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Hydra as a model organism to teach biology in secondary schools

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ABSTRACT

Aim: Using a personal narrative, highlight the influence that university research can have on secondary education.

Questions: How does the presence of algae in individual hydra affect hydra regeneration? What is the role of hydra body size in the response? How do hydra respond to red and blue light?

Organisms: Two strains of the green hydra, *Hydra viridissima*, one larger than the other.

Methods: Laboratory experiments with the simplest of materials and apparatuses.

Results: Brown hydra, which have no algae and are larger than green hydra, regenerate more successfully after food deprivation than do green algae. But green hydra in well-lit environments may lose control of their algae and find their intracellular spaces overgrown, leading to failure of regeneration and to disintegration of their cells. This fate was more likely in the larger strain of *H. viridissima*. Hydra are positively phototaxic to blue light and negatively phototaxic to red light.

Conclusions: There is a trade-off between body size and the ability of hydra to take advantage of an algal endosymbiont. Large hydra species and strains may be harmed by the algae within. Hydra have supported scientific awakenings in faculty and students of secondary schools.

Keywords: gene expression, hydra model, phototaxis, regeneration, teaching secondary school inquiry-based biology.

NARRATIVE

In 1980, I was midway through my high school teaching career. While on maternity leave, I registered for an introductory course in ecology and evolution taught by Lawrence Slobodkin at Stony Brook University. I found myself in a class surrounded by graduate students who appeared to understand the nuance of every statement. They actually laughed at his jokes. I was so lost I didn't know what questions to ask and, to make matters worse,

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I didn't even get the jokes. When I told him exactly that one day after class, he looked distressed (as only he can) and said, 'Oh dear this is unsettling but don't be discouraged. Most of them don't really get it either'.

Encouraged by this kindness, I volunteered to help maintain the hydra in his laboratory (Stony Brook University, 1980). At one point, I failed to feed the hydra stocks on schedule and so had inadvertently produced a lot of starving hydra. Such hydra are thin with elongated tentacles. They cease to produce buds. Then their bodies begin to shrink and their tentacles become short and stubby. If one severs their apical region – hypostome and tentacles – they regenerate owing to activation of a cellular reprogramming.

Some of my neglected hydra contained algal symbionts (green hydra) and some did not (brown hydra). My understanding of the relationship between the algae and the animal, at this time, was simply that being green must be good. I assumed that the algae would provide the host with some sort of carbohydrate supplement if normal prey were missing. This had already been demonstrated (Muscatine, 1965; Muscatine and Lenhoff, 1965; Mews, 1980; Mews and Smith, 1982), although at the time I did not know that.

I decided to see whether the regeneration potential was diminished in the hydra without algal endosymbionts. Indeed it was.

Seventy-two hours after amputation, the green hydra had regenerated completely. The brown hydra that did regenerate had fewer, shorter tentacles. Some brown hydra failed to regenerate any tentacles at all and soon disintegrated into a slurry of cells.

I repeated the experiment, prolonging the fast, looking for a point where the green hydra would also disintegrate after amputation. It didn't happen.

Determined to find a way to inhibit the green hydra's regeneration, I extended the fast further, amputated their hypostome and tentacles, and then put half in the dark and the other half in the light. Naturally, I assumed that green hydra placed in the dark would be unable to regenerate after a prolonged fast. But that didn't happen either. In fact, they regenerated better than those left in the light.

The hydras in the light were intensely green compared with those left in the dark and, although they could regenerate, their tentacles were stubby and they were subsequently unable to capture or ingest prey. Eventually, some of these intensely green hydras that could no longer capture prey also disintegrated. Using the methods of David (1973), I prepared slides of both sets of hydra and found significantly more algae per cell in the green hydra that regenerated in the light compared with those that regenerated in the dark.

It seemed to me at this point that being green was not without its downside. The cells of hydra amputees that I left to regenerate in the light after a long fast were completely filled with algae. The algae seemed to be growing beyond the capacity of the host cells to contain them, suggesting that they were detrimental to host survival, at least under these experimental conditions. I also noted that this algal overgrowth was more pronounced in the larger of the two green hydra strains I used. The green *Hydra viridissima* maintained in the laboratory at that time consisted of a variety of sub-strains, which, under steady-state conditions, where food intake and temperature were carefully controlled, reached significantly different body lengths (Bossert, 1987). Smaller strains of green hydra were better able to control this experimentally induced algal overgrowth. Larry suggested this should be written up and submitted for publication, which, with his help, I did, learning sentence by sentence how to write a scientific paper (Bossert and Slobodkin, 1983).

Only the smaller strains of hydra maintain relationships with algae (Bossert, 1987) and it is difficult to assume that this is because the larger strains have never encountered these algae,

which are relatives of the ubiquitous *Chlorella* (Oschman, 1967; Park *et al.*, 1967; Pardy, 1976a). The idea that whatever regulates the size of the hydra might have something to do with its ability to control the growth of its algal endosymbionts became a working hypothesis. The suggestion that larger host strains are less able to control algal growth, and that this might set a host size limit or developmental constraint on the evolution of the association, became the focus of my research.

At that time, two experiments supported the idea that hydra host cells controlled the growth of the algae within them. In one, Jolley and Smith (1978) showed that algal growth increased by a factor of 30 when cultured outside the host cell. Also, it was shown that there was a tight link between host and algal cell division such that algae could divide only when their host cell did. Algal division typically preceded host cell division, resulting in a transient increase in the number of algae per cell, but then, when the host cell divided, the number of algae per cell was restored to a steady state (McAuley, 1981, 1982).

I devised a series of experiments to test the hypothesis that the steady-state size of the green hydra would affect this tight link between host and algal cell mitosis. Green strains that achieved different sizes under controlled temperature and feeding regimes were forced to fast, then a single brine shrimp was placed onto the tentacles and observed until ingestion occurred. The experiment was repeated with two, three, and five brine shrimp systematically fed to replicates of individual hydra in each experiment. When I assayed host and algal cell mitotic index over a 24-h period, I found that the tight link between host and algal cell division existed only in the smallest of the green strains. As host size increased, the coordination broke down, so that in the larger strains I could see an increasing asynchrony in host and algal cell division and an increase in the number of algae per cell, which suggested that other mechanisms of control must be involved. Ken Dunn was also interested in mechanisms of regulation of algal endosymbionts and, using these experiments, he began to quantify his ideas that the host must also be capable of digesting the algae as a normal mechanism to prevent algal overgrowth of the host cell (Bossert and Dunn, 1986).

Since amputation of the apical region resulted in algal overgrowth under the experimental conditions described above, I became interested in what was happening in the cells of the regenerating stump. Grafting experiments with hydra had already established the distribution of positional information along the axis of the body (Browne, 1909; Webster and Wolpert, 1966) and others had shown that this organizer activity appears in the regenerating stump (Berking, 1979; MacWilliams, 1983a, 1983b). Chica Schaller (1973, 1975) and her colleagues (Schaller and Gierer, 1973) began to isolate and characterize (Schaller and Bodenmuller, 1981) the first of a series of morphogenic substances in hydra (Schaller *et al.*, 1979). In particular, a peptide they called 'head activator' was shown to have powerful mitogenic and morphogenic properties (Schaller, 1976; Schaller *et al.*, 1989). Another substance (still not as well understood but not a peptide) called 'head inhibitor' was shown to inhibit head formation (Schaller and Kemmner, 1984) [see Galliot *et al.* (2006) for a complete and recent review of the use of hydra to study developmental plasticity]. I used crude extracts of head activator and head inhibitor (Schaller and Kemmner, 1984; see also Schaller *et al.*, 1986) to study the effect they had on host and algal cell mitosis in hydra strains of different size. These studies became my doctoral dissertation (Bossert, 1987).

When I returned to teaching, I developed a research program for high school students in Northport, New York. Many of the research projects were done in student laboratories at Northport High School using hydra as a model to teach biology. Students used the hydra bioassay (Johnson *et al.*, 1982) to compare removal of heavy metals from water by various *Lemna* species, to detect remediation of guaiacol by enzymes on tomato plant roots (Adler *et al.*, 1994),

and to screen for toxicants in the Hudson River. Several students became Westinghouse and Intel Science Talent Search Semifinalists. Other of my high school students have investigated aspects of light in the biology of hydra based on Roosevelt Pardy's (1976b) phototaxis experiments. For example, Rushan Guan did the following, previously unpublished experiment.

Guan created phototaxis chambers by painting the sides of culture bowls black while leaving their bottoms clear. Each bottom was centred on top of a target consisting of four concentric circles with diameters of 8 cm, 4 cm, 6 cm, and 2 cm defining three rings and a central disk. The ring between 8 cm and 6 cm is Ring 1; Ring 2 lies between 6 and 4 cm, Ring 3 between 4 and 2 cm. The central disk is called Ring 4. The centre of Ring 4 was the centre of the target (Fig. 1). Finally, a metal plate containing a single hole was placed on the bowl, so that the hole was superimposed over the centre of Ring 4.

To study the effect of light colour on phototaxis behaviour, Guan placed Oriel-band and long-pass glass filters [either 450 nm (blue) or 700 nm (red)] over the hole. Then, he placed infrared filters over the coloured filters to minimize variation in temperature, and suspended low-watt, incandescent light bulbs over the bowls. To test the effectiveness of the infrared filters, he varied bulb height and recorded temperature. Water temperature in the bowls was 27°C regardless of bulb placement (Fig. 2).

Fig. 1. Phototaxis bowl. The student creates a 'bull's eye' and places the culture dish over it. Healthy hydra that have eaten 2 h before the start of the experiment are placed in Ring 2 at the start of each trial.

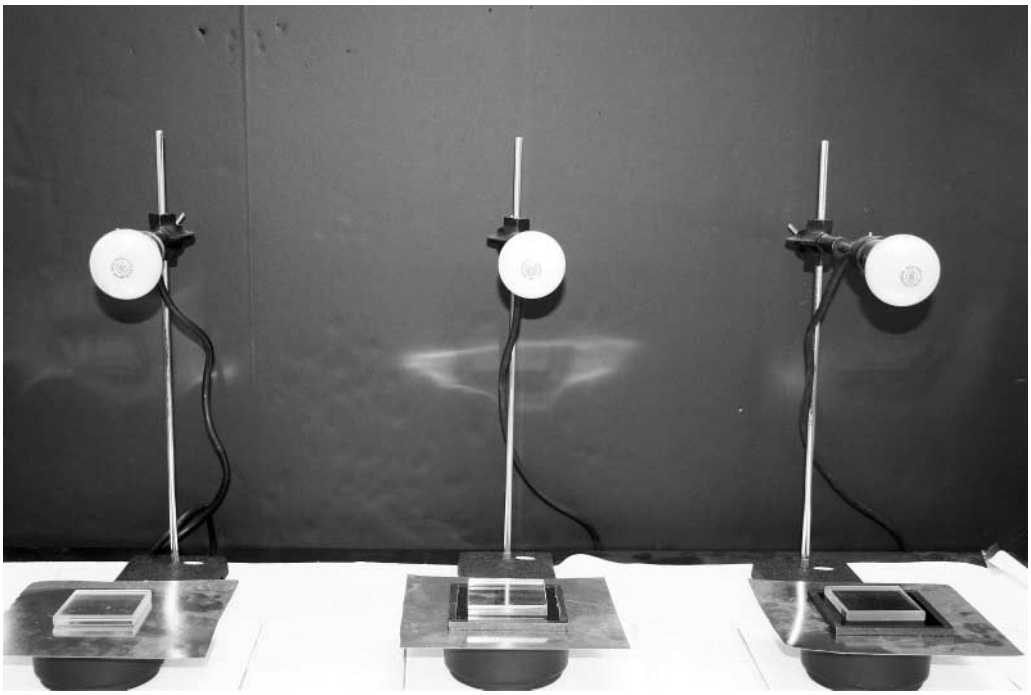

Fig. 2. Phototaxis experiment. Three phototaxis bowls are covered with a tight lid that allows light to enter only through the centre of the bull's eye. Each bowl has an infrared absorbing glass placed over the central hole. In this experiment, movement of hydra over the experimental interval is compared in hydra exposed to white light, blue light (450 nm filter), and red light (700 nm filter).

During each 4-day experiment, bowls were half-filled with hydra medium and either a blue or red filter laid over the bowl. At the start of an experiment (day 0), six hydras were placed in Ring 2. Hydras selected were always fed 2–3 h before placement. A selected hydra appeared healthy (i.e. had long tentacles), had food in its gastric cavity, and was not budding. Every day for 4 days, Guan recorded each hydra's Ring position and kept track of bud production. On the fourth day, he calculated an average final Ring position (AFRP). He replicated each 4-day experiment nine times.

Guan found that both green and brown hydra move away from red light and towards blue light (Table 1). Hydra move towards the outer ring when the red filter is in place but back towards the centre when it is replaced by the blue filter. Passano and McCullough (1962) showed that hydra are responsive to wavelengths at the blue end of the spectrum (450 nm), so we were not surprised by the strong positive phototaxic response to the blue light. But Passano and McCullough (1962) also reported that hydras are blind to red light, whereas in these experiments they showed strong negative phototaxis to red light. The evolution of extra-ocular perception has been recently reviewed by Santillo *et al.* (2006).

Even more surprising, red light seems to inhibit bud formation in green hydra. In red light, green hydra that have fasted produced only 0.3 ± 0.7 buds per hydra, whereas brown hydra produced 2.4 ± 1.8 (ANOVA, $P < 0.005$) (Table 2). We did not expect this result because green hydra survive a fast longer than brown hydra. We speculated that some aspect of algal metabolism, specifically activated by the red light, might inhibit budding. But we

Table 1. Results of the effect of light colour on phototaxis behaviour

	Wavelength			
	450 nm	700 nm	450 nm	700 nm
Experiment	Green hydra		Brown hydra	
1	4.0	1.2	2.0	1.3
2	4.0	1.7	3.6	1.1
3	3.3	1.0	2.5	1.0
4	3.6	1.0	2.3	1.1
5	2.9	1.0	3.1	1.0
6	3.8	1.6	2.6	1.4
7	3.6	1.1	2.4	1.7
8	3.9	1.3	3.4	1.9
9	3.5	2.3	1.7	1.3
Mean of means	3.6 ± 0.4	1.3 ± 0.4	2.6 ± 0.6	1.3 ± 0.3

Note: Both green hydra and brown hydra move strongly towards blue light (700 nm) (ANOVA; $P < 0.0001$ for each type of hydra). Green hydra move closer to blue light than do brown hydra ($P < 0.0007$). Both types of hydra move as far as possible away from red light (700 nm) in the experimental apparatus (1.0 is the minimum score possible). Experiments took place in a circular theatre (Fig. 1) with four numbered regions (or Rings): 'Ring 1' was farthest from light and 'Ring 4' closest to it. Each experiment lasted 4 days and involved six hydras that began in Ring 2. Numbers in the table report the average final Ring position (AFRP) of the six hydras. Numbers in the last row are the averages of 54 hydras.

Table 2. The number of buds produced by the hydra in the experiments of Table 1

	Wavelength			
	450 nm	700 nm	450 nm	700 nm
Experiment	Green hydra		Brown hydra	
1	4	0	1	0
2	3	0	3	2
3	5	0	5	3
4	1	0	1	6
5	3	0	3	1
6	5	0	5	2
7	3	1	3	4
8	1	0	1	1
9	2	2	2	3
Mean	3 ± 1.5	0.3 ± 0.7	4.3 ± 1.9	2.4 ± 1.8

Note: Hydra were not fed during the experiments. Green hydra produced significantly more buds in blue light than red light (ANOVA, $P < 0.0006$). The difference in bud production for brown hydra in the two light treatments is not significant (ANOVA, $P < 0.08$).

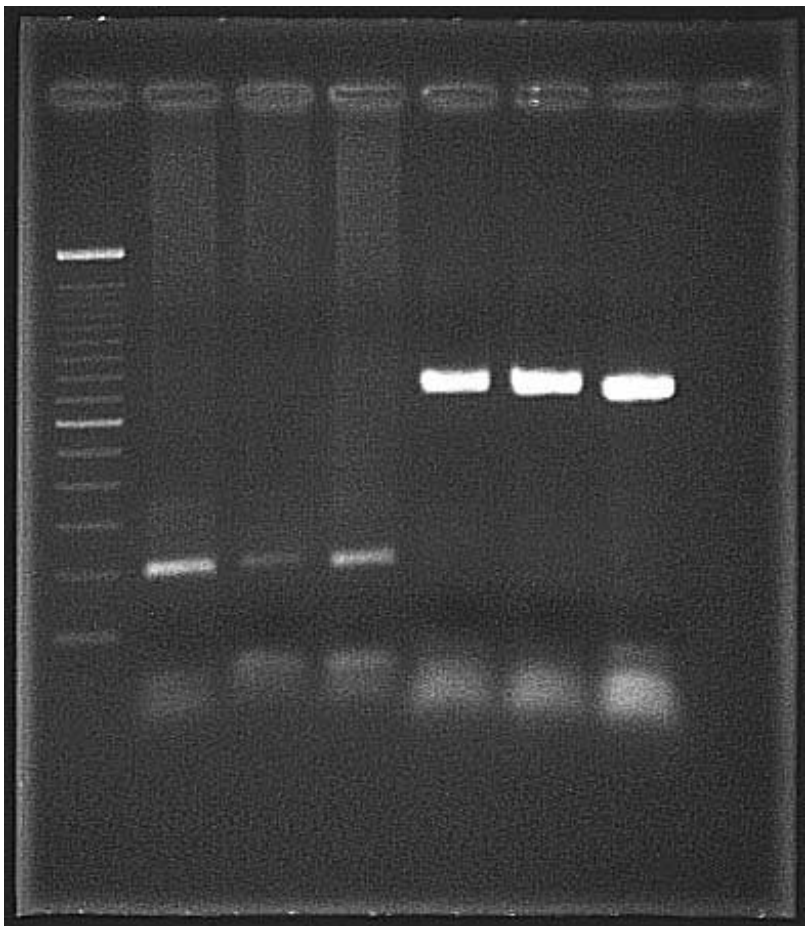

Fig. 3. Expression of CREB gene 3 h post-amputation in hydra exposed to lipoic acid during regeneration. Lane 1: molecular weight ladder standard. Lanes 2–4 show RTPCR products using CREB primer (targets 200-bp product). Lanes 5–7 show RTPCR products using Actin primer (targets 700-bp product). Lane 2: hydra regenerating in hydra medium. Lane 3: hydra regenerating in lipoic acid + hydra medium; Lane 4: non-regenerating hydra in hydra medium. Lane 5: hydra regenerating in hydra medium. Lane 6: hydra regenerating in lipoic acid + hydra medium. Lane 7: hydra regenerating in lipoic acid + hydra medium.

never tested our hypothesis: A student's science career in high school is short, and teachers retire!

Students at Brentwood High School in Brentwood, New York are currently replicating Guan's experiment with added controls for temperature variation and the addition of a white-light control. Their teacher, Rebecca Grella, was also a student of Larry's and is currently working on her PhD at Stony Brook University in the Department of Ecology and Evolution.

With Larry's encouragement, I used the diary of Abraham Trembley, translated by Lenhoff and Lenhoff (1986), to show that the study of biology became an experimental science in 1740 when Trembley, a Swiss native employed as a tutor, discovered the tiny

creatures we know as hydra in a pond in the Netherlands. Convinced that they were animals and therefore should not regenerate, green hydra surprised him also.

Today, the understanding that all organisms share essentially the same genes has placed hydra once again at the centre of experimental biology. Recently, Swiss scientist Brigitte Galliot (2005) noted that 'hydra regeneration should not be regarded as a curiosity for zoologists, but rather as a path towards understanding basic developmental and ageing mechanisms probably shared, more or less extensively, by all animals'. In other words, her view (quoted by Helen Pearson, 2001) is, 'it doesn't matter if you make a tooth or a tentacle, the problem is the same: How do you turn a pool of stem cells into a three dimensional structure?'

Thanks to help and advice from Stony Brook University faculty (Zuzana Zachar and Kate Dickman), my students have now also demonstrated an inhibitory effect of lipoic acid on CREB gene expression in regenerating hydra (Fig. 3).

Recently, another high school student obtained data suggesting that hydrogen peroxide can rescue hydra whose regeneration has been inhibited by lipoic acid (Dennis, 2007), thus supporting the hypothesis that hydrogen peroxide mediates one of the signal transduction cascades activated during the stem cell differentiation that precedes hydra regeneration (Galliot *et al.*, 1995, 2006; Galliot and Schmid, 2002; Galliot, 2005).

Today, I teach teachers to use hydra in the classroom. In partnership with Connecticut Valley Biological Supply Co., Inc., we are marketing a series of pedagogical 'kits' that use hydra as a model organism to teach biology. In the simplest of these, students compare what happens when some hydra are fed *Daphnia pulex* while others are fed *Artemia salina*. The brine shrimp succumbs immediately to the hydra attack, whereas the *D. pulex* sometimes escape. In another lesson, some hydra are fed *Simocephalus* while others receive *D. pulex*. *Simocephalus* co-exist with hydra in the littoral zone and are immune to hydra toxin while the planktonic *D. pulex* are not (Schwartz and Hebert, 1989). The kit is designed to generate questions about the role of natural selection in the evolution of predator–prey interactions.

Larry took the time to teach a teacher. And for me, my students, and the teachers I teach, that has made all the difference.

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