

Studies on Ethology of Planaria with Reference to Physico-Chemical Stimuli

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Abstract: Planaria are flatworms with unsegmented cephalisation, acoelomate dorso-ventrally flattened organisms constituting neoblasts responsible for characteristic process of regeneration. Anterior cephalic region comprises eyespots and brain interpreting various signals resulting in distinct animal behaviour. Present study focuses on such ethological responses with respect to artificially induced stimuli and way it induces the neuronal stimulation co-relating with regenerative physiological signaling in Planaria. Collection of Planaria was done from various freshwater stations such as Pashan lake, Sarasbaug and Yerawada, Pune. Culture was maintained using artificial pond water and aerator to prepare and cultivate Planarian cells in dark conditions and were fed with sheep liver and egg yolk. Photo-responsiveness was studied using monochromatic light generated from 500 lux white light. Planaria are negatively photo-tactic and tend to repel when exposed to artificially induced light. Planaria was treated 0.05 N formalin to observe its response to chemical stimulus. Eversion of pharynx was found out to be defence mechanism in Planaria. Planaria everted out its food feeding apparatus in response to formalin. Neoblasts were stained with acetocarmine, recorded to initiate process of regeneration were dispersed in response to formalin.. Present study infers that for undergoing regeneration Planaria has to degenerate itself. Such Behavioral responses and its canonical mechanisms can be leading to a path of cell signalling in cancer biology and stem cell research. Planarian diversity seems to be confined to certain trajectory path extensively around limnologic sources of Pune, Maharashtra. Owing to surplus reasons, we need to look forward to some ways of conserving Planaria.

Keywords: Planaria, Ethology, Chemotaxis, Thigmotaxis, Neoblasts.

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I. INTRODUCTION

Planarians are free living, unsegmented, acoelomate turbellarians. Anterior cephalic region comprises oriented eyespots and brain. Pharynx and Mouth is centrally located tapering into posterior tail region. They are triploblastic, soft bodied dorsoventrally flattened organisms (Rieger et al, 1977). Planarian Regeneration requires adult stem cells known as neoblasts (Reddien and Sanchez, Alvarado, 2004). They are small cells of 5-10 um size with large nucleus and restricted cytoplasm and chromatid bodies. Neoblasts are somatic cells that are mitotically active and well distributed throughout body except photoreceptors and centrally located pharynx (Reddien and Sanchez Alvarado, 2004). Signalling between the injury site and neoblasts is important to understand stem cells and role in regeneration. Planarian brain is an information collecting and processing centre well versed for interpreting various signals resulting in distinct animal behaviours. Neuronal Stimulus tend to be responsible for various behaviour responses.

Perception of stimulus and association to physiological response with cellular and molecular mechanisms tend to be associated with the behavioural responses (T Inoue *et al*, 2004). Planaria shows a stereotypical behaviour which is negative phototaxis. It is ideal model organism for study of Visual system (Deochand *et al*, 2018). Light sensing eyes are composed of pigment cells and visual neurons. Visual neurons integrate photosensory inputs from sides of animals. (Agata *et al.*, 2003) The distinctive pharynx located in center of body with regenerating posterior body fragment and undifferentiated neoblasts form rudiment. Regeneration in Planarian pharynx proceeds by accumulation of these neoblasts.

Many species tend to exhibit certain unique behavioural responses pertaining to survival of individual. Studies on ethology prove to be essential in understanding behaviour exhibited by organism in various normal conditions in order to differentiate it from the experimental observations.

Planaria tends to be neglected in scientific community hence few studies have been conducted on its ethology and stress mechanisms. Present study focuses on such ethologic responses with respect to some artificially induced stimuli and the way it induces the neuronal stimulation correlating it with regenerative physiological signalling in Planaria.

II. MATERIAL AND METHODS

A. Collection of Planaria

Survey of lentic bodies around Pune region was done for investigation of availability of Planaria. Planarians were collected from areas such as Pashan Lake, Sarasbaug, Yearawada Planarians were captured using wide mouth bottle along the roots and leaves of Eichornia and Pistia along with nutrient medium and settled for some time before culturing.

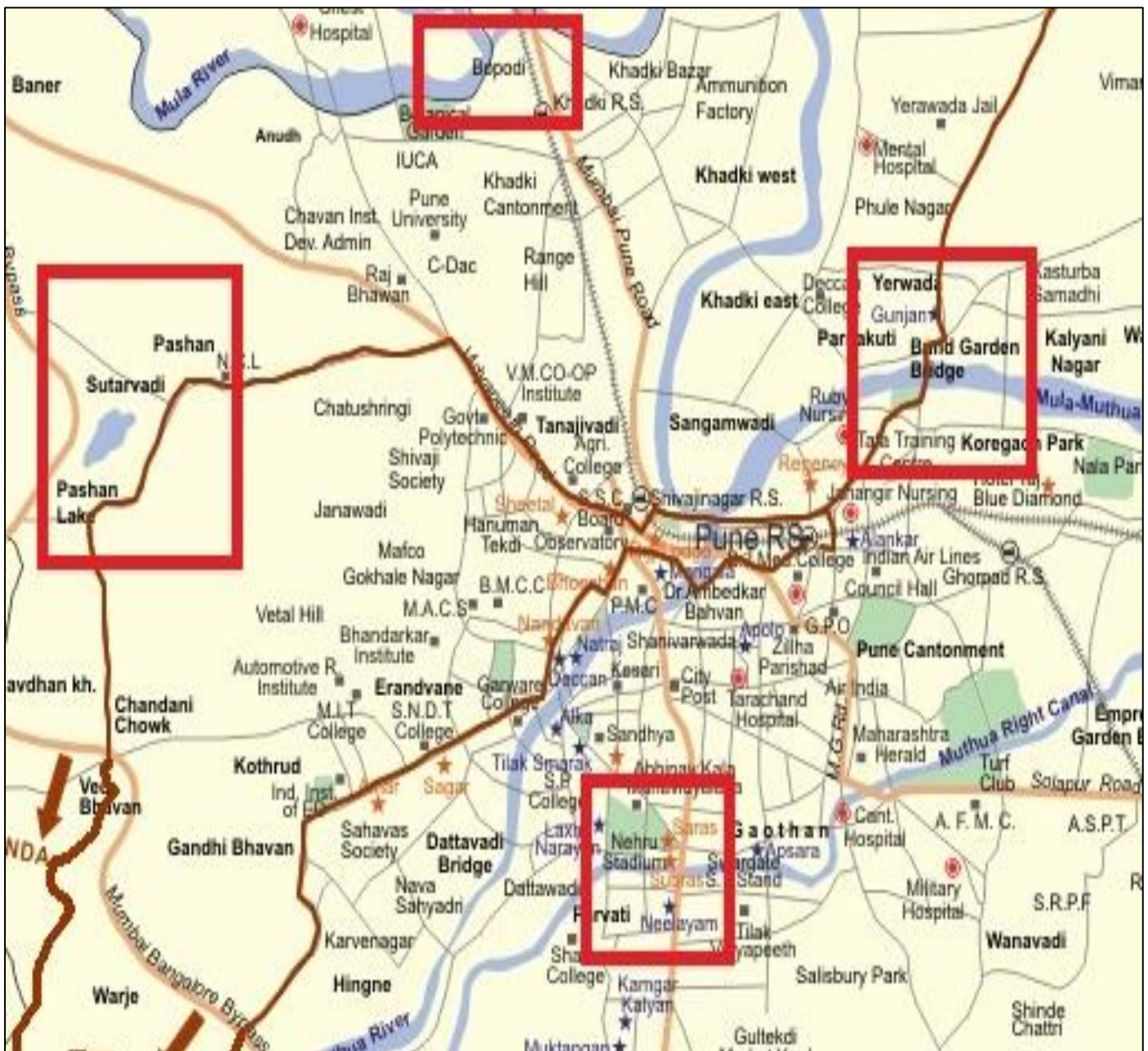


Fig 1 – Sampling Stations

B. Culturing of Planaria

Planarians were maintained in small tanks with careful handling in 1x Montjuic water (1.6 mol/l NaCl, 1.0 mol/l KCL, 1.0 m-mol/l MgCl₂, 0.1 mol/l MgSO₄, 0.1 mol/l CaCL₂ and 1.2 m-mol/l NaHCO₃ in water, pH 6.7-8.5) (Cebrià and Newmark, 2005). Planaria cultures were maintained at room temperature (20–24 °C). Planaria were fed with small pieces of raw liver or hard-boiled egg yolk.

C. Ethological studies

Phototactic assay was conducted using monochromatic light of generated from 500 lux white light. Observations were captured and examined for 12hrs with water change using customised video capturing device. Migration and directional pattern were studied constructing quadrants plates suspending Planaria in it. Response to piece of liver, solution of 0.05% formalin and 6-ODHA as an chemo attractant was examined using stereomicroscope and customised video capturing device to study chemotactic and thigmotactic behaviour of Planaria.

III. RESULTS

A. Occurrence

Occurrence of Planaria around Pune M.S. region seems to restricted to certain trajectory. Lentic freshwater sources

with high eutrophication and stagnant water have abundant planarian diversity confined to season from Dec–March. Fluctuating Physicochemical Properties along with changing climatic conditions have profound effects on availability of planaria.

B. Genus Identification

Two types of Planaria have been identified on lentic waters around Pune region viz. Brown and Black planaria. Using standard taxonomic keys Genus *Dugesia* has been identified and confirmed. (Knezovic *et.al*, 2015)

C. Ethologic Responses

Phototactic assay revealed negatively phototactic behaviour and it tends to repel when exposed to artificially induced light. Migration Patterns were observed to be restricted to Upper quadrants not exposed to monochromatic white light. Eversion of Pharynx followed by scattering of neoblasts was observed in response to the chemotactic assay in response to the chemo attractant. Regeneration mechanism was observed by proliferation of neoblasts and formation of Blastema in first 3 days but no planaria was developed after. Chemo attraction towards liver particle and subsequent eversion of pharynx to feed upon it was observed

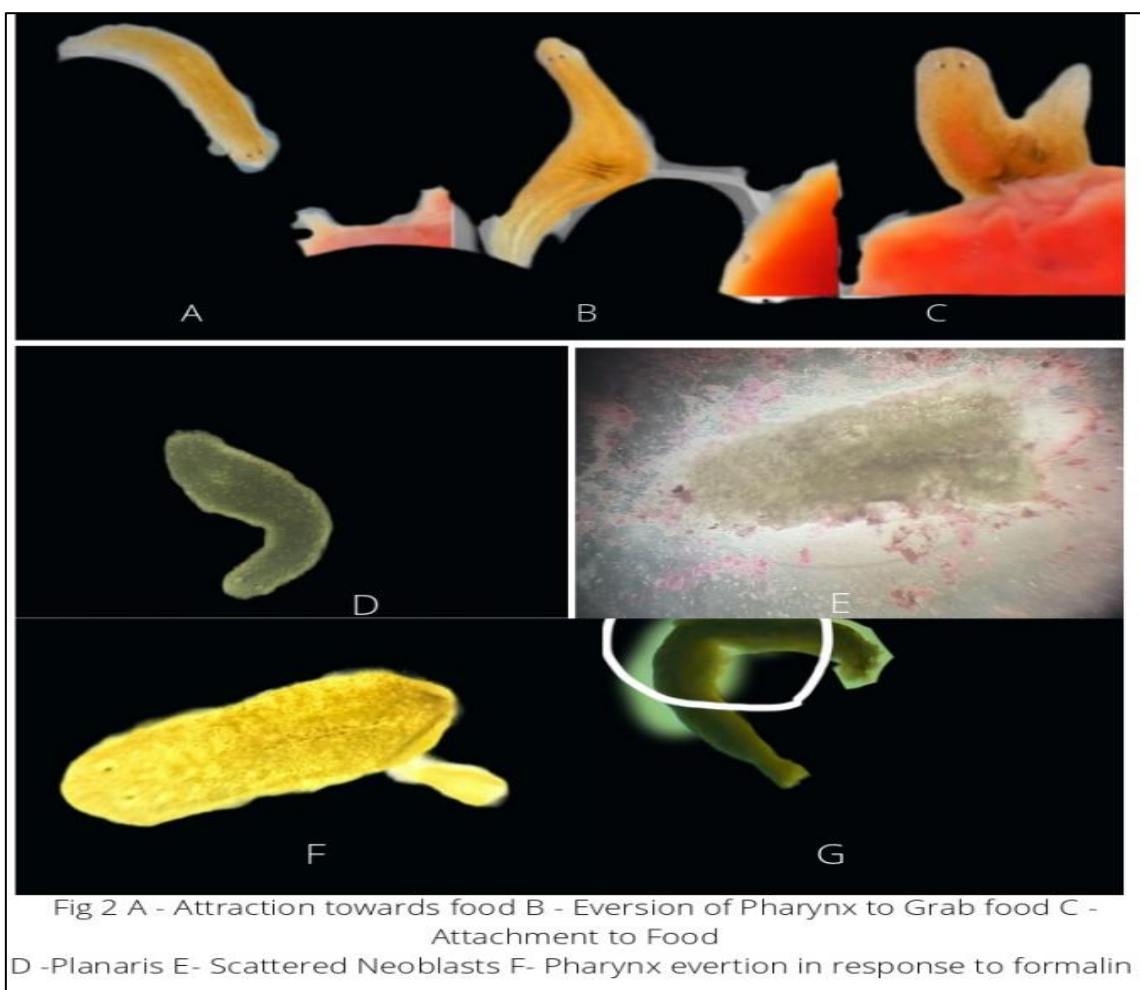


Fig 2 Observations : Fig 2A –Attraction towards food , B-Eversion of Pharynx to Grab food, C- Attachment to food, D-Planaris, E-Scattered Neoblasts, F- Pharynx eversion in response to formalin. G – Curvature due to Pharynx Eversion.

IV. DISCUSSION

Eversion of pharynx in response to chemicals was found out to be a defence mechanism in Planaria. Subsequent scattering of neoblasts and formation of blastema confirms initiation of epimorphic regeneration as a defence and survival mechanism. Present Study infers that for undergoing regeneration for survival Planaria has to degenerate itself. Such Behavioural responses and canonical mechanisms can lead to path of research and applications in cancer biology and stem cell research. Defence mechanisms can be a pathway of neuronal signalling studies correlating with neuronal stem cells in Humans. Due to above reasons and restricted diversity around Pune region Planarians must be conserved.

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