Culturing of the Ascidian, *Aplidium multiplicatum* & Its Dimorphic Larvae

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The temperate species *A. multiplicatum* is found to produce dimorphic tadpole larvae. Observations are made on the growth and colony formation of oozoids developed from big and small tadpoles with a view to presenting preliminary data on the possible occurrence of dualism of larvae in the species. A technique for laboratory culturing of the ascidians, the tadpoles of which readily settle on glass slides, has been described. The technique can be used in the bioassay of chemical biocides and in marine pollution survey using ascidians.

Development and budding of colonial ascidians like *Aplidium multiplicatum, A. solidum* and *Symplegma reptans* have been extensively studied. These ascidians are excellent material for several studies like tissue regeneration, immunology, budding and colony organisation and bio-accumulation of elements like vanadium, zinc, etc. Many species are clear and transparent and therefore permit close observations on the growth of organ systems and their functioning.

The ascidians can be induced to settle on glass panels almost at desired spots under laboratory conditions. This faculty has been fully exploited by Nakauchi and Kawamura to study the behaviour of buds with reference to chemical attractant during colony formation in some Pacific species.

Colonial ascidians found in the Indian seas have received very little attention as an experimental material. Karande who studied larval settlement and early growth and budding of *Ascidiella* sp. in laboratory aquaria has considered this species to be very ideal for controlled experiments.

This paper presents the duality of tadpole larvae of the ascidian *A. multiplicatum* and highlights the laboratory rearing technique perfected by Nakauchi which can be used for studying the Indian species. Earlier, reports on the techniques of rearing of ascidians in the laboratory have been made. The colonial ascidian *A. multiplicatum* abundantly found in shallow waters of Japan was used. The observations were made during May-June 1979 when the sea water temperature was around 20-22°C. The observation was confined to hitherto unknown phenomenon of duality of the tadpole larvae produced by the species. The early settlement and growth of 'big' and 'small' larvae were studied in the laboratory tanks using the method described by Nakauchi.

Collection of tadpoles—*A. multiplicatum* grows profusely on rocky substratum in shallow waters. Each colony, pearl white in colour, measures 2 to 3 in. in diam. It is flat, transparent and bears several hundred zooids. The 1st lot of colonies for the laboratory studies are obtained from the field from plastic or glass panels immersed in the floating raft in near shore waters. This ascidian can also be cultured on desired surfaces by transplanting whole or a part of the colony and also by inducing one or more tadpoles to settle and grow into adult colony.

During the active breeding season, free swimming tadpoles are liberated released. Two types of tadpoles, one measuring 2040 ± 20 µm and the other 1460 ± 15 µm are produced. To obtain the tadpoles, field colonies with mature oozoids are brought to the laboratory in suitable containers on previous evening and are kept near the window in sea water tanks of 4 to 5 litre capacity. The colonies release several hundred tadpoles in the morning hours and since these are positively phototrophic, they can be collected easily.

Glass-slide culture—Tadpoles released in the aquaria when given a proper surface settle within 30 min. The larvae allowed to remain in the aquaria for a longer duration generally do not settle on desired substratum. The larvae pipetted out must be utilised immediately so as to bring about a satisfactory adhesion and further metamorphosis. The settlement of the larvae on microscope glass slides or on 'Falcon' slides is very advantageous since it permits the examination of the growing colony both from its fixed and free sides under stereo-microscope as well as electron microscope.

In order to bring about the settlement on glass slides, individual tadpoles collected in the petriplate may be pipetted out gently and kept in another containing tap...
or distilled water for about 20 sec. When transferred in distilled water, it becomes motionless. It then may be pipetted out and released very close to the surface of the glass slide cushioned on 'U' shaped glass rod, in another petriplate containing normal sea water. Tadpole adheres to the glass surface immediately if the freshwater 'shock' is adequately timed. During the active breeding season of the species, 7 or 8 of every 10 tadpoles can be induced to settle using this technique.

Continuous culture—Depending on the experimental work, one may have to rear *A. multiplicatum* colony for a shorter duration of a few weeks or for an extended period covering several months. With the technique described so far, it was possible to study the metamorphosis and budding through strobilation leading to colony formation in this species within 15 days. For long term experiments where profuse growth and multiplication is desired, the culture colonies need to be kept on floating raft in the field environment.

For shorter duration experiments, freshly made slides bearing just metamorphosed tadpole or tadpoles should be kept in finger-bowls for a period of 24 to 30 hr. For the subsequent growth the slides should be slotted in a timber or a plastic rack-box and maintained in laboratory aquaria containing steady water. The sides of the slides bearing the growing oozoids should face the bottom of the tank since it helps to eliminate the accumulation of detritus on young colonies. Sea water in aquaria should be changed at least twice during 24 hr. If this is done no extraneous food is required for feeding the oozoids. For long duration experiments where the colonies need grow profusely and are also intended to be used as stock cultures for the fresh supply of tadpoles of the known parentage, the slide boxes should be immersed on a floating raft moored at the field habitat.

Colony formation—Tadpole larva of *A. multiplicatum* is 2040 μm long. Its trunk measures 600 μm and the tail including fin is 1440 μm. Detailed development of the species has been earlier described. The tadpole emerges into oozoid in a few hours. The budding in the species is by strobilation. The buds produced in abdomen and in post-abdomen regions of the oozoid move under its tunic and migrate towards the thorax to form a common cloacal system.

Fig. 1 illustrates the formation of buds and their behaviour towards the development of a colony. Strobilation, which precedes budding, commences after 11 days of metamorphosis. The number of buds produced is generally 6. More than 60% oozoids strobilate between 11 and 13 days. A colony of mother oozoid surrounded by its blastozooids is formed in next 48 to 60 hr. Nakauchi and Kawamura have examined the role of chemical attractant present in the tunic of oozoid that regulates the movements of blastozooids leading to colony formation.

Dimorphic tadpoles—During the present study *A. multiplicatum* produced 2 types of tadpoles differing in dimensions. About 80% tadpoles were 2040 μm long whereas the rest were 1460 μm long.

Rate of growth in two oozoids—Growth of big and small tadpoles during the first 16 days is shown in Fig. 2. Oozoids metamorphosed from big tadpoles grow...
rapidly during the first 48 hr. They grow steadily until 10th or 11th day at the end of which early signs of budding are noticeable. During the strobilation, the growth of oozoid is reduced but accelerates again when the blastozooids produced start moving to form a colony. It may achieve a length of over 4 mm by the time it becomes a mother zooid. Oozoids from small tadpoles grow generally at uniform and faster rate. They grow nearly as big as oozoids from big tadpoles though at no stage they are actually longer than the latter.

Onset of strobilation in two oozoids—Fig. 2 further illustrates that in big oozoid strobilation commences between 10th and 11th day when its length is around 3 mm, whereas small oozoid strobilates between 13th and 14th day when it is about 3.5 mm in length. Only in about 15% of small oozoids, strobilation commences this early and majority amongst them show signs of it only after 15 days growth. At the end of 16 days, big tadpole oozoid undergoes complete metamorphosis and budding and fully develops into a colony.

The present study suggests that in all probability *A. multiplicatum* like a few other ascidian species produces 2 types of tadpole larvae. This phenomenon of duality of the larvae needs to be confirmed keeping in view the utility of the species as an experimental ascidian material. One may have to choose either only big or small tadpoles for the intended experimental work. To establish the duality of the larvae, some of the aspects which need to be examined in the 2 zooids are possible differences in stigmatal rows and atrial langutes, relative growths of thorax and abdomen, re-organisation during strobilation and budding, number of blastozooids produced and their movements leading to colony formation, morphology and growth of blastozooids produced and bioaccumulation rates of vanadium, etc. Further it would be worthwhile to grow big and small oozoids separately and ascertain if each of them produces dimorphic larvae and if they do, in what proportions big and small tadpoles are produced.

The present technique of culturing ascidians on glass slides can be used with advantage in the assessment of chemical antifoulants (biocides) and also in harbour pollution studies involving monitoring of the sea water quality. In this regard marine pollution survey carried out by Kobayashi along the Japanese coasts where he used sea urchin plutei as the test organisms is worth mentioning. A prerequisite to such an investigation is to culture the ascidians encountered along the Indian coast and ascertain their utility as the test organisms.

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