Freshwater sponges as indicators of water pollution: an investigative undergraduate lab

Malcolm S. Hill and April L. Hill

Biology Department
Fairfield University
Fairfield, CT 06430
mhill@fair1.fairfield.edu

Malcolm Hill is an Assistant Professor of Biology at Fairfield University where he teaches General Biology and Evolution (both for majors). He also teaches an evolution course for non-majors. His research focuses on the evolutionary ecology of marine sponges.

April Hill is an Assistant Professor of Biology at Fairfield University where she teaches Genetics and Developmental Biology. She is an active proponent of incorporating information technology in her lectures. Her research focuses on the role developmentally-important genes play in early development, and she uses sponges as a model system.

Reprinted From: Hill, M. S. and A. L. Hill. 2002. Freshwater sponges as indicators of water pollution: an investigative undergraduate lab. Pages 385-389, in Tested studies for laboratory teaching, Volume 23 (M. A. O'Donnell, Editor). Proceedings of the 23rd Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 392 pages.

- Copyright policy: http://www.zoo.utoronto.ca/able/volumes/copyright.htm

Although the laboratory exercises in ABLE proceedings volumes have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibility for risk. The Association for Biology Laboratory Education (ABLE) disclaims any liability with regards to safety in connection with the use of the exercises in its proceedings volumes.

© 2002 Malcom S. Hill and A. L. Hill

Background

This lab uses freshwater sponges as model organisms to examine the biological effects of water pollution. Specifically, the focus of this laboratory module is on the effects that chemicals of environmental concern (*e.g.*, endocrine disrupters) have on sponge growth and development. Contamination of aquatic ecosystems is a serious issue in environmental science. Identifying which chemicals we should be concerned with, and determining the consequences of contamination by specific compounds, is a major area of current research. Undergraduates are constantly exposed to news of environmental deterioration, yet few have an idea of how bioassays can help set national drinking water standards or shape environmental guidelines/laws. Our laboratory activity represents

an introduction to this area of biological exploration with an unusual animal model.

Biology of Freshwater Sponges

Freshwater sponges are common animals of most aquatic ecosystems. They utilize flagellated choanocytes to pump water through a series of canals. Incoming water enters through ostia, passes through choanocyte chambers, and exits through the osculum. Bacteria are filtered from incoming water, and large volumes of water can pass through a sponge in a 24-hour period. Because of their simple morphological construction, many cells come into direct contact with the surrounding water as the sponge pumps. Thus, a sponge's mode of feeding results in high levels of exposure to any compound present in an ecosystem. Watanabe and colleagues have produced a beautiful film using time-lapse videography to document the life cycles of freshwater sponges (*Life of the Freshwater Sponge*). The running time of the film is 28 minutes, and it shows sponges in their natural environment and in the lab. The film was produced by Tokyo Cinema, Inc., and provides a nice introduction to the topic. (Copies can be ordered from the British Universities Film & Video Council's web page at *www.bufvc.ac.uk*.)

A useful aspect of freshwater sponge biology, particularly for the purposes of an undergraduate lab module, is the fact that they enter diapause as small gemmules. Gemmules are overwintering balls that are produced in the late summer/early fall by the adult sponge. They are the size of the period at the end of this sentence. Adult tissue disintegrates around the gemmule during the winter, and a new sponge emerges from the gemmules in the spring. The newly developing sponge exits the gemmule from a micropyle, and then quickly spreads around the gemmule. In a healthy sponge, a water vascular system is evident, and many sponges produce a long osculum. Gemmules may be stored for years at 4˚C and still remain viable.

There are several other reasons why sponges are a model laboratory organism to explore the biological consequences of environmental pollution. For the purposes of ease of set-up, freshwater sponges represent a cost- and time-effective study organism. Gemmules grow relatively quickly (within 3-5 days) and require very little equipment to grow. All of our sponges were grown in 24-well tissue culture plates. While our experiments were conducted in a growth chamber with constant temperature and photoperiod, we have found that the sponges grow well at room temperature using ambient light levels. Gemmules are readily available at a low cost from the major teaching supply companies (*e.g.*, Connecticut Valley Biological, Inc. Cat. # L14), and grow in spring water. While Connecticut Valley has been a reliable and inexpensive supplier, we now use a source of gemmules collected from sponges located less than a mile from campus.

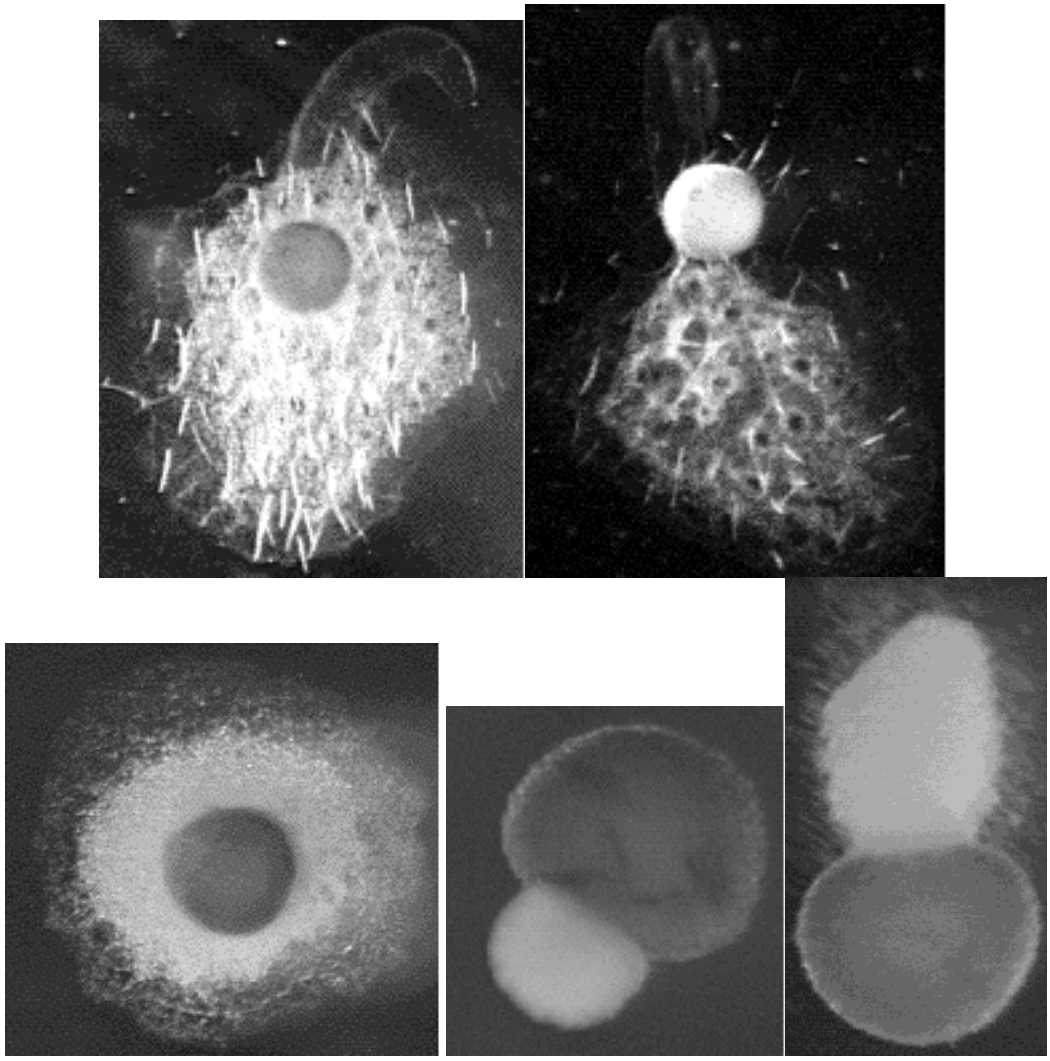
Laboratory Goals and Protocol

This lab will introduce students to a simple bioassay that will allow them to explore the effects that a chemical's concentration has on the level of toxicity. By relying on morphological examination of sponges hatching from gemmules that are smaller than a millimeter in diameter, this module will help students develop their microscopy skills. Our major aim, however, is to have students strengthen their ability to design and test their own hypotheses. We recently reported that several potential endocrine disrupters cause gross morphological defects in developing sponges (Hill *et al.* 2001). As can be seen in Figure 1, the effects of a number of chemicals can have a significant effect on sponge morphology. The compounds we used in our trials produced dramatic effects within 3 days. Compounds that we have tested include: nonylphenol, ethyl benzene, toluene, methyl paraben, benzo-a-pyrene, tolytriazole, and bisphenol-a. We attempted to choose compounds that would be commonly encountered in a student's daily life (*e.g.*, methyl paraben is used in sunscreens and in the color coatings of many candies, tolytriazole is a component of aircraft deicing/anti-icing

fluids). Countless other compounds would work equally well.

Students should work in teams of two. After the teams have familiarized themselves with the basics of the freshwater sponge life cycle (*i.e.*, seen the video), they should propose a hypothesis about the effects of pollutants on sponge growth and morphology. Teams may be given the freedom to examine any compound, but we have found it helpful to focus them on endocrine disrupters and have provided some background reading to channel their thought processes (Hill *et al.* 2001; Hutchinson *et al.* 2000; Krishnan *et al.* 1993; Routledge *et al.* 1998; White *et al.* 1999). Endocrine disrupters are also particularly useful for forcing the students to think about cell-to-cell signaling.

Figure 1. Growth in freshwater sponges in response to various chemicals. The sponges in the top row were in spring water or low concentration treatments and exhibit 'normal' development with well-developed water canals and oscula. The bottom row sponges show abnormal growth in response to ethyl benzene, nonylphenol and bisphenol-A from left to right.

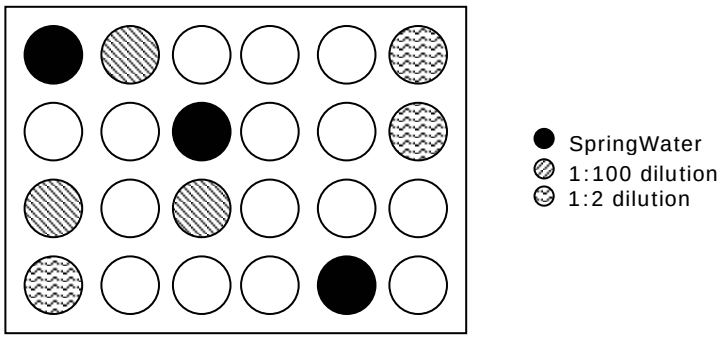


Once a hypothesis is decided upon, teams should design an experiment to examine 1) the effects of chemicals on sponge growth, and 2) the effects of pollutant concentration on sponge growth. The choice of chemicals will be at the discretion of the instructor and research team, but our work has focused on endocrine disrupters (Table 1). For each of the compounds tested, students should create a concentrated solution by placing a known amount of the compound in 100 ml of

spring water. If possible, allow the solution to mix at room temperature for 24 hours. The solubility of the compound will dictate how much goes into solution, and for some of the compounds we have tested (*e.g.*, nonylphenol) very little will dissolve.

Once the concentrated solution is prepared, students should prepare the following dilutions (1:2, 1:10, and 1:100). Spring water will serve as the negative control (*i.e.*, it should have no effect on sponge growth). We have found that students can always use practice making serial dilutions, and this activity helps them visualize what dilutions accomplish. We have collaborated with our Chemistry Department to determine the actual amount of a compound that ends up in the most concentrated solution, but this is not necessary for the hypothesis that students test. However, joining forces with the chemistry department provides students with the opportunity to see how important their developing chemical skills are for biological studies.

The five treatments should be replicated at least three times (depending on gemmule availability) by each team. That is, three separate gemmules should be placed in three separate wells for the control treatment and for the most concentrated treatment, and so on. The drawing below shows the placement of three replicates for three treatments, and teams can discuss the importance of randomization in experimental design. Once the solutions are added to the treatment wells, a single gemmule may be placed in each well. To reduce the possibility of evaporative contamination, we placed a sheet of Parafilm™ over the wells and sealed each well for the others.



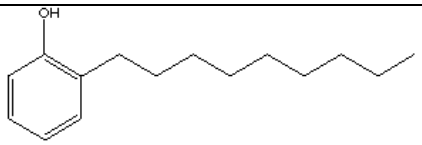
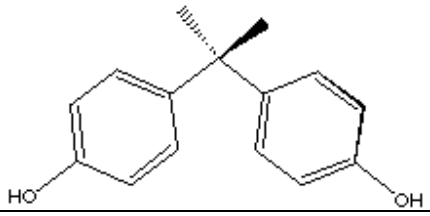
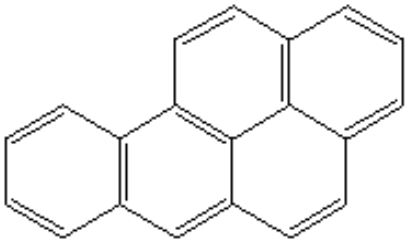
Students should check their experiments every day for signs of growth. Care should be employed when examining the sponges under a stereomicroscope since the gemmules may take more than a day to attach firmly to the bottom of the well. Magnification at 30 X or less should be sufficient for the visualization of growth. As the experiments progress, students will be able to detect major growth abnormalities immediately, but should also look for more subtle developmental abnormalities (such as the absence of a well defined water vascular system). As mentioned earlier, normal sponge growth typically includes the production of a distinct water vascular system with a less dense cellular construction. Comparing 'normal' sponge growth with the growth observed in chemical treatments will help hone observational capabilities. Figure 1 provides some examples of growth in various treatments.

Results from this laboratory can be used for a discussion of the biological consequences of pollution and will provide students with an appreciation for how dilution influences a chemical's biological effect. While endocrine disrupters are currently a 'hot topic,' other pollutants could also be tested. We tested some heavy metals (which are a perennial concern) and had some very interesting growth abnormalities show up. Chemical pollutants are not the only parameters that may influence sponge growth. The rate or success of gemmule could be tested as a function of temperature, light, food concentration, water oxygen content, etc.

Acknowdelgements

We thank ABLE for funding the development of this laboratory through a Laboratory Teaching Initiative Grant. C. Stabile conducted many of the preliminary experiments.

Table 1. Selected compounds of interested, their sources, toxic effects in humans and structural formulae. Information in this table was compiled from the following sources: Environmental Defense website www.scorecard.org, and the Environmental Protection Agency website www.epa.gov.

Compound	Sources	Toxic Effects	Structural Formula
Nonylphenol	Fuel stabilizer PVC tubing Plastics for food packaging	Suspected gastrointestinal and liver damage in humans	
Bis-phenol-A	Epoxy-resin & polycarbonate plastic production Food packaging	Causes exposed human mammary cancer cells to exhibit higher progeserone receptor levels	
Benzo [a] Pyrene	Car exhaust Coal, oil, and wood stove emissions Asphalt processing	Known carcinogen Suspected developmental, gastrointestinal, liver, respiratory, skin, or sense toxicant Suspected immuno-toxicant	

Literature Cited

Hill M.S., C.A. Stabile, L.K. Steffen, and A.L. Hill. 2001. Toxic effects of endocrine disrupters in freshwater sponges: common developmental abnormalities. Environmental Pollution. *In press*. Environmental Pollution.

Hutchinson T.H., R. Brown, K.E. Brugger, P.M. Campbell, M. Holt, R. Länge, P. McCahon, L.J. Tattersfield, and R. van Egmond. 2000. Ecological risk assessment of endocrine disruptors. Environmental Health Perspectives 108:1007-1014.

Krishnan A.V., P. Stathis, S.F. Permuth, L. Tokes, and D. Feldman. 1993. Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132: 2279-2286.

Routledge E.J., J. Parker, J. Odum, J. Ashby, and J.P. Sumpter. 1998. Some alkyl hydroxy benzoate preservatives (Parabens) are estrogenic. Toxicology and Applied Pharmacology 153: 12-19.

White P.A., S. Robitaille, and J.B. Rasmussen. 1999. Heritable reproductive effects of Benzo(*a*)Pyrene on the fathead minnow (*Pimephales promelas*). Environmental Toxicology and Chemistry 18: 1843-1847.