CAN'T CATCH MY BREATH!
A STUDY OF METABOLISM IN FISH

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Grade Level
High School

Subject area
Biology, Environmental, or Marine Science

This work is sponsored by the National Estuarine Research Reserve System Science Collaborative, which supports collaborative research that addresses coastal management problems important to the reserves. The Science Collaborative is funded by the National Oceanic and Atmospheric Administration and managed by the University of Michigan Water Center.

1. **Activity Title:** Can't Catch My Breath! A study of metabolism in fish

2. **Focus:** Metabolism (Ecological drivers); The Scientific Method (Formulating Hypothesis)

3. **Grade Levels/ Subject:** HS Biology, HS Marine Biology

4. **VA Science Standard(s) addressed:**

BIO.1. The student will demonstrate an understanding of scientific reasoning, logic, and the nature of science by planning and conducting investigations

(including most Essential Understandings and nearly all Essential Knowledge and Skills)

BIO.4a. The student will investigate and understand life functions of Archaea, Bacteria and Eukarya. Key concepts including a comparison of their metabolic activities.

Essential Understandings: For the body to use food for energy, the food must first be digested into molecules that are absorbed and transported to cells, where the food is used for energy and for repair and growth. To burn food for the release of energy, oxygen must be supplied to cells and carbon dioxide removed. The respiratory system responds to changing demands by increasing or decreasing breathing rate in order to maintain homeostasis.

The circulatory system, which moves all of these substances to or from cells, responds to changing demands by increasing or decreasing heart rate and blood flow in order to maintain homeostasis.

Essential Knowledge and Skills: identify the proper response an organism would exhibit in response to changes in the environment to maintain homeostasis.

BIO.2 The student will investigate and understand the chemical and biochemical principles essential for life. Key concepts included) the capture, storage, transformation, and flow of energy through the processes of photosynthesis and respiration.

Essential understandings: Organisms can tolerate only small changes in pH because every cell has a particular pH at which it functions best. For example, changes in pH cause changes in enzyme conformation, resulting in a change in activity. Most cells function best within a narrow range of temperature and pH. At very low temperatures, reaction rates are too slow. High temperatures or extremes of pH can irreversibly change the structure of proteins or alter their function.

Essential Knowledge and Skills: Recognize the equations for photosynthesis and respiration and identify the reactants and products.

Describe the role of ATP in the storage and release of chemical energy in the cell. Explain the interrelatedness of photosynthesis and cell respiration.

5. **Learning objectives/outcomes**

a. Students will be able calculate rate of oxygen consumption, metabolic rate, andaerobic scope
b. Students will be able to make inferences concerning the habitat and life history ofthe animals the data comes from
c. Students will design a project proposal outlining the method and necessary data thatwill need to be collected.

6. **Total length of time required for the lesson: 2 hrs**

7. **Key words, vocabulary:**

Scientific Method: The step-wise method or procedure that consists of systematic observation, measurement, and experiment, and the formulation, testing, and modification of hypotheses.

Metabolism: the chemical processes that occur within a living organism in order to maintain life.

Metabolic Rate: the amount of energy used by an animal per unit of time oxygen consumption rate (MO_2).

Maximum Metabolic Rate: The maximum rate of oxygen consumption possible. Usually obtained through exercise or feeding or a combination of both.

Standard Metabolic Rate: The metabolic rate (rate of oxygen consumption) of an animal at rest

Aerobic Scope: The difference between the maximum metabolic rate, and the standard metabolic rate (see attached Figure).

8. **Background information**

By now, all students should be familiar with the scientific process. It may be helpful to review these if students haven't had recent exposure to these steps. Traditionally, this starts with asking a question, formulating a hypothesis, running an experiment to test the hypothesis, doing statistical analysis to determine which hypothesis is correct, and interpreting your results.However, in practice, science is rarely this straightforward. One of the primary goals of this project is to get students to formulate questions on the fly, to challenge each other, and realize that science can be difficult to interpret.

To accomplish this, students will be given metabolic rate data (NOTE: For lower level classes it may be helpful to focus on reinforcing the scientific method in its step-by-step procedure. For higher level classes, it might be fun and interesting to incorporate more of the calculations and data processing. (See prompts within the procedure for both options).

Metabolism is how every living organism survives. It is the chemical processes that convert food, and water into the energy needed to move, to digest food, to grow, and to reproduce. Unfortunately, the exact amount of energy needed for all of the complex processes constantly occurring in our bodies is difficult to measure. Conveniently for us, [almost] every living thing needs oxygen to survive, so we can use oxygen consumption rate as a proxy for metabolism. When we are running, our bodies are working hard and a lot of energy is being expended, and our breathing rate goes up. Conversely, when we are asleep, our bodies are still working, but our breathing slows because our rate of energy consumption is slower.

When thinking about metabolism of animals, there are a number of hints we can get about its energy consumption from other characteristics. For example, animals that live in warmer environments are likely to have higher metabolic rates because all life processes happen faster with added heat energy. Therefore, we would expect a tropical hummingbird to have a higher metabolic rate than a pigeon from a temperate climate. We can also compare activity levels between species. A really active species that is always moving will likely have a much higher metabolic rate than a sedentary species because it expends more energy in its daily activities. The same thing applies to animals that are "stressed" because they are sick or because they are in an environment that is not conducive to their bodies needs. For example, a fish that is in acidic water might consume more oxygen because it is working harder to maintain its internal pH at desired levels.

These same principles are largely applied to studies of fish physiology. To measure fish oxygen consumption, individuals are placed in special containers called respirometers (respiration + meter) that are completely sealed. The oxygen content of the water is then measured with an oxygen probe in the chamber. Because the goal is not to harm the fish, oxygen content is only permitted to drop to ~80%, and then the chamber is flushed with fullyoxygenated water. This means that the oxygen consumption rate is measured in many small increments over a long time, and potentially at different temperatures, resulting in a range of consumptionrates.

If maximum metabolic rate and standard metabolic rate are calculated at a variety of temperatures, then researchers can calculate aerobic scope curves (see attached). These can be thought of as the available power to complete life tasks (growth, foraging, migrating, mating, etc.) for an animal.

9. Student handouts and other materials needed (worksheet, data tables, diagrams, websites, etc.)

- Instructions on how to calculate metabolic rate, and aerobic scope curves
- Example data
- Instructions for formulating hypothesis

10. Materials & Supplies, A/V/Tech Support

- Handouts listed above
- Rulers
- Graph Paper (depending on the technical prowess of the students, graphing can be done on a computer)
- Writing utensils

11. Classroom/Lab/Field Study Setup

Tables for small group work are ideal. Desks that can be pulled together into small circles would also suffice?

12. Procedure:

Advance preparation of lab materials – 20 minutes

The instructor should review background information, and be prepared to explain how these data were obtained using the attached respirometer diagram.

Lab Set-up – 5 minutes

Move desks into tables, or arrange tables so that students can form small groups (~4 people/group) for this activity.

ENGAGEMENT (Introduction) – 15 minutes

Shrimp on a Treadmill: LiveScience Article
http://www.livescience.com/4221-scientists-put-shrimp-treadmill.html
Teacher's note: This was a big deal several years ago, both because of the silly science, and because it was picked up as a hallmark of bad government spending. I'm not sure if your students will remember it, but the principle of this project was to measure the metabolic rate and swimming capacity of shrimp that were healthy and that had a disease, in different water quality treatments. This is similar to the project we're going to ask students to do further in the lesson.

Remind students of the scientific process by doing a quick "Think, Pair, Share" activity. Have student brainstorm what they think are the steps of the scientific method (hint: there are 7, see Appendix A), then share what they came up with with their neighbor. Also ask students to think of instances where you might not want to follow the scientific method (ex: repeating an experiment to confirm results, getting unexpected results in the middle may cause you to go back to the beginning before completing the experiment. Finally, go around the room and ask pairs to take turns coming up to the board to write out different steps and share the examples of when the scientific method may not work.

For this lesson, we are focusing on metabolism. Since this is included in the required curriculum, students should have a basic understanding already of what is metabolism. Spend several minutes going over the basics to refresh everyone's memory.

- Q: What is metabolism?
  - A: The processes that occur within a living organism in order to maintain life
  - A: The energy used by a living organism
- Q: Why would a scientist be interested in measuring metabolism?
  - A: All life functions (breathing, eating, avoiding predators, growth, reproduction, etc.) have to happen for an animal to live and have high fitness, and all these functions make up metabolism. So, by measuring an animal's metabolism, we can get an idea of how much energy they have to maintain their bodies, to grow, and to reproduce.
- Q: What factors might influence an animal's metabolism?
  - A: Examples include but are not limited to the following: size of an animal, temperature of their environment, the health of the animal, whether an animal is pregnant, whether the animal has just eaten, if the animal is sleeping or moving around.

EXPLORATION– 20 minutes

Pretend you are brand new scientists just hired in a new lab that studies fish physiology. You are tasked with reviewing some data (GENERAL) or processing data (ADVANCED) that was collected years ago as part of a respirometry experiment that fell to the way-side due to lack of funding (this happens all the time in real science). Your boss has asked you to do some initial processing of the data, and report back to him.

Describe what respirometry is (see Appendix B). These experimental set-ups are used to measure oxygen consumption over time. Since all activity requires oxygen, this is a measure of metabolism.

GENERAL: Give students the sample oxygen consumption curves (Appendix C), and the metabolic rates that were calculated from each curve (Appendix E). Tell that that some of the data comes from sandbar sharks, which are really active predators who need to swim to breath. Some of the data comes from smooth dogfish which are capable of resting on the seafloor without swimming, but still can actively hunt. The remaining data come from clearnose skates, which are relatively sedentary species who mostly get their food from foraging on the seafloor. Ask them questions such as:

- What might cause the metabolic rates of the fish these data were taken from to be so different?
    - A: See above for what factors might influence an animal's metabolism. For fish specifically, temperature plays a big role since they are cold-blooded animals (ectotherms).
- The labels have gotten mixed up on these data! Can you make any predictions about which data came from which species? How would you defend your choice?

A: Students should pick up on more active species having higher metabolic rates, since movement requires oxygen.

ADVANCED: Give students the sample oxygen consumption curves (1-3 per group depending on time; Appendix C), and have them calculate oxygen consumption rates for each one (see Appendix D and be ready to check over their methods before they begin calculations). If each group only has time to calculate metabolic rate from one graph, have them share their results with the class, then return to small groups to answer the following questions. If they have time to calculate metabolic rate from several different curves, they may want to answer the following questions before coming back to a full-class discussion.

- What might cause these curves to be different?
    - A: See above for what factors might influence an animal's metabolism. For fish specifically, temperature plays a big role since they are cold-blooded animals (ectotherms).
- What can we infer about the individuals from which these curves were calculated?
    - Animals with lower metabolisms may be sedentary animals, like a skate, or more active animals, like a sandbar shark. Animals with higher metabolisms may have just eaten, so their body is working hard to digest food. Female animals with a higher metabolic rate may be working on making eggs, or could be pregnant (depending on reproductive mode).

- What will you tell your boss these data say?
    - A: This will depend on the graphs that each teacher chooses to give their students. Students should be able to correctly identify which species their graphs come from, and make inferences about the within species differences observed in the graphs.

NOTE: Students may say they do not have enough information to answer substantial questions. They need more information about the species these experiments were conducted on, the temperatures the experiments were run at, if the animals were sleeping or exercised before being placed in the chamber, etc. This is ideal, and leads well into the next section. Maybe prompt students with discussion questions such as:

- Would you expect a difference in metabolic rate of a Sandbar shark in cold water and a smooth dogfish in warm water?
- What about a resting smooth dogfish or a clearnose skate that is avoiding a predator?

EXPLANATION- 20 (This may be a separate class, and/or an extended homework project)

GENERAL: Now that you've identified the species that each of these data sheets came from, you need to get ready to present your conclusions to your boss. He is known to ask hard questions, so you'll need to defend your choices. Keep in mind some other variables.
Maybe prompt students with discussion questions such as:

- Would you expect a difference in metabolic rate of a Sandbar shark in cold water and a smooth dogfish in warm water?
- What about a resting smooth dogfish or a clearnose skate that is avoiding a predator?
- Where in the scientific method did this confusion arise?
- What steps could you take to help get some clear answers? How would you apply the scientific method to this problem?

ADVANCED: Your boss at the lab isn't happy with your lack of conclusions, but is willing to listen to a proposal for a small pot of money to do more experiments. You and your team must work together to write a 1 paragraph proposal describing:

- The 5 trials you will conduct (Species and temperature)
- What information you have
- What information these new trials will give you
- What questions this new information (or total information) will answer

ELABORATION (Discussion) - 20 minutes

Have each group present their proposals or ideas on how to get more clear answers to the class, and then come together as a group to discuss what was the hardest part of the activity.

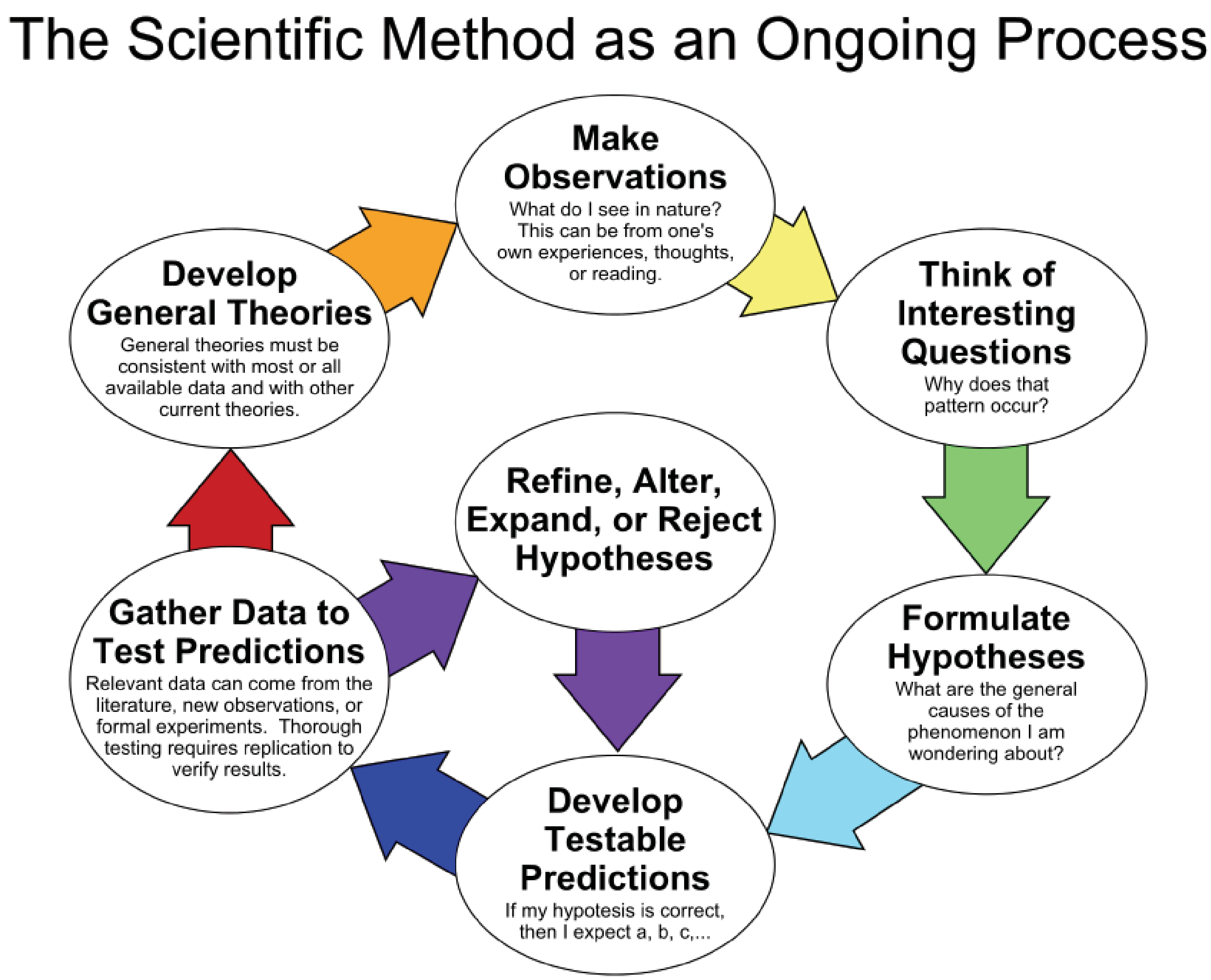
- Were you able to follow the scientific process?
- How were the steps you took different from the rote "scientific process" we put together at the beginning of class?
- What did you learn about metabolism?
- How do you think this compares to the job of a real scientist?

13. *Assessment (EVALUATION):*

Student success will be measured through the metabolic rate they calculated from the given graphs. Additionally, students will be evaluated on their ability to reconcile incomplete information and design a new set of experiments based on the knowledge they do have in-hand. This can be formally evaluated through a written proposal, or through written responses to questions. At the conclusion of the activity, students should be able to answer the following questions:

- What is metabolic scope and why is it important to understand?
- How does metabolic scope change, and what causes it to change?
- How do scientists deal with incomplete information?
- What are the challenges that arise when attempting to follow the scientific process?

Appendix A. Scientific Process Example



Appendix B. Respirometer Diagram

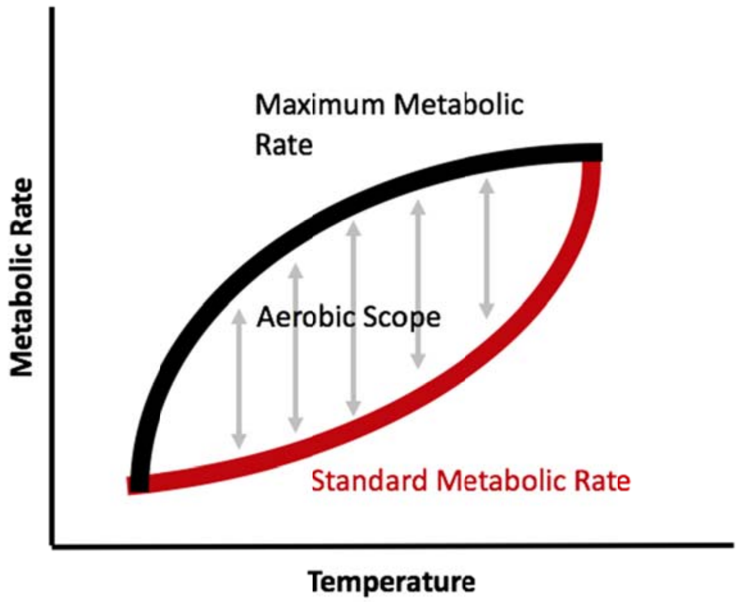


Figure 1. The effects of a controlling variable (in this instance temperature, T) on an individual's standard (red line) and maximum metabolic rates (black line) and metabolic scope or aerobic scope (range between standard and maximum active metabolic rate, grey).

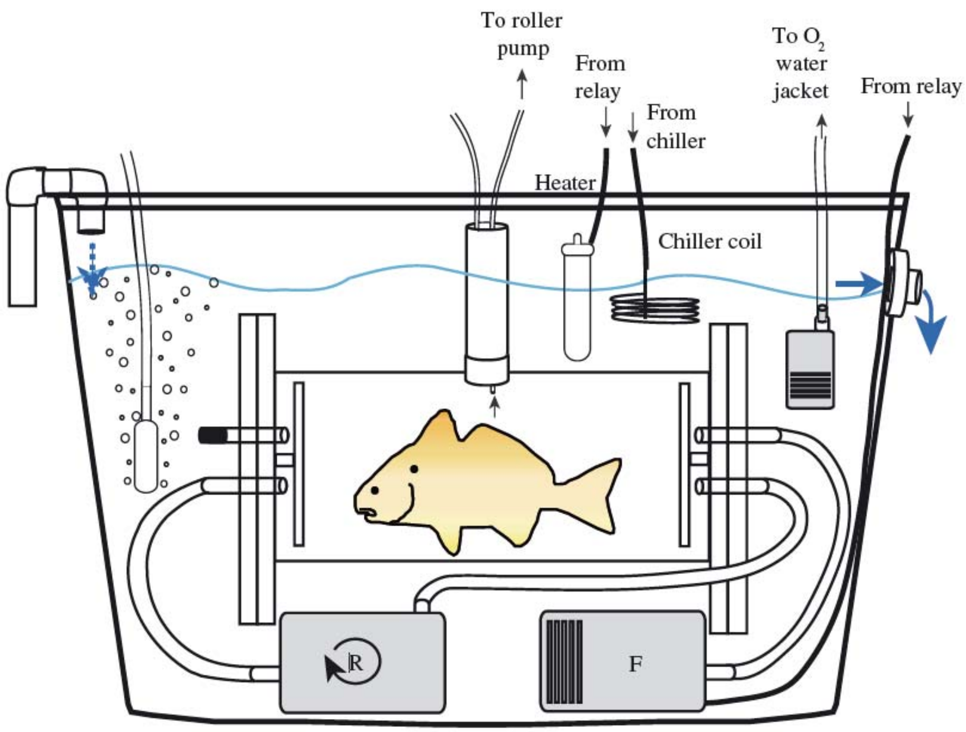
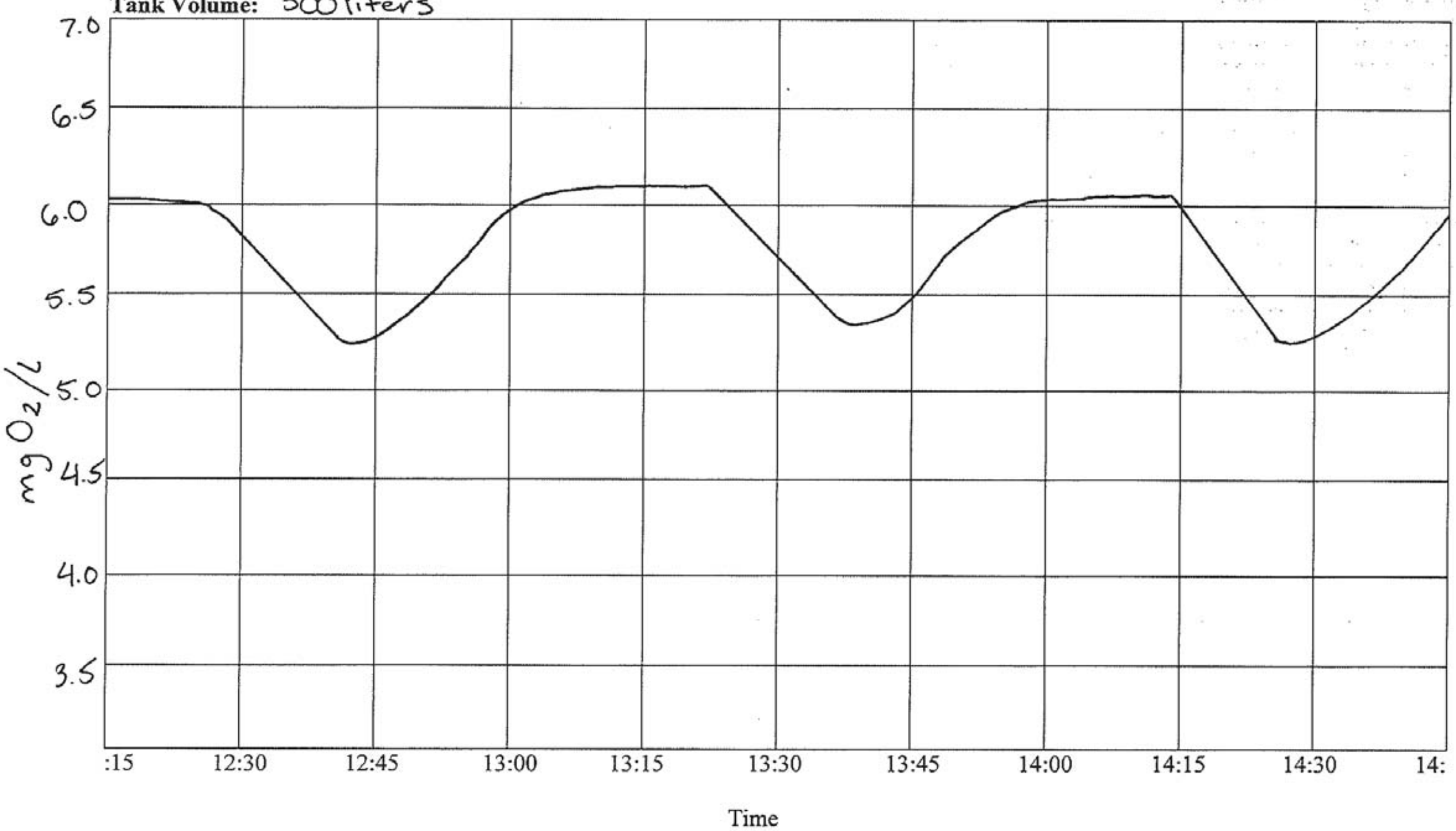


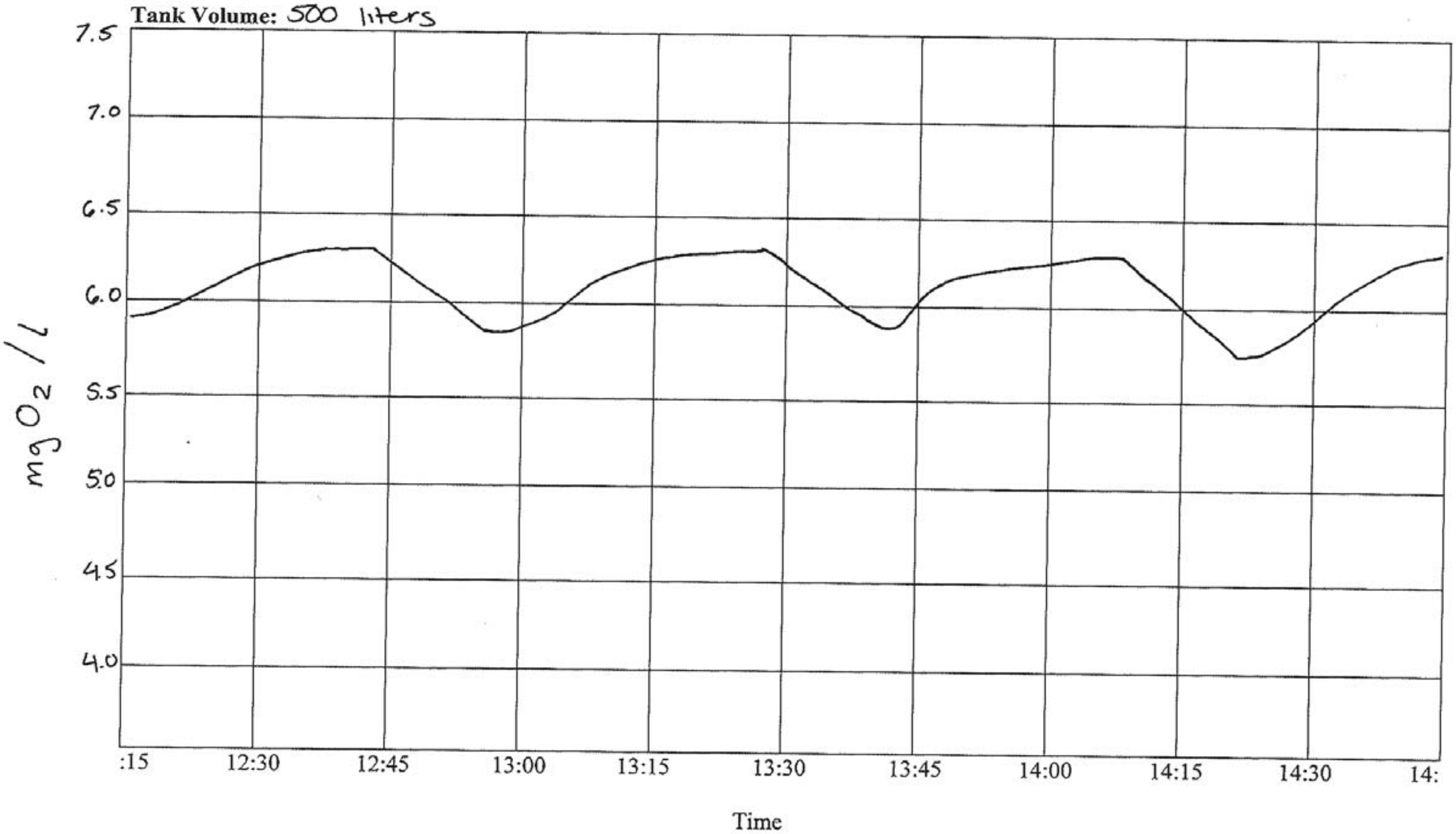
Figure 2. Experimental intermittent-flow respirometry chamber for metabolic rate measurements of winter flounder (figure reproduced from Horodysky et al. 2011). System will use automated intermittent-flow respirometry – periods of flushing the respirometer with water from the surrounding bath alternating with closed recirculation in the chamber for recording O_2 consumption by the fish.

Horodysky, A.Z., Brill, R.W., Bushnell, P.G., Musick, J.A., Latour, R.J. Comparative metabolic rates of common western North Atlantic Ocean sciaenid fishes. Journal of Fish Biology. 79(1): 235-255

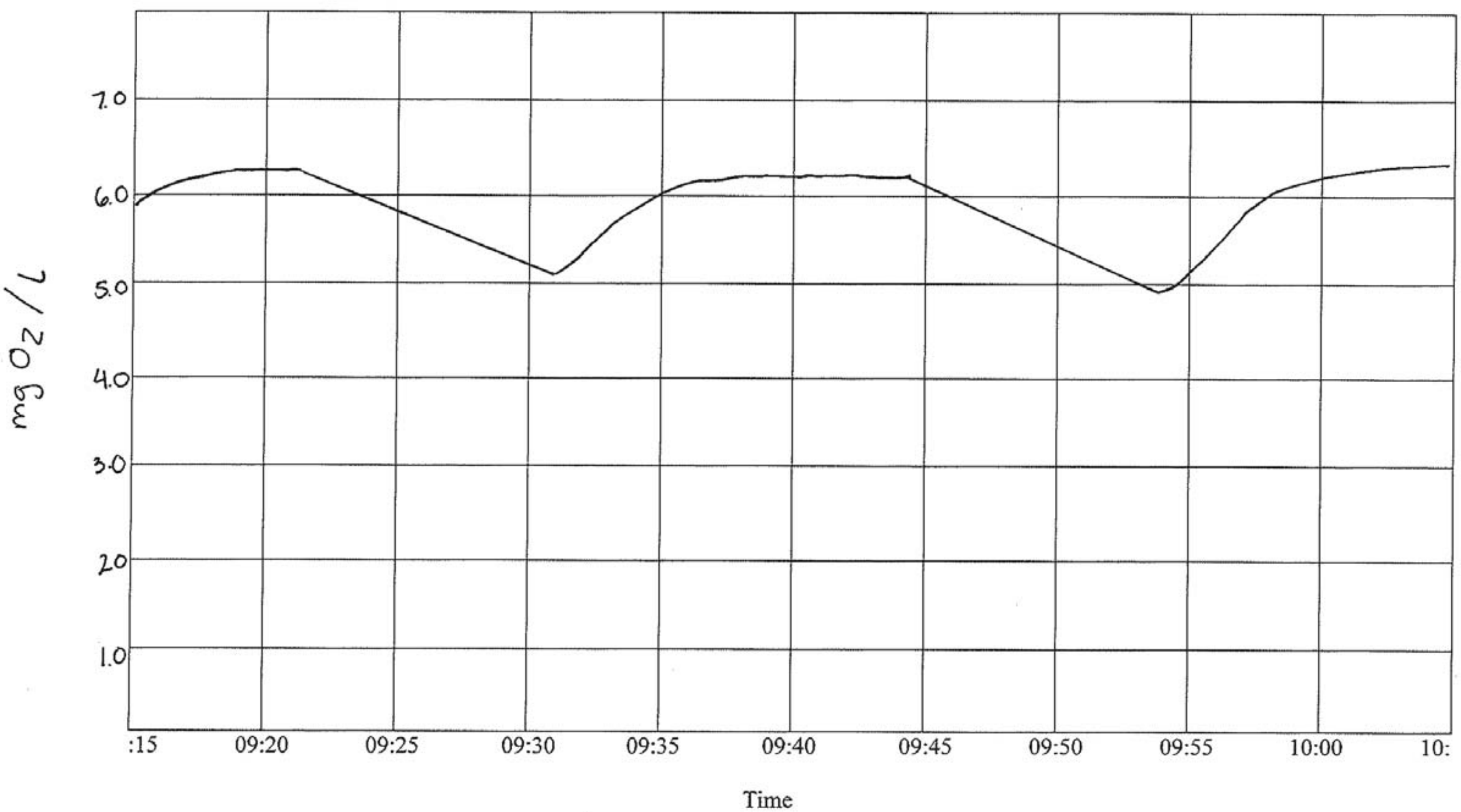
Appendix D. Examples of Raw Data
Animal A
Mass: 5 kg
Tank Volume: 500 liters



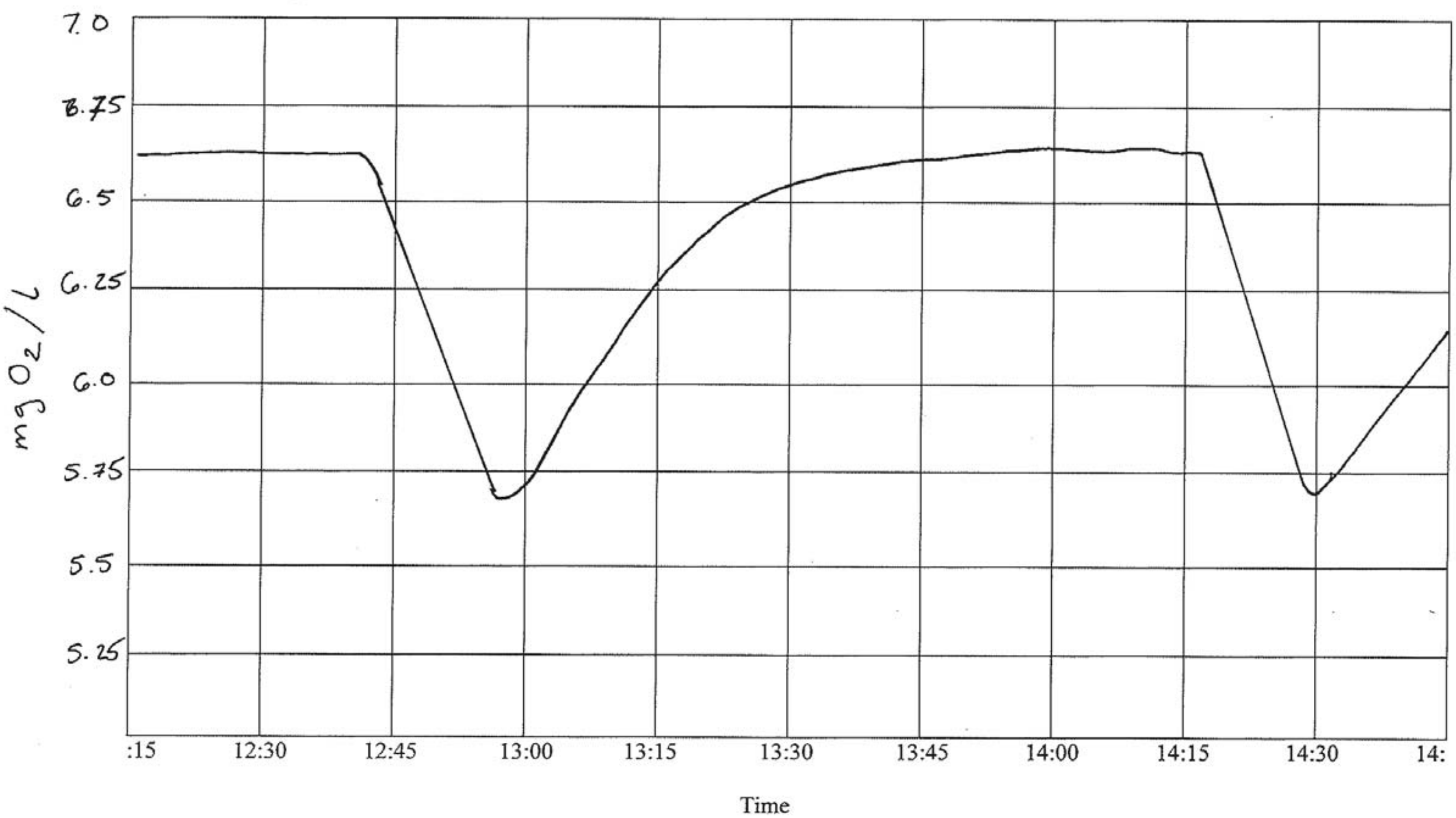
Animal B
Mass: 3.8
Tank Volume: 500 liters



Animal D
Mass: 1.8 kg
Tank Volume: 42.8 liters

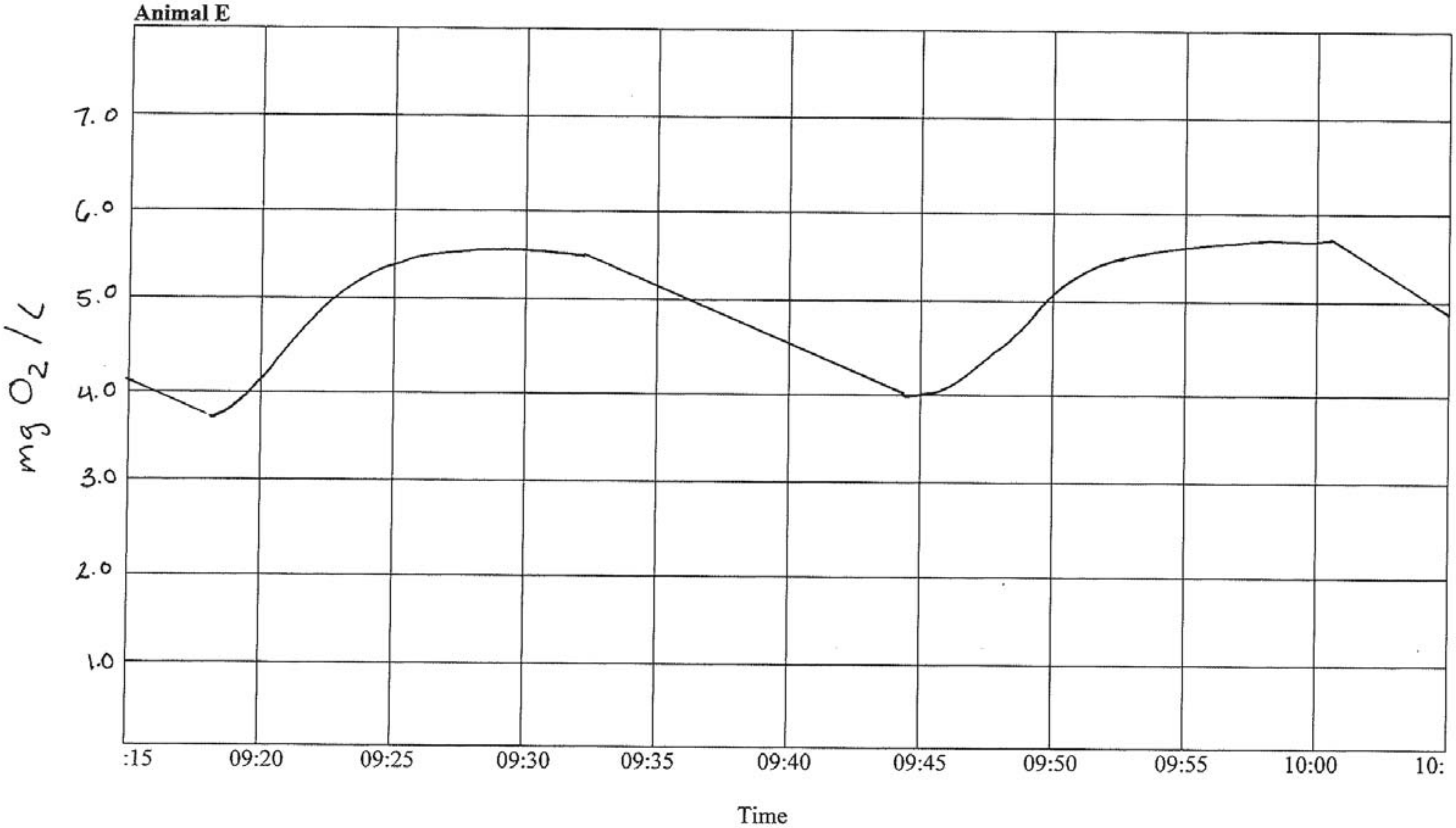


Animal C
Mass: 4.7
Tank Volume: 500 liters

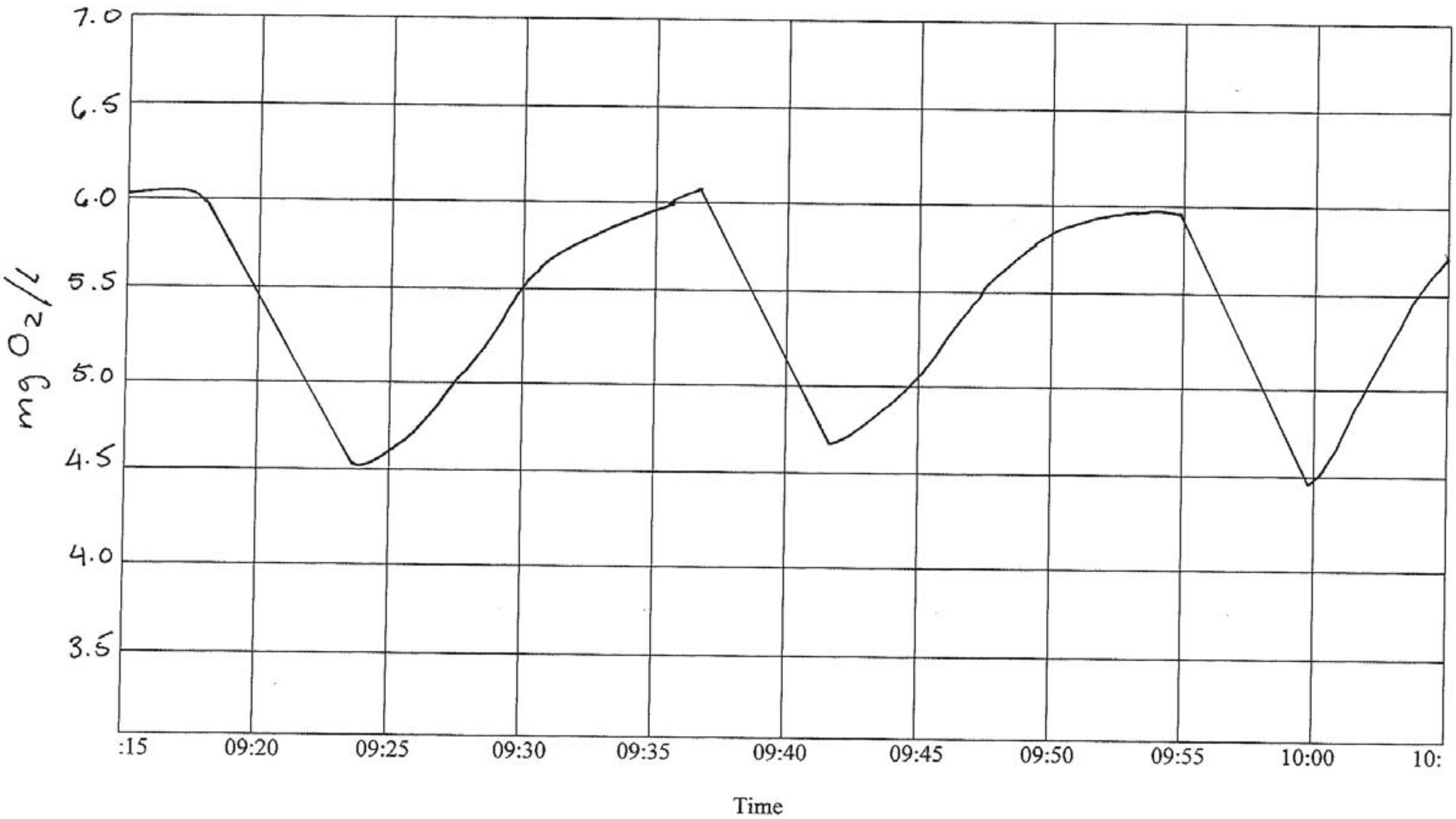


Mass : 2.1
Tank volume : 42.8

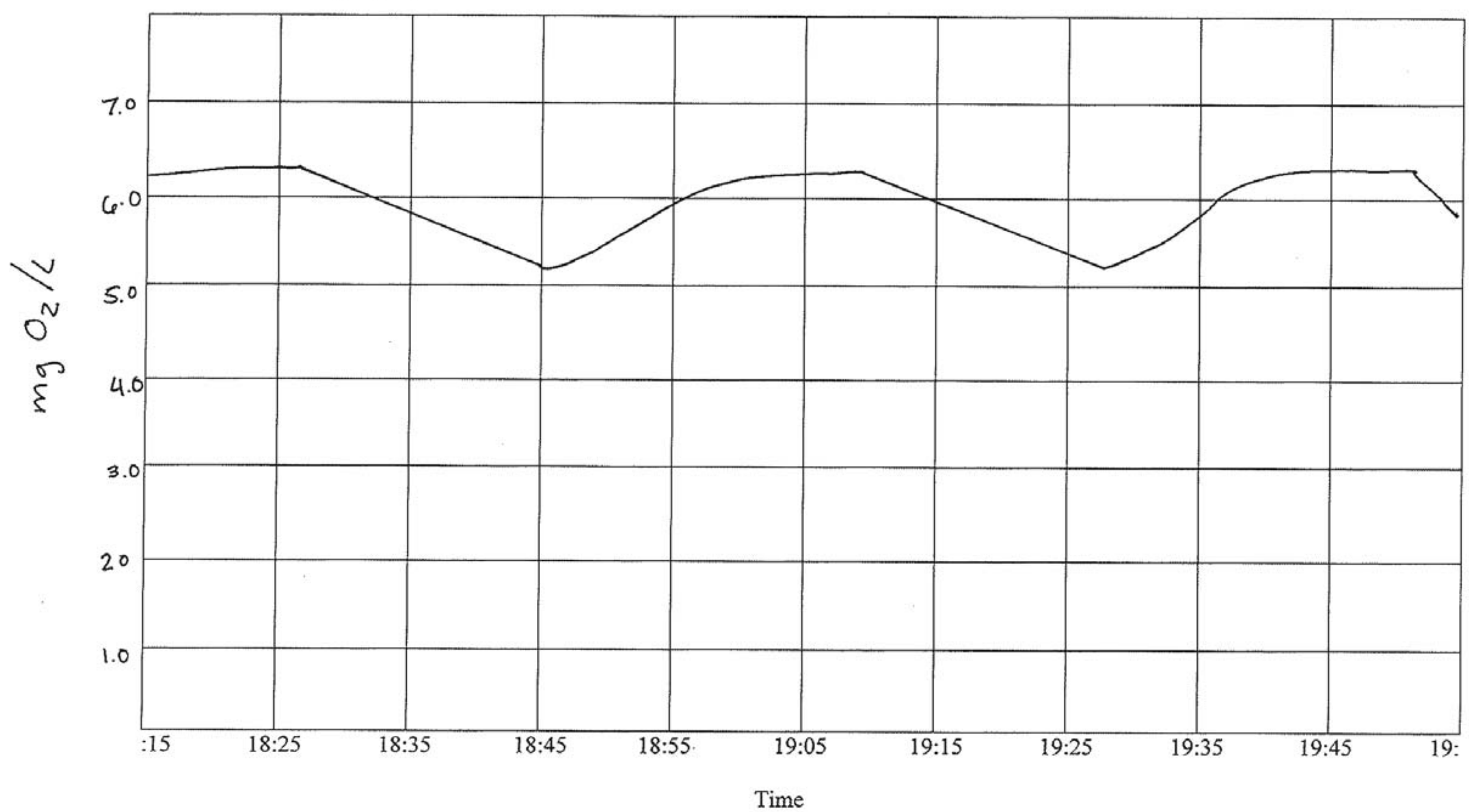
Animal E



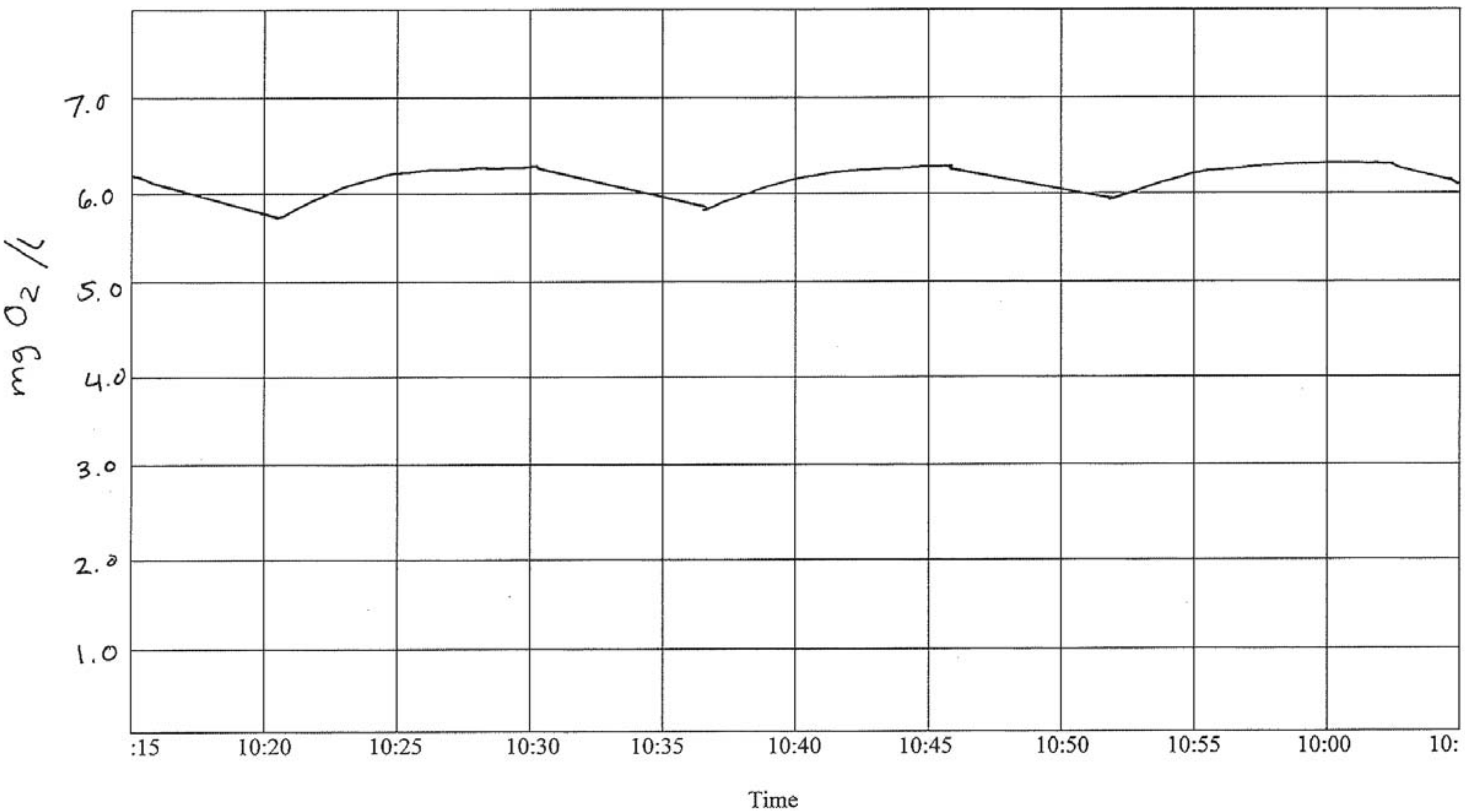
Animal F
Mass: 4.2
Tank Volume: 42.8



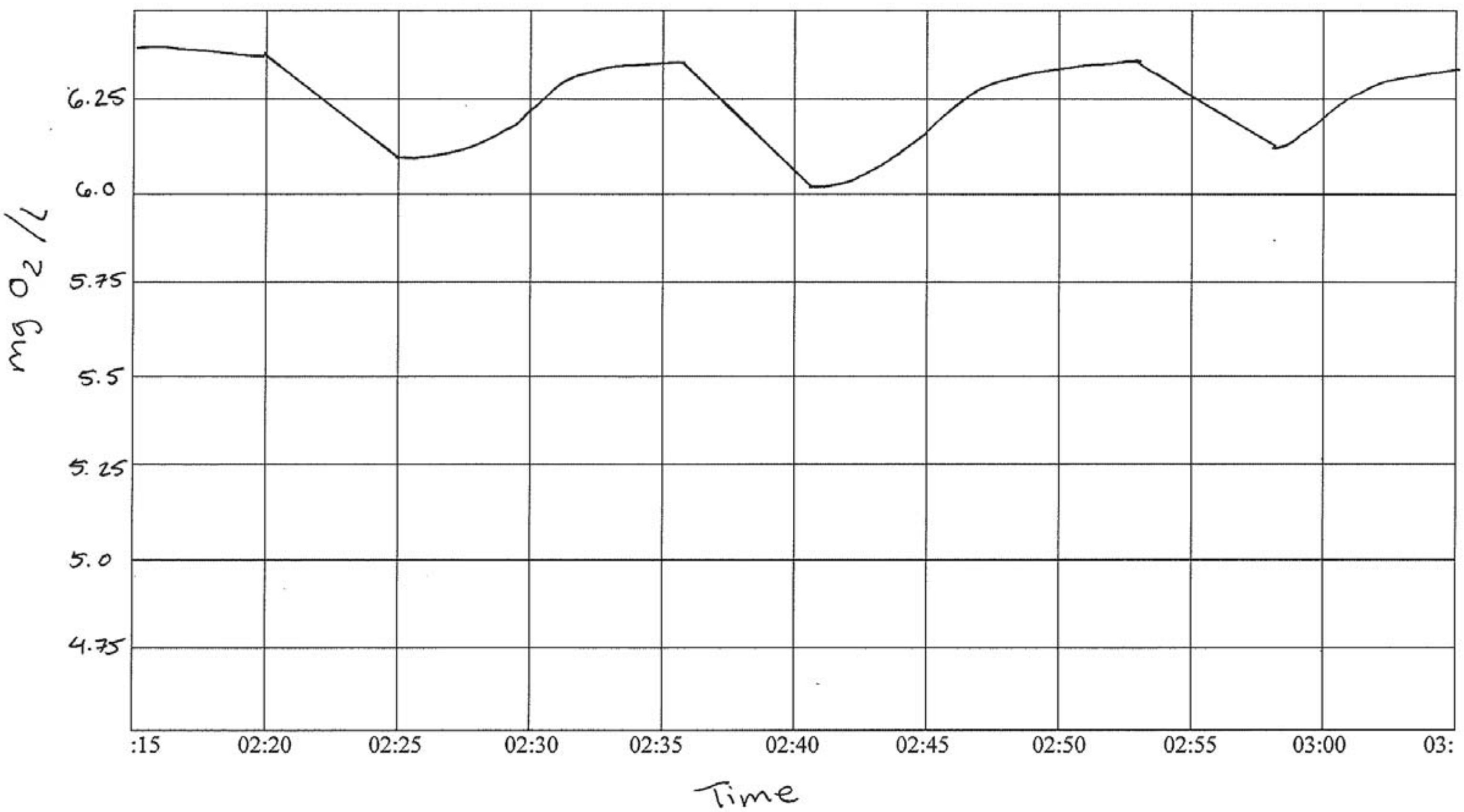
Animal G
Mass: 1.5 kg
Tank Volume: 36 liters



Animal H
Mass: 1.4 Kg
Tank Volume: 36 liters



Animal I
Mass: 0.8 kg
Tank Volume: 36 liters



Appendix D. Instructions for calculating Oxygen Consumption Rates

Calculating the rate of oxygen consumption:

$$\boldsymbol{MO_2} = \frac{\frac{\boldsymbol{O_{2(start)} - O_{2(end)}}}{\boldsymbol{T}} * \boldsymbol{V}}{\boldsymbol{W}}$$

where MO_2= is the rate of oxygen consumption (mg O_2/kg/hr), O_2= the readings at the beginning and end of the measurement period (mg O_2/l), T= time (h), V = respirometer volume (l), and W = weight of the fish (kg).

When calculating oxygen consumption, your group must decide how to calculate it. Is one period enough? Should it be averaged over multiple periods? Does the rate change over time? How should you account for that? Will you calculate it differently based on what question you are trying to answer?

Record the method your group decided upon, and present it to your boss (teacher) for approval before proceeding.

Appendix E. Metabolic Rates for Each Given Graph

Teacher Cheat Sheet

FYI These are made up data, but are similar to results that me or my colleagues have obtained. Animals A-C are representative of sandbar sharks. Sandbars are ram-ventilators, so they need to be constantly swimming in order to breathe. The data for animals D-F are representative of smooth dogfish, and animals G-I are representative of clearnose skates. Examples of answers are below, but students may get different values based on their estimates for start/stop oxygen concentration values and elapsed time.

A: 903.6 mgO_2/kg/h

Ex: $\boldsymbol{MO_2} = \frac{\frac{O_{2(start)} - O_{2(end)}}{T} * V}{W} = \boldsymbol{MO_2} = \frac{\frac{6.0-5.25}{(\frac{60}{5})} * 500}{5}$

Here the starting oxygen content was 6.0, the ending oxygen content was ~5.25. The time should be in hours, so I calculated what fraction of one hour 5 minutes is. The mass of the animal (5) and the volume of the chamber (500) is given at the top of each graph.

B: 792.6 mgO_2/kg/h

C: 1139.8 mgO_2/kg/h

D: 139.9 mgO_2/kg/h

E: 119.9 mgO_2/kg/h

F: 184.2 mgO_2/kg/h

G: 99.2 mgO_2/kg/h

H: 62.0 mgO_2/kg/h

I: 135.5 mgO_2/kg/h