

Lesson 1: Checkup Time – Give Your Monarch a Physical!
Master List of Documents

Teacher Resources:

- Overview (Introduction, Key Concepts, Student Level, Skills, Objectives)

Student Worksheets:

- *Checkup Time – Give Your Monarch a Physical*
 - *Background – Monarch Characteristic*
 - *Materials and Procedures*
 - *Patient Form*
 - *Discussion/Analysis – Part 1*
 - *Discussion/Analysis – Part 2 – MonarchHealth Methods*
- *Monarch Physical Rating Scales* (pages 1 and 2)

Lesson 1: Checkup Time – Give Your Monarch a Physical!

Overview

Introduction:

In this activity students from upper elementary through high school will use some of the same techniques that scientists in Dr. Altizer's lab at the University of Georgia routinely utilize when conducting actual scientific research with monarch butterflies and the obligate parasite, *Ophryocystis elektroscirrha* (OE). Students will also participate in a citizen science program called Project *MonarchHealth*. Through Project *MonarchHealth* students will contribute to an international scientific study of the prevalence of the parasite OE in monarch populations throughout North America. Working alone or in small groups students will practice standard sterile protocols found in disease research, but in a totally safe environment. (OE only parasitizes monarch and queen butterflies. OE does not harm humans). Teachers have the option of using one or two techniques for testing monarchs for parasites. The standard *MonarchHealth* swab technique is easy and accurate. It works well with all age groups, including elementary students. Classrooms are encouraged to first use the swabbing method and send off samples to Project *MonarchHealth*, then if resources are available to use the tape method as well. The tape method is more difficult and requires practice, but it allows middle and high school students to immediately check monarchs for OE using microscopes. Students will also make observations, collect data, and make inferences regarding the health of individual monarch butterflies.

For more detailed information on monarchs, OE parasites, and the *MonarchHealth* project, there are additional details online at <http://www.monarchparasites.org/>

Key Concepts:

- Physical characteristics of a monarch give clues to the health and prior experiences of the butterfly.
- Environmental conditions affect the physical characteristics of monarch butterflies.
- Scientists use standard safety procedures when conducting disease research.
- All monarch butterflies are not the same. Their physical characteristics vary.

Student Level:

- Upper elementary
- Middle school
- High school

Skills:

- Prediction
- Observation
- Measurement
- Data collection
- Data analysis

Objectives:

Students will predict, observe, measure, and record data about the physical characteristics of adult monarch butterflies while using sterile techniques. They will convert their qualitative observations to numeric values using standard conversion scales. Students will also make inferences about the health and physical condition of monarch butterflies.

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Background:

How healthy is your monarch? Could your butterfly make the long flight to Mexico? Are its wings in good condition? How much energy does it have stored for the future? Is it infected with parasites?

When scientists at Dr. Altizer's lab at the University of Georgia test monarchs for OE parasites, they also look at the general condition of each butterfly. They have discovered several relationships between OE infections and the physical characteristics of monarch butterflies. You can learn a lot about your monarch by just taking some basic measurements.

Parasitic infections harm monarchs in many ways. Energy that butterflies normally need for growth and flight may be used fighting infections. As a result of OE, monarchs are often small. Two ways that scientists measure the size of a butterfly are mass and forewing length.

OE infections also affect the amount of energy monarchs save for the future. Butterflies with OE frequently have less stored energy or fat than uninfected monarchs. An easy way to measure the amount of fat is to look at the thickness of the adult butterfly's abdomen. A monarch with a thick abdomen could have lots of fat, or could have just recently fed.

If your monarch was raised from a caterpillar, you know a lot about your butterfly's past. A wild monarch is really a mystery with many experiences before being caught. Where did the butterfly come from? Is it originally from your area or hundreds of miles away? How old is the butterfly? Why are the wings damaged or worn? Did it escape a predator as indicated by pieces missing from the wings? Has the monarch mated or laid eggs? Is it infected with parasites?

Scientists are able to get some ideas about a wild butterfly's past after looking at its wings. As a butterfly gets older its wings become worn and damaged. Mating also has a dramatic affect on butterfly wings. Male monarchs are very aggressive. During mating many scales rub off the wings of both male and female butterflies, and wings can become tattered.

Mating can also move OE spores from infected to uninfected monarchs. These adult butterflies can not get sick, but they can scatter parasites on milkweed plants or eggs. This increases the number of caterpillars that get OE infections.

If you are extremely careful, it is possible to learn if an adult female monarch has mated. Spermatophores are hard pea-like structures that form in the abdomen of a female monarch after mating. During mating, males transfer nutrients to the female and are stored in a special female organ called the bursa copulatrix. You can feel these hard structures by *gently* palpating or touching the female's abdomen.

Like the scientists at Dr. Altizer's lab you can give your monarch butterfly a physical examination. With a few simple tools, you can test the butterfly for parasites and look at its general condition. After the exam you can compare your results with others and draw conclusions about your monarch's health. Let's get started and give your monarch a checkup!

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Monarch Characteristics to Examine:

OE Infection:

Option 1 - Easy and Accurate – MonarchHealth Swab Method (required):

Use your *MonarchHealth* kit to check your monarchs for OE parasites. Be sure to send your samples to Dr. Altizer's lab.

Option 2 - Advanced Technique – Tape Method (optional):

The tape method can be tricky and requires practice, but it lets you immediately check your monarchs for OE parasites. Use the OE Infection Rating Scale to evaluate your results. Be sure to send your samples to Dr. Altizer's lab. We will confirm your results and you will make an important contribution to an international scientific study of OE parasites in monarch populations.

Size:

Scientists in Dr. Altizer's lab routinely collect data on the size of monarchs. The size of an adult monarch butterfly is controlled by many things. The amount of food that a caterpillar eats is a major factor affecting butterfly size. When there is not much milkweed available for caterpillars, the adult butterflies will be smaller. Genetics also play a role. Some monarchs are more likely to be smaller than others. Diseases and parasites, such as OE, harm monarchs and can produce smaller butterflies.

Two good indicators of butterfly size are forewing length and mass. The forewing length is the longest measurement from the point of wing attachment to the distal tip. Be sure to locate the small white dot at the base of the forewing. This white dot marks the place where the forewing attaches to the thorax. The distal tip is the farthest wing edge from this white dot. The easiest way to measure forewing length is using calipers, but a ruler works as well.

When measuring the mass of a monarch, always put the butterfly in a glassine envelope. The butterfly will not fly away or get damaged when it is stored in the envelope. The mass of the butterfly is determined by subtracting the mass of the envelope from the total mass of the butterfly in the envelope.

Wing Wear, Wing Damage, and OE:

Monarchs normally emerge from the chrysalis with beautiful wings. The longer a butterfly lives, the more likely scales will rub off and the wings will get tears or holes. Damaged wings may be the result of encounters with predators or other monarch butterflies. Mating is one of the most common causes of scale loss and wing tears.

In addition to harming wings, contact between monarch butterflies increases OE transmission. Infected monarchs can pass OE spores to uninfected monarchs. This is especially a problem during mating. An uninfected monarch may test positive for OE after mating with infected monarchs. This monarch is now a carrier of the OE parasite.

Scientists are interested in learning about the past experiences of wild caught monarchs. Raised monarchs may not have the wing wear or damage that a wild butterfly might have. If properly reared and handled, a raised butterfly will only have OE spores on its abdomen if it was infected as a caterpillar. That is not true of wild monarchs. These butterflies can get OE spores during mating and contact with other monarchs. By looking at the condition of the wings, scientists get some clues about the age of a butterfly and the chances that OE spores were picked up during mating.

Scientists at Dr. Altizer's lab separate wing condition into two categories: wing wear and wing damage. Wing wear is the loss of scales from the wing surface. This is measured using the

Wing Wear (Scale Loss) Rating Scale. Actual physical damage to the wings, such as tears, holes, and missing pieces of wing are considered wing damage. Wing damage is measured using the Wing Damage Rating Scale.

Sex:

There are several characteristics that can be used to determine if your butterfly is a male or a female. Beginners often look at the wings. Male monarch butterflies have a dark scent gland on each hind wing that females do not have. Wings of females tend to have darker thicker veins than wings of males.

The abdomens of males and females are different. A male has claspers at the tip of its abdomen. The claspers are used to hold the female during mating. Females do not have claspers, but an ovipositor at the end of the abdomen. The ovipositor is pointed and it is used to lay eggs.

Fat (Stored Energy):

The thickness of an adult butterfly's abdomen can be used to measure the amount of fat or stored energy available to a butterfly. The abdomen stores energy and swells after feeding, a large abdomen could indicate either recent or past feeding history.

Mating Status of Females:

Scientists can determine whether an adult female monarch has mated. After mating a female's abdomen contains spermatophores. Spermatophores vary in size. They often resemble peas or grains of rice. These hard spherical or oval objects can be felt through the abdominal wall. You can feel the hard spermatophores by *gently* palpating or touching the soft abdomen of a female butterfly.

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Materials:

- *Checkup Time – Give Your Monarch a Physical!*
 - *Patient Form*
 - *Discussion/Analysis – Part 1*
 - *Discussion/Analysis – Part 2 - MonarchHealth Results*
- *Monarch Physical Rating Scales* (pages 1 and 2)
 - *Wing Wear (Scale Loss) Rating Scale*
 - *Wing Damage Rating Scale*
 - *Sex Determination Table*
 - *Fat (Stored Energy) Rating Scale*
 - *OE Infection Rating Scale*
- *MonarchHealth* kit
- Live adult monarch butterflies
- Disposable vinyl or latex gloves
- 20% bleach solution or disinfecting wipes with bleach
- Calipers or metric ruler
- Electronic scale
- Glassine or small paper envelopes

Procedures (Day 1):

- 1) Getting started:
 - a) Put on your gloves!
 - b) Clean all work surfaces and tools with 20% bleach solution or disinfecting wipes. This sanitizes the area and helps kill parasites.
- 2) Record the initial observation on *Checkup Time – Give Your Monarch a Physical! – Discussion/Analysis – Part 1*.
 - a) Fill in the heading.
 - b) Get your monarch butterfly.
 - c) Carefully look at the monarch. Answer the pre-exam questions.
- 3) Record basic information on *Checkup Time – Give Your Monarch a Physical! - Patient Form*.
 - a) Record the sample number.
 - b) Was the monarch raised or was it caught as a wild butterfly? Record raised or wild in the space provided on the form.
 - c) Record where the monarch came from in the location box.
 - d) Record the collection date.
 - e) Fill in today's date as the date sampled.
- 4) Check for OE parasites.
 - a) *Swab Method*: Sample your monarch for OE parasites by following the instructions in your *MonarchHealth* kit.
 - b) Send your samples to the Altizer lab. (By sending all of your samples to Dr. Altizer's lab you will contribute to an international scientific study of monarch butterflies and OE parasites. The lab will also confirm your results.)
 - c) Record the OE infection results when you receive them from Dr. Altizer's lab.
- 5) Measure the forewing length of your monarch (from the point of attachment to the distal tip).
 - a) Use calipers or a metric ruler. Measure to the nearest tenth of a centimeter.

- b) Locate the base of the forewing. This is where the forewing attaches to the thorax. Notice the small white dot that marks the base of the forewing. Place the edge of the ruler on the white dot.



- c) Locate the distal tip of the forewing. This is the most distant edge of the forewing from the wing base.
- d) Record the length of the forewing from the point of attachment to the distal tip. Your measurement should be to the nearest tenth of a centimeter.
- 6) Determine the amount of wing wear.
- Carefully examine the wings. Look for scratches and areas on the wings where scales are missing.
 - Use the *Wing Wear Rating Scale* to measure the amount of scale loss.
 - Record the Wing Wear score.
- 7) Determine the amount of wing damage.
- Carefully examine the wings. Check for tears and holes. Look for missing pieces of wing along the wing edges.
 - Use the *Wing Damage Rating Scale* to determine the amount of wing damage.
 - Record the Wing Damage score.
- 8) Determine the amount of fat (stored energy).
- Examine the butterfly's abdomen. Look at the thickness of the abdomen. How fat is your butterfly?
 - Use the *Fat (Stored Energy) Rating Scale* to score your butterfly.
 - Record the Fat score.
- 9) Determine the sex of the monarch.
- Examine the butterfly's abdomen. A male monarch will have a pair of claspers at the tip of the abdomen. Claspers are used to hold on to the female during mating. A female will not have claspers, but will have a slit on the underside of the abdomen and an ovipositor at the tip. The ovipositor helps guide eggs out of the female's body.
 - Draw and describe the butterfly's abdomen in the appropriate space on the *Patient Form*.
 - Examine the wings of the butterfly. Females tend to look darker and have thicker black wing veins than males. Males have distinctive hind wings. Near the middle of each hind wing, a male monarch has a small black scent gland. Females do not have these black scent glands.
 - Describe the monarch's wings in the appropriate space.
 - Compare your observations with the descriptions and pictures on the *Sex Determination Table*.
 - Record the sex of your butterfly.

- 10) Determine the mating status of females.
 - a) This section only applies to female butterflies. You can not tell whether males have mated. If you have a male butterfly, proceed to step #11.
 - b) Take your female butterfly and gently palpate or touch the abdomen to locate any spermatophores. During mating, males transfer nutrients to the female and are stored in a special female organ called the bursa copulatrix. You can feel these hard structures by *gently* palpating or touching the female's abdomen. Spermatophores may feel like small peas or grains of rice. If you feel a spermatophore your female butterfly has already mated.
 - c) Record "yes" or "no" in the Mated column.
- 11) Determine the mass of the monarch.
 - a) Determine the mass of a glassine (or paper) envelope in grams using an electronic scale.
 - b) Place the butterfly into the envelope.
 - c) Determine the mass of the butterfly and envelope.
 - d) Calculate the mass of the butterfly by subtracting the mass of the envelope from the mass of the butterfly and envelope.
 - e) Record the mass of the butterfly in grams.
- 12) Answer the remaining questions on *Discussion/Analysis - Part 1*.
- 13) Send all of your OE samples to *MonarchHealth* at the Altizer lab.
- 14) **OPTIONAL: Advanced Technique – Tape Method:** Check for OE parasites in the classroom using the tape method after swabbing for parasites. The tape method requires practice, but it makes it possible to immediately check your monarch for OE parasites. Record the number of spores observed. Use the *OE Infection Rating Scale* on the *Monarch Physical Rating Scales* form to score the extent of the OE infection.

Procedures (Day 2 – After receiving OE results from *MonarchHealth*):

- 1) Record "yes" or "no" in the OE Infection box of the *Patient Form*.
- 2) Answer the questions on *Checkup Time – Give Your Monarch a Physical! – Discussion/Analysis – Part 2 # 1-15*.
- 3) If you also used the tape method, answer the questions on *Checkup Time – Give Your Monarch a Physical! – Discussion/Analysis – Part 2 # 16-21*.



Checkup Time – Give Your Monarch a Physical! Patient Form



Name _____ Class _____ Date _____

Sample Number:	Raised/ Wild:	Location:		
Date Collected:		Date Sampled:	OE (Tape method only) Spore #	OE Infection: (yes/no)
Forewing Length (cm):		Mass (g):	Score:	
Wing Wear (Scale Loss): Description:				Wing Wear: Score:
Wing Damage (Tears, holes, etc.): Description:				Wing Damage: Score:
Sex Determination: Abdomen Drawing/Description:		Sex Determination: Wing Description:		Sex: (male/female)
Fat (Stored Energy): Abdomen Thickness Drawing/Description:				Fat: Score:
Mating Status (Females Only): Abdominal Palpation Description:				Mating Status: Female mated? (yes/no)

Checkup Time – Give Your Monarch a Physical!
Discussion/Analysis - Part 1

Name _____ Class _____ Date _____
Sample Number _____

Pre-exam Questions:

1. Before beginning the checkup, what do you think about the monarch? Does it look like a large or small monarch?

2. Does the monarch look young or old?

3. Does it look healthy or sick? Explain your answer.

Post-exam Questions:

1. Look at the fat score of your monarch. Does the butterfly have a large supply of stored energy for the future?

2. Monarchs with OE parasites are often smaller and have less fat than healthy monarchs. After examining your monarch, do you think your monarch is infected with OE parasites? Why or why not?

3. Parasites can pass from one monarch to another monarch. Do you think your monarch has had contact with other monarchs? Explain your answer.

4. Do you think your monarch could make the long flight to Mexico? Explain your answer.

7. Why is it important to wear disposable gloves when testing a monarch for OE?

8. Was your monarch infected with OE parasites?

9. Did the OE results from *MonarchHealth* support your prediction about the health of your monarch? Explain your answer.

10. Did your monarch have any symptoms of being sick with OE parasites? (Was it small or thin with little stored fat?)

11. If your monarch was a female, was there evidence that she mated? (Were there spermatophores in the abdomen?)

12. What will happen to the offspring of a female monarch that is infected with OE parasites?

13. What will happen if an infected monarch is released into the wild?

14. Should an infected monarch be released into the wild? Explain your answer.

15. Answer the following question if your monarch was infected with OE parasites. Based on wing wear, wing damage, and female mating status, do you believe your monarch picked up OE spores from another adult monarch? Explain your answer.

If you also used the tape method, answer the following questions. If you did not use the tape method, you can stop here.

16. Were there any OE parasites on your monarch butterfly?

17. How many OE spores did you see?

18. What score did your monarch get on the *OE Infection Rating Scale*?

19. Describe the infection using the *OE Infection Rating Scale*. (Not infected, minimal infection, etc.)

20. A monarch with more than 20 OE spores (OE infection scores of 3 or more) is expected to have OE parasites within its body. OE spores are so tiny that they can accidentally transfer from infected to uninfected monarchs during testing. OE infection scores of 1 or 2 may be the result of testing errors. For this reason, MonarchHealth samples are only considered infected when there are more than 20 OE spores in the sample. Based on this information, would you expect to find OE parasites inside your monarch?

21. Did the OE results support your prediction about the health of your monarch? Explain your answer.

Monarch Physical Rating Scales

Wing Wear (Scale Loss) Rating Scale

Wing Wear Score	Description of Scale Loss
1	Newly emerged - no scale loss
2	Minimal scale loss – a few scratches
3	Moderate scale loss - scratching throughout the wings
4	Moderately severe scale loss
5	Severe scale loss - wings are almost clear

Wing Damage Rating Scale

A wing is damaged when there are tears, holes, or missing parts.





Wing Damage Score	Description of Wing Damage
0	No damaged wings. There are no tears, holes, or missing parts on any wing.
1	One wing has tears, holes, or missing parts.
2	Two wings are damaged.
3	Three wings are damaged.
4	Four wings are damaged.

Fat (Stored Energy) Rating Scale

The fat (stored energy) score is based on the shape and thickness of an adult monarch's abdomen.

Fat Score	Description of Adult Monarch Abdomen Width	Abdomen Shape
1	The abdomen is very thin. The skin collapses inward.) (
2	The normal abdomen shape of a newly emerged monarch.	I I
3	The abdomen is slightly swollen. Robust.	()
4	The abdomen is extremely swollen, like a grape.	()

Sex Determination Table

	Male	Female
Abdomen	 <p>-A pair of claspers at the tip.</p>	 <p>-Slit on the underside of the abdomen. Ovipositor at the tip.</p>
Wings	 <p>- A black scent gland in the middle of each hind wing. - Black wing veins are thin.</p>	 <p>- No scent glands in the hind wings. - Thick black wing veins. - Wings are dark.</p>

OE Infection Rating Scale (Taping Method only)

OE Infection Score	Number of OE Spores per Sample	Infection Description
0	0	No infection – Disease free
1	1	Minimal infection
2	2 to 20	Minimal infection
3	21 to 100	Mild infection
4	101 to 1000	Moderate infection
5	More than 1000	Severe infection