Symbiosis with Backbone

Introduction

According to Merriam-Webster, symbiosis is "the living together in more or less intimate association or close union of two dissimilar organisms." Classic examples of symbiosis include lichen**,** coral and zooxanthellae algae, clownfish and anemone, and ants and aphids. The list goes on.[1] Within our own bodies, the aptly named human microbiome represents the relationship with bacteria within our gut and on our skin [2, 3]. Our bodies contain and are covered with trillions of bacteria and other microbes. In fact, the ratio of microbial cells to human cells is approximately 10:1.

Three types of symbiosis exist that further define these relationships, but these definitions may be too simplistic to describe these complex interactions. Below are the standard definitions:

- In a ***mutualistic*** relationship, both organisms benefit equally. For example, a fish called a goby does not burrow well but can see. A blind shrimp can burrow. By themselves, these animals are easy prey. But, working together, the shrimp digs a burrow and the goby watches for predators that could eat them both.
- In a ***commensal*** relationship**,** only one organism benefits. For instance, a barnacle gets food and travels by attaching itself to a whale. There may be no advantage for the whale.
- In a ***parasitic*** relationship, one organism benefits while the other suffers. A tick benefits by feeding off the blood of a host, for example a dog. The dog can be harmed because the tick may transmit disease to the dog.

Figures 1 & 2. A lichen (l) illustrates the symbiosis of an algal species and fungus, the clownfish and anemone (r) serves as another classic example of symbiosis. Lichen image, J. Adam Frederick. Clownfish and anemone image, PixaBay.

Although first defined by Heinrich Anton de Bary in 1879 in his published lecture *"Die Erscheinung der Symbiose,"* (*The Phenomenon of Symbiosis)*, scientists are still learning about the relationship among and between organisms known as symbiosis[4]. These intricate relationships seem to provide more questions than answers. Scientists and naturalists agree that there is still much to learn. In fact, amid this growing field of knowledge, there is now

evidence—or rather rediscovery and affirmation—of a unique symbiosis between an animal with a backbone (vertebrate) and a photosynthetic unicellular organism.[5]

The "Back" Story

Early spring in the Northeast United States brings about many pleasures for those of us channeling fervent thoughts of melting snow, warmer temperatures, and longer daylight hours. For naturalists, herpetologists, and nature lovers, this is a peak time of year for refreshing rainfall, the sights and fragrance of early blooms, and trips to vernal pools…*what*?

Figure 3. A vernal pool forming from snow and ice melt in the Frederick County Municipal Forest, Frederick, Maryland. J. Adam Frederick.

Vernal or "springtime pools" are temporary bodies of water formed by snow and ice melt and rain. Some look at these pools that form in forests, along roadsides, and near coastal areas and see only large puddles. The well-informed however know that nocturnal visits are a veritable hotspot of activity. These pools provide opportunities to witness events that, depending on the weather, only happen for a few days to weeks. The emergence of spring peepers and wood frogs after the snowmelt are two of the most common, and very audible, harbingers of the annual "amphibious race."

Nature enthusiasts seek vernal pool formations to observe amphibian activity or a chance to glimpse that special toad, frog, salamander, or newt. If their timing is right, they'll find a few, or possibly a few hundred, creatures. These night treks make use of LED lights for visibility in the darkest forest or watershed landscape; weather and waterproof phones and video cameras are used to capture remarkable events below the surface. Many naturalists and organizations have shared images and video of these events.

Check out the Maryland Sea Grant video "Into the Vernal Pool."

Modern Research

In 2011, Ryan Kerney, now an Assistant Professor of Biology at Gettysburg College, started to ponder century-old suspicions of symbiosis about one such vernal pool denizen, the spotted or yellow-spotted salamander, *Ambystoma maculatum*.

Figure 4. A spotted salamander on the move across a road. Photo, J. Adam Frederick

Spotted salamanders, commonly found from Maine to Georgia and extending into the Midwest, are difficult to sight since they are strictly nocturnal; and, after mating season, they spend most of their time underground in the quiet confines of small mammal burrows and tunnels. These ability to burrow under leaves and logs in search of food, like earthworms and slugs. In early spring, amid cool temperatures and rainy nights, “spotteds” begin their annual migration to vernal pools to start the reproductive process. At its peak, hundreds of these salamanders trek to the pool’s icy cold waters. Male salamanders leave behind a spermatophore, or packet of sperm, attached to a stick or leaf.

Figure 5. Spermatophores deposited by male salamanders attached to twigs and leaves in the vernal pool. Photo, J. Adam Frederick

Females pick up the packets of sperm and fertilization occurs internally. But, this is not the part of the lifecycle that caught Kerney's eye. Dating back to 1888, Tulane University biologist Henry Orr documented his findings—a spotted egg case, a whitish thick gelatin, turned greenish during development, presumably because it was covered with a species of algae. Orr suspected a symbiotic relationship. In his paper *Note on the Development of Amphibians*, he stated:

> *"I have not discovered how the algae enter the membrane, nor what the physiological effect they have on the respiration of the embryo, but it seems probable that in this latter respect they may have an important influence."*[6]

Orr's hunch was a good one. A spotted salamander egg case is dense and can house over 100 individual eggs. In a vernal pool with little water circulation, it would be difficult for embryos to get oxygen and food (since they have little yolk).

By the 1940s, it was widely accepted that these organisms had developed a symbiotic relationship consisting of algae colonizing the egg case of the spotted salamander with each being a potential beneficiary.[7]

NOMENCLATURE NOTES

Note the different spelling of the genus name of the salamander (Ambystoma) and the species name of the algae (amblystomatis). Ambystoma, a misspelling by Swiss naturalist Johann Jakob von Tschudi in 1838, was intended to translate into "blunt jaw" when he described the genus of what is known as mole salamanders. He repeatedly used the misspelling in publication; and, by taxonomic rule, the original spelling stands. However, the correct spelling amblystomatis (note the addition of l), was given to the name of the symbiotic algae in 1927 by Lambert Ex Printz.[8]

Although the suspicion of a mutualistic relationship continued, no other experimentation delved further into the exact nature and intimacy of the relationship until Kerney probed a bit further in 2011. Kerney postulated that the algal species, *Oophila amblystomatis* (Oa), was actually intertwined within the spotted salamander, *Ambystoma maculatum* (Am) embryo and not just on the surface of the egg or egg case.

Figure 6. Egg cases attached to sticks. Sometimes female "spotteds" produce clear egg cases. These clear cases could also be from another species, the Jefferson salamander. Photo, J. Adam Frederick

To prove his point, Kerney used a simple fluorescence technique to check for photosynthetic activity, or chlorophyll, in the tissues of the embryo. Voila! Under fluorescence microscopy, the embryos exhibited small red dots, telltale signs of photosynthetic production. These algae had not only colonized the egg, but they had managed to colonize the salamander. (The alga was

not on the outside of the egg but *inside* the animal.) How is that possible in a vertebrate animal?

Figure 7. Developing larva with notable green tint on the egg cases. Photo, J. Adam Frederick

Kerney continued his experiments to see if the algae had in fact penetrated the cells of the embryo. He carefully prepared the cells for transmission electron microscopy (TEM). Using the TEM, he validated that the algae cells were *inside* the cells of the salamander.[9] But, how and when does this colonization take place? And, what is the true nature of a potentially mutualistic relationship between an algal species and a vertebrate animal?

Kerney's experiments confirmed the presence of Oa in the Am embryo as an endosymbiont (an organism that lives within the cells of another organism).

Erin Graham, a graduate student at Temple University in Philadelphia, took up the evidence provided by Kerney and others to figure out if there was a "direct relationship" between the presence of the alga and growth of the salamander. She also wanted to find out if the alga provided other benefits to the growing salamanders, beyond supplying oxygen. She developed an experiment to compare salamander "egg masses maintained in light, darkness, and with a photosynthesis inhibitor" thinking she would find a direct association between more algal cells and the growth of the salamander embryos related to the amount of light.[10]

Graham postulated that the benefit for Oa was the nitrogen-containing waste products coming from the Am embryo, which help fuel algae growth. But, the question lingered about sugar (composed of carbon, hydrogen, and oxygen) produced from photosynthesis by the algae. Oxygen production from photosynthesis had been shown to benefit the embryos in other studies done since 1940 but linking the benefit of algal sugar production to the embryo would break new ground in vertebrate symbiosis. Gilbert (1944) had also established that Oa is able to penetrate the egg cases within hours of deposition by the female and congregate in the inner membrane of the eggs.[7]

As a reminder, in the process of photosynthesis, plants use energy from sunlight, carbon dioxide gas from the atmosphere, and water to produce sugar (glucose) and oxygen.

$$\mathbf{CO_2 + H_20 + light\ energy \text{ ---------}\rightarrow Sugar\ (C_6H_{12}0_6) + 0_2}$$

Hypothesizing that oxygen may not be the primary benefit for the growing salamanders, Graham focused on carbon. She tracked radioactively labeled carbon to define the pathway

from algae to embryo and to investigate whether the main benefit for the embryo was the production of oxygen or the production of sugar.[10]

Graham's study shows a direct positive relationship between alga and salamander embryo growth. The relationship is potentially mutualistic since the algae use the salamanders as a host and the growing salamanders benefit as the alga increase oxygen, provide nutrition from the photosynthetic process, and may even remove waste in the egg. Her study is also the first to clearly show that algal produced sugar is translocated into the cells of salamander embryos. It is estimated that there is production of some sugars that translocate to the embryo but only about 13% of production. This pales in comparison to zooxanthellae algae that can produce sugars in coral and up to 90–90% is shared.

Kerney and Graham's work shows that this is a unique form of symbiosis between an algal species (Oa) and a vertebrate (Am) animal.

New research by Burns et al (2017)[11] sheds new light on this relationship and delves into greater detail and compares the algae, *O. amblystomatis* (Oa), and it's reactions and interactions with the salamander by looking at Oa that colonizes space within an individual egg (intracapsular space) and the cells of the salamander embryo (intracellular space).

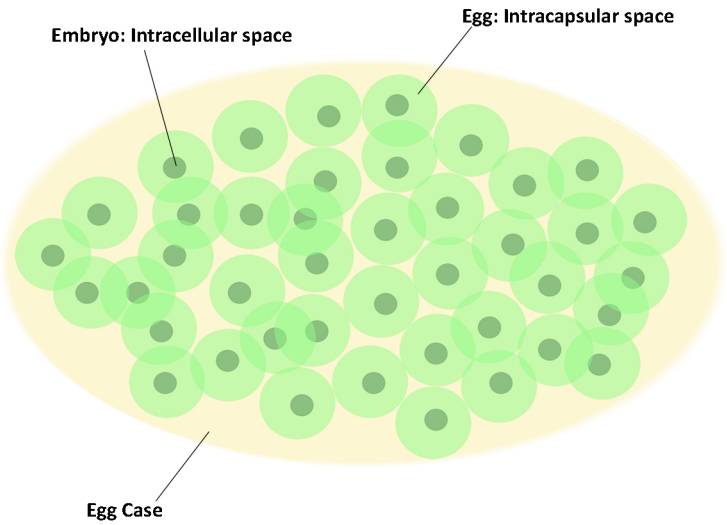


Figure 8. Drawing of egg case illustrating the location of the algae cells.

The research findings are fascinating because it is possible that the algal interaction or symbiosis is more akin to an "infection" with the salamander and the reaction of the algal cells and embryo are intriguing. Below are some of the major points of the research to ponder:

The research findings are fascinating because it is possible that the algal interaction or symbiosis is more akin to an “invasion”, the reaction of the algal cells and embryo are intriguing. Below are some of the major points of the research to ponder:

Algal cells that colonize the intracapsular space (figure 8) of an egg react the following ways:

- Exhibit more normal functions of respiration than those occupying intracellular spaces
- Maintain the ability to photosynthesize at reduced levels compared to cells outside the egg case

Algal cells that colonize the intracellular space (figure 8) of the embryo react the following ways:

- Demonstrate signs of stress due to low oxygen and as a result switch to fermentation as a way to support metabolism and energy production.
- Are shown to use glutamate from the host salamander as a nitrogen source.

Salamander cells that are colonized react in the following ways:

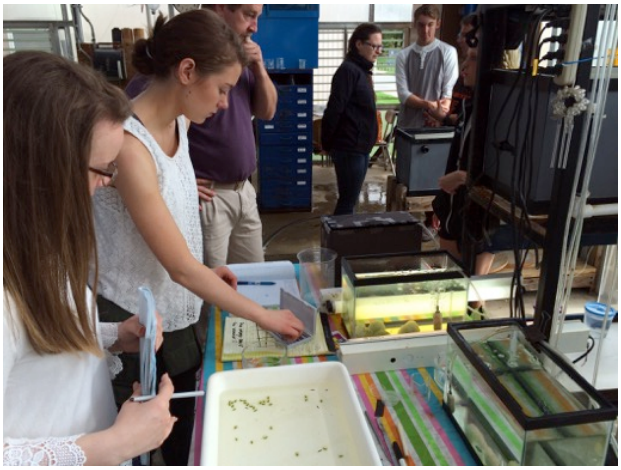
- Express genes that control immune response suppression thereby making it possible for Oa to colonize or invade the cells.
- Do not demonstrate similar stress responses as algal symbionts.
- Are provided with energy sources from photosynthetic products although they are at low levels.

Some of this information can be a bit confusing at first since many of us have stumbling blocks from basic biology and life science. A good example is, in many instances, middle school and high school students are taught about respiration from the “animal” perspective (animals use oxygen during respiration) and are taught about plants from the “photosynthesis” perspective (plants produce oxygen during photosynthesis). A missing component is that plants (and all other organisms) perform some type of respiration that uses oxygen or a type of anaerobic respiration or a variety of fermentation for the production of energy. So when plants and fermentation (a way to produce energy under low oxygen conditions) are mentioned together it results in confusion.

The research of this unique symbiosis is on-going and new information will be interpreted when it is available. This symbiosis provides a unique opportunity to integrate many concepts in biology, ecology, and project-based learning in the classroom.

Classroom Inquiry and Project-Based Science

Starting in 2014, Carroll County Public Schools (CCPS) in Maryland integrated this unique symbiotic relationship into their Science Research course as another means of exposing students to hands-on research focused on a local species. The initial work started with night treks (led by Maryland Sea Grant educator J. Adam Frederick) to observe the annual spotted migration in the Frederick Municipal Forest, Frederick County, MD. The sights and sounds of those treks served as a highly motivating factor for the educators as they were up close and personal with hundreds of spotted salamanders coming to local vernal pools. Following these night treks, teacher professional development meetings were held to stimulate thoughts about the focus of classroom-based activities related to the uniqueness of the spotted salamander life cycle.



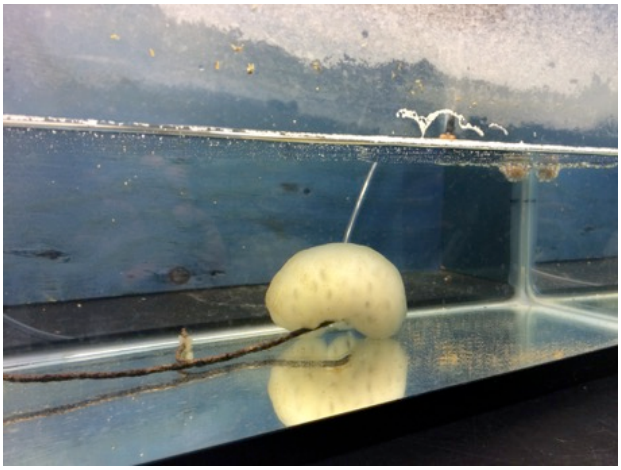


Figure 9 and 10. Students from CCPS examine spotted salamander eggs and developmental stages (top). An egg case in an aquarium (bottom). J. Adam Frederick.

That same year, Maryland Department of Natural Resources (MD-DNR) granted permission for teachers and students to collect spotted salamander egg cases from the wild. Students and teachers set up simple holding tanks. They studied the chemical composition of water used to house aquatic amphibians using information from the Ambystoma.org website, leading to a better understanding of the water quality and needs of the spotted salamander in the larval phase.

Students and teachers designed simple experiments to observe differences in salamander development related to the amount of light exposure provided for the egg cases in the aquariums. Once eggs began to hatch, they gathered data on the number that hatched and their length to make comparisons to the different light exposures. Student-driven projects like these are the hallmark of CCPS's Science Research courses led by Science Supervisor Jim Peters.

These projects expose students to the ideas behind the process of science and the multiple disciplines, skills, and collaboration required.

In 2015, CCPS students and teachers took their project to another level by hosting a "family science night" at a local elementary school so young students could see their work and get excited about science, reading, and writing. High school students worked side-by-side with elementary students to teach them microscopy skills, measuring techniques, and the similarities and differences of different animal larvae.

In partnership with Jennifer Keats Curtis and J. Adam Frederick, students were able to experience some of the sights and sounds of recent night treks to the vernal pools through video and images taken by Frederick. Curtis read to the students from the newly published book *Salamander Season* (Arbordale Press, 2015), co-authored with Frederick, to simulate ideas about the salamander life cycle and the importance of caring for our environment. Overall, the outreach project was a great success. Teachers from the high school and elementary school benefited from seeing a vertically integrated activity as an excellent learning experience for all students.

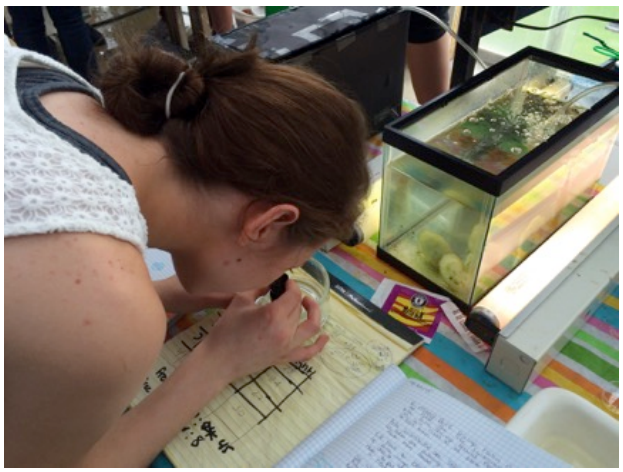


Figure 11. A student from CCPS examines an egg for developmental traits. J. Adam Frederick

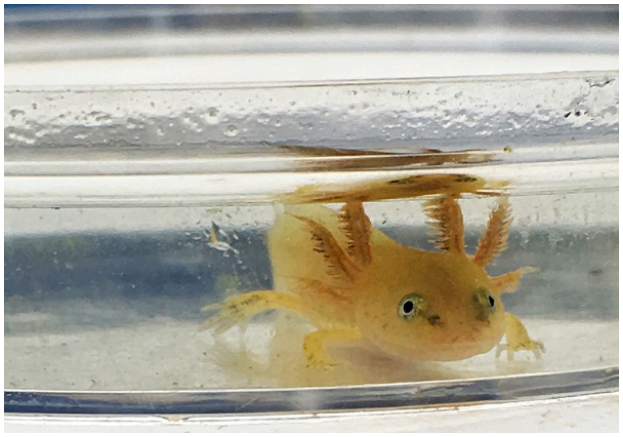


Figure 12. A spotted salamander after 4 weeks of development at Westminster High School, Westminster, MD (CCPS). Don Adams.

Works Cited

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10. Graham, E. R., S. A. Fay, A. Davey, and R. W. Sanders. "Intracapsular algae provide fixed carbon to developing embryos of the salamander *Ambystoma maculatum*." *Journal of Experimental Biology* 216.3 (2013): 452–59. Web.
11. Burns, John A., Huanjia Zhang, Elizabeth Hill, Eunsoo Kim, and Ryan Kerney. "Transcriptome analysis illuminates the nature of the intracellular interaction in a vertebrate-algal symbiosis." eLife, 2017;6:e22054.

For Further Study

- **Obligate and facultative relationships**
  Symbiotic relationships may also be described as obligate (completely dependent on the relationship) and facultative (not completely dependent on one another).
- **Immune system development**
  How does the immune system develop in early stages of development?
- **Innate and adaptive immunity**
  Review innate immunity and adaptive immunity.

Articles

- Phylogenetic Analysis of Algal Symbionts Associated with Four North American Amphibian Egg Masses (PLOS ONE research article on algal symbionts, Kim et al, 2014)
- "Salamander's Hefty Role in the Forest" (New York Times, April 7, 2014)
- "The trophic role of a forest salamander: impacts on invertebrates, leaf litter retention, and the humification process." (Ecosphere article on woodland salamanders, Best et al, 2014)

Video

- Spotted Salamander Migration and Reproduction
  This Maryland Sea Grant video shows the spring migration of the spotted salamander and parts of the reproductive cycle in a vernal pool in the Frederick Municipal Forest, Frederick County, MD.
- The Natural Heritage of Indiana: Life in the Water
  This documentary takes viewers into water habitats, where they will witness the diversity of life that exists. Sometimes, the life cycles take place on a nearly microscopic level. Elsewhere, the program reveals prehistoric looking fish and amphibians that still inhabit the landscape, as well as waterfowl. *Vernal pool footage: 10:42–20:00*
- Algal Endosymbiosis in Salamanders
  An overview of a study of intracellular invasion of green algae in a salamander host (Kerney et al. 2011)
- PBS News Hour: A New Genetic Map That Could Make Your Skin Crawl
  Scientists with the Human Microbiome Project have completed the first microbial map of healthy humans. Jeffrey Brown speaks with Dr. Eric Green, director of the National Human Genome Research Institute.
- BBC's The Trials of Life: Episode 7, Living Together
  David Attenborough looks at how, for better or worse, many animals form surprising partnerships. Birds rid larger animals of itchy hangers-on, hermit crabs enlist stinging anemones to repel predatory octopuses and others use their hosts in less benign ways. *Note: video no longer available*
- Ant and Butterfly Symbiosis
  A National Geographic video on the symbiotic relationship between ants and butterfly caterpillars.
- Symbiotic Relationships: Mutualism, Commensalism & Parasitism

In this lesson, learn the many types of symbiosis in biology, and how these relationships can have a positive, negative, or neutral effect on the individual species.

- Jonathan Bird's Blue World: Symbiosis In The Sea
  In this webisode Jonathan explores different types of symbiosis in the ocean, including mutualism, commensalism and parasitism, and how animals use it for survival.

Websites

- Maryland DNR: Discover Maryland's Herps
  Maryland Department of Natural Resources website focusing on the amphibians and reptiles that make their home in Maryland.
- Maryland Amphibian and Reptile Atlas (MARA)
  The Maryland Amphibian and Reptile Atlas (MARA) is a five-year, joint project of the Natural History Society of Maryland and Maryland Department of Natural Resources. The goal of the MARA project was to document the current distributions of Maryland's amphibian and reptile species using a systematic and repeatable approach.
- Natural History Society of Maryland
  A Maryland-based non-profit that focuses on conserving natural history collections, educating citizens, and inspiring youth to study the natural sciences.
- Amphibian Survival Alliance (ASA) and Amphibian Specialist Group (ASG)
  The Amphibian Survival Alliance and the IUCN SSC Amphibian Specialist Group work with over 100 partners around the world to develop projects and invest in the protection of amphibians.
- Society for the Study of Amphibians and Reptiles (SSAR)
  A not-for-profit organization established to advance research, conservation, and education concerning amphibians and reptiles.
- Short guide to axoloti husbandry
  Husbandry techniques for larval salamanders.