearing surfaces intact, resulted in almost complete regeneration of the lost parts.

However death resulted if any of the following parts were excised: neural ganglion/neural gland complex; dorsal tubercle complex; lower part of the branchial sac/endostylar complex; even damage to the intestinal/renal caecae complex resulted in the death of the animal.

Discussion

It is of interest to note that many predominantly Mediterranean species have established themselves in the Red Sea and Makrân (now in Pakistan) Coasts, as seen from the surveys made by Pérès (1962) and Lall (1938) (for the Karachi area). It is possible that this phenomenon has occurred partly due to the great shipping activity in the post-1960 period (by countries located in the Mediterranean and Black Sea areas and Indian ports), resulting in many species (particularly those that 'foul' on ship's bottoms) in being carried into Indian tropical waters and over years, succeeding in colonizing these waters: one of these species appears to be *Ascidiella aspersa*. Millar (1970) had mentioned that this species prefers warm, sheltered waters; may be its success in extending its known distribution into boreal waters, like the British Coasts and Norwegian Fjörds, could be attributed to the fact that the warm waters of the Gulf Stream Drift (across the vast Atlantic) wash these otherwise inhospitable coasts creating conditions warm enough to facilitate this species colonization there.

In our tropical waters, *Ascidiella aspersa* occurs in the Madras coastal waters (and as far as our observations indicate) occurring only in the marine habitat; we have failed to collect this species from brackishwater habitats, although it has been reported from such habitats, in addition to the marine one.

Our data agree well with previous information on this species from European waters, the results of such work have been summarized by Berrill (1950). However, there are some aspects which are highlighted in this paper which add to our knowledge about the biology of this species in our waters, and that already known for its European distribution.

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