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Original Article

The Art of Dye Expulsion in an Indian Freshwater Sponge

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Abstract

Sponges, regarded as 'living fossils,' provide important models for studying primitive mechanisms of environmental tolerance and immune defense. In freshwater sponges, adaptational plasticity reflects their capacity to modulate physiological, cellular, and immunological responses to environmental challenges, including the recognition and rapid elimination of foreign materials, thereby contributing to survival in biologically vulnerable environments that are often contaminated by pollutants. Their remarkable adaptational plasticity and immunological resilience are associated with an efficient innate immune system, mediated by specialized immunocytes. Microscopical observations of freshwater sponge cells employing phase-contrast, bright-field, confocal and scanning electron microscopy revealed the existence of heterogeneous populations of polyphenotypic cells, which are presumed to be involved in physiological defense, self–nonself recognition, and tolerance to environmental stress. In the present article, the filter-feeding mechanism and rapid expulsion of foreign materials by the Indian freshwater sponge *Eunapius carteri* are discussed, with particular emphasis on its innate immune reactivity *in situ*. To demonstrate the movement and expulsion of foreign material, small volumes of Alta and blue water-soluble dyes were administered directly into the sponge body using a syringe. Following administration, the sponge rapidly expelled the foreign dyes through the osculum proximal to the injection site, indicating a localized and prompt canal mediated expulsion reflex, in which flagellated choanocytes generate water currents that facilitate filter feeding, gaseous exchange, and excretion. The present paper would help the researchers to identify cellular and immunophysiological mechanisms governing filter-feeding and the precise mechanisms of dye uptake, intracellular processing, and subsequent expulsion in freshwater sponges.

Keywords: *Biological Pumping; Dye Expulsion; Eunapius carteri; Filter Feeding*

Introduction

Physiological and Ecological Significance of Freshwater Sponges

Sponges are sessile metazoans that lack true muscular, nervous, and digestive systems but possess a porous body architecture that facilitates biological pumping. Because respiration, feeding, excretion, and reproduction in sponges are all mediated by filtered water, regulation of pumping activity is essential for understanding sponge physiology (Morganti *et al*., 2019). As suspension feeders, sponges depend on flagellated collar cells, or choanocytes, to pump and filter large volumes of water, many times greater than their own body volume per unit time (Vogel, 1977). As ambient water passes through the complex canal system within the sponge body, it is thought to generate a pressure gradient across the sponge body wall, although the precise mechanism underlying this process remains unresolved (Leys *et al*., 2011). Thakur *et al*. (2005) recorded water filtration rates ranging from 0.002 to 0.84 $cm^3\ s^{-1}$ per cm^2 of sponge tissue. Despite their relatively simple body plan, tissue-like organization, and non-specialized outer epithelial sheets, Wagner *et al*. (1998) hypothesized that

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sponges possess sophisticated cellular mechanisms and greater physiological capacity to respond to environmental stressors. However, information remains limited regarding biological pumping activity, filter-feeding mechanisms, and the mechanisms underlying the expulsion of xenobiotic dyes from the sponge body.

Sponges are potential sources of biosilica, pharmaceutical substances, and bioactive compounds with significant applications in ecology, cell biology, immunology, biotechnology, and evolutionary biology (Manconi *et al*., 2013; Mukherjee *et al*., 2015a). Freshwater sponges, members of the Family Spongillidae, represent important aquatic bioresources in Indian lentic ecosystems. Owing to their simple morphology and sessile nature, freshwater sponges are often overlooked by researchers and are consequently regarded as 'neglected bioresources' of aquatic ecosystems (Mukherjee *et al*., 2018). Freshwater sponges have also been identified as 'ecological hotspots' because of their close functional associations with diverse aquatic organisms, including microarthropods, nematodes, annelids, molluscs, snakes, small fishes, and crabs (Mukherjee *et al*., 2015b) (Figure 1A–C). In some cases, sponge colonies serve as favorable sites for breeding, parental care, and nesting for neighboring aquatic organisms (Soota, 1991). During periods of declining water levels in summer, the dried skeletons of freshwater sponges, composed primarily of spicules, often remain attached to aquatic weeds (Figure 1D).



Figure 1: *The association of the freshwater sponge Eunapius carteri with mollusc Bellamya bengalensis (indicated by arrows) (A, B) and a snake (C) in its natural habitat. The dried skeletons of sponges are attached to aquatic weeds (D) as the water level of the pond declines during the summer season.*

Bauplan and Morphofunctional Uniqueness of Sponges

Sponges are among the earliest evolving multicellular animals and serve as model organisms for studying morphogenesis and physiology (Ogawa *et al*., 2025). According to Ogawa *et al*. (2025), the architecture of the sponge choanocyte chamber is a key determinant of biological pumping and filter-

feeding efficiency. The diameter of the choanocyte chamber, the number of flagella per chamber, and the angle of the outlet opening are among the factors that influence filtration efficiency. Thus, a close relationship exists between the morphogenesis of the choanocyte chamber and the physiology of biological pumping. Heterogeneous populations of polyphenotypic sponge cells are responsible for a wide range of physiological functions, including food capture and digestion, immune responses, cellular reorganization, filter-feeding, and self–nonself recognition (Mukherjee, 2017) (Figures 2–5). Poriferans primarily rely on innate immune responses for biological defense (Wiens *et al*., 2007; Mukherjee *et al*., 2021) and lack a sophisticated adaptive immune system. Adaptational plasticity and immunological resilience in sponges remain relatively understudied, with limited information available on these processes (Mukherjee *et al*., 2016a). The cellular organization and body plan of sponges are thought to exhibit various physiological processes, including filter-feeding, digestion, morphogenesis, gas exchange, and the maintenance of internal water circulation.

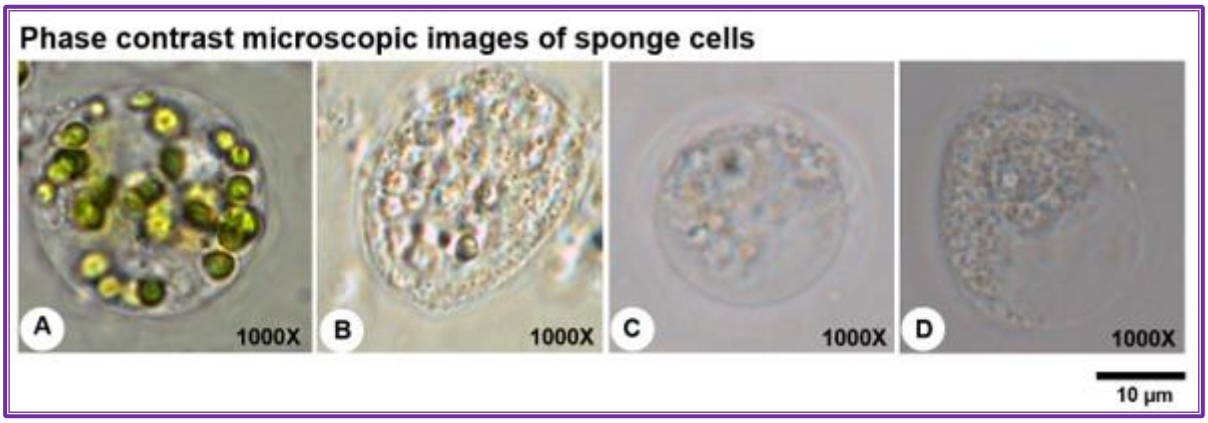


Figure 2*: Microscopical examination and morphological analysis of unstained, live freshwater sponge cells were performed using phase-contrast optics (BX–51; Olympus, Japan) attached with the light microscope. For, phase-contrast microscopical examination of sponge cells, tissue samples were obtained from five independent, healthy sponge specimens (n = 5).*

According to Elliott and Leys (2007), sponges have evolved a sophisticated network of aquiferous canals that permeates the body and facilitates the circulation of ambient water along a predetermined pathway. Consequently, sponges are continuously exposed to potentially toxic substances present in their surrounding environment and may therefore be particularly vulnerable to the accumulation of hazardous compounds (Schröder *et al*., 2006). The highly permeable body architecture and continuous filtration of ambient water expose sponges to dissolved and suspended contaminants, which may subsequently be taken up and accumulated within their tissues (Müller *et al*., 1998). Consequently, their efficient biofiltering capacity makes sponges valuable biomonitors of aquatic pollution (Negri *et al*., 2006), while also raising important questions regarding the mechanisms by which potentially harmful substances are processed, retained, and expelled from the sponge body.

Filter Feeding Mechanism and Biological Pumping Activity of Sponges

Weisz *et al*. (2008) reported that sponges are capable of filtering water at a rate of 0.5 L s^{-1} kg^{-1} dry mass. Hoffmann *et al*. (2008) demonstrated a positive correlation between active biological pumping and oxygen saturation within sponge tissues, thereby contributing to the maintenance of a well-oxygenated internal environment. Choanocytes internalize bacteria, microorganisms, and other suspended food particles from the surrounding water through phagocytosis and subsequently transfer them to archaeocytes for intracellular digestion. In *E. carteri*, phagocytosed zoochlorellae are frequently observed as cytoplasmic inclusions within archaeocytes (Mukherjee, 2017) (Figure 3). As sessile filter feeders, sponges incur energetic costs associated with filter feeding, which may vary depending on the availability of food in the surrounding environment. Tsubaki and Kato (2014) described a unique form of mutualism, termed ‘filtering mutualism’, between a host sponge and its endosymbiotic bivalves. According to their findings, the close association between sponges and

bivalves enables the sponge to utilize water exhaled by the bivalves, demonstrating the morphological adaptability of the host sponge to receive this water source. A similar kind of association between the freshwater sponge *E. carteri* and the mollusc *Bellamya bengalensis* was observed (Figure 1A, B). The potential functional significance of such an association, particularly in relation to filtering mutualism between *E. carteri* and *B. bengalensis*, demands further investigation.

To monitor the *in situ* pumping pattern under relatively undisturbed natural conditions, Pfannkuchen *et al*. (2009) injected a blue, water-soluble tracer dye into the body of the marine sponge *Aplysina aerophoba*. To minimize stress to the experimental sponge, the authors refrained from placing sensors or other instruments in close proximity to the specimen. Immediately after dye application, the dye was expelled through the 'chimney', indicating continuous water flow through the sponge body. This observation prompted us to investigate the biological pumping activity and dye-expulsion pattern of the freshwater sponge *Eunapius carteri*.

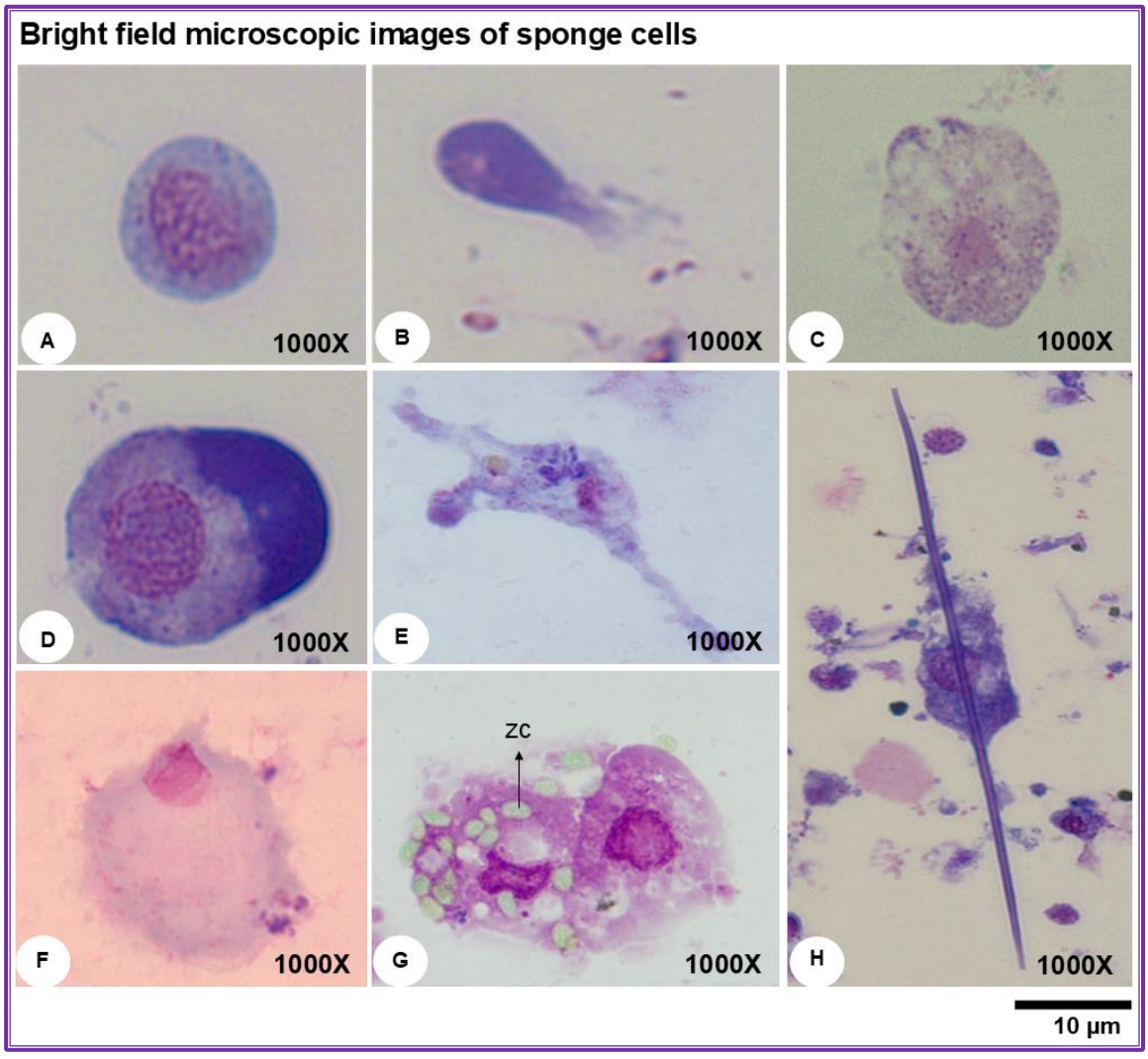


Figure 3: *Heterogeneous and polyphenotypic freshwater sponge cell subpopulations were examined under bright-field optics. The cells were air dried for 10 min, fixed in methanol for 5 min, and stained with Giemsa's stain (Mukherjee et al., 2015a). Photodocumentation of cells were carried out using a CCD camera (ProgRes C5; Jenoptik, Jena, Germany) mounted on an Olympus BH-2 microscope (Tokyo, Japan). At least 200 cells per slide were examined to identify distinct subpopulations, including blast-like cells (A), choanocytes (B), small amoebocytes (C), granular cells (D), pinacocytes (E), large amoebocytes (F), archaeocytes containing phagocytosed zoochlorellae (zc) (G), and sclerocytes (H). For, bright-field microscopical examination of sponge cells, tissue samples were obtained from five independent, healthy sponge specimens (n = 5).*

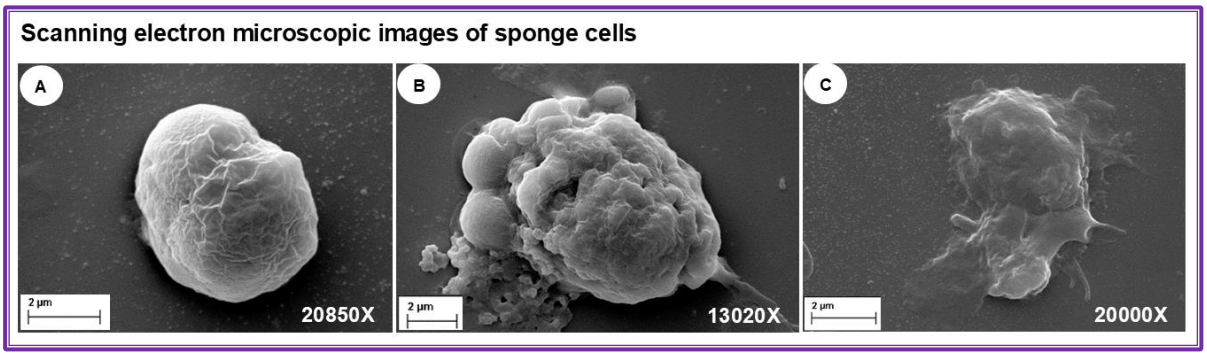


Figure 4: *Ultrastructural analysis of freshwater sponge cells was performed using scanning electron microscopy (A–C). Cell suspension monolayer was allowed to adhere to glass surfaces for 30 min, followed by fixation in 3% glutaraldehyde (Sigma, USA) in 0.1 M sodium cacodylate buffer containing 12% glucose (pH 7.8) for 2 h at 4 °C (Mukherjee et al., 2015b). Cells were then postfixed in 1% osmium tetroxide (Sigma, USA) for 1 h, dehydrated through graded ethanol, air dried, sputter coated with gold, and examined under a scanning electron microscope (Zeiss EVO 18 special edition, Germany). For, scanning electron microscopical examination of sponge cells, tissue samples were obtained from five independent, healthy sponge specimens (n = 5).*

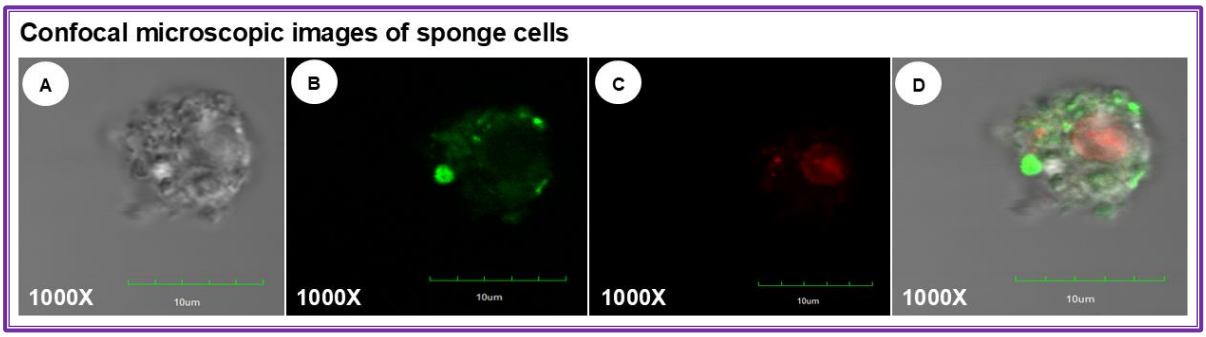


Figure 5*: Immunofluorescent detection of apoptotic and necrotic cells of freshwater sponge after staining with annexin V- FITC and propidium iodide (PI). Stained cells were immediately examined under differential interference contrast (DIC) (A) and confocal optics (B-D) attached with a confocal laser scanning microscope (IX81; Olympus, Singapore). Cells with green fluorescence (FITC positive) (B), red fluorescence (PI positive) (C) and colocalization effect (FITC and PI both positive) (D) were identified microscopically and photodocumented (Mukherjee et al., 2015c). Image calibration and analyses were performed using Olympus FV10-ASW Viewer Version 3.0 software attached with the microscope. For, examination of sponge cells employing confocal microscopy, tissue samples were obtained from five independent, healthy sponge specimens (n = 5).*

Materials and Methods

Identification of Freshwater Sponge Population for Our Study

An extensive field survey was conducted during the pre-monsoon (February – April), monsoon (May – July) and post-monsoon (August – January) seasons of the year for the last fifteen years in and around the districts of North 24 Parganas and Kolkata of the State of West Bengal, India, to identify the natural habitat, distribution pattern and abundance of freshwater spongillids (Mukherjee *et al*., 2016b). A total of three experimental ponds (designated as fields 1, 2 and 3) were selected to collect the specimens of freshwater sponge throughout the year. Among these three fields, field 1 was a pond geographically located near Zoological Survey of India, Park Street, Kolkata (22° 33′ 23.4″ N and 88º 21’ 05.6″ E). Fields 2 (22° 51' 21.5 " N) and 3 (88° 25' 32.7 " E) were located in the North 24 Parganas district, approximately 3.3 km and 3 km away from Kankinara railway station, respectively. A GPS navigator (Etrex 30×, Garmin, Kansas, USA) recorded the location, and Google Earth Pro software (Maxar Technologies, USA) analyzed satellite images for location confirmation. Many

emergent aquatic weeds and submerged plant twigs were present in these perennial aquifers, which provided suitable substrates for sponge colonization. The study sites were free from anthropogenic disturbances, including applications of pesticides, heavy metals, washing soda, netting, bathing, or aquacultural activities. Regular observations and monitoring of the ecological state and health status of spongillid sponges were carried out in these experimental ponds since 2011. Freshwater sponges were predominantly observed along the shallow peripheral margins of the ponds, generally at a water depth of approximately 6–8 cm (Figure 6A–D).

A small fragment of approximately 5 cm^3 in volume was surgically excised from the body mass of healthy freshwater sponge specimens showing no visible signs of bleaching or discolouration (Mukherjee *et al*., 2018). The fragments were surgically excised from submerged twigs using a sterile scalpel during daytime and immediately transported to the laboratory, with an adequate volume of freshly collected pond water procured from their natural habitat. During collection, the sponges were handled carefully to minimize morphological damage and collection-induced physiological stress (Figure 6E). Experimental specimens were gently detached from the substratum and slowly lifted from the water using a specially designed cotton net to minimize structural damage and mortality. Photographs of the live sponges were captured in their natural habitat using a digital camera (Nikon COOLPIX L820, Nikon Corporation, Tokyo, Japan). During specimen collection, the temperature, pH and dissolved oxygen concentration of the pond water were recorded as 25°C, 7.6 and 14 mg L^{-1}, respectively.

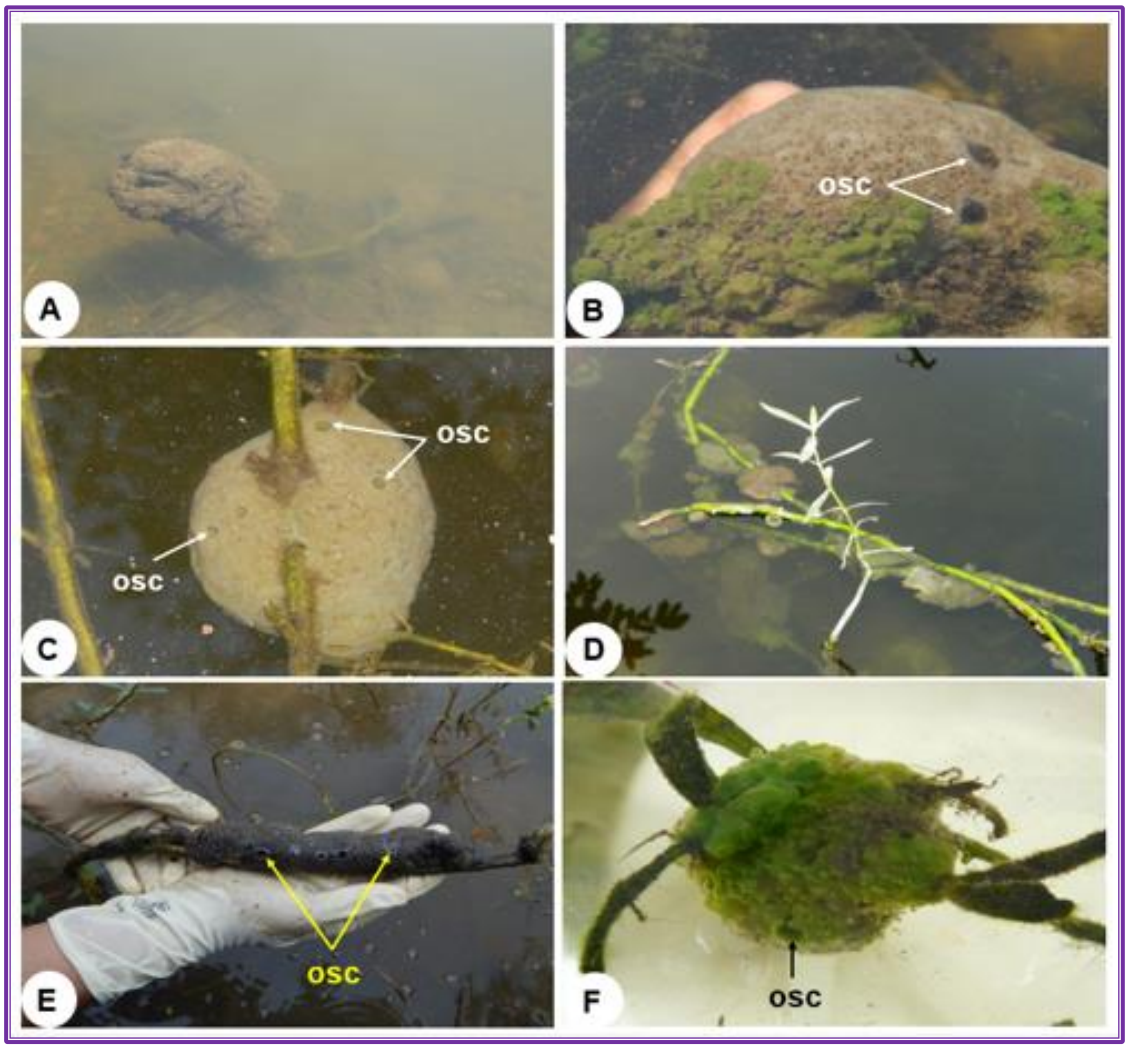


Figure 6*: Live and healthy freshwater sponges attached to submerged aquatic weeds and plant twigs (A–D) were manually collected (E) and acclimated in the controlled laboratory conditions (F) before taxonomic identification.*

Freshly collected healthy sponges were dissected into small pieces, each approximately 2 × 2 × 2 cm in dimension and bore at least one osculum (Hansen *et al*., 1995). The sponge fragments were acclimated in glass aquaria measuring 60 × 45 × 55 cm and supplied with continuous aeration using an electrically operated aerator (SB-648A, SOBO, India) under controlled laboratory conditions for 7 days (Figure 6F). This acclimation period was intended to minimize physiological stress and facilitate reorganization of the canal system (Duckworth & Pomponi, 2005). During acclimation, the temperature, pH, and dissolved oxygen (DO) concentration of the aquarium water were maintained at 25 °C, 7.6, and 14.5 mg L^{-1}, respectively (Mukherjee *et al*., 2021). A controlled photoperiod of 12 h light:12 h dark was maintained throughout the experiment. The aquarium water was replenished every 24 h with freshly collected pond water to provide suspended food and minimize the accumulation of excretory products and metabolic waste. All handling of freshwater sponge specimens was conducted in accordance with the institutional animal care guidelines of the University of Calcutta. Following 7 days of acclimation, representative specimens from the collected material were transported to the Zoological Survey of India (ZSI), Kolkata, for taxonomic identification. Permission for the collection of sponge specimens from their natural habitats was granted by the West Bengal Biodiversity Board, Department of Environment, Government of West Bengal, India (Reference No. 219/IL(Bio)-4/2007). The specimens were taxonomically identified by the Zoological Survey of India, Kolkata, under the Ministry of Environment, Forest and Climate Change, Government of India (Reference No. GNC/I & A Services/917), as *Eunapius carteri* (Porifera: Demospongiae: Spongillidae). Specimens collected from the three field sites did not exhibit any significant differences in the diagnostic taxonomic characteristics observed through the examination of spicules and gemmules.

Results

Experimental Expulsion of Xenobiotic Dyes by Sponge

The experiment aimed to investigate the filter-feeding mechanism of the sponge in its natural habitat. A 5 mL aliquot of each of two water soluble dye solutions, Alta and blue fountain pen ink (purchased from Sulekha outlet, India), was injected separately into the body of the sponge through a syringe (Figure 7A-D, E-H). The dye solutions were prepared by suspending 5 mL of the commercial dye in 5 mL of filtered pondwater (1:1, v/v). The experimental specimen was under scientific surveillance, and the pumping activity of the sponge was documented using photographs and videography. Following dye administration, the introduced dye was observed to be rapidly expelled through the nearest osculum associated with the site of injection in a perpendicular fashion. This observation was interpreted as evidence of the active water-current system of the sponge. Within 5 minutes, *E. carteri* expelled approximately 95% of the introduced dyes, demonstrating its effectiveness in eliminating xenobiotic dyes (Figure 7). The experiment was qualitative and was conducted using the specified dye concentrations; therefore, the observation does not imply that the sponge would expel dyes at all possible concentrations.

The dye-injection experiment was repeated five times using five independent, healthy sponge specimens under comparable experimental conditions. In each experiment, 5 mL of the respective dye solutions (both Alta and blue fountain pen ink) were injected separately into the sponge tissue, and the subsequent movement and expulsion of the dye were documented photographically and by videography. Consistent expulsion of the introduced dyes through the nearest osculum was observed in all five experimental trials. Using different dye solutions on different experimental replicates exhibited no noticeable variation in the expulsion of the introduced dye through the nearest osculum following injection. Furthermore, the purpose of the experiment was to visually demonstrate the direction of water movement and the association between the injection site and the nearest osculum, rather than to quantitatively assess the differential response exhibited by different individuals.

Alta is a traditional cosmetic typically applied to the hands and feet of married Hindu women during religious or social ceremonies. This vibrant red dye is traditionally employed in Indian ceremonial practices, notably in the northern, central, and eastern regions. Traditionally, Alta, also known as

Alakta, was prepared by dissolving or extracting colourants from lac, a reddish-brown resinous substance secreted by the scale insect *Laccifer lacca* (Sharma *et al*., 2020). Lac, also known as lakh or laksha, is a natural resinous secretion composed primarily of resin, wax, and other associated components. Historically, crushed stick lac was dissolved in water to produce the characteristic red colour of Alta. Plant-derived colourants, including extracts of *Alkanna tinctoria* (commonly known as Ratanjot), have also been used traditionally to produce red dyes. However, the traditional red coloured Alta is often replaced by synthetic alternatives. A synthetic version of the dye offers superior colour stability, whereas the traditional form is relatively non-toxic and rarely causes skin irritation. Because of its intense colour and water-dispersible properties, Alta was selected as one of the dyes for visualizing water movement and expulsion in the sponge.

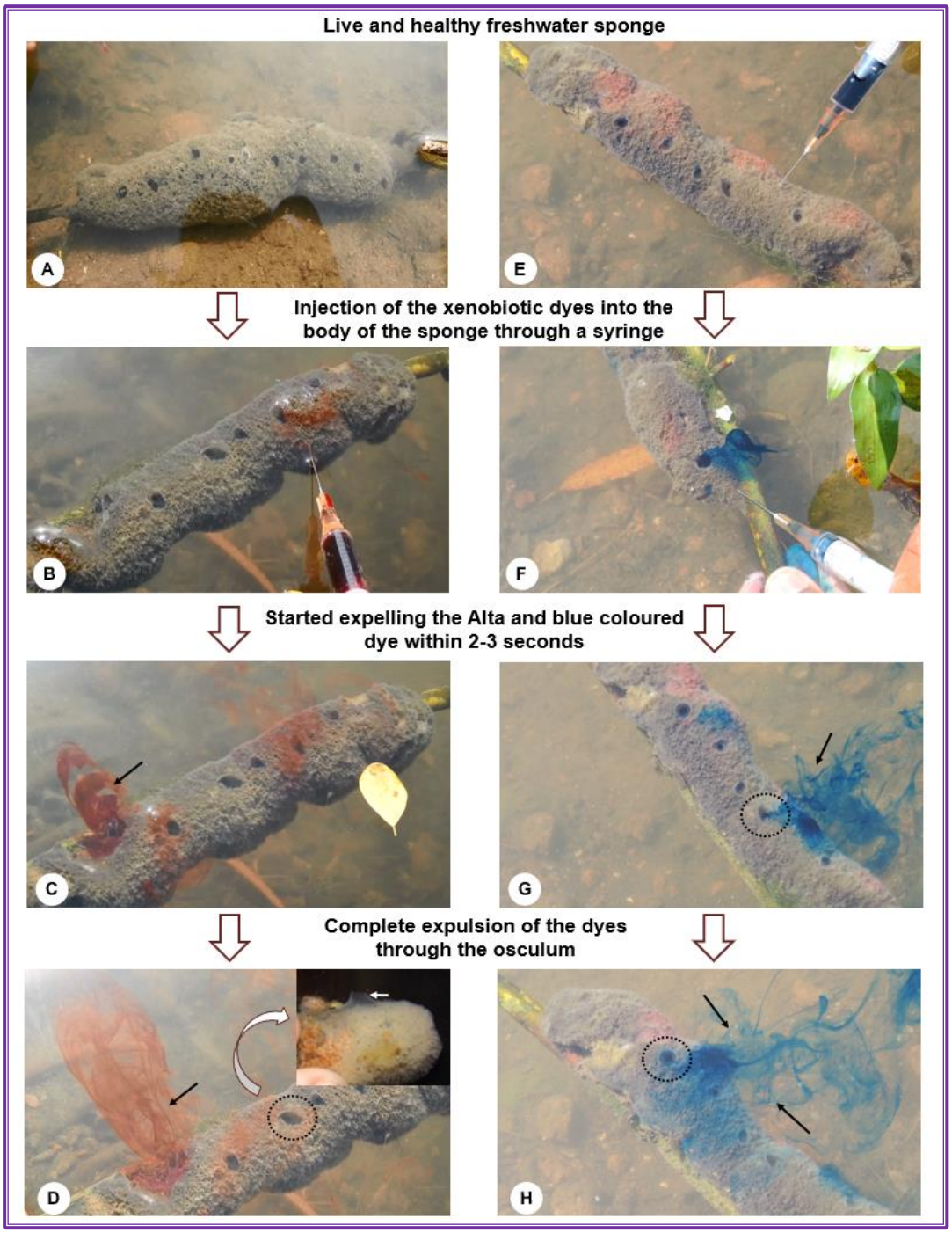


Figure 7: *Sponges make filter feeding visible by expelling foreign dyes through the nearest osculum after they have been injected.*

Dye Expulsion as an Immunophysiological Reflex Evolved in Sponges

For many years, scientists have attempted to understand the movement of food particles and water flow within a sponge's body during feeding and digestion. Consequently, tracer dyes have been used to visualize water flow and trace the movement of particles through the canal system of sponges (Patterson *et al*., 1997; Morganti *et al*., 2019). Filter feeding is a specialized feeding strategy exhibited by many aquatic invertebrates and is crucial for feeding, digestion, respiration, and survival of sponges. Because water movement through the sponge canal system is not readily visible to the naked eye, the application of tracer dyes provides an effective means of visualizing and tracking water flow within the sponge body. Accordingly, several researchers have used tracer dyes to investigate this continuous but otherwise invisible physiological process. Tracer-based approaches have also been employed to estimate the volume of water passing through the sponge body over a defined period and, consequently, to assess its pumping rate. The pumping rate is directly related to the volume of water pumped through the sponge per unit time and provides valuable insights into the filtration and biofiltering capacity of sponges.

While investigating pumping activity in the glass sponge *Aphrocallistes vastus*, Stevenson *et al*. (2020) introduced the fluorescent dye calcein near the sponge body and estimated the ‘minimum residence time’ and ‘pumping strength’. According to the authors, ‘minimum residence time’ refers to the time required for the dye to emerge from the oscula following its introduction, whereas ‘pumping strength’ encompasses characteristics of dye expulsion, including its volume and velocity. Sponges can remove and retain dissolved and suspended substances from the surrounding water, including particles and chemical contaminants of varying composition and toxicity, thereby influencing water quality. Through their efficient filter-feeding activity, sponges contribute to clearing the surrounding water and may facilitate the persistence of other aquatic organisms. However, this same filtration capacity can also result in the uptake and bioaccumulation of potentially hazardous substances and pathogens within sponge tissues. Despite the importance of tracer-based approaches for visualizing sponge pumping, the dynamics of dye uptake, transport, and expulsion in freshwater sponges remain poorly understood.

Discussion

Leys *et al*. (2011) estimated the energetic cost of pumping and discussed its evolutionary significance in the glass sponge *Aphrocallistes vastus* under *in situ* conditions. Sponges have an inherent capacity to minimize the energetic cost of pumping by taking advantage of passively driven water flow generated by ambient currents. As sessile suspension feeders, sponges can adjust their energy allocation to pumping, particularly in environments with limited food availability. Unlike mobile filter feeders, sponges cannot relocate from areas of low food availability to those with higher food concentrations. Nevertheless, they can modulate their pumping activity and optimize their energy budget for suspension feeding under food-limited conditions. Sponge pumping rates are influenced by various environmental factors, including temperature, food availability, suspended sediments, and the day–night cycle (Morganti *et al*., 2019). However, temperature and food availability have been reported to exert only minor effects on pumping activity, whereas sponge size is considered a principal determinant of filtration efficiency. Kornder *et al*. (2022) identified a novel mechanism in the sponge *Aplysina archeri*, whereby the sponge produces mucus to trap foreign particles and subsequently expels the mucus against the internal water flow into the surrounding environment. Although the mechanisms underlying waste removal and self-cleaning in sponges remain poorly understood, sponges are thought to possess mechanisms that prevent solid waste from accumulating and clogging their internal filtration systems (Bell *et al*., 2015; Cummings *et al*., 2020). Because metabolic waste products are continuously removed by the flow of water through the sponge body, sponges appear to have limited capacity for prolonged accumulation of self-generated waste. Thus, continuous filter feeding and biological pumping represent essential physiological processes that require sustained energetic investment (Pfannkuchen *et al*., 2009).

Sponges are continuously exposed to a wide variety of xenobiotic substances and environmental pathogens present in the surrounding water owing to their structural simplicity, functional uniqueness, porous architecture, and ability to generate microcurrents (Mukherjee *et al*., 2015c, 2016c). These characteristics facilitate the entry of pathogenic microorganisms, suspended particles, and potentially toxic substances into the sponge body, thereby posing potential ecotoxicological risks to freshwater sponge populations. Sponges readily retain suspended particles (Fraissinet *et al*., 2023) and may consequently accumulate xenobiotic substances and chemical residues (Ribeiro *et al*., 2024).

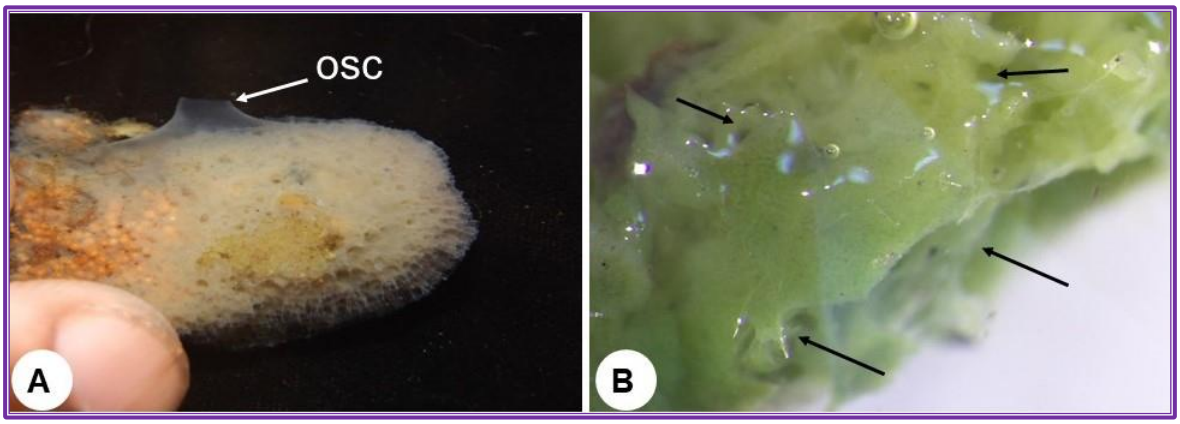


Figure 8: *Sponges eliminate xenobiotic compounds through the osculum (osc) (A) and produce abundant mucus (arrows) to trap foreign particles (B).*

Therefore, sponges are considered as valuable model organisms for investigating the toxicological effects of both naturally occurring and anthropogenically derived chemical compounds (Wagner *et al*., 1998; Ledda *et al*., 2013). The filter-feeding nature of sponges facilitates the uptake of nutrients but also promotes the internalization and retention of foreign particles. The activity of flagellated choanocytes generates microcurrents that facilitate the transport and retention of microscopic particles within the aquiferous canal system. Once introduced into the sponge body, these particles may obstruct water channels or clog ostia (Figure 8A), potentially leading to physiological disturbances, cellular stress, and alterations in ecological interactions (Hombing *et al*., 2025). These functional characteristics make sponges effective ‘sentinel organisms’ for assessing aquatic pollution. It has been hypothesized that, following ingestion, foreign particles may either be phagocytosed by sponge archaeocytes or trapped within mucus (Figure 8B), before being processed and eventually expelled from the sponge body.

Conclusion

Indian freshwater ecosystems are an abode of a wide array of aquatic life forms; many of which are important bioresources with considerable ecological, evolutionary, and cell biological significance. The apparent absence of specialized nervous and muscular systems, together with their cellular polymorphism and lack of well-defined organs, represents one of the distinctive features of sponge biology. The sponge body is structurally adapted for filter feeding, with an internal architecture comprising a complex network of canals and pores that facilitates the continuous circulation of water through the organism. Sponges combine active pumping generated by choanocytes with passively driven water flow to facilitate the capture of suspended food particles, a feeding strategy that differs from that of many motile filter feeders. Filter feeding provides several ecological and evolutionary advantages to sponges. Sponges can conserve energy by minimizing the need to actively search for food particles, thus reducing their energy budget. Sponge filtration also provides an advantage over other organisms in terms of resilience, as it enables them to survive and thrive in nutrient-deficient environments. Sponges act as natural water purifiers by absorbing organic particles, thereby helping to maintain water quality. Additionally, they assist in nutrient cycling and provide shelter to surrounding organisms, thereby maintaining the ecological balance and stability of freshwater ecosystems. Furthermore, additional research is needed to investigate the significant role of sponges

as bioindicators for evaluation of water quality. In addition, sponges can be considered as a model organism for studying filter feeding and regeneration in lower invertebrates.

From the experiment, it is thus concluded that the dye movement primarily indicates the passage of water through the sponge body. However, several factors should be considered when interpreting this observation. These include the precise location where the dye was injected within the sponge body, the distance between injection points and the nearest osculum, the volume of dye injected and expelled, hydrological parameters of the pond, the pumping rate, the sponge's health condition during the experiment, the time between dye injection and expulsion, and the path of dye migration through the sponge's cross-section are some of the areas requiring further research. Furthermore, additional research would contribute to a more comprehensive understanding of the precise mechanism of filter feeding and dye expulsion by the sponge in the future.

Conflict of Interest

There is no conflict of interest that may have influenced either the conduct or the presentation of the research.

Acknowledgement

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