



Culturing of *Hydra viridissima* fed with *Moina macrocopa* under varying conditions of light and feed

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Abstract

Hydra viridissima (formerly *Chlorohydra viridissima*) appears green, owing to its symbiotic relationship with the green alga, *Chlorella vulgaris*, which are present in the body of the Hydra. *Hydra viridissima* are carnivores and are able to consume animals roughly the volume of their own bodies. The objective of this experiment was to study the symbiosis between *Chlorella* and *Hydra* along with the population regeneration of *Hydra viridissima* in varying conditions of feed of *Moina macrocopa* and light. The Hydras were maintained in dechlorinated water under five conditions viz. Light conditions:- no feed - only light, feed as well as light, Dark conditions:- only feed – no light, no feed- no light, excessive feed – no light. The set up was observed for 14 days continuously. *Moina macrocopa*, which is a crustacean, was cultured separately in bottles. It was observed that the best regeneration of *Hydra viridissima* occurred in feed as well as light conditions. It was also observed that *Chlorella* leaves hydra when there is insufficient light and Hydras became pale coloured.

Keywords: *Hydra viridissima*, *Chlorohydra viridissima*, *Moina macrocopa*, *Chlorella*

Introduction

Hydra viridissima have symbiotic association with *Chlorella* alga, which are found in vacuoles within the gastro-dermal cells, where they are protected from host digestive enzymes (Muscatine, 1965) ^[7]. Up to 20 algal cells may coexist in the same animal cell (Douglas, 1995). The photosynthetic alga supplies nutrients such as maltose (Muscatine, 1965) ^[7] or glucose-6-phosphate (Lenhoff and Muscatine, 1963; Roffman and Lenhoff, 1969; Kelty and Cook, 1976) ^[5, 8, 4] to the Hydra. *Chlorella* can also be found living freely outside of hydras. During times of starvation, hydra will rely on their *Chlorellae*, which are classified as a genus of algal symbionts that provide energy to their host through photosynthesis (Hamada et. al 2018) ^[2].

Hydra viridissima is typically 10 mm long and have tentacles that are about half of their length. They are strictly carnivorous and typically feed on small crustaceans, insects and annelids. *Hydra* are normally sessile and live on aquatic vegetation. They secrete mucous to attach to substrate using their basal disc. Hydra reproduces both asexually and sexually. The first, asexual method, involves budding new individuals from the body wall. This is the usual method of reproduction in warmer months.

Hydra are exceptionally interesting due to their regenerative ability and that they appear not to age or die of old age. However, they can still die from means other than aging (senescence), such as through injury, disease, starvation, or unsuitable conditions in the habitat. They are easy to culture and maintain in the laboratory, reproduce rapidly and can be used in toxicity tests that are both simple to perform and low-cost (Trottier et al., 1997) ^[11].

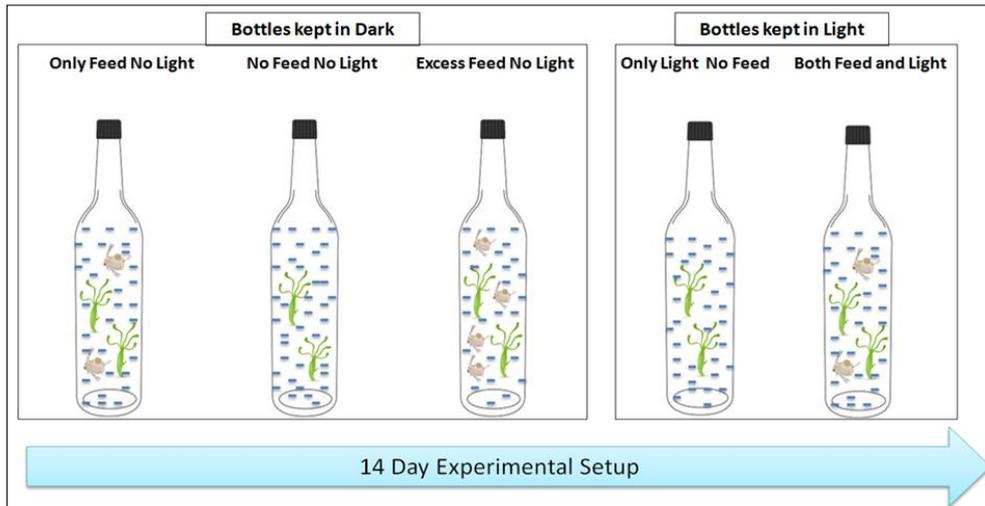
The feed, *Moina macrocopa*, referred to as “Water fleas”, although they are not true fleas, are tiny crustaceans, measuring 0.2-0.5mm in length as adults. *Moina* are fed to Hydras as they are easy to culture compared to other animals like *Artemia*. The experiment was carried out to study the growth of Hydra in varying conditions of light and feed and to find out what happens to Hydras when they are starved and what is the status of *Chlorella* in dark condition.

Materials and Methods

Maintenance of Hydra cultures: *Hydra viridissima* was procured from Homi Bhabha Centre for Science Education, Mumbai. Cultures were maintained in dechlorinated water. 1000 ml of tap water was taken in a beaker, kept the water in open for dechlorination. 10 glass bottles of 10ml capacity were taken and 5ml of dechlorinated water was taken. Two hydras were added to each of the glass bottles. The Hydras were daily fed with *Moina macrocopa*. Five sets were maintained: Light conditions:- I) no feed - only light, II) feed as well as light, Dark conditions:- III) only feed – no light, IV) no feed- no light, V) excessive feed – no light. (Normal feed -1Hydra: 1 Moina, Excess Feed- 1Hydra: 2 Moinas).

The cultures were maintained at room temperature 20 ± 2 °C, in the month of February 2023 when the daylight was available for approximately 10 hours. Every day the animals were fed with *Moina macrocopa*. (Fig 3 showing Hydras feeding on Moina). For dark, the bottles were wrapped with aluminum foil. These animals were exposed to no more than 2-4 minutes of light daily for counting, feeding and routine maintenance. A separate culture of *Moina* was maintained in transparent plastic bottles.

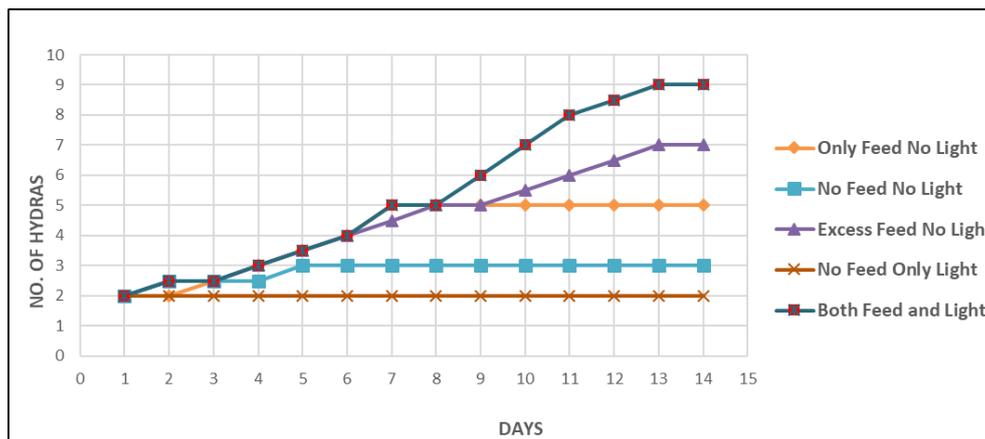
Experimental setup to study *Chlorohydra*



Each set had duplicates. The set up was observed for 14 days and observations recorded. The experiment was started with 2 Hydras in each bottle. A value of 2.5 indicated that one of the Hydra produced a Hydranth/ bud. A value of 3 indicated that the bud separated from the mother Hydra.

Hydra that could not be seen or observed were categorized as 0 and were assumed to have died and disintegrated. The photos of hydras were taken by Samsung Monile camera everyday.

Results



Graph 1: Showing Population growth curve of *Hydra viridissima* cultured in dechlorinated water at 20 ± 2 °C, in light and dark fed on *Moina macrocopa*

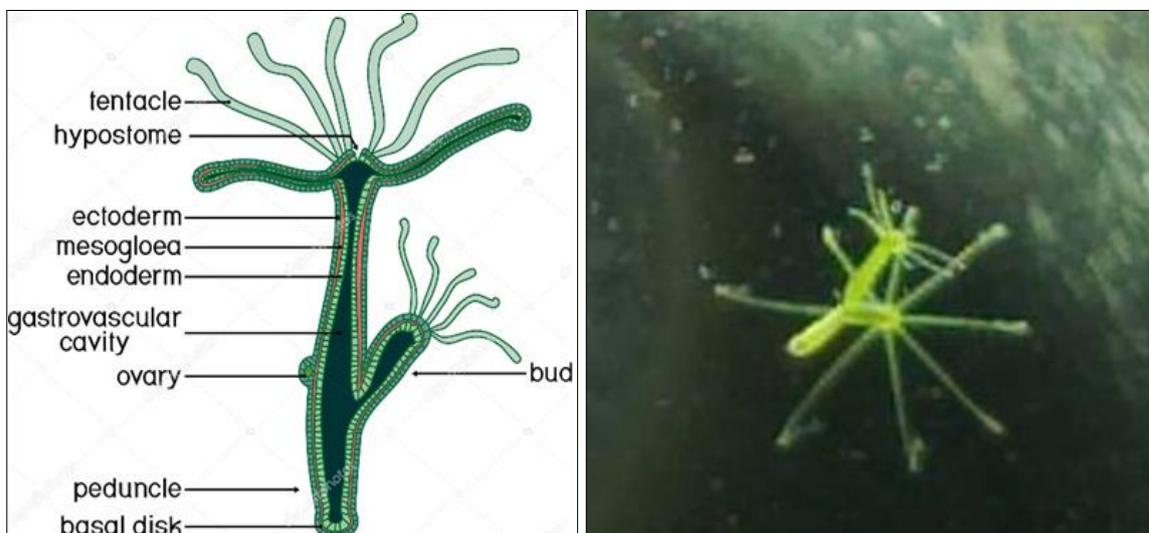


Fig 1: *Hydra viridissima* Diagrammatic and Photo of *Hydra viridissima* by mobile camera



Fig 2: *Hydra viridissima* feeding on *Moina macrocopa* by Hypostome

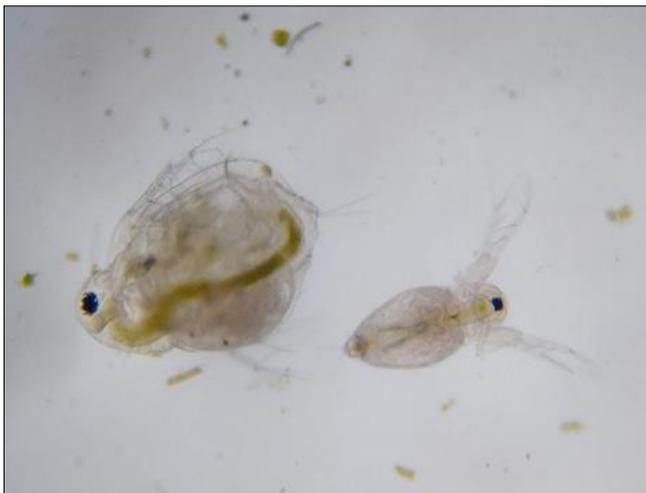


Fig 3: *Moina macrocopa*



Fig 4: *Chlorella* isolated from the base of the bottles

Discussion

Experiment was conducted for about 14 days in the month of February 2023 when the temperature was 20 ± 2 °C. It was observed that hydras which were kept in light conditions were healthy, green colored and survived for 14 days. Those which were fed under light conditions reproduced and increased in number compared to those which were not fed.

Hydras which were kept in dark (given feed in the ratio 1:1, excess feed 1:2 and no feed) started budding on the 4th day and flourished well but eventually lost *Chlorella* and

became pale coloured. Similar results were obtained by Rosevelt L. Pardy (1974) [9]. In the absence of light, *Chlorella* came out of Hydra, which accumulated at the bottom of the glass bottles as shown in Fig.4.

According to Rosevelt L. Pardy (1974) [9] Green hydra survive and bud longer than algal free hydra under starvation conditions. The assumption is that maltose is translocated to the host by the algae *in vivo* and serves as nutritional supplement for the host during periods of starvation.

The effect of light and feeding on the growth of algae in hydra can be viewed as the consequence of two distinct but interrelated factors: the necessity of light for maximum algal multiplication, and tissue growth in hydra. Light has been shown to be a necessary condition for a variety of algal processes (photosynthesis, organic and inorganic nutrient assimilation, ion uptake) including division in certain strains of *Chlorella vulgaris* (Griffith, 1961) [1]. Algal multiplication in green hydra is strongly light dependent. Under constant light and feeding, the number of hydra increased exponentially (Graph 1).

In starved hydra the rate of animal growth decreases (Muscatine, 1961) [6] and with time the hydra cease multiplying altogether.

It was observed that Moinas were feeding on *Chlorella*, which left the Hydras in dark conditions. The intestine of Moina appeared green coloured in the culture of Hydras (Fig.3.) *Chlorella vulgaris* is commonly used in *M. macrocopa* culture (Jose *et al.*, 2015) [3]

From the experiment it is concluded that the *Hydra* has a great symbiotic association with *Chlorella*. When the *Hydra* was kept in dark and not given enough sunlight *Chlorella* abandons *Hydra* and comes out of it leaving *Hydra* pale/colorless. The best growth of Hydra occurred when enough feed and light was given to the Hydras. When the Hydras are given feed and kept in dark, they grow fairly well but when they are not given any feed and only light is provided, they survive but growth is severely hindered (Graph: 1).

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