Volume 3 | Issue 2, 2023 | Pages 66 - 72

KRISTU JAYANTI JOURNAL OF CORE & APPLIED BIOLOGY

REVIEW ARTICLE

Hydra – A Powerful Lab Model for Life Science Teaching and Research

Prasanna Kumar C

Department of Life Sciences, Kristu Jayanti College (Autonomous), Bangalore, Karnataka- 560077, India

Abstract

Hydra, a unique freshwater diploblast, not only boasts a well-defined body plan and an intricate nervous system but also stands out for its possession of stem cells. Revered as one of the oldest model organisms in biological research, Hydra defies aging and retains embryonic traits even in adulthood. Its remarkable regenerative prowess adds to its mystique, making it an invaluable resource for unraveling the evolution of complex animal forms. This article sheds light on Hydra's extraordinary features, emphasizing its potential significance in both scientific exploration and life science education.

Keywords

Hydra culture, Regeneration, Toxicity assay, Artemia, Alternative animal

Article History
Received: 17 July 2023
Revised: 22 October 2023
Accepted: 24 October 2023
Published online: 29 December 2023

Author for Correspondence
prasanna.kc@kristujayanti.com

1. Introduction:

Early attempts in the eighteenth century to study marine organisms in simulated natural conditions, focusing on corals and mollusks takes a turn in 1740 when Abraham Trembley, with a background in mathematical infinity, began his pioneering investigations into aquatic insects (Ratcliff, 2012). Trembley's remarkable findings included the discovery of unique mobile tubes in water, which he carefully studied and successfully maintained in glass jars, even transporting them over significant distances via the postal system. Trembley conducted a series of experiments, leading to the regeneration of entire polyps and the creation of multiheaded hydra. Despite its mythological name, hydra captivates observers with its beauty and delicacy. Trembley's work, published in 1744, is widely recognized as the 'Trembley' effect, a pivotal moment in marine biology that encouraged the study and laboratory manipulation of marine creatures, despite hydra's freshwater habitat (Ratcliff, 2012).

Hydra, a model organism in biology (fig. 1), is a diploblast with two cell layers—ectoderm and endoderm. Inhabiting freshwater environments, it belongs to the Cnidarian phylum, characterized by a simple yet well-defined body structure. Unlike sponges lacking a distinct body axis, hydra surpasses them in structural complexity. In contrast to humans with three primary body axes and bilateral symmetry, hydra exhibits an oral-aboral axis and radial symmetry.

Morphological and cellular characteristics of Hydra

Hydra, a freshwater diploblast, exhibits radial symmetry and an oral-aboral axis (fig. 2).

Cite this article as: *Prasanna KC (2023). Hydra – A Powerful Lab Model for Life Science Teaching and Research. Kristu Jayanti Journal of Core and Applied Biology. 3(2):66-72*

Fig. 1: Hydra (H. vulgaris) polyps thriving in a laboratory setting.

With a conical 'hypostome' and 5-7 tentacles, it captures prey using nematocytes. The aboral end has a foot-like structure with a basal disk for attachment. The body column houses a gastric cavity for digestion. Reproducing asexually through budding, hydra can also reproduce sexually in unfavorable conditions. With 20 to 25 cell types, the organism's ectoderm and endoderm are mainly composed of epithelial cells. A nerve net aids in prey detection, while gland and mucous cells in the endoderm facilitate digestion.

Unlocking the secrets of immortality: Hydra's unique resistance to aging processes

Hydra, in its peculiarity, defies the norm by not experiencing organismal senescence; it remains potentially immortal. While conventional wisdom links aging to cellular aging, with cells in culture also undergoing this process, hydra escapes aging through the remarkable resilience of its three robust stem cell populations. Constantly dividing and differentiating, these stem cells replace aging cells, ensuring a perpetual youthfulness in hydra polyps. Notably, some hydra species do undergo aging post-gametogenesis. It's intriguing to highlight that reports of hydra developing tumors are exceedingly rare, and the occurrence of natural mutants is scarce. Inducing stable mutations in hydra is also notably challenging. Nevertheless, scientists have achieved a breakthrough by successfully introducing foreign genes into hydra, paving the way for the creation of transgenic strains with tremendous potential in the field of hydra biology.

Hydra's Regenerative Marvel: Unveiling the Secrets of 'Morphallaxis' and Cellular Resilience

Hydra's remarkable regenerative prowess stands out as one of its most captivating features (fig. 3). Able to regenerate lost body parts with almost limitless potential, hydra exhibits a unique process termed 'morphallaxis.' Unlike epimorphosis observed in amphibians, which involves growth, morphallaxis enables hydra to regenerate without an increase in cell numbers, resulting in a smaller regenerate compared to the original polyp.

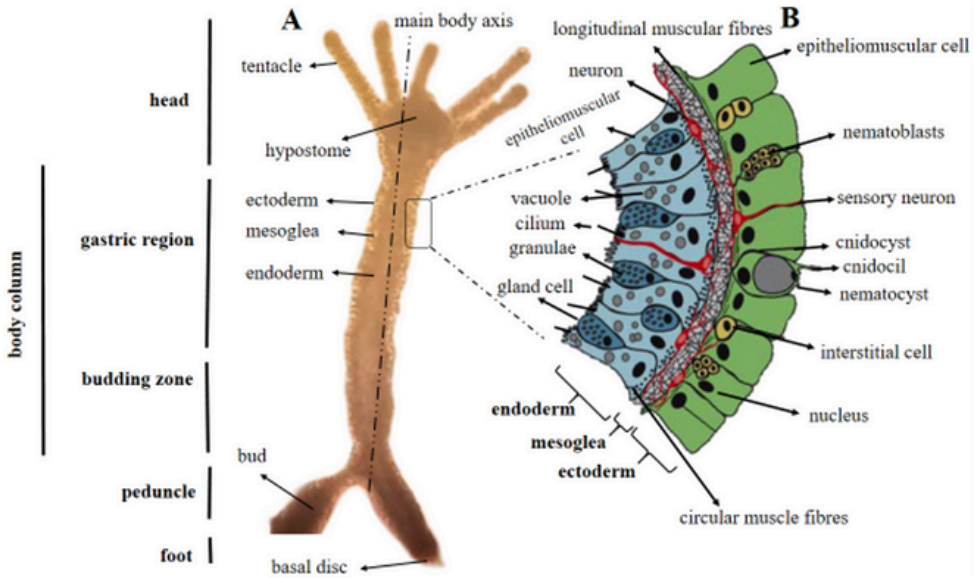

Fig. 2: Anatomy of a hydra polyp. [A] Hydra polyp is a tubular structure with two layers, featuring a circlet of tentacles encircling the mouth opening at the hypostome's tip. Asexual budding takes place in the lower body column. Interstitial stem cells and nematoblasts are evenly dispersed throughout the body column, situated beneath the tentacle ring and above the peduncle border, which connects the budding region to the pedal disc. [B] The Hydra polyp exhibits a bilayered cellular arrangement with ectoderm and endoderm separated by the acellular mesoglea matrix (gray). All Hydra epithelial cells are myoepithelial, featuring basal myofibers (red). Longitudinally oriented fibers are present in ectodermal cells (green), while circumferentially oriented fibers (ring muscle) are observed in endodermal cells (pink). Interstitial cells and nematoblast clusters are predominantly found among the ectodermal epithelial cells, while neurons are present in both the endoderm and ectoderm layers. Sensory neurons connect to ganglion neurons (shown in purple), which are located at the base of the epithelium above the myofibers and occasionally cross the mesoglea. Various gland cells, mostly located in the endoderm, are scattered among the epithelial cells.

Virtually all body parts of hydra, excluding tentacles and the basal disk, can regenerate to varying degrees, even from a cell pellet. Notably, cutting a hydra into three pieces reveals the middle piece's ability to regenerate both head and foot precisely where they were originally located. This indicates the presence of information within the cells directing regeneration in the original orientation. Lewis Wolpert, inspired by hydra experiments and limb regeneration in chick embryos, proposed the theory of morphogen gradients and positional information in the late 1960s (Vargesson, 2019). Morphogens, chemical entities driving morphogenesis, form concentration gradients that guide cell fate, ensuring proper regeneration. For instance, in hydra, a gradient of head-forming molecules exists, with the highest concentration at the cut end nearer the original head.

Wolpert's theory suggests that cells in the gradient 'sense' their position based on morphogen concentration, a concept now recognized as crucial in developmental processes across organisms. Hydra's regenerative capacity offers a unique platform to study pattern-forming mechanisms at morphological, cellular, and molecular levels. Understanding hydra's regeneration mechanisms may unlock insights into why more complex organisms exhibit limited regenerative abilities. In the future, this knowledge could potentially enhance regenerative capacities in other organisms.

Application of Hydra in Life Science Teaching and Research

Hydra polyps offer a valuable educational tool for teaching fundamental biological processes, including asexual reproduction and tissue regeneration. Simple experiments can be designed to engage students in colleges and schools, capitalizing on hydra's ease of collection and maintenance in comparison to more complex animals (Kovačević et al., 2024). Furthermore, hydra enables the development of short and long-term research projects, making it accessible for places with limited infrastructure. For example, experiments can focus on the asexual reproduction of hydra, studying the rate of budding and tentacle emergence patterns during this process which also act as a significant taxonomic key for species identification (Londhe et al., 2017). Additionally, hydra serves as a model for studying the impact of unnatural culture conditions, such as varying temperatures or the introduction of exogenous chemicals, providing insights into sensitivity and environmental responses (Patwardhan and Ghaskadbi, 2013). Its aquatic nature makes hydra a sensitive indicator of pollution in freshwater bodies, allowing for the assessment of chemical toxicity (Murugadas et al., 2019; Yoganathan and PrasannaKumar, 2022; Fathima et al., 2024). Protocols for hydra culture maintenance and basic life science experiments are provided at the end of this article.

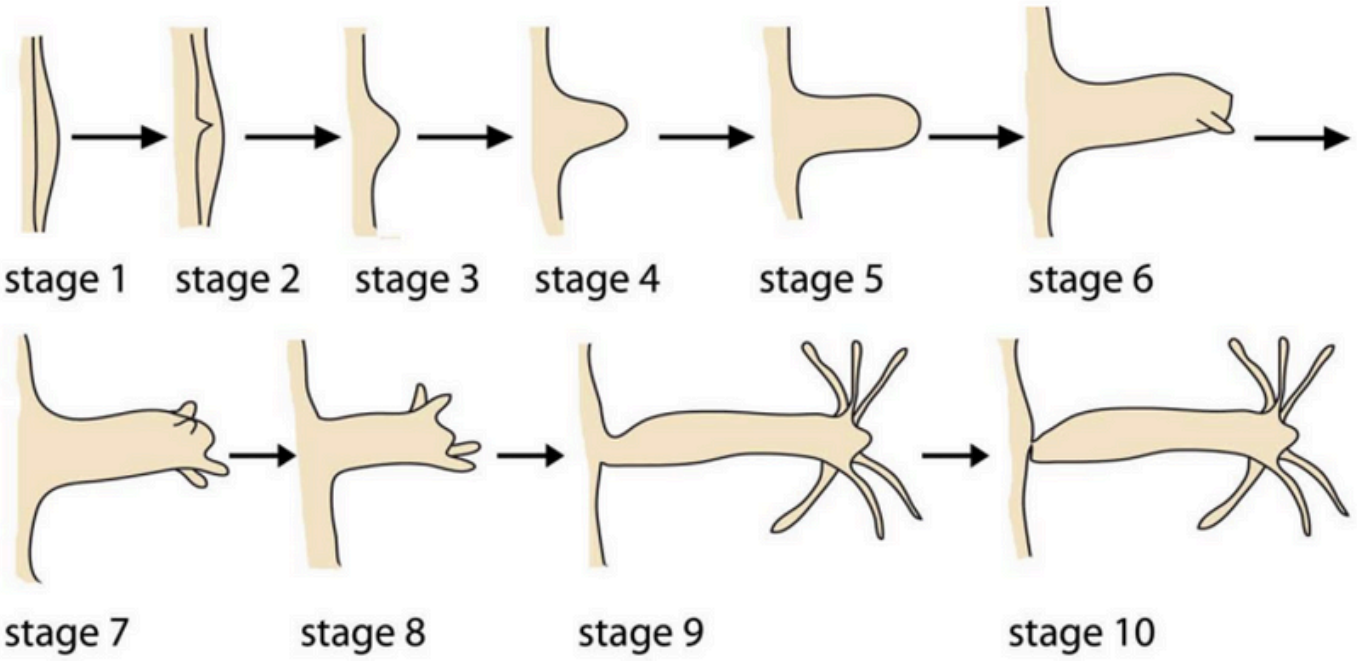

Fig. 3: A schematic representation illustrates the asexual or vegetative reproduction of Hydra through budding almost from any body parts (adapted from Otto and Campbell, 1977). During stages 1-5, both the ectoderm and endoderm undergo evagination, while stages 6-8 involve the evagination of tentacles. In the final two stages (9-10), differentiation occurs in the peduncle region and the basal disc.

Based on my own experiences, I want to emphasize a crucial observation. While providing Hydra to various educational institutions across Tamil Nadu, India, I have noticed that only a few have managed to sustain Hydra cultures over an extended period. Despite encountering numerous challenges, I have continued to support these institutions in the hopes of cultivating robust Hydra cultures. Unfortunately, I have seen a pattern where initial enthusiasm wanes, resulting in the neglect of Hydra, leading to their starvation and eventual demise. It is essential to understand that Hydra requires constant care and daily feeding with live prey, similar to caring for a pet. Just as we prioritize feeding our pets before ourselves, Hydra needs daily attention unless an experiment involves intentional fasting. The typical workweek structure, mandatory Sunday holidays, and public holidays should not prevent researchers and students from accessing the lab for Hydra maintenance. Some teachers and students might lose motivation during long weekends and vacations, but for those aiming to use this delicate and fascinating organism to inspire a love for biology in students, these sacrifices are minor. Despite the difficulties, the benefits of establishing a meaningful connection with Hydra and fostering a genuine interest in life science are undeniably valuable.

PROTOCOLS

1. Hydra culturing and maintenance

Culture a clonal population of hydra in glass crystallizing dishes filled with 1X hydra medium at a consistent temperature of 18 ± 1˚C, following a 12-hour day and night cycle. Nourish the polyps with recently hatched Artemia salina nauplii and replace the medium six hours after feeding (Sugiyama and Fujisava, 1977).

1.1. Composition of 1X Hydra Medium

KCl – 0.1 mM
NaCl – 1.0 mM
MgSO4.7H2O – 0.1 mM
CaCl2.2H2O – 1.0mM
Tris Base – 1.0 mM (Use pH 8.0 Tris-Cl solution)

1.2. Preparation of 1X Hydra Medium

Dissolve all the listed components, excluding MgSO4 (to prevent precipitation), in distilled water. Add the separately dissolved MgSO4 solution to this mixture. Adjust the pH to 8.0. Then, reach the final volume of 1 L and autoclave for 40 minutes. It is recommended to create a 100X hydra medium stock and dilute it with distilled water to achieve a 1X concentration for practical use.

1.3. *Artemia nauplii* hatching

Artemia nauplii hatch in a broad range of salinities, provided there is sufficient oxygen (fig. 4); however, their survival depends on the appropriate ionic balance.

1. Start the hatching process by adding 500 ml of tap water to Griffe beaker, followed by the addition and thorough mixing of 2g of NaCl.
2. Subsequently, place the aeration stone at the beaker's bottom and connect it to the low-pressure air pump.
3. Sprinkle a pinch of dried Artemia eggs over the surface, mix the eggs by stirring with a glass rod, and initiate aeration by turning on the aquarium air pump.
4. Depending on the surrounding temperature, cysts will typically hatch within 24 to 48 hours.

1.4. Harvesting, desalting and feeding

1. After 24 hours, turn off the air pump to collect the hatched Artemia.
2. Since Artemia larvae are photophilic (attracted to light), expose one side of the beaker to intense light to gather them easily.
3. Within minutes, the hatched Artemia will congregate near the light at the bottom of the container, while the unhatched eggs will float to the surface.
4. The hatched Artemia will appear as an orange mass, which can be collected using a 25 ml long glass pipette. It is important to harvest only the hatched Artemia, as Hydra cannot digest the unhatched eggs.
5. To remove the salt from the hatched Artemia, transfer them to a petri dish containing Hydra medium (HM) and allow them to settle at the bottom.
6. Repeat this transfer through two additional petri dishes pre-filled with Hydra medium.

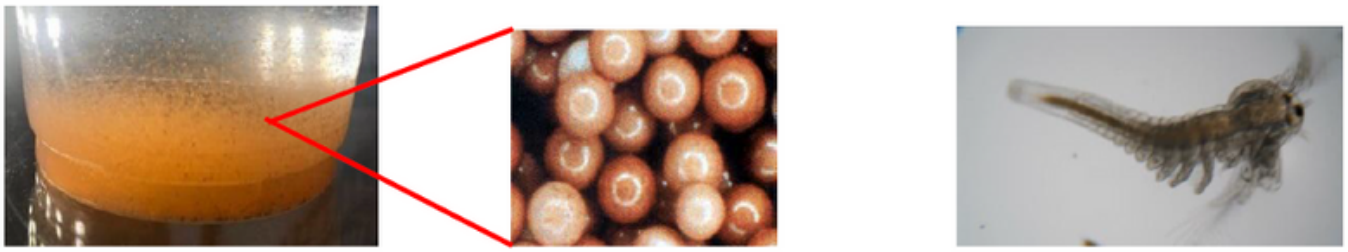

Fig. 4: (A) The Artemia cyst after absorbing water, (B) Newly hacted Artemia nauplii

7. The entire process takes around 20 minutes.
8. Finally, transfer the fully hatched and desalted Artemia to a small beaker for feeding Hydra.
9. Provide approximately 20 Artemia per Hydra.
10. After 6 hours, refresh the container by replacing the Hydra medium.

2. Hydra regeneration experiment

Experiment on Hydra regeneration can be conducted in following steps;
1. Place hydra that has been starved for 24 hours on a clean glass slide to allow them to relax.
2. Use a sharp blade or needle to cut the hydra into two or three pieces.
3. Separate the pieces (head, middle, and base) into individual wells of a six-well plate filled with hydra medium.
4. After 24 hours, monitor the regeneration of the head and foot.

Head segments will regenerate a foot, middle segments will regenerate both a head and a foot, and foot segments will regenerate a head. The regeneration process is easily observable, with tentacle rudiments indicating head regeneration and a sticky secretion indicating foot regeneration. Foot-specific staining can be conducted 48-72 hours after regenerating head and middle segments to observe functional foot formation. To assess toxicity, two sets of bowls can be maintained: one with the test substance and one without. The scoring system developed by Ambrosone et al. (2012) could be adopted to evaluate the influence of toxic compounds on Hydra's regenerative capacity (fig. 5).

2.1. Foot-specific Staining

The basal disk of hydra possesses mucous cells that harbor a peroxidase-like enzyme, enabling the selective staining of these cells using peroxidase substrates. In this context, ABTS (azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) is utilized as a peroxidase substrate. This enzyme serves as a reliable indicator for distinguishing mucous cell differentiation in the regenerating foot (Hoffmeister and Schaller, 1985).

2.2. ABTS Solution

Citric Acid – 65.5 mM
Tri-sodium citrate – 34.5 mM
ABTS – 0.1%
Hydrogen peroxide – 0.003%.

Between 48 and 72 hours, pieces regenerating the foot can undergo staining to observe the development of a fully functional foot.

2.3. Procedure for staining the regenerating foot:

1.Take head pieces in a six-well plate, approximately 10 pieces per well, that are undergoing foot regeneration.
2. Completely remove the hydra medium and add 2–3 ml of ABTS solution with hydrogen peroxide
3. Cover the plates with aluminum foil and incubate at room temperature for 5–15 minutes until a pink color appears.
4. Stop the reaction by adding 1X PBS (pH 5.0) or by washing the pieces with distilled water.
5. After washing for 15–20 minutes, transfer the head pieces to fresh PBS and observe.
6. A purple ring can be seen at the tip of the regenerating foot.

2.4. Acute toxicity testing

Hydra are well-suited for conducting acute toxicity tests (example, against heavy metals), and one can adopt the methodology proposed by Zeeshan et al. (2016).

1. Place ten polyps without buds in a small petri dishes containing 8 mL of Hydra medium, and incubate them in a Bod incubator. The quantity of petri dishes can correspond to the number of distinct concentrations of the toxic substance being tested.
2. Conduct all the exposures consistently in triplicates.
3.Continuously expose the polyps to different concentrations of heavy metal ranging from 1µg/L to 65µg/L (prepared in 1X hydra media) for 96 hours.
4.Document the evolving changes in morphology at 24-hour intervals using a stereo-zoom dissecting microscope equipped with a camera.
5.Assign a score of 10 to healthy polyps and a score of 0 to animals that have disintegrated (table 1 & fig. 6).
6.Animals with scores ranging from 9 to 1 exhibit varying degrees of morphological changes. The median lethal concentration 50 (LC50) for each time point is determined based on the observed median score and calculated using PROBIT analysis.

Determining the LC50 helps establish sub-lethal concentrations to evaluate the low-dose level toxicity of heavy metals on Hydra's regeneration and feeding behaviour.

4. Measuring toxicity induced apoptosis

Apoptosis, a programmed cell death, entails distinct morphological changes. Reliable cell death detection is vital due to its implications in various conditions. Acridine orange, a cationic acidophilic dye, selectively stains apoptotic cells within phagocytic vacuoles with low pH (Clerc and Barenholz, 1998). It fluoresces green/yellow, differentiating live, early apoptotic, late apoptotic, and necrotic cells based on distinctive nuclear features in hydra. For measuring toxicity induced apoptosis in hydra;

1.Treat a group of 10 Hydra animals with two different concentrations of the target substance and incubate them for 48 hours.
2.Wash the treated animals in Hydra medium at least five times, each time for 5 minutes.
3.Transfer the animals to a glass slide.
4.Apply a drop of acridine orange (3.3 µM) to the polyp and incubate it in the dark for 15 minutes.

Table 1: The morphological damage assessment scoring system introduced by Wilby (1988).

SCORE	MORPHOLOGY OF POLYP
10	Extended tentacles and body responsive
9	Partially contracted with slow reactions
8	Clubbed tentacles and slightly contracted body
7	Shortened tentacles with slightly contracted body
6	Tentacles and body both shortened
5	Fully contracted with visible tentacles
4	Fully contracted with no visible tentacles
3	Expanded with visible tentacles
2	Expanded with no visible tentacles
1	Dead but intact
0	Disintegrated

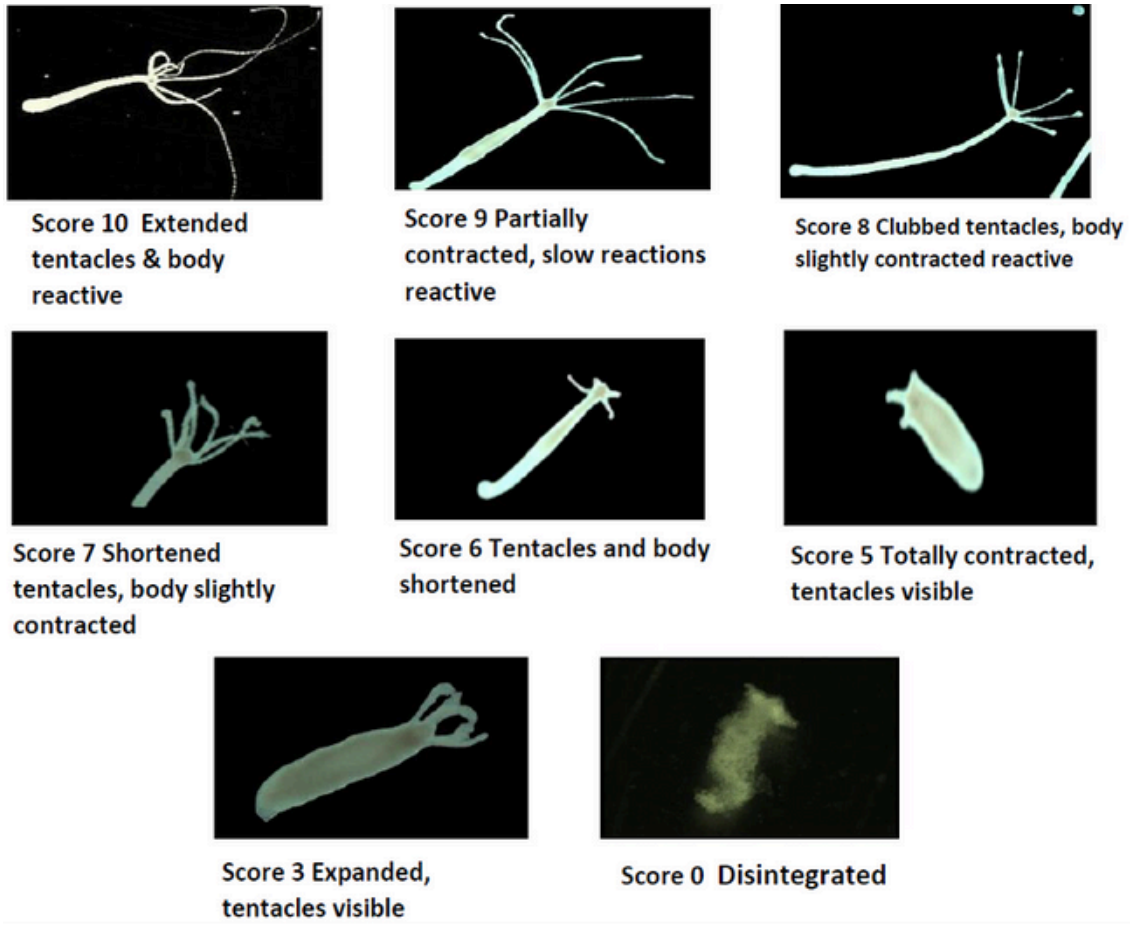

Fig. 6: Evaluating the impact of toxicity on hydra morphology based no Wilby's scoring system

5. Wash the stained animals four times in Hydra medium, each time for 5 minutes.
6. Add 2% urethane to the slide to relax the animals.
7.Observe the animals under an epifluorescent microscope and capture photographs.

References:

Ambrosone A, Mattera L, Marchesano V et al (2012) Mechanisms underlying toxicity induced by CdTe quantum dots determined in an invertebrate model organism. Biomaterials 33(7):1991–2000. https://doi.org/10.1016/j.biomaterials.2011.11.041

Clerc, S., & Barenholz, Y. (1998). A Quantitative Model for Using Acridine Orange as a Transmembrane pH Gradient Probe. In Analytical Biochemistry (Vol. 259, Issue 1, pp. 104–111). Elsevier BV. https://doi.org/10.1006/abio.1998.2639

Fatima, J., Ara, G., Afzal, M., & Siddique, Y. H. (2024). Hydra as a research model. In Toxin Reviews (Vol. 43, Issue 1, pp. 157–177). Informa UK Limited. https://doi.org/10.1080/15569543.2024.2306544

Hoffmeister, S., & Schaller, H. C. (1985). A new biochemical marker for foot-specific cell differentiation in hydra. In Wilhelm Roux's Archives of Developmental Biology (Vol. 194, Issue 8, pp. 453–461). Springer Science and Business Media LLC. https://doi.org/10.1007/bf00868146

Kovačević, G., Korać, P., Želježić, D., Sertić Perić, M., Peharec Štefanić, P., Sirovina, D., Novosel, M., & Gottstein, S. (2024). Hydra for 21st Century—A Fine Model in Freshwater Research. In Water (Vol. 16, Issue 15, p. 2114). MDPI AG. https://doi.org/10.3390/w16152114

Londhe, R., Krishnapati, L. S., & Ghaskadbi, S. (2017). Description and Phylogenetic Characterization of Hydra from Naukuchiatal (Uttarakhand, India) and Comparison with other <i>Hydra</i> Strains. In Current Science (Vol. 113, Issue 09, p. 1739). Current Science Association. https://doi.org/10.18520/cs/v113/i09/1739-1745

Murugadas, A., Mahamuni, D., Nirmaladevi, S. D., Thamaraiselvi, K., Thirumurugan, R., & Akbarsha, M. A. (2019). Hydra as an alternative model organism for toxicity testing: Study using the endocrine disrupting chemical Bisphenol A. In Biocatalysis and Agricultural Biotechnology (Vol. 17, pp. 680–684). Elsevier BV. https://doi.org/10.1016/j.bcab.2019.01.009

Patwardhan, V. and Ghaskadbi, S. (2013). Invertebrate alternatives for toxicity testing: Hydra stakes its claim, ALTEX Proc., 2, 69–76.

Ratcliff, M. J. (2012). The Trembley Effect or the birth of marine zoology. In The International Journal of Developmental Biology (Vol. 56, Issues 6-7–8, pp. 425–436). UPV/EHU Press. https://doi.org/10.1387/ijdb.123520mr

Sugiyama, T., & Fujisawa, T. (1977). Genetic analysis of developmental mechanisms in hydra i. Sexual reproduction of Hydra magnipapillata and isolation of mutants. In Development, Growth and Differentiation (Vol. 19, Issue 3, pp. 187–200). Wiley. https://doi.org/10.1111/j.1440-169x.1977.00187.x

Vargesson, N. (2019). Positional Information—A concept underpinning our understanding of developmental biology. In Developmental Dynamics (Vol. 249, Issue 3, pp. 298–312). Wiley. https://doi.org/10.1002/dvdy.116

Wilby, O. K. (1988). In The Hydra regeneration assay, Proceedings of the workshop organised by association francaise de teratology. pp 108-124.

Yoganathan, P.R.S. and PrasannaKumar, C. (2022). Toxicological assessment of chromium using Hydra vulgaris as model organism. Unpublished. https://doi.org/10.13140/RG.2.2.13484.54403

Zeeshan, M., Murugadas, A., Akbarsha, M.A., 2014. Alternative model organisms for toxicity testing and risk assessment, in: Mathur, P.P. (Ed.), In Contemporary Topics in Life Sciences. Narendra publishing house, Delhi, pp. 259-275