NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Peek-a-Bamboo!

Embryonic Development and Toxins

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Part I – Natural History

A. Surprise!

One afternoon a group of visiting undergraduates studying at the University of Singapore were collecting data on bamboo plants. The biology students needed to determine the height of running versus clumping bamboo as part of a study to determine if plant growth form (running or clumping) has any correlation to overall height. They were also studying if there were patterns of bamboo height that varried according to their proximity to surrounding farmland. One side of the bamboo forest ran alongside a series of farms, while the other did not and was thus free from the same potential for agricultural interaction.

Dr. Keefe, the leader of the team, divided the students into pairs and assigned them to different regions of the forest. "Kristin and Billy, you begin collecting data from the bamboo in this area of our research field. Nicole and Cameron, start with collecting data on the bamboo on the opposite side of the research field. One person in each team can take measurements while the other records, and then halfway through you can switch so that each of you has a chance to take measurements and record data."

As they made their way to their assigned area, Nicole asked Cameron what type of growth he thought the bamboo in their section had. "Well," he replied, "this looks like running bamboo since it's spread out all across our research field. Remember that running bamboo expands by having 'runner' bamboo that spreads out and then more bamboo fills in the area later on. And clumped bamboo is when a few shoots grow around the edges of the bamboo to gradually expand in circumference."

After they had measured about 10 trees, Nicole observed, "It looks like our bamboo stalks have about an average circumference of 12 centimeters. Now we can calculate our estimated height by using the equation: height = circumference × constant, and our constant is 60."

As they continued the calculation, they heard Billy, who had been measuring bamboo with Kristen, call out excitedly from across the field, "Hey guys, check this out! There's a frog inside this bamboo!"

"No way Billy," said Kristen. "You must be mistaken." But sure enough, when she peered inside the plant, she too saw the frog.

Question

1. Why would a frog be inside a bamboo stem? Try to come up with at least three different ideas.

B. Benefits and Threats

Quickly the four students gathered around to discover a small green frog hidden inside the internode of the bamboo plant. The internode is the smooth space between the nodes of a plant (Figure 1). A node is the connecting part of the plant where the new stems or leaves emerge. In the case of bamboo, the internode is usually hollow while the node is solid. The frog could only be seen by looking through a small hole in the plant.

"This is so strange," said Kristen. "I wonder what the frog is doing inside the bamboo?"

Billy, who had been observing the frog the longest, responded, "Look closer Kristin, the frog is in the plant because it laid her eggs there."

While the other students took turns peeking at the little frog, Billy began looking into other pieces of bamboo in search of more frogs with eggs. He soon discovered a few more frogs with eggs in the area of the bamboo forest where he and Kristen had been working.

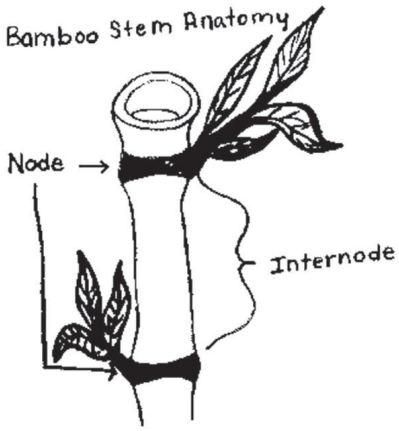


Figure 1. Bamboo stem anatomy.

Questions

2. What benefits would the frog's eggs gain from being inside the bamboo? Are there other benefits for the frog to be inside the bamboo?

3. What threats can you envision for the frogs inside the bamboo?

C. Comparing Species

Dr. Keefe knew that frogs liked to hide in small areas but he didn't know of frogs laying their eggs inside bamboo. Once learning of this, Dr. Keefe was delighted and set off the next morning with his students to conduct a formal survey of the area. They decided to set out a 50 meter transect line and all the trees that fell along this line in the four different areas would be sampled.

Billy asked, "Dr. Keefe, how many more frogs do you think we'll find?"

"That's a very good question, it's difficult to say. These frogs you found could be very rare and only occupy one small area of the research field or they could actually cover a larger area. My guess would be the first since I haven't discovered them before, but I obviously couldn't see them since they were in the bamboo this whole time!"

At the end of a long day, the team found these bush frogs present in only about 2% of the trees sampled, and what a rare discovery to make! "I never knew frogs could lay eggs in a tree," said Billy.

"I know what you mean," commented Dr. Keefe, "we usually learn at an early age that frogs lay their eggs in water, males come and fertilize them, and then the eggs hatch into tadpoles in the water."

"Yes," agreed Billy, "I can remember catching bullfrog tadpoles in our pond when I was young."

Dr. Keefe smiled. "I remember catching tadpoles when I was young too. It was so exciting to see the changes that occurred over time in the pond in my neighborhood."

Dr. Keefe then asked, "Did you notice how few eggs there were in the bamboo compared to the thousands of eggs a bullfrog might lay in the water? A female bullfrog will release as many as 20,000 eggs into the water for a male bullfrog to fertilize."

"Wow," said Billy, "I didn't realize they laid so many eggs. That large amount must be a thousand times the amount of eggs that are in these bamboo plants!"

Suddenly the mood changed when Billy noticed two frogs in the bamboo plants that seemed to be deformed in some way. It was almost as if their hearts had expanded and were bleeding. "Hey Dr. Keefe! Look at this!"

Dr. Keefe noticed the problem right away and the professor and students started brainstorming over causes. Some thought it was a mutation, others thought something might have happened during development, and others thought that there was a toxin in the bamboo plant itself. Billy was determined to learn as much as he could about embryological development, frogs, and environmental toxins.

After returning to the lab and conducting a literature search, Billy learned that male bush frogs and male bullfrogs actually had some similarities. Bullfrogs occupy a certain territory and during mating season, they attract females to their territory through mating calls. Then the males will climb onto the females and release their sperm as the females release the eggs. On the other hand, male bush frogs climb into tiny openings in the internodes of bamboo plants before the females. The males actually attract the females, through mating calls, to the bamboo so that they can mate. This is similar to the bullfrog mating in that the females may be selecting their males based on certain attributes or traits. Females could select for a better environment (pond or bamboo) for their offspring. Female bush frogs then lay their eggs inside the nodes of bamboo. They usually lay about 5 to 8 eggs and other females may lay eggs in the same internode. Furthermore the male will then tend to the eggs, leaving every so often to get food, until they hatch directly into little frogs.

Billy thought of the tadpoles from his childhood; he was astonished that these bush frogs were never tadpoles. A very different strategy than the bullfrogs back home! Billy wondered out loud, "I wonder which trait is more primitive—laying eggs in water, which then hatch into tadpoles, or laying eggs in the bamboo plant that hatch directly into froglets?"

Questions

4. Billy mentioned primitive traits. He wondered if having a tadpole stage or lacking a tadpole stage (hatching directly into froglets) was the primitive trait. Compare and contrast primitive and advanced (or derived) traits in general. What do these terms mean?

5. Natural selection can help develop traits, such as egg laying or reproductive behavior, over evolutionary time. What is the definition of a selective pressure and how does it work with natural selection?

6. Why do frogs reproduce in different environments (such as bamboo plants and ponds)? Do you think there are selective pressures that caused the bush frog to adapt its reproduction method to reproducing inside bamboo (assuming laying eggs in water is the primitive trait)? If so, name a few. If not, justify your response.

7. Consider a typical bullfrog who lays thousands of eggs in water. Because there typically is no standing water inside bamboo, do you think the bush frog has adaptations in place to prevent the embryos from dying due to lack of water? Explain. What ideas can you think of to deal with the stress of dehydration?

8. Knowing that the male bush frogs attract their mates into the bamboo, and bullfrogs attract their mates to a territory, do you think that one type of frog has a reproductive advantage over another one due to their specific living environment? Explain.

9. The bush frog has no tadpole stage and eggs hatch directly into froglets. The bull frog has a tadpole stage that is followed by metamorphosis into an adult frog. Is it reasonable to think the development of these eggs into either froglets or tadpoles is the same? In other words, do you think that the stages of embryonic development and change would be the same because these species are both frogs or do you think they would be different somehow because of their habitat? Defend your answer.

Part II – Embryonic Development

An embryo begins to develop as soon as a sperm enters an egg. The spot where the sperm enters the egg will become the head of the embryo and produce (head-tail) *polarity* for the organism. The vegetal pole develops directly opposite the sperm point of entry.

The egg doesn't need multiple sperm (or there would be problems due to too much DNA) so it creates two blocks or barricades for the remaining sperm. One method used is a fast block in which a calcium signal is released throughout the cell. As calcium rushes across the fertilized egg it changes the properties of the egg such that other sperm cannot penetrate the egg. The second method is a slower process where an actual membrane is formed around the egg so that no other sperm can penetrate. This is called the *fertilization membrane*.

After the egg is fertilized, the cytoplasm begins to rotate to form two poles or two divided sections (Figure 2). One will be the *animal pole*, where there is more cytoplasm present, and will give rise to the animal. The other is the *vegetal pole,* which has more yolk present and will act as a food source for the developing animal/embryo.

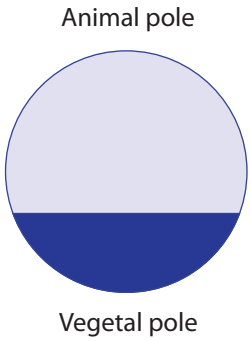


Figure 2. Polarity.

The next step in development is called *cleavage*. Cleavage is the name for mitosis in embryonic development. The major idea of cleavage is to make more cells rapidly. Many vertebrates, such as frogs, and some invertebrates go through *radial cleavage,* which divides the animal and vegetal poles equally and results in cells that are all the same size. If you were to look at the embryo from above at its animal pole, you could "slice" the embryo in any dorso-ventral slice (that is back-belly slice) and get mirror images (Figure 3). The cells that result are said to be *indeterminate* in that any cell can invidually give rise to a complete embryo if provided the right cues and environment. The fate of these cells is not dictated early in embryonic development and thus have the capacity to become many potential cell types.

Another type of cleavage is *spiral cleavage*, which causes the cells, also called *blastomeres*, to be uneven in size. Some will be larger in size *(macromeres)* and some will be smaller in size *(micromeres)*. This is because, unlike radial cleavage, the cleavage planes found with spiral cleavage are not parallel or perpendicular to the animal-vegetal pole axis of the developing embryo. Cleavage takes place at oblique angles, which forms a "spiral" pattern of daughter blastomeres. The cells (blastomeres) that result have a determined embryological fate early during development and are referred to as *determined* cells.

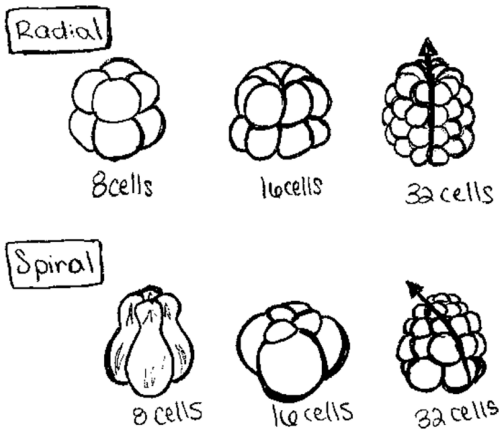
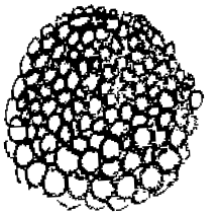


Figure 3. Radial vs. spiral cleavage. Deuterostomes use radial cleavage, protostomes use spiral cleavage.

When animals go through these different types of cleavage, maternal DNA, mRNA and proteins (that were placed in the original egg by the mother) are very important to how the cells/embryo divides. Eventually, the embryo will rely on its own genetic information as time goes on to take over its own development.

Cleavage will continue until the embryo consists of hundreds of cells. The term *morula* is used to describe this solid ball of cells. The cells are clustered close to one another and water is prevented from entering the spaces between the cells. This changes, however, toward the end of the morula stage. At this point, when you might have 128 cells, the morula will change from being impermeable to water, to becoming permeable to water. This is accomplished when NaCl is pumped into the very center of the morula. This changes the concentration of water inside the morula so now water will follow the NaCl into the center and fill the morula. This creates a hollow sphere of cells called a *blastocoel* and marks the *blastula* stage, which can be seen in Figure 4 (next page).



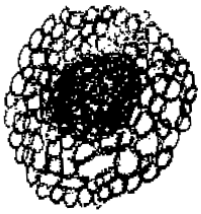


Figure 4. Blastocoel.

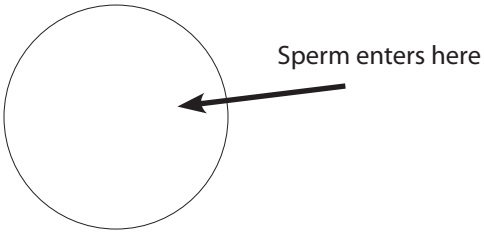


Figure 5. Egg.

Questions

10. Label/Draw the following parts on the egg in Figure 5: *head-tail polarity, animal pole,* and *vegetal pole*.
11. Label the blastocoel in Figure 4.
12. Which ball of cells/embryonic stage is impermeable to water? Which is permeable to water? What does it mean to be impermeable or permeable?

13. When NaCl moves inside the morula, it changes the water concentration there (Figure 6). Because the water concentration has _____ (*decreased, increased*) additional water will follow and we find a shift to the next stage of development. What is the term used to describe such passive movement of water? _____ What term is correct when describing the morula compared to an environment of pure water: *isotonic, hypertonic* or *hypotonic?* (Consider the solute concentration of the embryo in reference to the solution it is in when choosing your answer.)

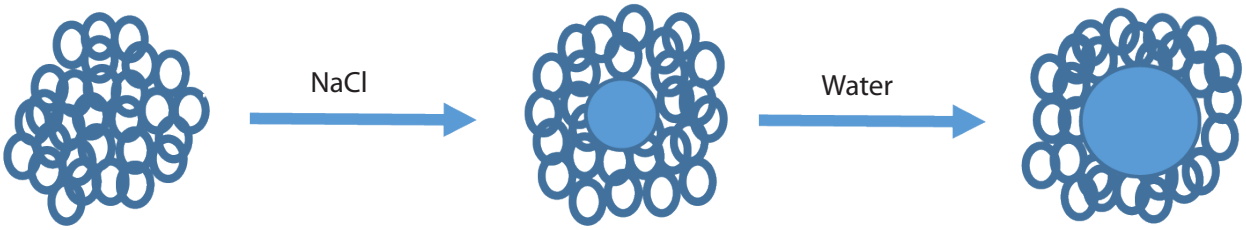


Figure 6. The progression from the morula stage to the blastula stage.

When comparing concentration of solutes inside the cells to that outside the cells, the following terms can be used:

- *Hypertonic*—a solution that has a higher solute concentration compared with another. For example, if the extracellular fluid has greater amounts of solutes than the cytoplasm of a cell, the extracellular fluid is said to be hypertonic.
- *Hypotonic*—a solution that has a lower solute concentration compared with another.
- *Isotonic*—a solution that has the same tonicity as some other solution with which it is compared

The *gastrula* stage follows the blastula stage and is very important for the embryo because during this stage, the three germ layers of the embryo will form. These germ layers will later help give rise to all the organs or body parts of the animal. During the start of the gastrula stage, some of the surface cells of the embryo begin to move inside the blastocoel; this is called *invagination* and can be seen in Figure 7. Once this movement of cells is finished, the cells that are now on the outside of the embryo are called *ectodermal cells*. The new cavity or area that was formed by invagination is called the *archenteron* or the primitive gut. As these cells on the outside of the embryo, the ectodermal cells, move inside they create a second layer of germ cells, the *endoderm*. The ectoderm will eventually give rise to the future epidermis and nervous system, and the endoderm will become the lining of the stomach and the intestines. The third germ layer that forms is called the *mesoderm* and these cells stem from the endodermal cells spreading out in the blastocoel off of that primitive gut. The mesoderm will eventually give rise to the muscles, skeleton, and reproductive organs of the animal. The mesoderm is not evident on Figure 7, but will result when the endodermal cells begin to spread out within the space next to the endoderm.

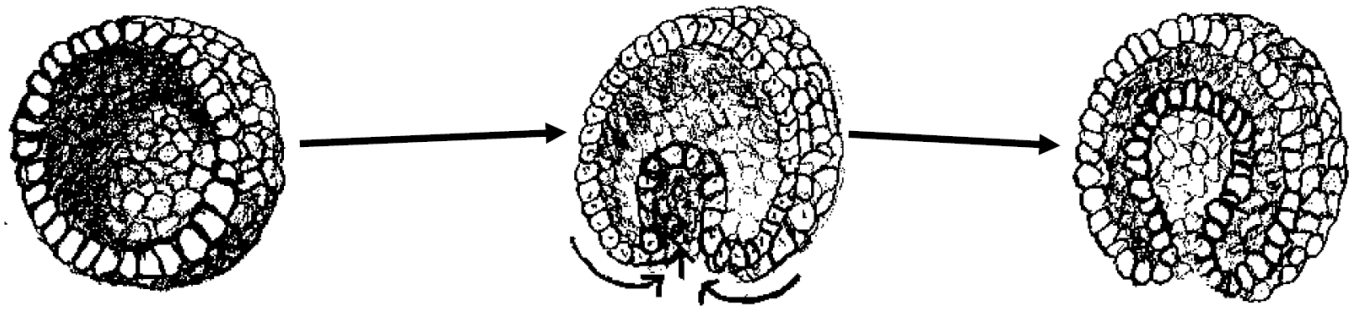


Figure 7. Once the blastula stage is complete with a blastocoel (left), invagination will begin (center) and three layers form along with a new cavity known as the archenteron (right).

Question

14. Label the following in Figure 7 above: *archenteron, ectoderm*, and *endoderm*.

After the gastrula stage, the *neurulation* stage begins. With this stage, we find formation of the *neural tube*. This tube eventually becomes the spinal cord and the brain. The neural tube will develop down the embryo as the gastrula elongates.

When neurulation begins we are on the outside of our embryo on the ectodermal layer. To help grasp this step, picture a basketball that is hollow inside (blastocoel cavity). The part of the ball you can touch is the ectoderm. This is where neurulation will take place (Figure 8A).

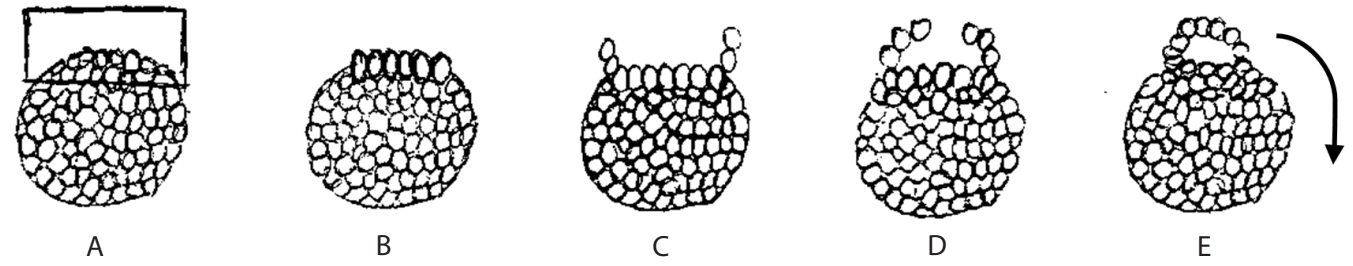


Figure 8. The process of neurolation.

The first part of neurulation is the formation of a *neural plate*, which is a thickening of these ectodermal cells (Figure 8B). This happens initially at the *cranial* (head) end of the embryo and moves towards the *caudal* (or tail or posterior) end. So, to return to our basketball analogy, imagine that the part of the ball where your hand is touching is the cranial end and this part of the ball begins to "thicken up" (the boxed area of Figure 8A). The ectoderm will ascend to a pair of distinct ridges that look like two mountains called the *neural fold* (the ball gets "thick" and starts to rise as

shown in Figure 8C). The valley in-between the two mountains is called the *neural groove*. As the neural folds continue to rise (Figure 8D), they will eventually connect to one another and fuse together (Figure 8E). This is when the *neural tube*, the opening between the fused folds, is created. The neural tube will consist at one end of the brain and at the opposite end, the end of the spinal column.

When the neurulation process starts (again boxed area of Figure 8A), it would be as if a small hollow circle is sitting on top of your basketball (the start of the neural tube—the brain). In the embryo, this now formed neural tube pinches off becoming completely closed. As this is happening, some extra cells are detaching and forming a new cell population called the *neural crest*. The neural crest goes on to create many structures of the developing embryo. As one part of the neural tube is forming (again like a small hollow circle on top of your basketball), the basketball itself (the embryo) is elongating, and the next section of the neural tube is forming to continue elongating this neural tube. The process is repeated (Figure 8) with cells thickening, rising, fusing, and forming more of the neural tube. It is as if the tube is being built in little sections and being fused as one larger unit; this is similar to a hollow rope running along the top of the basketball. This sectional building will continue along the embryo (the basketball) until it gets to the caudal (tail) end, almost like zipping up a zipper. You start at one point and move along the zipper to the other end. Once an embryo has a neural tube it is then called a *neurula*.

Questions

15. Why do you think that this stage of embryonic development is so complex compared to the rest of the stages?

16. Now that you know some information about the stages of embryonic development, do you think that the stages (cleavage, morula, blastula, gastrula, neurula) are this similar between all species of frogs? Why or why not?

17. Do you think the embryonic development of other animals such as reptiles or mammals (other vertebrates) are also similar in embryonic development? Why or why not? Be sure to explain your answer fully.

Part III – Toxins and Development

A. Circulation

After the nerve cord has been formed, the organs will begin to develop. One of the first organ systems to form is the circulatory system, which deals with all the parts of the heart and blood movement circulating through the body of an organism. There are arteries and veins within the circulatory system. Veins pump blood into the heart from various areas of the body while the arteries actually pump blood out of the heart to areas that need the oxygen. The circulation of blood also helps to regulate heat to organs and/or extremities. After the circulatory system is in place, the other organ systems will form in the embryo.

Question

18. Why do you think that the circulatory system is the first major organ system to develop? List a few reasons.

B. Atrazine

Billy was intrigued with these frogs and how their embryos developed the same way even though they had very different reproduction patterns. He now wanted to explore the idea his peers had proposed that toxins might be responsible for the deformities of the froglets in the field. Billy decided to tackle the scientific references again, but first he wanted to relax a bit. He was browsing through some science-related articles for fun when he stumbled upon one that was quite interesting and related to what his group was considering. It was an article discussing recently deceased Professor Andrés Carrasco's research throughout his lifetime. One piece of research was fascinating. Dr. Carrasco found evidence that small amounts of glyphosate, which is a key ingredient for common weed killer, cause neurological damage in frog embryos. Billy could see this possibly being an issue with the bamboo bush frogs as well since there were farms nearby that might have applied weed killer to the fields. This motivated Billy to begin searching for peer-reviewed literature about common agricultural toxins and their effects on development in frogs and other animals, and it wasn't long until he came upon something that seemed directly relevant, and alarming.

The research article (Lenkowski *et al.,* 2008) documented a series of experiments on *Xenopus laevis*, the African clawed frog, with atrazine. Atrazine is a common agricultural herbicide that is used to control broadleaf and grassy weeds in crops; it controls growth of these plants by blocking the electron transport chain during photosynthesis and therefore prevents the plants from being able to make their own food. This chemical is applied to crops multiple times in the growing season. It is used very often in the United States. If the chemical is used extensively, water contamination can occur as the chemical will wash off the land and into rivers, streams and ponds. Many crops are grown in the spring, the same season when many amphibians are mating and embryos are developing. Billy read that the scientists used *in vitro* tadpoles to determine the effect that atrazine has on development of various organs. The experiment evaluated malformations of the intestines, circulatory system, and body axis using three different doses of atrazine (10, 25, and 35 mg/L).

Question

19. Before looking at the results of this experiment, create your own hypothesis as to what will happen to the development of the tadpoles after exposure to these doses of atrazine.

C. Experimental Data

Below are the data from the experiments (graphs modified for simplicity).

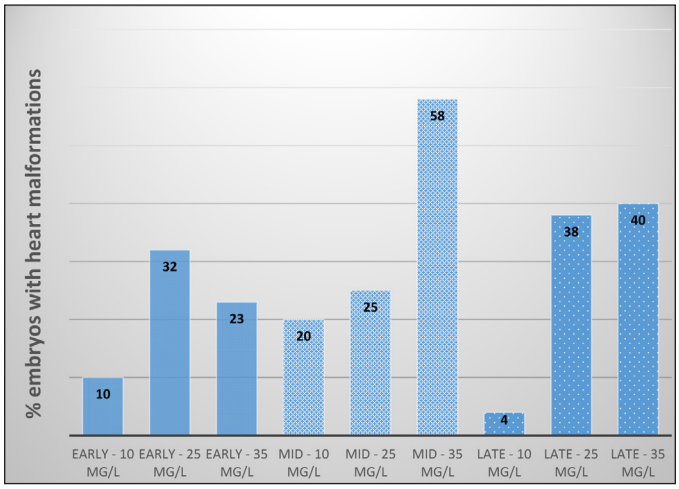


Figure 9. Incidence of reduced and enlarged hearts in tadpoles exposed to 10, 25, or 35 mg/L atrazine from early, mid or late stages of development.

Questions

20. Looking at Figure 9, which pieces of data should you compare to one another to understand the effects of atrazine on tadpole heart formation? There is more than one way to compare these data (keep in mind: time intervals, control groups and dosage groups). Name at least two ways. Is there anything missing that you wish were present that could help clarify the results?

21. Determine the conclusion of this experiment based on the data found in Figure 9.

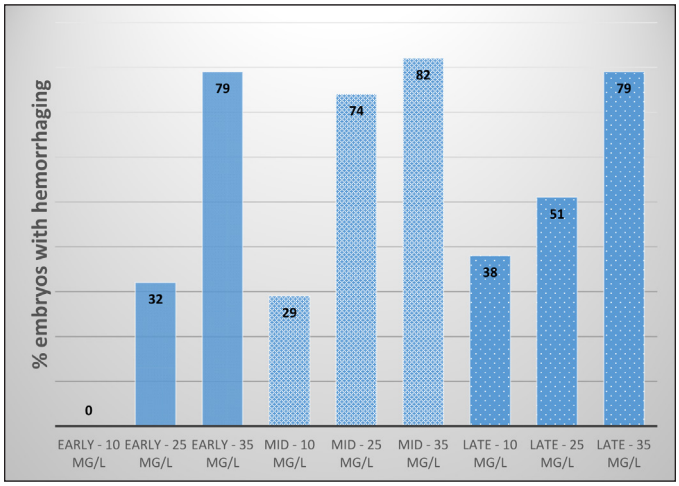


Figure 10. Incidence of visceral hemorrhaging in tadpoles exposed to 10, 25, or 35 mg/L atrazine during early, mid or late stages of development.

Questions

22. Figure 10 involves internal hemorrhaging (bleeding) found in tadpoles with atrazine levels of 10, 25, and 35 mg/L. After viewing the data, what do you determine to be the result of this hemorrhaging on the species overall?

23. Knowing this information about the impacts of atrazine on development for African clawed frogs, do you think that this chemical or others like it can also be harmful to other vertebrates (including humans) or organisms? Why, or why not?

D. Human Impacts

Upon considering what he learned from this research, Billy grew increasingly alarmed at what this meant for the bush frogs they had discovered in the bamboo. Billy approached Dr. Keefe and summarized the research. "Let me see those graphs and results, please," stated Dr. Keefe with concern. Billy handed the data to his professor. After studying the pages for several minutes, Dr. Keefe looked up at Billy. "We need to figure out if atrazine is used here. It's possible this is what caused the deformities you saw in the field. After we study this here, we need to alert our colleagues in the States about this issue. Atrazine is so widely used in the U.S. that I'm concerned about what it means for the bullfrogs and other animals that depend on water for their reproduction and development."

Billy nodded in agreement and added, "Didn't you tell us about bioindicator species the other month? I remember you stating that these are animals that can act like a canary in a coalmine alerting us to potential problems. If the frogs are being negatively affected, what about other wildlife?" Billy paused to catch his breath and added, "Oh boy ... what might atrazine do to humans?"

Dr. Keefe decided to update the other students on what Billy had discovered. They then formed teams to learn more about atrazine use in Singapore, how it might affect bush frogs, and whether or not frogs could be seen as bioindicator species.

A few days later there was much discussion when the teams reconvened for a lab meeting. Nicole began by stating her concern that atrazine might be doing damage to other species, not just frogs. "And," she continued, "it could be in the drinking water and therefore potentially affecting humans! I found an article discussing atrazine global use and the fact that it is the number one contaminant found in U.S. drinking water. This came from a well-respected scientist, Dr. Tyrone Hayes at the University of California Berkeley, who has been studying the problem as it relates to frogs for years. He found in his research that testosterone can be converted into estrogen in the presence of atrazine and that this pesticide is connected to frog development problems."

Another member on Nicole's team added, "We learned that in the United States, the acceptable level of atrazine in the drinking water is set at 3 parts per billion and that in Canada this value is set to 5 parts per billion. I'm concerned about any atrazine in the water because it is considered a possible carcinogen to humans."

Another team picked it up from there and summarized how widely used atrazine is, but how interesting it was that Europe has banned its use due to environmental concerns. They stated that most of the studies done have involved amphibians but that there are some studies showing correlations between the pesticide and low sperm counts in humans or low fertility. There are some studies indicating an increased risk of cancers too, like breast and prostate.

Dr. Keefe broke in at this point and reminded the students that the United States Environmental Protection Agency (EPA) generally does a good job researching and monitoring the environment and it feels atrazine is alright to use and did not feel the evidence was strong enough to link atrazine to human cancers.

The last group reported that they were excited to discover that there is a portable colorimeter test kit that can test for atrazine. Cameron declared, "We need to purchase this kit and start testing the bamboo plants in the field." Dr. Keefe thought this was a great idea and then started working with the students on a design for their next field research project.

Questions

24. Using the portable colorimeter test kit, what overall field design would you recommend to determine whether atrazine is present in the bamboo plants?

25. If atrazine were detected in the bamboo plants, what would you do next to determine if atrazine was negatively affecting the bush frogs? When designing an experiment remember the importance of incorporating control groups into the project.

Part IV – Environmental Toxins and Their Impact

As a follow-up assignment, write an opinionated letter declaring your point of view on the issue of toxins present in our environment and their overall harm. You may want to consider amphibians, such as the African clawed frog, as well as other indirect harm to other species coming in contact with the toxin.

Be sure to choose a recipient for your letter who can properly address the issue. Be creative with this assignment and include scientific details to support your argument. Write this letter as if you are really going to send it in the mail.

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