Monarchs in Space:
The Challenges of Microgravity

The following text has been prepared for teachers participating in the Monarchs in Space program. This text is meant to be educational and to be used for instruction. Teachers working with early elementary students might wish to simplify the details and the questions outlined below to better communicate with their students.

Introduction

Why send monarchs into space? What do we expect to learn from such experiments?

Sending monarchs into space offers an opportunity to teach children many intricacies of monarch biology. Further, it will be instructive to see how monarchs function in a microgravity environment in which near weightlessness is the prevailing condition. As you will see in the text that follows, there are at least five stages during the monarchs' development where the absence of normal gravity could be a factor in how well they function. The observations made during this experiment will provide some insights concerning basic monarch biology and will add more to our knowledge of how organisms, other than humans, might function in space. It is a win-win experiment. If the monarchs cannot perform certain tasks, such as successfully pupating, we will learn something about their limitations but also that gravity is essential for this function. On the other hand, if monarchs perform all life functions normally in spite of the near weightless conditions, it will tell us that there are aspects of the monarch's nervous control and physiology that allow for adjustments under adverse conditions.

Background: Monarch Biology

To fully understand the challenges that monarchs face in a microgravity environment we need to know how they function in the presence of gravity on Earth.

The monarch rearing chambers will be loaded with three 4^{th} instar monarch caterpillars, which will be raised on an artificial diet. When placed in these chambers the caterpillars aboard the space shuttle (and those that will remain here on earth) will be attracted to the trays with the diet and will begin feeding. To get an idea of how the caterpillar moves, place a caterpillar on the stick that came with your kit. Hold the stick horizontally and watch how the legs move. Notice that to move forward, the anal prolegs are brought forward first then each of the abdominal prolegs is moved forward from back to front and finally the caterpillar moves the thoracic legs forward. You will also notice that, as the caterpillar moves forward, the head is moved from side to side. As the head moves, a thin strand of silk is produced from the spinneret just behind and below the head capsule. The caterpillar is laying a silk trail and the legs, particularly the abdominal and anal prolegs, are equipped to grab onto the silk with a series of hooks or crochets on the bottom of each leg. The silk path and the gripping ability of the prolegs allow the caterpillars to cling to milkweed plants to feed and anchor them in place during wind and rain.

If we place a monarch caterpillar on a potted milkweed plant, it will crawl to the top of the plant to begin feeding - but why does it go up? Is it because this is where the best leaves are or where the light is most intense? How would you find out? Here's how: wrap the bottom of the plant with plastic wrap to keep the dirt in place and turn the plant upside down and place the caterpillar on the plant. What happens next? The caterpillar goes up to what is now the bottom of the plant. So, what does that tell us? It eliminates the idea that leaf quality is the issue, doesn't it? So, is it light or could it be gravity? Now we need to be able to distinguish between geotaxis and phototaxis. The term "taxis" refers to a behavioral response to a stimulus, and such responses can be positive or negative with respect to the stimulus. To go further, we can conduct the experiment again in the dark. Again the caterpillar goes up and this eliminates positive phototaxis as an explanation for why the caterpillar heads to the top of the plant. That leaves geotaxis, and it appears that the caterpillars are strongly negatively geotaxic. In other words, they have a sense of up and down based on gravity and they prefer to go up. You can conduct these tests in class with the stick provided by holding the stick vertically. You can also test for photo taxis by placing the stick with the caterpillar horizontally with a dark area at one end and a light at the other.

Note: Students have tested the above principles many times. If you challenge your students to conduct these tests, use at least ten replicates so that the trends are clear.

When the fourth instar caterpillar is ready to molt to become the 5th and last caterpillar stage, it seeks out a vertical or horizontal surface and spins a bed of silk. When the bed is completed, the caterpillar anchors itself to the bed with the crochets on the abdominal and anal prolegs. Once the development of the new skin is completed beneath the old, the caterpillar forces fluid forward causing the skin behind the head to split. The new 5th instar caterpillar then "walks" out of the old skin and after resting for some time, it turns around and eats this skin. Shortly thereafter, the caterpillar will resume feeding on the artificial diet and will do so until it has completed development in 4-6 days.

At the end of the 5th instar, a caterpillar has reached its maximum length and is noticeably plump. A signal that a caterpillar is finished developing is the appearance of reddish frass in the container. This frass represents the last food material in the gut of the caterpillar, and the red color is due to the nitrogenous wastes and possibly uric acid removed from the blood and secreted into the gut by the Malpighian tubules, excretory organs that function in a manner similar to kidneys.

The J Shape

After emptying its gut, the mature caterpillar enters a wandering phase that may last for hours. It is searching for a sheltered place, usually the underside of a horizontal surface - a leaf, stick, fence rail or, in the classroom, the top of a container in which it is being reared. In nature most sites selected are somewhat dark and well sheltered. Once a "suitable" site has been located and after resting for a short time, the caterpillar begins to spin a bed of silk by extruding silk from the spinneret and weaving its head back and forth. When a suitable bed has been created, the caterpillar moves to its center where it produces a distinctive white silk button by moving its head back and forth hundreds of times over a small area. The caterpillar then fastens its anal prolegs into the silk button. In most cases, the caterpillar is now more or less horizontal. To get into the J position, the caterpillar first loosens its grip with the thoracic legs, starting with the first pair, and then the abdominal prolegs, proceeding from front to back. The release progresses slowly, perhaps to keep the force of gravity and the mass of the caterpillar from pulling the crochets of the anal prolegs from the silk button. The arc of the final release, when the last of the abdominal prolegs releases from the silk, is only about half of what the arc would be if all the legs were released at once (usually 90 degrees). If the caterpillar falls at this stage, and some do, it will not be able to pupate properly. The final release happens so rapidly that most people who rear monarchs haven't seen this happen. The duration of the J stage is temperature dependent but is usually 12-16 hours. Unless disturbed, the J caterpillar does not move until just before the formation of the chrysalis.

The Chrysalis

Near the end of the J stage, when the J shape starts to straighten, the filaments become limp and twisted. The color of the caterpillar begins to change as well, due to the color of the cuticle of the developing chrysalis beneath the last caterpillar skin. In what appears to be an effort to loosen the old skin from the new cuticle beneath it, the caterpillar begins a series of "pull ups" that bring it back into the J position for a few seconds. The "pull ups" signal that pupation is just minutes away. Pupation starts with rhythmic contractions that start just below the anal prolegs. These contractions force fluid forward causing the skin to split between the thoracic filaments dehind the head. With continued contractions, the split enlarges and the old caterpillar skin begins to move upward toward the silk attachment point. Curiously, this movement of the skin is not uniform; rather, the skin is shed along the dorsal (back) aspect more rapidly than along the ventral (front) aspect of the abdomen. The result is that the when the dorsal split is complete the ventral side of the skin is still attached. Within seconds of when the skin reaches the end of the abdomen on the dorsal side, a remarkable thing happens – the end of the abdomen contracts just enough to pull the cremaster free from within the anal prolegs in which it had formed. The cremaster is immediately thrust into the silk pad. It usually takes two or three jabs to firmly implant the cremaster in the silk, and this is typically accomplished within seconds but can take many attempts and 30 seconds or more. Once firmly attached to the silk, the new chrysalis begins to twist, the effect of which is to wind the silk threads around the hooks and pegs of the cremaster thus holding the chrysalis firmly in place. The twisting usually causes the shed skin to fall away. You can find this old skin and head capsule at the bottom of the rearing chamber.

A remarkable aspect of this process is how the chrysalis is held in place while the cremaster is brought out from beneath the anal prolegs. At this moment in time the skin of the anal prolegs is the only attachment to the silk pad, and the only skin attached to the chrysalis is along the ventral aspect – perhaps two or three segments – of the abdomen. What holds this skin in place, and how is it that the anal prolegs can support the weight of the chrysalis when separated from the end of the abdomen? A partial answer may lie in the form of the end of the abdomen during this process. During pupation, the ventral aspect of the last three abdominal segments project outward forming shelves or ridges. At the end of the abdomen there are also two raised black knobs. It is these ridges and the knobs that hold the skin to the chrysalis when the cremaster is stabbing at the silk pad. Once the cremaster is attached, the ridges and black knobs contract rapidly. The former knobs remain as the largest black dots on the ventral side of the abdomen of the chrysalis.

After the caterpillar skin is shed, the soft yellowish-green chrysalis begins to change shape and harden. After several hours the chrysalis is jade green in color with metallic-looking gold spots.

Monarchs: The Challenges of Space

Challenge #1
Crawling and Clinging to the Substrate

Even though monarch caterpillars walk with the aid of 16 legs and spin silk threads to cling to the walls of the chamber, it is fair to ask - what will happen if they lose their grip? What will happen on lift off of the shuttle when the force of gravity is several times the normal level? Will they float in the chamber and, if so, can they twist their body in a manner that will help them reconnect with the substrate, or do they have enough mass to slowly sink to the floor of the chamber? If you place a caterpillar in water, what does it do? If you have an extra caterpillar, fill a shallow bowl with water and place the caterpillar on the surface of the water. Does the caterpillar "swim" and have direction or does it just twist in a random fashion? (If you conduct this experiment, remove the caterpillar from the water after a minute or so and place it on a paper towel, then on its food once it starts to crawl.) The actions of the caterpillar in water are likely to be similar to those of a caterpillar that loses its grip and begins to float in the capsule. The question is whether twisting (swimming) is effective in getting the caterpillar to reconnect with the surface or whether the caterpillars have enough mass to slowly sink.

Along these same lines, how do astronauts move in space when not in contact with the substrate (i.e., untethered)? Can they move their limbs and muscles to twist their body (torque) and "swim" so as to connect with solid surfaces of the spacecraft?

What will happen to the frass? Will the frass stay where the caterpillar excretes it or will it float in the capsule?

Caterpillars normally show strong negative geotaxis when feeding on plants – meaning they go to the top of the plants. What will happen in the microgravity of the capsule? Will the caterpillar be able to sense which way is up?

Challenge #2
Selection of a Pupation Site and Forming the Silk Bed & Button Prior to Pupation

Sensing which way is up is also important for the next stage. When a caterpillar has finished feeding it usually moves upward (negative geotaxis) and seeks a horizontal surface on which to lay down a bed of silk. In the middle of this silk bed it swings its head back and forth hundreds of times to create a silk button. When this task is completed, it crawls forward and positions its anal prolegs over the silk button. Once the anal prolegs are firmly attached to the button, it lets go of the silk bed first with the thoracic legs and then with the abdominal prolegs, and the weight of the caterpillar swings it into a J position. What will happen in absence of normal gravity when the caterpillar lets go? Without gravity to help it swing into the J position, will the caterpillars actually form Js? Even if they do form Js, will they hang perpendicular to the substrate on which the silk pad was woven?

Challenge #3
Shedding the Last Caterpillar Skin During Pupation

To what extent is gravity involved in shedding the last caterpillar skin? To shed the skin, the J stage caterpillar uses the muscles in the abdomen to force fluid forward and since the caterpillar is hanging upside down this means forcing fluid downward could be aided by gravity - a force in the same direction. The contractions begin in the last abdominal segments and move forward (downward) in waves. The force of the fluid moving into the thorax appears to cause the caterpillar skin to split between the thoracic filaments, and once the split has begun, the "chrysalis to be" begins a series of movements that push the skin upward toward the silk attachment point. (It's a bit like wiggling out of your clothes with your feet firmly glued to the ceiling.) So, the questions here are: Does gravity aid in shedding the skin, and to what extent are the movements of the caterpillar and "chrysalis to be" influenced by gravity?

Challenge #4
Jabbing the Cremaster into the Silk Button

Imagine this: You are hanging upside down from the ceiling with the use of socks made of Velcro* and you are wiggling out of your old clothes (you have some new clothes underneath) and just as your old clothes reach your ankles you have to pull your feet out from your socks and jab your Velcro covered feet into a patch of Velcro on the ceiling that is right next to your socks – and you only have seconds to do it and you can't see what you are doing. Basically that's what monarchs are faced with at the last stage of pupation. While somehow hanging on with the anal prolegs and the old skin that clings to a portion of the abdomen, the chrysalis has to pull a new foot, in this case the cremaster, out of the old skin of the anal prolegs and jab it into the silk button. The end of the cremaster contains hooks (and pegs) that become entangled in the loops formed by the silk. If the process of hooking into the silk fails, the chrysalis falls and like Humpty Dumpty can't be put together again. If this process should fail in space, the chrysalis will float and then the question becomes whether it will be able to mature and contract into its normal shape. Actually, it may not be able to do so. Pupae that fall at this stage in our lab are sometimes unable to contract and assume their normal shape. But, getting back to the first problem, how will the new chrysalis be able to sense when and where to jab the cremaster? Will the end of the chrysalis' abdomen "know" where it is through feedback provided by proprioceptors? And, what about all that wiggling in microgravity? Will the gyrations be exaggerated in this environment?

*(Students all know Velcro but they probably don't know that one piece contains hooks while the other contains loops of strands of material that wrap around the hooks when the two pieces are brought together. In fact, the idea for Velcro was inspired by seeds equipped with hooks that help attach them to the fur of passing animals.)

Challenge #5
Emerging from the Chrysalis and Expanding the Wings

Does gravity aid emergence of the adult from the chrysalis, and do the wings require gravity to expand normally? Remembering that the developing adult butterfly is hanging upside down within the skin of the chrysalis, does gravity aid in splitting the skin? The prevailing idea is that the new adult takes in air through the spiracles (seven pairs) of the abdomen thus increasing the size of the abdomen and that it then pushes downward causing the cuticle (skin) of the chrysalis to split behind the head on the thorax. Within seconds of this split the new adult pushes its legs forward causing a V shaped split to develop on the opposite side along the mouthparts, antennae and legs. The effect of both splits is to create an opening that allows the butterfly to move downward and to free its legs so that they can grab onto the cuticle. Once the legs have a good grip, you can see the abdomen moving within the chrysalis, and when free of this cuticle, the abdomen "flips" downward and the new butterfly is separated from the cuticle that once contained it. The entire process takes 1 to a few minutes. What will happen in space? Does the mass of the adult and gravity aid in emergence? Will the new butterfly be able to get into the proper position to expand its wings?

Expanding the wings is the next problem faced by the butterfly. Within a minute of separation from the chrysalis cuticle, the abdomen begins to contract forcing fluid into the wings. The wings expand with this force but the mass of the fluid, and the wings, help them expand in the presence of gravity. What will happen under low gravity conditions? Will the wings be able to fully expand within the usual 10 minutes as they do on earth or will the process take longer? And, will the butterflies be able to detect whether the tips of their wings are in contact with a surface? This stage is also critical, if the wings become crumpled, the butterfly will not be able to fly, feed, or mate and reproduce.

Outcomes

What will it mean if monarchs cannot cling to the substrate, hang up to pupate, shed their skin, pupate, emerge from the chrysalis or expand their wings normally?

If there is failure at any stage, it can show us how monarchs are limited in their ability to perform life-sustaining tasks in the absence of earth's gravitational field. To be sure such assessments are correct, all of the specimens should fail at the same task – such as failing to expand the wings properly - and ideally, the experiment should be repeated so that more individuals are tested. In other words, the three individuals used in this experiment can give us a clue as to limitations, but we would need to test a larger number to be sure of our observations.

What will it mean if all the normal life functions from walking and feeding by the caterpillars to emergence as normal adults are performed successfully?

If monarchs perform all their life tasks successfully in the microgravity environment of the shuttle, it might mean that gravity is not as important to monarchs as we anticipated. Or, it may mean that the force of the microgravity can be detected and that the monarchs make adjustments accordingly. If the latter is the case, it raises an interesting question. How might organisms make adjustments to near weightless conditions? Suppose that the nervous system that controls the muscles and senses is connected through a feedback system such that the body “knows” the differences between “normal” and “abnormal’ body positions and functions. Most species of higher organisms have such a system, and the feedback is provided by proprioceptors, nerve endings that relay the position of muscles, etc., to other parts of the body. So, if monarchs perform all functions normally, is it possible that proprioceptors are important in allowing them to make adjustments to microgravity?

Here is an example of “knowing” that probably involves proprioceptors.
When a monarch emerges from the chrysalis and expands its wings, the tips of the wings are very soft and will become crumpled for life if they are in contact with a substrate that keeps them from expanding fully. The tips of the wings do not contain nerves yet, if the tip of the wing touches a surface, the butterfly will move away, usually upward, until the tip of the wing is no longer in contact with that surface. In the absence of functional nerve endings in the ends of the wings, how does the butterfly “know” that the tips of the wings are in touch with a surface - probably through a series of proprioceptors at the base of the wings.

Lower organisms such as monarchs seem simple don’t they? They have simple systems, tiny brains and yet they can do some amazing things such as migrating to the mountains of central Mexico where they overwinter, only to move north again in the spring to start the cycle for another year. We can learn a lot about life from these “simple” creatures.

Timeline

If you participate in the Monarchs in Space program, your kit will contain third and possibly new fourth instar larvae upon arrival. All the larvae we send will be 3^{rd} instars. Examine the cups containing the larvae upon arrival for small hard black dots, these are the head capsules of the third instar larvae and can be easily distinguished from frass. If there are no head capsules, all your larvae are still in the third instar. Fourth instar larvae have noticeably larger heads and the filaments are much longer than those of the third instar.

The larvae are all very close in age so, if you see a fourth instar larva in the cup, they are all ready to be transferred to your habitat. The protocol for the shuttle calls for the introduction of early 4^{th} instar larvae into the capsule on the 15^{th} of November. If you introduce your larvae into your habitat at about the same time and use temperatures that are similar to those on the ISS, your larvae will develop at a similar rate.

To transfer your larvae from the cup to the new chamber loaded with diet, lift up the larvae from the side with a small paintbrush and gently place them in the new habitat.

We have tested the growth of monarchs in a simulated capsule in our laboratory. Here is what you can expect in your habitat over the next 17-18 days. Your results might vary 1-2 days either way due to the temperatures in your classroom.

Day	Life Stage
1	Early fouth instar
2	Mid fourth instar
3	Late fourth/pre fifth – sitting on a pad with the new head capsule seen beneath the old one
4	Early fifth instar
5	Mid fifth instar
6	Late fifth instar
7	Late fifth instar
8	Late fifth/prepupa – searching for a place to pupate or spinning a bed/button or even making a J
9	J stage to chrysalis
10	Chrysalis
11	Chrysalis
12	Chrysalis
13	Chrysalis
14	Chrysalis
15	Chrysalis
16	Chrysalis – could be changing color
17	Chrysalis – could be changing color or emerging – most emergences occur early in the morning
18	Chrysalis or emergence
19	Chrysalis or emergence

Note: If started on the 15th, pupation should occur from the 22nd through the 24th, with emergence of the butterflies occurring after Thanksgiving.

Glossary

Abdomen – the third body region of an insect, primary center for food processing in the caterpillar and reproduction in the adult

Caterpillar – the second stage in the development of a butterfly or moth, also referred to as a larva

Cremaster – a post like structure at the end of the abdomen of a chrysalis bearing hooks and pegs that attaches the chrysalis to a substrate

Chitin – a tough polymer, a major component of the cuticle of insects and other arthropods

Chrysalis - the third stage in the development of butterfly – egg, caterpillar (larva), chrysalis (pupa), adult; the transformation that occurs during this stage is termed metamorphosis

Crochets –a ring of curved hooks on the abdominal and anal prolegs of most caterpillars, used to cling to surfaces and silk

Cuticle – the skin of a caterpillar, chrysalis or adult – composed of proteins and chitin

Ecdysis – the process of replacing an old skin with a new one that develops beneath it

Eclosure – to emergence of an adult form (butterfly or moth) from the chrysalis/pupa

Exuvia – the old skin shed in the process of ecdysis or molting

Filaments – the fleshy tentacles on the second thoracic and the eighth abdominal segment of the monarch caterpillar

Frass – the digested leaves or, in this case, artificial diet expelled by the caterpillars

Free fall – falling through an atmosphere with acceleration provided by gravity, in space the astronauts are in continuous free fall due to the speed and arc of the orbit of the space station or shuttle

Gravity – a force of attraction that brings objects together accounting for the existence of the earth and the planets or more simply, an explanation for why an apple falls to earth (as in Newton) rather than floats off into space; alternatively, as the force that causes objects to fall toward earth at a rate of 32 feet per second per second

g-force – a measurement of the force of gravity under conditions of acceleration, e.g. an object on the Earth's surface experiences 1 g, while an object in free fall experiences 0 g, on lift off the astronauts experience about 3 gs, for a short time they weigh 3 times as much as they do on Earth.

Head capsule – the hard exoskeleton of the head region of the body, head capsules are shed along with the skin during each molt

Head - the first body region of an insect, the head contains mouthparts, eyes, simple eyes (ocelli), antennae and is the site of most sensory input and processing

Instar – the developmental stage of a caterpillar between each molt, monarchs have 5 instars and five molts - becoming a chrysalis/pupa at the end of the 5^{th} instar

J stage – the J shape of a monarch caterpillar that is preparing to form a chrysalis, the head is downward

Larva – a wormlike immature form, a term most often used to describe immature beetles, flies, ants and bees as well as butterflies and moths, in the latter case larvae are referred to as caterpillars

Malpighian tubules – organs that excrete wastes and water absorbed from the blood (hemolymph) into the intestine

Metamorphosis – a change in form, e.g. a process by which an organism changes from caterpillar to chrysalis and chrysalis to adult

Microgravity – an environment where the force of gravity is present but has a minimal effect

Negative geotaxis – moving away (upward) from the stimulus (gravity)

Negative phototaxis – moving away from a source of light

Orientation - a response to a directional stimulus

Positive geotaxis - moving toward (downward) the stimulus (gravity)

Positive phototaxis – moving toward a source of light

Proboscis – the coiled feeding tube of the adult butterfly, a "pump" at the base of this straw-like structure aids in feeding on liquids such as nectar and water

Prolegs – legs found on the abdomens of caterpillars/larvae of most butterflies and moths, aid in clinging to surfaces, there are 4 pairs of abdominal prolegs and a pair at the end of the caterpillar that are termed anal prolegs.

Proprioceptors – nerve endings that signal the positions of body parts giving the organism a "sense of self"

Pupa – the third stage in the development of a butterfly, also known as a chrysalis

Pupation – the process of transition from the last caterpillar/larval stage to the formation of the chrysalis/pupa, characterized by the shedding of the last larval skin to reveal the cuticle of the chrysalis beneath it

Silk – a protein secretion of the salivary glands that hardens immediately upon contact with the air, monarch larvae lay down silk strands as they move and create beds of silk that they anchor to prior to molting

Spinneret – the opening just behind and below the head of a caterpillar through which liquid silk is secreted

Spiracles – pairs of external openings that connect to airways (trachea) in the caterpillar, chrysalis and adult, caterpillars have a pair of spiracles in the first thoracic segment and eight pairs along the sides of the abdomen

Taxis – a behavioral response such as moving away from or toward a directional stimulus

Thorax – the second body region of an insect, primary function is locomotion, site of the true legs in caterpillars and both wings and legs in the adults

Thoracic legs – caterpillars have three pairs of thoracic legs as do adults but monarchs only use the second and third pair for walking; the first pair is reduced and held close to the body behind the head

Torque – a force (in this context a muscle movement) that causes a body to twist about an axis

Weightlessness – a term used to describe the sensation described by astronauts experiencing continuous free fall – see free fall and zero gravity

Zero gravity – an unfortunate term, often used interchangeably with weightlessness, that implies a lack of gravity – a condition that does not exist on the shuttle or the International Space Station, the g force while in orbit is about 95% of that on the Earth's surface